SPRINGER NATURE
Reference

Mukteswar Prasad Mothadaka
Murugadas Vaiyapuri
Madhusudana Rao Badireddy
Chandragiri Nagarajrao Ravishankar
Rajesh Bhatia
Joykrushna Jena
Editors

Handbook on Antimicrobial Resistance

Current Status, Trends in Detection and Mitigation Measures

Springer

Handbook on Antimicrobial Resistance

Mukteswar Prasad Mothadaka •
Murugadas Vaiyapuri •
Madhusudana Rao Badireddy •
Chandragiri Nagarajrao Ravishankar •
Rajesh Bhatia •
Joykrushna Jena
Editors

Handbook on Antimicrobial Resistance

Current Status, Trends in Detection and Mitigation Measures

With 76 Figures and 100 Tables

Springer

Editors
Mukteswar Prasad Mothadaka
ICAR-Central Institute of Fisheries Technology
Kochi, Kerala, India

Murugadas Vaiyapuri
ICAR-Central Institute of Fisheries Technology
Kochi, Kerala, India

Madhusudana Rao Badireddy
Visakhapatnam Research Centre
ICAR-Central Institute of Fisheries Technology
Visakhapatnam, Andhra Pradesh, India

Chandragiri Nagarajrao Ravishankar
ICAR-Central Institute of Fisheries Education
Mumbai, Maharashtra, India

Rajesh Bhatia
Food and Agriculture Organization
New Delhi, India

Joykrushna Jena
Indian Council of Agricultural Research
New Delhi, India

ISBN 978-981-19-9278-0 ISBN 978-981-19-9279-7 (eBook)
https://doi.org/10.1007/978-981-19-9279-7

This Springer imprint is published by the registered company Springer Nature Singapore Pte Ltd.
The registered company address is: 152 Beach Road, #21-01/04 Gateway East, Singapore 189721, Singapore

Foreword

The incidence of antimicrobial resistance (AMR) takes place when bacteria, viruses, fungi, and parasites are unresponsive to antimicrobial agents. The development of resistance to antibiotic, antifungal, antiviral, and antiparasitic drugs poses immense threat to human health and food security across the world. AMR results in infections unmanageable to treat, enhanced hazards in disease transmission, and severe sickness and fatalities in humans and animals. The impact of AMR on the farming of terrestrial animals, aquatic animals, and crops is severely threatening global food security.

As on today, the AMR-related mortalities worldwide is estimated to be 1.3 million per annum. In the absence of stringent measures to contain this scourge, it can cause incalculable financial losses, especially the economically underprivileged nations face serious indentation in their GDP to such an extent that tens of millions of populations can be pushed into abysmal poverty. The Food and Agriculture Organization of the United Nations (FAO), the UN Environment Programme (UNEP), the World Health Organization (WHO), and the World Organisation for Animal Health (WOAH), known as the Quadripartite, have joined forces to address the threat posed by AMR to humans, animals, plants, ecosystems and livelihoods.

We are left with unit time of 7 years to reach the destination of 17 Sustainable Development Goals (SDGs) of United Nations by 2030. Apart from the challenges of climate change and pandemics, AMR is another impediment to reckon with as it adversely impacts 12 of the 17 SDGs. At this juncture, understanding the basics and intricacies of AMR is the need of the hour. Our journey of final destination of containing the global threat of AMR looks arduous and we have a long way to go. In this context, it is noteworthy to mention that the Indian Council of Agriculture Research (ICAR), India, too is contributing to the global fight against AMR in collaboration with major international organizations.

The task of containing AMR is cutout for everyone on the planet, and the dire need is for a totalizer for scholars, students, academicians, and researchers planning to start work on AMR as none of the books available dealt AMR across the sectors. Taking this cue, editors with vast experience and expertise in the field of AMR research took an onerous task of bringing out a Major Reference Work *Handbook on Antimicrobial Resistance: Current Status, Trends in Detection and Mitigation Measures*.

The distinctiveness of this handbook is exploring AMR across the sectors and facilitating alleviation stratagems. The subjects encompass in this academic endeavor included Antimicrobial Resistance, Aquaculture, Fisheries, Animal Science, Human Health, Environment, and Mitigation Strategies. The handbook provides information not only to the targeted stakeholders but also to persons of all walks of life on antimicrobial resistance and its control. This MRW facilitates opening of new vistas for research that will be a frame of reference for academicians and policy makers.

The editors offered the MRW in 50 chapters and as 5 diverse and evocative sections. The primary part of the handbook offers an understanding of AMR across sectors, viz. human health, terrestrial animals, aquatic animals, and food and environment sectors. The second section centered on antimicrobials application in human health care and faunae of food source. The third portion delivers deep discernments on modes of action of drug obduracy in microbial pathogens. The fourth section defines the drivers of AMR and gives an overview of recent trends in detection and characterization of AMR pathogens. The quinary probes the mitigation measures across the sectors in dealing the menace of AMR with stress on increased responsiveness of antibiotic literacy. The aim is enhanced levels of human-centric investment than on finances to reach the destination of world with minimum burden of AMR. In this book, it is very well portrayed that each sector must contribute in unison in the form of "One Health Approach" in containing the threat of antimicrobial resistance.

I wholeheartedly congratulate the entire team involved in the publication of the *Handbook on Antimicrobial Resistance: Current Status, Trends in Detection and Mitigation Measures*.

Secretary (DARE) & Director General (ICAR) Dr. Himanshu Pathak
Ministry of Agriculture and Farmers Welfare
New Delhi
India

Preface

The antimicrobial resistance (AMR) is transcending all forms of life that include humans, animals, and environments sans consideration for class, race, and gender. This is recognized as the greatest challenge to the humanity's battle against infectious diseases. AMR has the potential to negate contemporary advances in health technologies for humans and animals. AMR impacts several Sustainable Development Goals (SDGs) that have been adopted by all the United Nations member states and thereby poses huge threat to global peace, development, and prosperity. The SDGs, viz. no poverty (SDG 1), no hunger (SDG 2), good health and wellbeing (SDG 3), quality education (SDG 4), clean water and sanitation (SDG 6), decent work and economic growth (SDG 8), industry, innovation, and infrastructure (SDG 9), reduced inequalities (SDG 10), sustainable cities and communities (SDG 11), responsible consumption and production (SDG 12), life below water (SDG 14), and partnerships for goals (SDG 17), are negatively affected, either directly or indirectly by AMR.

A small SARS-CoV-2 virus caused a pandemic that has affected the entire world. Apart from causing substantial mortality, morbidity, economic loss, and social chaos, the pandemic saw extensive misuse of antibiotics. Trends worsened further during the treatment due to unwanted application of antibiotics as demonstrated by the fact that 70–80% of hospitalized COVID-19 patients in the USA received antibiotic treatment despite the fact that only <10% had secondary bacterial infections. Likewise, in Italy, of those who were administered antibiotics, only 12% had secondary bacterial infection. Unlike COVID-19 and other pandemics that draw significant attention, the AMR is an unfelt tormentor.

AMR affects the health and wellbeing of people, including newborns, as AMR bacteria were responsible for 1.27 million deaths in 204 countries and territories in 2019 and is estimated that 300 million people would die prematurely because of drug resistance by 2050. In India alone, 58,000 babies died due to infection with resistant pathogens. The world is expected to incur an economic loss of USD 100 trillion by 2050 if AMR is not addressed by the global fraternity, severely compromising the fight against poverty by low- and middle-income countries (LMICs). AMR has cost the European Union more than € 1.5 billion per year in healthcare expenses and productivity losses. It is estimated that antimicrobial use in food animal production would increase by 70% by 2030 especially in BRICS nations, namely Brazil, Russia,

India, China, and South Africa. Moreover, 73% of the antibiotics used in human medicine are also used in animal agriculture. Overuse, misuse, or improper use of antibiotics in animal agriculture leads to the emergence of AMR in animal pathogens and threatens food security leading to hunger issues. The increase in per capita income drives the consumer demand for terrestrial and aquatic animal meats that might drive the use of antibiotics in commercial animal agriculture.

Concerted efforts are needed to prevent contamination of potable water resources with pharmaceutical wastes and drug-resistant microorganisms. Developing a new antibiotic is a long drawn process, and industry needs to incur an expenditure of over €850 million to bring a new drug to the market. In this regard, stewardship in antibiotic prescription by the clinicians and veterinarians, especially in LMICs, is essential to prevent the emergence of spread of AMR. Educating the general public and school-going children on the implications of uncontrolled AMR on human health and food security is vital. Research and innovations to develop alternatives to antibiotics for use in agriculture is the need of the hour to preserve all the critical antibiotics for human health care. Inaction against AMR jeopardizes human and animal health and takes us back to the pre-antibiotic era making minor operations life threatening. New and strong partnerships are required to regulate the use of existing antimicrobial drugs and innovate new drugs to ensure human health and assure global prosperity.

Antimicrobial resistance (AMR) is not problem specific to any single sector. The menace is felt across sectors, viz. human health, animal, agriculture, food, and environment. This signifies the requirement of inter-disciplinary approach, i.e., the one health approach envisaged by WHO-FAO-WOAH. A strong need was felt for a ready reckoner for students and academicians for thorough understanding of AMR across the sectors, bestowing deep insights and global picture on AMR for policy makers. The need was also to offer substantial information that enables anyone to initiate the research on AMR in respective sectors. Further, it needs to throw deep insight on molecular mechanisms and antibiotic resistance genes of very important AMR pathogens, regulatory frameworks available across the globe, mitigation strategies across the sectors which includes probiotics, prebiotics, antimicrobial peptides, bacteriophages, phytochemical compounds, immunostimulants, vaccines, bacteriocins, CRISPR, etc., and their pros and cons of employment in each sector.

The book shall cater to the needs that purposes as an actuary for researchers who are new to the field of AMR. In this regard, books are available on AMR, albeit there is no single book that comprehends the AMR across sectors in one health context, current methodologies to detect AMR, and mitigation strategies to control AMR across the sectors. Regulatory frameworks and other aspects in the context of AMR are also necessitated.

Keeping in mind all the necessitates, a book proposal was made way back in June 2020 to Springer Nature with an intended completion of manuscript by 31.12.2020 as a book of 300–400 pages. However, gradually it has transformed into voluminous major reference work (MRW) of 1000 pages with 50 chapters as *Handbook on Antimicrobial Resistance* encompassing all aspects of AMR that is useful to both academics and administrators. All six editors of this MRW have varied experience

and expertise in AMR and affiliated fields ranging from a decade to near four decades. It is important to note that all the chapters of the book are confined to antibacterial resistance only.

The present MRW has chapters authored by interdisciplinary experts from human health, terrestrial animal health, aquatic animal health, food, and environmental sectors. The book provides substantial information on AMR that enables anyone to initiate research on AMR in human health, animal agriculture, food, and environment sectors.

This MRW is intended for workers from human health, animal health, food, and environment. They range from students, academicians, and researchers planning to start work on AMR. This book is also ready reckoner for policy makers for devising extenuation strategies as this aspect too dealt at length. Uniqueness of this document is understanding AMR at the core of all sectors and designing mitigation strategies. None of the books available in all domains have AMR description across various sectors. The subjects encompassed in this academic endeavor includes antimicrobial resistance, aquaculture, fisheries, animal science, human health, environment, and mitigation strategies. This MRW will open new vistas for research and will be a frame of reference for policy makers and the public to be aware.

This MRW is presented as five distinct and meaningful sections. The first section provides an understanding of AMR across different sectors such as human health, terrestrial animals, aquatic animals, food, and environment sectors. The second section focusses on antimicrobial use in human health care and food-producing animals. The third section provides deep insights on the resistance mechanisms in pathogens. The fourth section delineates the drivers of AMR and gives an overview of recent trends in detection and characterization of AMR pathogens. The last section delves on the mitigation measures across the sectors in tackling the menace of AMR with an emphasis on increased awareness namely antibiotic literacy. The aim is enhanced levels of human-centric investment than on finances to get desired results of world with lessened burden of AMR.

The editors are thankful for all the specialists and experts in the related fields for their invaluable contributions in the form of chapters. They are hopeful that this reference work will be of use in mitigating the impact of AMR through better understanding of this complex and multisectoral challenge.

Kochi, India — Mukteswar Prasad Mothadaka
Kochi, India — Murugadas Vaiyapuri
Visakhapatnam, India — Madhusudana Rao Badireddy
Mumbai, India — Chandragiri Nagarajrao Ravishankar
New Delhi, India — Rajesh Bhatia
New Delhi, India — Joykrushna Jena
July 2023

Contents

About the Editors

Dr. Mukteswar Prasad Mothadaka is Emeritus Scientist, former Principal Scientist, and Head of Microbiology, Fermentation and Biotechnology, Central Institute of Fisheries Technology (CIFT), under Indian Council of Agricultural Research, Ministry of Agriculture and Farmers Welfare, Govt. of India. He did his MSc and PhD in Microbiology with vast experience of 36 years on human health hazard bacteria and their control in fish and fishery products and initiated flagship programs on AMR. Developed, designed, installed, and popularized community fish smoking kilns in North Eastern states of India. Transferred many a technologies on processing, hygiene and microbial safety of fish and fishery products benefiting millions of fishers across the country. He has a total of 326 publications that included 10 Books, 134 peer-reviewed national and international research papers, 10 conference proceedings, 3 training manuals, 17 reports, 27 training manual chapters, 12 popular articles, and 113 abstracts. Recognized as guide for postgraduate and doctoral programmes in three nationally important universities. He has provided research guidance to 17 students for PhD, MPhil, and Master degree programs. Reviewer for many peer-reviewed international and national journals. Served as member of Research Advisory Committee of ICAR-CIFRI, Chaired ICAR-CIFT Institute Biosafety Committee, and served as member of Animal Ethics Committee of ICAR-CIFT. Presently serving as member of National Expert Scientific Panel on Fish and Fishery Products Committee and as subject matter specialist in different advanced selection programs and technical committees of central and state governmental

organizations. Carried out outstanding works in all of eight north Eastern states of India for 20 years by undertaking field studies, conducting National Training Programs, to students at all levels, NGOs, and governmental agencies. Elected Fellow of Society of Fisheries Technologists of India and Life Member for seven national scientific organizations. Travelled extensively while participating in national and international seminar/symposia and workshops. He is actively involved in NAP AMR (National Action Plan of AMR, India), INFAAR (Indian Network for Fisheries and Animal Antimicrobial Resistance), KARSAP (Kerala Antimicrobial Resistance Strategic Action Plan), and APAPCAR (Andhra Pradesh Action Plan for Containment of Antimicrobial Resistance).

Dr. Murugadas Vaiyapuri working as Senior Scientist at Microbiology, Fermentation and Biotechnology Division, ICAR-Central Institute of Fisheries Technology. He did his BVSC and MVSC in Veterinary Microbiology and PhD in Biotechnology and has experience in seafood and aquaculture safety since 2010. He has expertise in the microbiological examination of seafood for safety and spoilage, antimicrobial resistant (AMR) pathogen isolation, molecular characterization, and resistant genes. Specifically worked in the methicillin-resistant *Staphylococcus aureus* and extended spectrum beta lactamase-producing *E. coli*. Since 2018 December, he has been working on the assessment of Vembanad and Sasthamkotta Lake for AMR in India. Involved in the testing of fish and fishery products for OIE-listed pathogens. Internal auditor and assessor as per ISO 17025: 2017 for biological testing as per NABL. He is a Co-investigator of INFAAR and KARSAP projects of AMR. Member of the Institutional Biosafety Committee since 2017 and Institutional Animal Ethics Committee member since 2019. He has more than 40 peer-reviewed publications to his credit. He has presented more than 50 abstracts in the national and international conferences. Published 1 book chapter and 15 training manual chapters for various programs. Ad-hoc reviewer for seven international journals and four national journals.

Dr. Madhusudana Rao Badireddy presently works as Principal Scientist at the Visakhapatnam Research Centre of ICAR-Central Institute of Fisheries Technology (ICAR-CIFT). He did MVSc in Veterinary Microbiology and PhD in Microbiology and has over 25 years of experience in the research areas of antimicrobial resistance (AMR) in bacteria, molecular characterization of pathogenic bacteria, bioprospecting of aquatic microbial resources, use of bacteriophages as natural antimicrobials, and innovative fish processing technologies. He has several credits to his research output. He has published 62 research papers in international and national peer reviewed journals, 50 popular articles, 10 book chapters, 15 training manuals, several extension pamphlets, brochures, edited 10 books, and made more than 100 presentations in various seminars, workshops, and other fora. He has completed 22 projects as principal investigator/co-investigator. He received five awards during his academic and research career.

Dr. Chandragiri Nagarajrao Ravishankar is presently Director and Vice Chancellor, ICAR-Central Institute of Fisheries Education (CIFE), under Indian Council of Agricultural Research, Ministry of Agriculture and Farmers Welfare, Govt. of India, and has 33 years of contribution to fisheries sector. He completed his Bachelor degree in Fisheries Sciences, postgraduation in Industrial Fisheries Technology, and PhD in Fish Processing Technology from the University of Agricultural Sciences, Bangalore. He has participated in the First Indian Antarctic Krill Expedition. He has developed, popularized, and transferred many processing and packaging technologies to the food processing industries and established India's first Business Incubation Centre in the field of Fisheries for the benefit of entrepreneurs. He has more than 260 publications to his credit which includes international and national research papers; authored 7 books and guided 38 students for doctoral and Master degree programs. He is representing as subject expert in many technical committees of central and state governmental organizations. He is the elected Fellow of National Academy of Agricultural Sciences, New Delhi, Association of Food Scientist and Technologists, and Society of Fisheries

Technologists and has received many awards for excellence in the field of fisheries science and technology. He is a reviewer for many peer-reviewed international and national journals and also recognized as guide for post-graduate and doctoral studies in many universities. He delivered number of talks as an invited speaker for several international organizations.

Dr. Rajesh Bhatia is specialized in communicable diseases and is International Consultant with FAO on AMR and with ADB on Public Health. He is the former Director of Communicable Diseases, WHO Regional Office for South-East Asia Region and Regional pivotal theme for AMR (2001–2015) and is currently working with FAO (2017–till date) as Regional Consultant on AMR. Earlier, for 15 years, he worked at the National Centre for Disease Control and as national focal point for AMR. He has authored several books and 145 scientific publications in peer-reviewed journals, of which 40 are on AMR. Dr. Bhatia has edited special issues on AMR of *Indian Journal of Medical Research* (IJMR) and *Regional Health Forum* of WHO. He contributed to development of WHO Global Action Plan on AMR, National AMR Action Plans for India, Jordan, Egypt, and Palestine, and Indian Network for Fisheries and Animal Antimicrobial Resistance. He has been awarded Gold Medals for his contributions by Indian Vaccinology Association (1993), Indian Society for Malaria and Other Communicable Diseases (2012), and Delhi Chapter of IAMM (2018)

Dr. Joykrushna Jena is currently serving as Deputy Director General (Fisheries Science), Indian Council of Agricultural Research, Ministry of Agriculture and Farmers Welfare, Govt. of India. Prior to this, he served as the Director of the ICAR-National Bureau of Fish Genetic Resources, Lucknow, India, for over 5 years. Dr. Jena possesses over 30 years of research experience in different fields of freshwater aquaculture and conservation genetics. Working in different aspects of freshwater aquaculture at the ICAR-Central Institute of Freshwater Aquaculture, Bhubaneswar, India, during his initial career in different capacities, including as National Fellow and Head of the Division, Dr. Jena

has contributed to the development of several technologies and research findings, including breeding, seed production and farming of diversified carp species, multiple cropping of carps, intensive carp culture, treatment of domestic sewage through aquaculture, cryopreservation of fish milt, and applied nutrition. He has operated over 20 research projects, including 10 externally funded projects, as Principal Investigator. As National Coordinator, presently he is operating the National Surveillance Programme for Aquatic Animal Diseases (NSPAAD) in India with the involvement of over 30 partner organizations. He is also leading the Indian Network of Fisheries and Animal Antimicrobial Resistance (INFAAR) of ICAR for the control of AMR in livestock and fisheries sectors in India. Dr. Jena has published over 200 research papers in national and international journals of repute, besides several books and other publications. He is also serving as the Chairman/Member of several national and international level committees.

Contributors

R. Ambily Department of Veterinary Microbiology, College of Veterinary and Animal Sciences, Mannuthy, Thrissur, Kerala, India

P. Amruth Biochemistry and Nutrition Division, ICAR-Central Institute of Fisheries Technology, Cochin, India

P. Anand Kumar Department of Veterinary Microbiology, NTR College of Veterinary Science, Sri Venkateswara Veterinary University, Gannavaram, Andhra Pradesh, India

K. Asha Department of Veterinary Public Health, KVASU, Pookode, Wayanad, Kerala, India

Till Bachmann Infection Medicine, Edinburgh Medical School: Biomedical Sciences, The University of Edinburgh, Edinburgh, UK

Madhusudana Rao Badireddy Visakhapatnam Research Centre of ICAR-CIFT, ICAR-Central Institute of Fisheries Technology (ICAR-CIFT), Visakhapatnam, Andhra Pradesh, India

Yamuna Devi Bakthavatchalam Department of Clinical Microbiology, Christian Medical College, Vellore, Tamil Nadu, India

Sarita G. Bhat Department of Biotechnology, Cochin University of Science and Technology, Cochin University, Cochin, Kerala, India

Rajesh Bhatia Communicable Diseases, WHO Regional Office for South East Asia, New Delhi, India

Niladri Sekhar Chatterjee ICAR-Central Institute of Fisheries Technology, Kochi, Kerala, India

Goutam Chowdhury Division of Bacteriology, ICMR-National Institute of Cholera and Enteric Diseases, Kolkata, West Bengal, India

Pavan Kumar Dara Biochemistry and Nutrition Division, ICAR-Central Institute of Fisheries Technology, Kochi, India

P. S. Divya Department of Marine Biology, Microbiology and Biochemistry, School of Marine Sciences, Cochin University of Science and Technology, Cochin, Kerala, India

Shanta Dutta Division of Bacteriology, ICMR-National Institute of Cholera and Enteric Diseases, Kolkata, West Bengal, India

Paula J. Fedorka-Cray Population Health and Pathobiology Department at the College of Veterinary Medicine, North Carolina State University, Raleigh, NC, USA

Sumithra T. G. Marine Biotechnology, Fish Nutrition and Health Division, Fish Health Section, ICAR-Central Marine Fisheries Research Institute, Kochi, Kerala, India

Vikas Galav Department of Veterinary Pathology, Post Graduate Institute of Veterinary Education and Research (PGIVER-RAJUVAS), Jaipur, India

Prasannan Geetha Preena Peninsular and Marine Fish Genetic Resources Centre, ICAR National Bureau of Fish Genetic Resources, CMFRI Campus, Kochi, Kerala, India

S. S. Greeshma Microbiology Fermentation and Biotechnology Division, ICAR-Central Institute of Fisheries Technology (ICAR-CIFT), Kochi, Kerala, India

Mumbai Research Centre, ICAR-Central Institute of Fisheries Technology, Mumbai, India

Renu Gupta Microbiology, Institute of Human Behaviour and Allied Sciences, New Delhi, India

Muneeb Hamza Microbiology, Fermentation and Biotechnology Division, ICAR-Central Institute of Fisheries Technology, Cochin, Kerala, India

Faculty of Sciences, Cochin University of Science and Technology, Cochin, Kerala, India

Ziming Han State Key Laboratory of Environmental Aquatic Chemistry, Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences, Beijing, China

University of Chinese Academy of Sciences, Beijing, China

National Engineering Research Center of Industrial Wastewater Detoxication and Resource Recovery, Beijing, China

Nagendra R. Hegde National Institute of Animal Biotechnology, Hyderabad, India

Rosemol Jacob M. Biochemistry and Nutrition Division, ICAR-Central Institute of Fisheries Technology, Cochin, India

Joykrushna Jena Division of Fisheries Science, Indian Council of Agricultural Research (ICAR), New Delhi, India

Toms C. Joseph Microbiology Fermentation and Biotechnology Division, ICAR-Central Institute of Fisheries Technology (ICAR-CIFT), Kochi, Kerala, India

Jyoti Kayesth Infectious Diseases Detection and Surveillance, ICF, Delhi, India

Shivaramu Keelara Population Health and Pathobiology Department at the College of Veterinary Medicine, North Carolina State University, Raleigh, NC, USA

Pankaj Kishore ICAR-Central Institute of Fisheries Technology, Cochin, Kerala, India

Abhay Kumar Microbiology, Fermentation and Biotechnology Division, ICAR-Central Institute of Fisheries Technology (ICAR-CIFT), Cochin, Kerala, India

Mumbai Research Centre of CIFT, Vashi, Navi Mumbai, India

Sanath H. Kumar Post-Harvest Technology, ICAR-Central Institute of Fisheries Education, Andheri (W), Mumbai, India

Arumugam Kumaresan Southern Regional Station, ICAR-National Dairy Research Institute, Adugodi, Bengaluru, Karnataka, India

Ahamed Basha Kusunur Visakhapatnam Research Centre of ICAR-Central Institute of Fisheries Technology (ICAR-CIFT), Visakhapatnam, Andhra Pradesh, India

Kuttanappilly Velayudham Lalitha Microbiology Fermentation and Biotechnology Division, Central Institute of Fisheries Technology, Cochin, Kerala, India

Manjusha Lekshmi Post-Harvest Technology, ICAR-Central Institute of Fisheries Education, Andheri (W), Mumbai, India

Ayyasamy Manimaran Southern Regional Station, ICAR-National Dairy Research Institute, Adugodi, Bengaluru, Karnataka, India

Suseela Mathew Biochemistry and Nutrition Division, ICAR-Central Institute of Fisheries Technology, Cochin, India

M. Mini Department of Veterinary Microbiology, College of Veterinary and Animal Sciences, Mannuthy, Thrissur, Kerala, India

V. A. Minimol Microbiology, Fermentation and Biotechnology Division, ICAR-Central Institute of Fisheries Technology (ICAR-CIFT), Cochin, Kerala, India

A. A. Mohamed Hatha Department of Marine Biology, Microbiology and Biochemistry, School of Marine Sciences, Cochin University of Science and Technology, Cochin, Kerala, India

Mukteswar Prasad Mothadaka Visakhapatnam Research Centre of ICAR-CIFT, ICAR-Central Institute of Fisheries Technology (ICAR-CIFT), Visakhapatnam, Andhra Pradesh, India

Microbiology, Fermentation and Biotechnology Division, ICAR-Central Institute of Fisheries Technology (ICAR-CIFT), Cochin, Kerala, India

Susmita Mukherjee Fish Processing Technology, Faculty of Fisheries Sciences, West Bengal University of Animal and Fishery Sciences, Kolkata, India

Asish Kumar Mukhopadhyay Division of Bacteriology, ICMR-National Institute of Cholera and Enteric Diseases, Kolkata, West Bengal, India

Dhiviya Prabaa Muthuirulandi Sethuvel Department of Clinical Microbiology, Christian Medical College, Vellore, Tamil Nadu, India

T. Muthulakshmi MFB Division, ICAR-Central Institute of Fisheries Technology, Willingdon Island, Cochin, Kerala, India

Ranjit Kumar Nadella MFB Division, ICAR-Central Institute of Fisheries Technology, Willingdon Island, Cochin, Kerala, India

Prejit Nambiar Centre for One Health Education, Advocacy, Research and Training (COHEART), KVASU, Pookode, Wayanad, Kerala, India

Sreejith Viyyokkaran Narayanan Microbiology Fermentation and Biotechnology Division, Central Institute of Fisheries Technology, Cochin, Kerala, India

Lekshmi Narendrakumar Peninsular and Marine Fish Genetic Resources Centre, ICAR National Bureau of Fish Genetic Resources, CMFRI Campus, Kochi, Kerala, India

Binaya Bhusan Nayak Post-Harvest Technology, ICAR-Central Institute of Fisheries Education, Andheri (W), Mumbai, India

Shivasharanappa Nayakvadi ICAR-National Institute of Veterinary Epidemiology and Disease Informatics (NIVEDI), Bengaluru, India

Ezhil S. Nilavan Microbiology, Fermentation and Biotechnology, ICAR-Central Institute of Fisheries Technology (ICAR-CIFT), Cochin, Kerala, India

Kerala University of Fisheries and Ocean Studies, Cochin, Kerala, India

Subhendu Kumar Otta ICAR-Central Institute of Brackishwater Aquaculture, Chennai, India

Satyen Kumar Panda ICAR-Central Institute of Fisheries Technology, Kochi, Kerala, India

Shaheer Peeralil Microbiology Fermentation and Biotechnology Division, Central Institute of Fisheries Technology, Cochin, Kerala, India

Devika Pillai Kerala University of Fisheries and Ocean Studies, Panangad, Kerala, India

Anna Sherin Pulithara Sebastian Microbiology Fermentation and Biotechnology Division of ICAR-Central Institute of Fisheries Technology (ICAR-CIFT), Cochin, Kerala, India

Thandavarayan Ramamurthy Division of Bacteriology, ICMR-National Institute of Cholera and Enteric Diseases, Kolkata, West Bengal, India

Kalaivanan Ramya Department of Veterinary Microbiology, Veterinary College and Research Institute, Namakkal, Tamil Nadu, India

Anandan Rangasamy Biochemistry and Nutrition Division, ICAR-Central Institute of Fisheries Technology, Kochi, India

G. S. Rao Department of Veterinary Pharmacology & Toxicology, NTR College of Veterinary Science, Sri Venkateswara Veterinary University, Gannavaram, Andhra Pradesh, India

Pushpendra Singh Rathore Department of Veterinary Microbiology and Biotechnology, Post Graduate Institute of Veterinary Education and Research (PGIVER-RAJUVAS), Jaipur, India

Karthika Raveendran Microbiology, Fermentation and Biotechnology Division, ICAR-Central Institute of Fisheries Technology, Cochin, India

Chandragiri Nagarajarao Ravishankar ICAR-Central Institute of Fisheries Technology (ICAR-CIFT), Kochi, Kerala, India

ICAR-Central Institute of Fisheries Education (ICAR-CIFE), Maharashtra, Mumbai, India

Aravind Reghukumar Department of Infectious Diseases, Govt. Medical College, Thiruvananthapuram, India

S. Remya Microbiology Fermentation and Biotechnology Division, ICAR-Central Institute of Fisheries Technology (ICAR-CIFT), Kochi, Kerala, India

Krupesha Sharma S. R. Marine Biotechnology, Fish Nutrition and Health Division, Fish Health Section, ICAR-Central Marine Fisheries Research Institute, Kochi, Kerala, India

Sanjeev Saini Infectious Diseases Detection and Surveillance, ICF, Delhi, India

Devi Sanjeev Microbiology, Fermentation and Biotechnology Division, ICAR-Central Institute of Fisheries Technology (ICAR-CIFT), Cochin, Kerala, India

Palanisamy Sankar Department of Veterinary Pharmacology and Toxicology, Veterinary College and Research Institute, Namakkal, Tamil Nadu, India

T. V. Sankar ICAR-Central Institute of Fisheries Technology, Cochin, Kerala, India

Veerasamy Sejian ICAR-National Institute of Animal Nutrition and Physiology, Bengaluru, Karnataka, India

Sandeep Kumar Sharma Department of Veterinary Microbiology and Biotechnology, Post Graduate Institute of Veterinary Education and Research (PGIVER-RAJUVAS), Jaipur, India

Sangeeta Sharma Neuropsychopharmacology, Institute of Human Behaviour and Allied Sciences, Delhi, India

B. R. Shome ICAR-National Institute of Veterinary Epidemiology and Disease Informatics (NIVEDI), Bengaluru, India

Welile Sikhondze Infectious Diseases Detection and Surveillance, ICF, Delhi, India

Reshma Silvester School of Industrial Fisheries, Cochin University of Science and Technology, Cochin, Kerala, India

Visnuvinayagam Sivam Microbiology, Fermentation and Biotechnology Division, ICAR-Central Institute of Fisheries Technology (ICAR-CIFT), Kochi, India

G. K. Sivaraman Microbiology, Fermentation and Biotechnology Division, ICAR-Central Institute of Fisheries Technology, Cochin, Kerala, India

K. S. Sritha Department of Biotechnology, Cochin University of Science and Technology, Cochin University, Cochin, Kerala, India

Jerusha Stephen Post-Harvest Technology, ICAR-Central Institute of Fisheries Education, Seven Bungalows, Andheri (W), Mumbai, India

Sudama Swain ICAR-Central Institute of Brackishwater Aquaculture, Chennai, India

Thangaraj Raja Swaminathan Peninsular and Marine Fish Genetic Resources Centre, ICAR National Bureau of Fish Genetic Resources, CMFRI Campus, Kochi, Kerala, India

Nivedita Thass Infectious Diseases Detection and Surveillance, ICF, Delhi, India

Murugadas Vaiyapuri Microbiology Fermentation and Biotechnology Division of ICAR-Central Institute of Fisheries Technology (ICAR-CIFT), Cochin, Kerala, India

Manuel F. Varela Department of Biology, Eastern New Mexico University, Portales, NM, USA

Balaji Veeraraghavan Department of Clinical Microbiology, Christian Medical College, Vellore, Tamil Nadu, India

Karunakaran Vijayakaran Department of Veterinary Pharmacology and Toxicology, Veterinary College and Research Institute, Namakkal, Tamil Nadu, India

Ardhra Vijayan Microbiology, Fermentation & Biotechnology Division, ICAR-Central Institute of Fisheries Technology, Cochin, Kerala, India

Min Yang State Key Laboratory of Environmental Aquatic Chemistry, Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences, Beijing, China

University of Chinese Academy of Sciences, Beijing, China

National Engineering Research Center of Industrial Wastewater Detoxication and Resource Recovery, Beijing, China

Yu Zhang State Key Laboratory of Environmental Aquatic Chemistry, Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences, Beijing, China

University of Chinese Academy of Sciences, Beijing, China

National Engineering Research Center of Industrial Wastewater Detoxication and Resource Recovery, Beijing, China

Part I

Status of AMR Across Sectors

Antimicrobial Resistance in India: The Road Ahead

Renu Gupta and Rajesh Bhatia

Contents

Abstract

Antimicrobial resistance (AMR) is a global public health challenge, requiring immediate actionable strategies to prevent the next pandemic due to untreatable multi- and pan-resistant microorganisms. The antibiotic overuse and misuse with poor infection prevention and control are the major reasons for accelerated resistance to antimicrobials. The problem of AMR is widespread across humans, plants, food, animals, and environment and does not recognize any geographic

R. Gupta (✉)
Microbiology, Institute of Human Behaviour and Allied Sciences, New Delhi, India
e-mail: renugupta2929@gmail.com

R. Bhatia
Communicable Diseases, WHO Regional Office for South East Asia, New Delhi, India
e-mail: drrajesh.bhatia1953@gmail.com

M. P. Mothadaka et al. (eds.), *Handbook on Antimicrobial Resistance*,
https://doi.org/10.1007/978-981-19-9279-7_1

borders. AMR is like a ticking time bomb that requires urgent, comprehensive, coordinated, collaborative actions between human health, animal health, and environment sectors based on "One Health approach" for deferring this disaster.

In recognition of this crisis, the Global Action Plan on AMR was developed and endorsed by the 68th World Health Assembly in 2015, followed by adoption by other international bodies. In alignment with the global action plan, India developed its National Action Plan on AMR (NAP-AMR) in 2017. The NAP-AMR is a comprehensive plan that addresses the strategies and priorities for AMR containment across all sectors encompassing all dimensions of antibiotic use and disposal. Several initiatives have been undertaken to implement NAP-AMR, but the gains have yet not been consolidated due to highly complex and competing national priorities. The plan is highly resource intensive and requires integrated, cohesive governance between human, animal health, and environment to bring out any perceivable change.

Keywords

Antimicrobial resistance · Global action plan · National action plan · One health approach · Antibiotics

1 Introduction

Antimicrobial resistance (AMR) is a phenomenon in which microorganisms stop responding to antimicrobial agents (antibiotics) intended to inhibit/kill them. Antibiotics are meant to kill microbes, and microbes fight back to evade antibiotics by different strategies and in due course become tolerant/resistant to the effect of antibiotics. Resistance to antibiotics is not only restricted to the older and much more frequently used antibiotic classes, but the resistance is increasing at alarming rates among the newer and more expensive drugs like carbapenems and colistin. Available data indicate alarming rates of AMR across multiple pathogens of clinical importance with almost one fourth of all isolates studied in India being resistant to multiple antibiotics (World Health Organization, 2020; Laxminarayan & Chaudhury, 2016). AMR is an equally devastating threat with much higher magnitudes and almost similar patterns of resistance in animal population due to higher animal biomass (Van Boeckel et al., 2019). Antibiotic residues, AMR pathogens, and resistant genes from humans, food-producing animals, agriculture use, and from pharmaceutical manufacturing units find their way into the environment through sewage, hospital wastewater, rivers, and surface and ground waters (Pareek et al., 2015). This leads to antibiotic pollution with AMR pathogens/genes seeding into environment, and subsequent recycling in food chain (Pareek et al., 2015).

AMR is a natural biological unstoppable phenomenon directly linked to antibiotic use and misuse. Resistance in microorganisms does not occur suddenly. It is the outcome of long-time misuse of antibiotics in different settings. Accumulation of resistance in one bacterium over the period makes it multidrug-resistant organism

(MDR) or as is called as "superbug" in layman term. Cross-resistance against other antibiotics and metals provides greater lethality to the microorganisms. The detection of superbugs or MDR pathogens reflects just the tip of the iceberg, consequential to long-term neglect of rational use of antibiotics in various sectors. All antibiotics have the potential to select drug-resistant bacterial populations with varying frequencies depending upon class of antibiotic, dose administered, and the bacterial strain specificities. Not only overuse but underuse of antibiotics due to lack of access in many settings is also a perpetuator for AMR. Once developed, AMR is largely irreversible or may reverse very slowly to susceptibility status if antibiotic use is withheld for a very long time.

AMR is a complex problem. It is not only a technical challenge but has several other dimensions. It is a regulatory issue, an educational problem, has behavioral dimension, and carries huge economic and social impact. It needs a multiprong attack. Engagement and ownership of all sectors with "One Health approach" is essential to address this challenge.

2 AMR: A Global Public Health and Economic Challenge

AMR is a global problem as infections caused by the antibiotic-resistant pathogens are much more difficult to treat than those caused by antibiotic-susceptible pathogens and result in increased morbidity, longer stay in hospitals, and forced use of expensive diagnostic tests/toxic drugs. These infections are also associated with worst clinical outcomes and consume much more healthcare resources (Table 1).

Global estimated mortality data due to AMR have emerged, but developing country-specific information on mortality, morbidity, and economic losses is yet not available. Among Asian countries, in Thailand alone, more than 38,000 people are being killed annually by antibiotic-resistant pathogens with a loss of US$ 1.3 billion/year (Thamlikitkul et al., 2015). It is estimated that globally ~ten million deaths will occur annually with a cumulative loss of US$ 100 trillion to the global economy by 2050 (O'Neill, 2014). The O'Neill report has also highlighted the impact of AMR on human development, including deaths, hospitalization, and

Table 1 Impact of antimicrobial resistance on human health

Longer illness
Longer treatment duration
Higher mortality
Treatment with expensive drugs
Greater use of diagnostics
Increased burden on health system
Nullify technological advances
Transmission of drug-resistant organisms in community
Impact on economy and global human development

food security (O'Neill, 2014). In one world, a catastrophe is waiting to happen because of our inactions.

Unfortunately, this war is increasingly being won by the resistant pathogen and the world is now approaching a "post-antibiotic era" where humanity will not be able to manage even small wounds and infectious diseases will again become major killers. The well-established and life-saving advances in complex medical surgeries, viz., transplantation, cardiac repairs, etc., shall be negated because of the post-surgery untreatable infections due to resistant pathogens.

Post-antibiotic era is not far ahead at the current pace of pan-resistant microorganisms spreading across the globe and not many new antimicrobials are likely to be developed in near future due to their cost, low return on investment, short shelf life, and long development period (Bhatia & Walia, 2017; Bhatia & Narain, 2010).

It is well known that the development of a new antibiotic class will take an investment of more than US$ 1 billion over a time span of more than a decade. These efforts do not ensure appropriate return on investment because of rapid emergence of resistance. According to a World Bank report, AMR shall be responsible for a fall of up to 3.5% in global exports, with diminishing of livestock production by 7.5% and likewise an increase in healthcare-related costs of US$ 1 trillion by 2050. If left uncontrolled, AMR shall push 28 million people into poverty (The World Bank, 2016).

Global leaders and leading inter-country organizations (FAO, WOAH, WHO, UNEP, OECD, G7, G20, G77, ASEAN, etc.) have recognized that AMR has serious implications not only on human health but more so on economy, food security, and a serious negating influencer for overall human development (WHO, 2016).

3 Key Drivers of AMR

Extensive, irrational, and indiscriminate use of antibiotics is the biggest driver of AMR, and this common thread for the emergence of AMR pathogens runs across various sectors. Antimicrobial use is in turn dependent on sanitation, hygiene, access to clean water, vaccination coverage, and quality healthcare services (CDDEP et al., 2021). Prevalence of AMR pathogens varies depending upon levels and volumes of the antimicrobials consumed in humans, animals, and environment.

Antimicrobials are inappropriately consumed in human health not only for treatment of infections but also for prophylaxis, treatment of self-limited, and noninfective diseases both in community settings and hospital-admitted patients (Versporten et al., 2018; Center for Disease Dynamics, Economics, & Policy et al., 2021). Antibiotics are prescribed without adhering to standard treatment guidelines and a large number of factors like high patient attendance (resulting in less counseling time for appropriate antibiotics use), unjustified patient demand, lack of diagnostics, poor sanitation, hygiene and infection control practices, fear of losing patients, unethical practices, and not keeping pace with advances in antibiotic prescribing practices are main factors for irrational prescribing practices. Self-medication, over-the-counter availability of antibiotics,dispensing by pharmacies and registered medical practitioners, and easy access to higher generation antibiotics,

broad-spectrum antibiotics and irrational antibiotic fixed-dose combinations, sub-standard antibiotics, also contribute to overuse of these precious molecules and thus emergence of AMR (Morgan et al., 2011; Laxminarayan & Chaudhury, 2016).

All classes of antimicrobials important for human medicine are used in much larger quantities in animals in veterinary practices and animal husbandry and factors driving antibiotic use in animals are more or less similar to human use. Antimicrobial agents are also extensively used in otherwise healthy livestock and poultry to overcome the issues of inadequate biosecurity and sanitation in farms and provide prophylaxis to animals against infectious diseases that may either kill them or stunt their growth (Klein et al., 2018). Besides, antimicrobials are added in low doses for growth promotionand improved feed conversion efficiency to promote faster growth in food animals (Van Boeckel et al., 2019). The use of antimicrobial agents as growth promoters has been successfully discontinued in Western countries. It still continues as a major intervention in developing nations where it is considered a cheap alternative to an improved biosecurity system. In India, economic prosperity and population growth have resulted in increased demand for animal protein with a massive increase in egg and broiler production, resulting in over-reliance on indiscriminate antibiotic use as a growth promoter in farms (Ministry of Animal Agriculture and Farmers welfare, 2018). Fish production systems have also become much more intensive to meet the growing demand with India becoming the largest producers of aquaculture products globally, along with China and Vietnam (Bostock et al., 2010). In parallel to increased demand of food/food products of animal origin, antibiotic consumption has also doubled between 2000 and 2015 in animals in India (Klein et al., 2018).

4 Responding to AMR Threat

In 2015, 68th World Health Assembly (WHA) endorsed the global action plan to tackle AMR (including antibiotic resistance) as the most urgent response to spiraling drug resistance trends (WHO, 2015). The goal of the global action plan (GAP-AMR) is to ensure successful prevention and treatment of infectious diseases as long as possible, with effective and safe quality-assured medicines used in a responsible way, and accessible to all those who need them. The WHA resolution 68.7 has also set a target that all WHO member states should develop respective National Action Plans on AMR (NAP-AMR), aligned to the principles outlined in the GAP-AMR by May 2017. International development partners, mainly the World Health Organization (WHO), World Organisation for Animal Health (WOAH), and the Food and Agriculture Organization of the United Nations (FAO), also recognized AMR as the top priority on their respective agendas and called all its member states to develop their nation-specific action plans to combat AMR within 2 years (WHO, 2015; World Organization for Animal Health, 2015; Food and Agriculture Organization of the United Nations, 2015). United Nations General Assembly in September 2016, in an unprecedented special session, recognized AMR as an immediate challenge and called for commitment by global leaders toward an aggressive and highly coordinated intercountry, multisectoral “One Health approach” (WHO, 2016).

4.1 One Health Approach

One Health is a simple, validated, powerful, integrated, and holistic approach advocated by the WHO, WOAH, and FAO, where human health, animal health, and environment sectors work together in a coordinated way to prevent the emergence of AMR and its spread (WHO, 2015; World Organization for Animal Health, 2015; FAO, 2015). To advocate the use of "One Health approach" in national health programs and provide guidance on its implementation, a tripartite agreement between these three organizations has been in vogue since 2010 (WHO, 2017a). The key elements of the implementation framework for One Health approach for AMR are shown in (Table 2). FAO in 2020 has developed a national framework to implement One Health to assist countries in initiating the implementation of One Health activities for AMR as well as the growing challenge of zoonoses.

It is crucial to bring about a change in the narrative in national response to zoonoses, improving food security and ensuring environmental integrity so that AMR can be effectively countered. The success of "One Health" is quite unlikely if it remains a purely governmental endeavor. Awareness, engagement, and active participation of individuals and civil societies shall augur well for its success (Bhatia et al., 2019; Bhatia, 2019). The engagement of top political leadership and the international development partners with intersectoral collaboration is essential to accomplish the benefits (Bhatia, 2019).

5 AMR Containment in India

Efforts for AMR containment in India started taking shape in 2010 with the establishment of National Task Force for AMR Containment with enactment of National Policy on containment of AMR in 2011 (Ministry of Health and Family Welfare, 2011). "Jaipur Declaration on AMR," which calls for comprehensive action against the irrational use of antibiotics, was adopted in September 2011 by India, along with the health minister of all member states of the WHO South-East Asia

Table 2 Key elements of implementation framework for One Health approach for AMR

Political commitment
Policy formulation
Sustainable financing
Program development
Knowledge sharing
Institutional collaboration
Capacity enhancement
Engagement of civil society
Active participation of the communities

Adapted from Food and Agriculture Organization, National Framework for One Health, New Delhi, 2021

Region WHO, 2011). The "National Programme on Containment of Antimicrobial Resistance" was launched under the 12th five-year plan (2012–2017) with the aim to regulate antimicrobials usage in humans and animals with requisite labeling requirements in food, along with the establishment of a laboratory-based surveillance system in the country (National Centre for Disease Control, 2012).

5.1 National Action Plan on AMR, India

AMR has been identified as a national priority by the Government of India, and the customized National Action Plan on AMR (NAP-AMR) was developed in 2017 in alignment with GAP-AMR (MoHFW, 2017). The NAP-AMR is built on an efficient multisectoral, multidisciplinary, and multipronged "One Health approach" incorporating strategic activities for human health, animal health, and environment sectors with the ultimate aim of combating AMR for global health security. NAP-AMR aims to reduce the impact of AMR in India by establishing and strengthening governance mechanisms and enhancing capacity of all stakeholders to work toward combating AMR.

The plan incorporates six strategic priorities, out of which five are aligned with the Global Action Plan on AMR and the sixth priority highlights India's leadership, commitment, and collaborations at the international level to implement AMR containment at the ground level. (Table 3) outlines the six strategic priorities outlined in NAP-AMR (MoHFW, 2017).

Each of the strategic priorities with defined focus areas is briefly discussed below.

5.1.1 Strategic Priorities

Priority 1

This strategy focuses on improving awareness and understanding of AMR through effective communication, education, and training, and has two focus areas:

1. To raise awareness among all stakeholders through information, education, and communication
2. Education and training to improve the knowledge and behavior of professionals in all sectors

Table 3 Strategic priorities under India's NAP-AMR (2017–2021)

To improve awareness and understanding of AMR through effective communication, education, and training
To strengthen knowledge and evidence through surveillance of AMR
To reduce the incidence of infection through effective infection prevention and control
To optimize the use of antimicrobial agents
To promote investments in AMR activities, research, and innovations
To strengthen India's leadership in AMR through international collaborations

Adapted from National Action Plan on Antimicrobial Resistance, MoHFW, 2017

This strategic priority aims to increase awareness among the users and providers regarding the emergence of AMR due to unnecessary antibiotic use and its socio-economic impacts. The first and foremost requirement before implementing any communication program is to understand and assess the level ofunderstanding and knowledge about the antibiotic use and AMR among the general public and other stakeholders to design comprehensive programs.

Besides improving general awareness, there is an urgent need for rigorous, regular, restructured, standardized education and training programs specifically tailored for policymakers, regulators, doctors, nurses, pharmacists, veterinarians, animal farmers, agriculturists, and environmentalists for upgradation of their knowledge, skill, and aptitude regarding the use of antibiotics.The plan envisages revision of curriculums in schools, colleges, and medical and veterinary schools with incorporation of basic and advanced learning about antibiotics and AMR. In addition to training, deep insight and brainstorming are required to bring in human factor engineering to inculcate sustained behavior modification for effective implementation of correct practices.

Priority 2

This priority focuses on strengthening knowledge and evidence through surveillance and has two focus areas:

1. Strengthening laboratories in human, animal, food, and environment sectors
2. Ensuring surveillance of AMR in human, animal, food, and environment sectors

Generation of quality-assured microbiology data to understand the trends in pathogens implicated and antibiotic susceptibility patterns is of paramount importance to design evidence-based interventions to combat AMR. This priority aims to strengthen the microbiology laboratories for pathogen identification and antimicrobial susceptibility testing with generation of robust quality-assured surveillance data in humans, animals, food, and environment (in wastewaters generated from healthcare settings, factories, and farms). The plan also envisages collection, compilation, analysis, and information management of AMR surveillance data in a standardized and coordinated manner at central, state, and district levels across all sectors.

Priority 3

Strategic priority 3 focuses on reducing the incidence and spread of infections by improving sanitation, hygiene, and infection prevention and control in

1. Healthcare
2. Animal health
3. Community and environment

This priority aims to promote sanitation, hygiene, and infection control practices in healthcare, veterinary practices, animal husbandry, dairying, aquaculture, food,

environment, and community to reduce the transmission of infections. Simple evidence-based interventions in the form of hand hygiene, biosecurity, and cleanliness drives can play an unparalleled role in reducing the spread of infections, thereby decreasing antibiotic use and emergence and spread of drug-resistant pathogens.

Priority 4

This priority focuses on optimizing the use of antimicrobial agents in health, animals, and food by

1. Strengthening regulations, ensuring access, and surveillance of antimicrobial use
2. Antimicrobial stewardship in healthcare
3. Antimicrobial stewardship in animal health and agriculture

Antibiotic use is the key driver for AMR, and this strategic priority focuses on optimizing antibiotic use based on evidence-based treatment guidelines and locally generated antibiograms in all sectors using antimicrobials in any form. Developing and strengthening the regulatory framework for rationalized antibiotic use for humans, animals, and food industries with enforcement of regulations and standards for preventing environmental contamination from waste effluents is critical to reduce the spread of antibiotic-resistant pathogens/genes. Ensuring uninterrupted access to quality-assured antimicrobials wherever indicated is an equally important prerogative for effective timely treatment and control of infection transmission. Surveillance of antimicrobial use with measurement of total antibiotic use/consumption, patterns, and rationality of antibiotic use allows for tracking and comparison of consumption statistics across different settings with designing interventions to regulate irrational prescribing.

Antimicrobial stewardship programs in healthcare facilities, animal facilities, agriculture, and food processing units are essential to ensure safe, effective, economic, and rational use of antimicrobials to reduce with increased life span of existing antibiotics.

Priority 5

This priority aims to promote investments in AMR activities, research, and innovations through

1. New medicines and diagnostics
2. Innovations to develop alternative approaches to manage infectious diseases
3. Sustainable financing to ensure adequate resources for containment of AMR

The focus of this strategy is to promote operational research and support innovations to find implementable solutions to contain AMR across human, veterinary, and environment sectors. The thrust is to identify research priorities and innovations for new antibiotics, alternatives to antibiotics, vaccines, new diagnostic modalities, and novel infection prevention and control remedies in human and animal health to tackle AMR. Identification of financial implications with resource mobilization for

sustained funding for AMR interventions is foremost for continued research, innovations, and implementations of interventions in all sectors.

Priority 6
This strategy focuses on strengthening India's leadership in AMR through

1. International collaborations
2. National collaborations
3. State-level collaborations

The prime focus of this strategy is to promote India's leadership and commitment through inter−/intra-country collaboration and coordination for AMR-related activities. Strengthening of national collaborations integrated with vertical disease control programs and implementation of action plan at ground level by development of state action plan is the ultimate requirement.

Implementation of NAP-AMR requires the establishment of relevant governance mechanisms with clear lines of accountability to strategize the outcomes across all settings. Stringent monitoring using descriptive, qualitative, and quantitative metrics for outcome measures is critical for the evaluation of the initiatives undertaken.

5.1.2 Initiatives under NAP-AMR, India

The major initiatives that have been undertaken under NAP-AMR toward combating AMR are summarized below.

5.2 Awareness, Education, and Training

Campaigns to raise awareness about antibiotic use and harms resulting from the misuse of antibiotics have gained momentum with sensitization of general public and school children amalgamated with the "World Antibiotic Awareness Week," which is observed every November (World antibiotic awareness week, 2019). Mass media campaign to raise awareness about Swachh Bharat Abhiyan initiative, redline campaign to identify drugs that need dispensing against a prescription from a licensed doctor, and prime minister radio address in Mann ki baat on faulty and unnecessary antibiotic use have provided a kick start to the program (Swachh Bharat Mission, 2014).

Customized education and training programs have gained impetus with the development and dissemination of guidelines, and standard operative procedures on various aspects of AMR, by the National Centre for Disease Control (NCDC), Indian Council of Medical Research (ICMR), Indian Network for Fisheries and Animal Antimicrobial Resistance (INFAAR) based on World Health Organization (WHO), and Centre for Disease Prevention and Control (CDC) guidelines. Capacity building through offline and online training programs has gained impetus for rational prescribing practices, basic identification and susceptibility testing in bacteriology, surveillance of antibiotic consumption and AMR, quality assurance, data capture,

and data management by several national-and state-level professional bodies and civil societies.

5.3 Strengthen Knowledge and Evidence Through Surveillance of AMR

AMR surveillance networks have been established and progressively strengthened with hand-holding of participating laboratories to generate quality data to determine the magnitude and trends of AMR for priority bacterial pathogens in both human and animal sectors.

ICMR and NCDC have started AMR surveillance networks to capture AMR data of priority pathogens. These organizations are supporting teaching and training programs, and development of resources (standard operating procedures) for isolation and antimicrobial susceptibility testing besides supporting laboratory infrastructure.

ICMR initiated the Antimicrobial Resistance Surveillance Research Network (AMRSN) in 2013 to generate a nationally representative reliable data on AMR to guide treatment strategies and rationalize AMSP in India (ICMR, 2013). The network started with six reference labs located in four tertiary care medical institutions each for 6 priority pathogens and 16 regional centers in tertiary care hospitals. The NCDC also initiated a National AMR Surveillance Network in 2017 for capturing AMR, which currently has around 29 sites across the country. The NCDC network sites have also started capturing AMU data. The NCDC as the national coordinating center for AMR surveillance is reporting aggregated AMR surveillance data to the Global Antimicrobial Surveillance System (GLASS) to contribute toward global understanding of the AMR trends (WHO, 2020).

INFAAR was established and operationalized as a joint Indian Council of Agricultural Research (ICAR) and FAO's initiative to generate structured, quality data on AMR in fisheries and animal health sector in order to strengthen knowledge and better understanding of AMR (FAO, 2017). The network has been expanded to include a total of 18 ICAR and 3 university members. ICAR-National Institute of Veterinary Epidemiology and Disease Informatics, Bengaluru (NIVEDI), is responsible for coordinating the overall technical and data management operations of the network, and ICAR-National Bureau of Fish Genetic Research, Lucknow (ICAR-NBFGR), collaborates with ICAR-NIVEDI in coordinating technical activities of labs from fishery sector.

5.4 Infection Prevention and Control

Initiatives like Swachh Bharat Abhiyan and Kayakalp award scheme have accelerated the efforts toward universal sanitation with safe management of solid and liquid waste across the entire nation with equivalent focus at the community and healthcare level (Swachh Bharat Mission, 2014; MoHFW, 2015). Sustained IPC activities have been recognized as a core component toward certification of health facilities by

many international and national healthcare organizations like Joint commission International (JCI), National Accreditation Board of Hospitals (NABH), and National Quality Assurance Systems (NQAS). Hand hygiene day is observed nationwide on 5 May every year as the most effective infection prevention method.

National guidelines for infection prevention and control in healthcare facilities, implementation manuals, and assessment frameworks have come into existence for effective dissemination of knowledge and translation at ground level (MoHFW, 2020). National Patient Safety Implementation Framework has also identified infection prevention and control as a strategic priority for patient safety (MoHFW, 2018–2025).

ICMR-AIIMS HAI surveillance, India, network has come into existence with the involvement of ~40 sites pan India to strengthen the national capacity for surveillance of HAIs.This network aims to develop trained workforce by strengthening HAI surveillance using standardized criteria, along with the generation of reliable AMR data (HAI surveillance, India, 2020).

5.5 Optimizing Antibiotic Use

Antimicrobial stewardship has been recognized by ICMR and NCDC as the backbone for optimizing antibiotic use and combating AMR. Treatment guidelines for antimicrobial use in common clinical syndromes and National Treatment Guidelines for Antimicrobial Use in Infectious Diseases have been released by ICMR and NCDC, respectively, to streamline antibiotic prescribing practices (ICMR, 2017; MoHFW, 2016). WHO has updated and published Critically Important Antimicrobials list (CIA) for human medicine (sixth revision, 2018) and Essential Medicines List (20th Edition, 2017) with categorization of antibiotics into Access, Watch and Reserve category (AWaRe) to improve the quality of antibiotic prescribing based on indications for use and potential for resistance development (WHO, 2017b, 2018). National Essential Diagnostics List with access to diagnostic tests from primary healthcare to district level has been rolled out and is being implemented toward integration of diagnostic stewardship with AMS activities (ICMR, 2019).

Measurement of antimicrobial consumption (AMC) using standardized tools is essential to design any AMS intervention.World Health Organization (WHO) Defined Daily Dose (DDD) methodology is an aggregate method to capture antimicrobial consumption and allow comparison within and across the facilities.Capacity building for AMC estimation has begun. National workshops on surveillance of antibiotic consumption are being organized for laboratories enrolled in NCDC Network across India with attempts to integrate antimicrobial use with AMR to establish a relationship of antimicrobial use with resistance.

Several regulations have been strengthened to streamline antimicrobial prescribing in humans, animals, and food industry. Schedule H1 came into existence to check over-the-counter indiscriminate use of 47 drugs, including several antimicrobials. The Food Safety and Standards Authority of India (FSSAI) under Food Safety and Standards (contaminants, toxins, and residues) Regulations 2011 laid the (a) limit of antibiotic and other pharmacologically active substances in the fish and

fisheries products; (b) antibiotics prohibited in fish farming system with (c) antibiotics limits in honey (FSSAI, 2011). Besides, a recent amendment (March 29, 2019) in the 2011 regulation has also specified the tolerance limits for 43 antibiotics and veterinary drugs for foods of animal origin (FSSAI, 2019). Also, the Bureau of Indian Standards 2007 laid the poultry feed specification with the prohibition of systemic use of antibiotics like chloramphenicol, doxycycline, tetracycline, nitrofuran, and furazolidone as food additives for growth promotion. Drugs and Cosmetics Rules, 1945, 2013, lay down the requirement of labeling the medicine container for the treatment of food-producing animals with the withdrawal period of the drug. The Central Pollution Control Board has recently drafted standards for antibiotic residues in pharmaceutical industrial effluent and common effluent treatment plants.

5.6 Investments in AMR Activities, Research, and Innovations

WHO published a list of priority pathogens for which new antibiotics are urgently needed, and the Indian Priority Pathogen List aligned with WHO global priority pathogen list has been released under DBT's Mission AMR to guide research, discovery, and development of new antibiotics (WHO, 2017©). Global Antibiotic Research and Development partnership (GARDp) in collaboration with WHO was launched in May 2016 to address global public health and needs of LMICs to target products that industries are not likely to develop to ensure new antibiotics are affordable to all and pilot use of alternative incentive models with delinking the cost of research and development from volume-based sales and prices.

5.7 Collaborations

The essence of One Health is coordination, collaboration, and communication between several stakeholders to plan and work together to achieve the shared objectives of combating AMR. Health is a state subject under the Indian Constitution, and all states and UTs need to develop their own state action plan on AMR depending on their situation and challenges in coordination and collaboration with all stakeholders. Kerala, followed by Madhya Pradesh and Delhi, has rolled out its state action plan and is striving to roll out operational plans to implement it at ground level.

5.7.1 Barriers to Implementation

Although attempts are ongoing for the implementation of NAP-AMR, in India but the gains have not been perceptive due to many barriers that are more or less alike in several developing countries because of its complexities and various competing national priorities.

Some of the barriers hampering implementation (Bhatia, 2018a; Queenan et al., 2017) include long-time ingrained practices of silo and sector-specific approaches

Table 4 Barriers to the implementation of One Health approach

Financial constraints
Disintegrated governance between human, animal health, and environment
Ambiguity about the concept and scope of one health approach
Underestimation of economic benefits
Discordance between professional on ways ahead
Inadequate training and capacity-building activities

with disintegrated governance between human, animal health, and environment. Besides, there is ambiguity about the concept and scope of "One Health approach," underestimation of its economic benefits, discordance between professionals on ways ahead, inadequate training, and behavior modification initiatives (Table 4).

6 Way Forward: AMR and Universal Health Coverage

It is evident that a resource-intensive stand-alone AMR containment program shall not be feasible in contemporary times. One of the possible alternatives is to pillion-ride another approach/program, which is already a national priority. One such endeavor and possible entry point for AMR plan implementation may be synchronizing it with Universal Health Coverage (UHC) by 2030, which has already been recognized as a sustainable development goal (United Nations General Assembly, 2012; Sustainable Development Goals, 2015).

UHC can provide an ideal enabling platform since it can support several sensitive and specific AMR interventions (Bhatia, 2018b; Tayler et al., 2019). UHC is the aspiration that all people obtain quality promotive, preventive, curative (including treatment of infections), rehabilitative, and palliative healthcare without suffering financial austerity. AMR containment program also aims at ensuring the prevention and treatment of infectious diseases with efficacious, quality-assured medicines easily accessible to those who need them. Both initiatives have to run in unison with an objective to bring equity, quality, efficiency, accountability, sustainability, and resilience in order to strengthen the health system. Integration of UHC and AMR by national governments and key stakeholders carries a tremendous potential to enhance economic growth and in turn neutralizing the major impact of AMR of pushing people into avoidable poverty (WHO, 2018).

7 Conclusion

AMR is not only a patient-oriented issue but is the biggest threat to the control of infectious diseases faced alike by the developed and developing world (Bhatia, 2018a). The unjustified, inappropriate antibiotic use in humans, animals, and agriculture needs intensive scrutiny with deliberations to curb it as an urgent global and national priority. Combating and countering AMR requires continuous uninterrupted

funding, worldwide collaboration, and national efforts toward rational use of antimicrobials with a concentrated focus on preventing infectious diseases with appropriate infection control measures and good animal husbandry techniques rather than using antimicrobials for prophylaxis and growth-promoting agents.

There is an urgent need to generate the resources needed to create newer diagnostic and therapeutic tools to effectively diagnose and manage infectious diseases without undue reliance on antimicrobials. It is a battle that must be fought aggressively and won; otherwise, inaction of today may culminate in a horrendous post-antibiotic era of tomorrow. Collaborative honest implementation of "One Health approach" with inviolable political commitment at the highest level is a prerequisite and the key to success (Bhatia, 2019). A strong political will and determination to contain this complex challenge, sustained funding, and an efficient programmatic well-coordinated "One Health approach" are validated and globally accepted practices. These need to be implemented across the country to minimize the impact of AMR on human development and preserve the efficacy of antimicrobial agents for the next generations.

The world must collaborate with India in strengthening its efforts to combat AMR and also in building capacity of other developing countries. Given the presumed high burden of AMR, availability of sufficient skilled human resource, numerous well-equipped institutes, and growing awareness of the implications of AMR on the national economy, especially cost to health system and export potential of animal products, India is the ideal country for greater technical support by the global community to reap abundant benefits with potential global implications in the context of AMR.

References

Bhatia, R. (2018a). Antimicrobial resistance: Threat, consequences and options. *National Medical Journal of India, 31*, 133–136.

Bhatia, R. (2018b). Universal health coverage framework as a tool to combat antimicrobial resistance. *Indian Journal of Medical Research, 147*, 228–232. https://doi.org/10.4103/ijmr.IJMR_1462_17

Bhatia, R. (2019). Implementation framework for One Health approach. *Indian Journal of Medical Research, 149*, 329–331.

Bhatia, R., & Narain, J. P. (2010). The growing challenge of antimicrobial resistance in the South-East Asia Region–Are we losing the battle? *Indian Journal of Medical Research, 132*(5), 482–486. https://doi.org/10.4103/0971-5916.73313

Bhatia, R., & Walia, K. (2017). Combating antimicrobial resistance in India: Technical challenges & opportunities. *Indian Journal of Medical Research, 146*, 683–687.

Bhatia, R., Katoch, V., & Inoue, H. (2019). Creating political commitment for antimicrobial resistance in developing countries. *Indian Journal of Medical Research, 149*(2), 83. https://doi.org/10.4103/ijmr.ijmr_1980_17

Bostock, J., McAndrew, B., Richards, R., Jauncey, K., Telfer, T., Lorenzen, K., Little, D., et al. (2010). Aquaculture: Global status and trends. *Philosophical Transactions of the Royal Society, B: Biological Sciences, 365*, 2897–2912. https://doi.org/10.1098/rstb.2010.0170

Center for Disease Dynamics, Economics & Policy, Sriram, A., Kalanxhi, E., Kapoor, G., Craig, J., Balasubramanian, R., Brar, S., et al. (2021). *State of the world's antibiotics 2021: A global analysis of antimicrobial resistance and its drivers*. Center for Disease Dynamics, Economics & Policy.

Food and Agriculture Organization. (2021). National framework for one health. Available at https://doi.org/10.4060/cb4072en. Accessed 25 Feb 2022.

Food and Agriculture Organization of the United Nations. (2015).The FAO Action Plan on Antimicrobial Resistance 2016–2020. Available at http://www.fao.org/fsnforum/resources/fsn-resources/fao-action-plan-antimicrobial-resistance-2016-2020. Accessed 5 May 2021.

Food and Agriculture Organization of the United Nations. (2017). FAO facilitates operationalization of India's Antimicrobial Resistance (AMR) surveillance network. Available at http://www.fao.org/india/news/detail-events/en/c/1026526/. Accessed 5 May 2021.

Food Safety and Standards Authority of India. (2011). Available at https://www.fssai.gov.in/upload/uploadfiles/files/Contaminants_Regulations.pdf. Accessed 20 Sept 2020.

Food Safety and Standards Authority of India, Government of India. (2019). Revised List of Antibiotics and Veterinary Drugs Food Safety and Standards Authority of India. https://apps.fas.usda.gov/newgainapi/api/report/downloadreportbyfilename?filename=Revised%20List%20of%20Antibiotics%20and%20Veterinary%20Drugs_New%20Delhi_India_4-5-2019.pdf. Accessed 15 May 2021.

Healthcare associated infection surveillance in India. (2020). Available at https://www.haisindia.com. Accessed 20 Sept 2020.

Indian Council of Medical Research. (2013). Antimicrobial Resistance Surveillance and Research Network (AMRSN). Available at http://iamrsn.icmr.org.in/index.php/amrsn/amrsn-network. Accessed 20 Sept 2020.

Indian Council of Medical Research. (2017). Treatment guidelines for antimicrobial use in common clinical syndromes. Available at http://bic.icmr.org.in/iamrsn/images/pdf/STG270217.pdf. Accessed 19 Sept 2020.

Indian Council of Medical Research. (2019). National essential diagnostic list. Available at https://main.icmr.nic.in/sites/default/files/guidelines/NEDL_2019.pdf. Accessed 20 Sept 2020.

Klein, E., Van Boeckel, T., Martinez, E., Pant, S., Gandra, S., Levin, S., et al. (2018). Global increase and geographic convergence in antibiotic consumption between 2000 and 2015. *Proceedings of the National Academy of Sciences, 115*(15), E3463–E3470.

Laxminarayan, R., & Chaudhury, R. (2016). Antibiotic resistance in India: Drivers and opportunities for action. *PLoS Medicine, 13*(3), e1001974. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4775002/

Ministry of Agriculture & Farmers' Welfare. (2018). Report of the Committee for Doubling Farmers' Income (Volume VIII). Available at https://agricoop.gov.in/Documents/DFI%20Vol-8D.pdf. Accessed 9 January 2023.

Ministry of Health and family welfare, Government of India. (2011). National Policy for containment of antimicrobial resistance, India. https://main.mohfw.gov.in/sites/default/files/3203490350abpolicy%20%281%29.pdf. Accessed 20 Sept 2020.

Ministry of Health and Family Welfare, Government of India. (2015). Guidelines for implementation of "Kayakalp" initiative. Available at http://www.nhm.gov.in/images/pdf/infocus/Implementation_Guidebook_for_Kayakalp.pdf. Accessed 29 Mar 2019.

Ministry of Health and Family Welfare, Government of India. (2016). National treatment guidelines for antimicrobial use in infectious diseases. Available at AMR_guideline7001495889.pdf. Accessed 19 Sept 2020.

Ministry of Health and Family Welfare, Government of India. (2017). National Action Plan on Antimicrobial Resistance (NAP-AMR). Available at https://www.ncdc.gov.in/WriteReadData/linkimages/AMR/File645.pdf. Accessed 19 Sept 2020.

Ministry of Health and Family Welfare, Government of India. (2018–2025). National patient's safety implementation framework. Available at: https://www.nhp.gov.in/national-patient-safety-implementation-framework-(npsif)_pg. Accessed 5 Feb 2020.

Ministry of Health and Family Welfare, Government of India. (2020). National guidelines for infection prevention and control in healthcare facilities. National Centre for Disease Control. Available at https://ncdc.gov.in/showfile.php?lid=431. Accessed 22 May 2020.

Morgan, D. J., Okeke, I. N., Laxminarayan, R., Perencevich, E. N., & Weisenberg, S. (2011). Nonprescription antimicrobial use worldwide: A systematic review. *The Lancet Infectious Diseases, 11*, 692–701.

National Centre for Disease Control. (2012). National Programme on AMR containment. Available at https://ncdc.gov.in/. Accessed 20 Sept 2020.

O'Neill J. (2014). Review on antimicrobial resistance: Tackling a crisis for the health and wealth of nations. Available at: https://amr-review.org. Accessed 9 Aug 2020.

Pareek, S., Mathur, N., Singh, A., & Nepalia, A. (2015). Antibiotics in the environment: A review. *International Journal of Current Microbiology and Applied Sciences, 4*(11), 278–285.

Queenan, K., Garnier, J., Rosenbaum, N., Buttigieg, S., De Meneghi, D., Holmberg., et al. (2017). Roadmap to a one health agenda 2030. *CAB Reviews: Perspectives in Agriculture, Veterinary Science, Nutrition and Natural Resources, 12*(014), 1–12.

Sustainable Development Goals. (2015). Transforming our world: The 2030 agenda for sustainable development. Available from: https://www.sustainabledevelopment.un.org/post2015/transformingourworld. Accessed 27 Mar 2019.

Swachh Bharat Mission. (2014). Available at https://swachhbharatmission.gov.in/SBMCMS/about-us.htm. Accessed 20 Sept 2020.

Tayler, E., Gregory, R., Bloom, G., Salama, P., & Balkhy, H. (2019). Universal health coverage: An opportunity to address antimicrobial resistance? *The Lancet Global Health, 7*(11), e1480–e1481.

Thamlikitkul, V., Rattanaumpawan, P., Boonyasiri, A., Pumsuwan, V., Judaeng, T., Tiengrim, S., et al. (2015). Thailand antimicrobial resistance containment and prevention program. *Journal of Global Antimicrobial Resistance, 3*(4), 290–296.

The World Bank. (2016). By 2050, drug-resistant infections could cause global economic damage on par with 2008 financial crisis. Available from: http://www.worldbank.org/en/news/press-release/2016/09/18/by-2050-drug-resistant-infections-could-cause-global-economic-damage-on-par-with-2008-financial-crisis. Accessed 10 Aug 2019.

United Nations General Assembly. (2012). Adopting consensus text, General Assembly encourages member states to plan, pursue transition of national health care systems towards universal coverage, 2012. Available from: https://www.un.org/press/en/2012/ga11326.doc.htm. Accessed 14 Aug 2019.

Van Boeckel, T., Pires, J., Silvester, R., Zhao, C., Song, J., Criscuolo, N., et al. (2019). Global trends in antimicrobial resistance in animals in low- and middle-income countries. *Science, 365*(6459), eaaw944.

Versporten, A., Zarb, P., Caniaux, I., Gros, M. F., Drapier, N., Miller, M., et al. (2018). Global-PPS network. Antimicrobial consumption and resistance in adult hospital inpatients in 53 countries: Results of an internet-based global point prevalence survey. *The Lancet Global Health, 6*(6), e619–e629. https://doi.org/10.1016/S2214-109X(18)30186-4. Epub 2018 Apr 23. Erratum in: Lancet Glob Health. 2018 Sep;6(9):e968. PMID: 29681513.

World antibiotic awareness week. (2019). Available at https://www.who.int/news-room/campaigns/world-antibiotic-awareness-week/world-antibiotic-awareness-week-2019/landing. Accessed 19 Mar 2020.

World Health Organization. (2011). Jaipur declaration on antimicrobial resistance. Available at http://origin.searo.who.int/entity/antimicrobial_resistance/rev_jaipur_declaration_2014.pdf. Accessed 20 Sept 2020.

World Health Organization. (2015). Global action plan on antimicrobial resistance. Available at http://www.who.int/antimicrobial-resistance/global-action-plan/en/. Accessed 13 Aug 2019.

World Health Organization. (2016). At UN, global leaders commit to act on antimicrobial resistance. Available from: http://www.who.int/mediacentre/news/releases/2016/commitment-antimicrobial-resistance/en. Accessed 13 Aug 2019.

World Health Organization. (2017a). The Tripartite's Commitment providing multi-sectoral, collaborative leadership in addressing health challenges. Available at http://www.who.int/zoonoses/tripartite_oct2017.pdf. Accessed 2 May 2019.

World Health Organization. (2017b). WHO model list of essential medicines. Available at https://www.who.int/medicines/publications/essentialmedicines/20th_EML2017.pdf?ua=1. Accessed 20 Sept 2020.

World Health Organization. (2018). Critically Important Antimicrobials list (CIA) for human medicine. Available at https://apps.who.int/iris/bitstream/handle/10665/312266/9789241515528-eng.pdf?ua=1. Accessed 20 Sept 2020.

World Health Organization. (2020). Global Antimicrobial Resistance and Use Surveillance System (GLASS) report – Early implementation 2020. Available at https://www.who.int/glass/resources/publications/early-implementation-report-2020/en/. Accessed 15 May 2021.

World Health Organization publications. (2017 c). Global priority list of antibiotic resistant bacteria to guide research, discovery and development of new antibiotics. World Health Organization publications. Available at: https://www.who.int/medicines/publications/global-priority-list-antibiotic-resistant-bacteria/en/. Accessed 10 Aug 2020.

World Organization for Animal Health (OIE). (2015). World Assembly of Delegates of the OIE Resolution No. 26: Combating antimicrobial resistance and promoting the Prudent Use of antimicrobial agents in animals. Available at http://www.oie.int/fileadmin/Home/eng/Our_scientific_expertise/docs/pdf/AMR/A_RESO_AMR_2015.pdf. Accessed 8 Dec 2018.

Antimicrobial Resistance in Animal Sector

P. Anand Kumar

Contents

Abstract

The antimicrobial resistance (AMR) phenomenon in bacteria is in existence well before the discovery of present-day antibiotics, but the rapidity of its development in bacteria is a cause of great concern as it renders the antibiotics ineffective for therapeutic use in human health and animal health. Antimicrobial use (AMU) is one of the main drivers for AMR in bacteria. The global consumption of antimicrobials in the animal sector is phenomenally increasing at great proportions, especially in low- and middle-income countries. The use of antibiotics as growth promoters in animal feeds for improved animal productivity is a cause of great concern. The AMR is transferrable among bacterial species across the human, animal, and environmental sectors. The AMR in animals has not received much-required attention compared to the human counterparts. As global AMR surveillance network for animals is not available, point prevalence surveys are employed to map AMR in animals. Considerable geographic variation in antibiotic resistance levels is observed in foodborne pathogens, viz., *Escherichia coli*, *Campylobacter* species, non-typhoidal *Salmonella* serotypes, and *Staphylococcus aureus*. Certain

P. Anand Kumar (✉)
Department of Veterinary Microbiology, NTR College of Veterinary Science, Sri Venkateswara Veterinary University, Gannavaram, Andhra Pradesh, India
e-mail: p_anandkumar@yahoo.com

M. P. Mothadaka et al. (eds.), *Handbook on Antimicrobial Resistance*,
https://doi.org/10.1007/978-981-19-9279-7_4

classes of antibiotics are listed as critically important in both human medicine and veterinary medicine. Therefore, the rational use of antimicrobials is the need of the hour as antibiotics are indispensable tools in animal health too. In animal sector, the increased AMR is responsible for the financial losses due to higher mortality of animals, reduced productivity, and early culling of breeding and production animals, effecting the livelihoods of livestock and poultry farmers.

Keywords

Antimicrobial resistance (AMR) · Antimicrobial use (AMU) · Point Prevalence Surveys (PPS) · Low-and-middle-income countries (LMICs) · World Health Organization (WHO) · World Organization of Animal Health (WOAH/OIE) · Food and Agriculture Organization (FAO)

1 Introduction

Antimicrobial resistance (AMR) is a fast-evolving phenomenon and a cause of global concern (O'Neill, 2016). World Health Organization (WHO), World Organization of Animal Health (WOAH), Food and Agriculture Organization (FAO), and United Nations Environment Programme (UNEP) are working together as quardipartite to find strategies to mitigate the AMR. The term AMR includes the resistance developed in infectious agents against antibiotics, antiprotozoal, antihelminths, and antivirals. However, in this chapter only the resistance of bacteria against antibiotics in veterinary/animal settings will be reviewed.

The AMR phenomenon in bacteria exists since ancient times, well before the discovery of modern antibiotics. But the great significance that is attached to AMR in the present scenario is due to the rapidity of its development in bacteria, thus rendering the antibiotics ineffective for therapeutic use. Slow and long evolutionary process is responsible for innate AMR, whereas selective pressure due to antimicrobial use (AMU) is responsible for adaptive AMR in bacteria. (Giedraitiene et al., 2011; Perry et al., 2016; Palma et al., 2020). Increased demand for animal protein in human nutrition with intensive livestock farming practices is responsible for increased AMU, which is one of the significant drivers for the development of AMR in animal settings, predominantly in low-and-middle-income countries (LMICs). In humans, antibiotics are used for health, whereas, in animals, antibiotics are used for both health and productivity. The antibiotic growth promoters in animal feeds for improved animal productivity are a cause of great concern (Van Boeckel et al., 2017).

The AMR in animals has not received much-required attention compared with the human counterparts. There is a growing body of evidence that the majority of the infectious diseases of humans are zoonotic in origin (Jones et al., 2008). The AMR is transferrable among bacterial species across the human, animal, and environmental sectors. Therefore, AMR in animals is a double-edged weapon as it may cause untreatable bacterial infections in animals and humans. In LMICs like India, China, Kenya, Uruguay, and Brazil, more resistance is observed in bacteria against commonly used antibiotics in farm animals (Van Boeckel et al., 2019). The quick spread

of plasmid-mediated mobilized colistin resistance-1 gene (MCR-1) globally within a short span of its first report in 2014 from a pig farm in China (Wang et al., 2018) further highlighted the urgent need to tackle the AMR issue in the animal sector.

Antibiotic use in animals drives the selection of AMR in animal bacterial pathogens and commensals. Evidence is also growing on antibiotic use in companion and food animals, leading to the spread of antibiotic-resistant bacterial pathogens or their resistant genes to other animals and humans, which makes the treatment of these infections more difficult. In contrast, animals may amplify the antibiotic-resistant bacteria acquired from their owners and act as reservoirs of human infection (Prescott, 2008). *Campylobacter jejuni*, extraintestinal pathogenic *Escherichia coli*, methicillin-resistant *Staphylococcus aureus* (MRSA) or *Staphylococcus pseudintermedius*, vancomycin-resistant enterococci, carbapenemase-producing enterobacteria, and extended-spectrum beta-lactamase (ESBL)-producing Gram-negative bacteria are examples for such infections (Pomba et al., 2017).

Many of the antibiotics used in animals are the same as those used in human medicine, though some of these antibiotics were rejected in human medicine due to toxicity issues (e.g., bacitracin). However, one of these antibiotics (colistin) is now being reclaimed for systemic use in humans. However, documentation of antibiotics use in animals is relatively poor, especially in LMICs (Prescott, 2017). The emergence and spread of antimicrobial (antibiotic) resistance in veterinary medicine/animal sector arising from the antibiotic use in this sector can be linked to individuals' economic behavior and institutional context (Raboisson et al., 2020).

2 Antimicrobial Use

It was estimated that in 2010 globally 63,151 (±1560) tons of antimicrobials were consumed in food animal production. It is projected to reach 105,596 (±3605) tons by 2030, thus a 67% increase in consumption of antimicrobials is expected in a span of 20 years (Van Boeckel et al., 2015). However, it is interesting to note that the evidence linking the reduced use of antibiotics in food animals to that of AMR in humans is very limited. This suggests more intricate pathways between AMR in food animals and human health (Tang et al., 2017). However, as most classes of antibiotics used for treating the bacterial infections in humans are shared with the veterinary sector, cumulative selective pressure will be exerted on the bacteria, resulting in reduced efficacy of antibiotics-based treatment in both human and veterinary medicine (Aarestrup et al., 2008). The resistance to antibiotics in the animal sector is not only a threat to animals' health, productivity, and welfare but also affects the livelihoods of millions of people who depend on animal husbandry for their income.

3 Antibiotic Growth Promoters (AGPs)

The role of antibiotics in the prevention, control, and treatment of bacterial diseases in animal health is significantly and critically acclaimed. However, unlike in human health, the antibiotics played a different role in animal production as growth

promoters in feed for improving the productivity of animals. Appreciable growth enhancement and feed efficiency were observed in animals that are fed AGPs (Teillant & Laxminarayan, 2015). Use of streptomycin in animal feed for growth improvement in chickens was reported by Moore et al. (1946). Improved growth performance in chickens and pigs that were fed chlortetracycline (Aureomycin) further augmented their commercial application in animal feeds (Jukes et al., 1950). The antibiotics have been used at subtherapeutic-level concentrations as AGPs. Swann (1969) raised the possible correlation between the development of antibiotic resistance in bacteria with the use of antibiotics as AGPs in animal feeds.

It was hypothesized that AGPs would have effects on microbiota of the animals and alter their density in favor of improved feed efficiency by improving the absorption in the gut (Gaskins et al., 2002). The AGPs also significantly inhibit the inflammatory responses in the intestine, thus promoting feed absorption and enhancing the growth (Niewold, 2007). Many reports were published on the role of AGPs in improving the feed efficiency and growth rates in animals (Cromwell, 2002). Though the normal microbiota are helpful to the host (animals) in preventing the colonization of pathogenic bacteria, competition for nutrients of the host for maintainability of gut microbiota taxes the costs of nutrition in farm animals (Dibner & Richards, 2005).

Among different classes of antibiotics, ionophores, macrolides, penicillins, and tetracyclines have been mainly used as AGPs in animal feeds, especially in monogastric animals. As the antibiotics are used as AGPs at subtherapeutic concentration levels that are most likely well below the minimum inhibitory concentration (MIC) levels reported for that particular antibiotic, the mode of action of AGPs was doubted many times (Broom, 2017). However, it was reported that the antibiotic naficillin at its sub-MIC values increased the susceptibility of *S. aureus* to phagocytosis in the host (Friedman & Warren, 1974). Antibiotic ampicillin at its sub-MIC values reduced the attachment and colonization (Sandberg et al., 1979), and the antibiotics aztreonam, gentamicin, clindamycin, and trimethoprim at their sub-MIC values reduced the expression of virulent factors in *E. coli* (Hacker et al., 1993). In *Pasteurella multocida,* the antibiotics amoxicillin, chlortetracycline, and enrofloxacin at their sub-MIC values inhibited the growth kinetics and modified protein expression, which were contemplated to increase the antibiotic sensitivity (Nanduri et al., 2006). Research reports are available on the reduced biofilm formation and quorum sensing in certain bacteria on treatment with sub-MIC values of different antibiotics (Cerca et al., 2005; Starner et al., 2008). Furthermore, sub-MIC levels of vancomycin, metronidazole, amoxicillin, clindamycin, cefoxitin, and ceftriaxone were reported to increase the initial lag phase of growth of certain strains of *Clostridium difficile* (Drummond et al., 2003). Similarly, significantly reduced growth rates were observed in *Mannheimia haemolytica* and *Haemophilus somnus* treated with sub-MIC values of chlortetracycline (Reeks et al., 2005).

Perhaps due to all the reasons associated with the use of antibiotics at their subtherapeutic levels such as increased sensitivity of the bacteria to the host immune system and reduced inflammatory response in the intestine to improve gut absorption, etc., antibiotics as AGPs have been extensively used in animal feeds, especially

in intensive farming practices. Though over the period it was reported that increased AMU in food animals is one of the chief drivers for increased AMR, there is only a little evidence that AMR originates only from the food animals (Allen & Stanton, 2014; Xiong et al., 2018).

4 Antibiotic Resistance Scenario in the Animal Sector

AMR surveillance is the most significant system to assess the AMR burden in livestock. However, there is no such system for the collection of AMR data in animals at the global level on the lines of WHO's Global Antimicrobial Resistance Surveillance System (GLASS), which estimates the global burden of AMR. Such types of AMR surveillance systems in the animal sector are available in a few European countries, where the AMR data of zoonotic and indicator bacteria in food animals and their products are collected from different age groups of food animal species annually (Magnusson et al., 2021). However, such types of systemic AMR surveillance in animals are not followed especially in LMICs where the antibiotic consumption in the animal sector is high.

In the absence of global AMR surveillance systems in the animal sector, the point prevalence surveys (PPS) were found to be helpful to map the trends in AMR in animals. Identification of indicator bacteria for such surveys is crucial. In the animal sector since the AMR from foods of animal origin is the highest priority for public health, the foodborne bacterial pathogens as indicated by the WHO Advisory Group on Integrated Surveillance of Antimicrobial Resistance (AGISAR) were selected by Van Boeckel et al. (2019) as indicator bacteria in AMR PPS. Therefore, antibiotic resistance in *Escherichia coli*, *Campylobacter* species, non-typhoidal *Salmonella* species, and *Staphylococcus aureus* were studied under PPS to report AMR in animals and foods of animal origin. It was reported that, in LMICs, the rapid increase in AMR from 2000 to 2018 in chickens and pigs is mainly due to increased intensive farming practices adopted for these two species compared to cattle.

Van Boeckel et al. (2019) also reported considerable geographic variation in antibiotic resistance levels. About 56% of pigs and 54% of chickens in the world are found in Asia. Therefore, the largest hotspots of AMR in animals are found in Asia. In regions such as central India and Kenya, where the meat consumption is low and still intensive farming practices have not picked up, the resistance to multiple antibiotics has not yet reached 50%. In the global scenario, the major hotspots for AMR are not found in Africa, except in Johannesburg.

Van Boeckel et al. (2017) reported the highest resistance rates in the antimicrobials such as sulfonamides, tetracyclines, and penicillins, which are commonly used in animal production. Ciprofloxacin and erythromycin have the highest resistance rates of 20–60%, third- and fourth-generation cephalosporins have moderate resistance rates of 10–40%, and linezolid and gentamicin have lower resistance rates of $<$20% in LMICs. However, different resistance rates were observed for quinolones and aminoglycosides.

When geographic-wise AMR patterns in the foodborne bacterial pathogens were assessed, the quinolone resistance in *E. coli* and *Salmonella* species in LMICs was comparable to the European levels, but the resistance to gentamicin was found to be higher in LMICs. In the United States, the resistance to quinolones was found to be higher than LMICs (Van Boeckel et al., 2019).

The highest rates of colistin resistance (about 18–40%) were found in Asia and the Americas. The research publications on plasmid MCR-1 gene isolated from pigs in China (Liu et al., 2016; Wang et al., 2018) have generated a significant interest in AMR research in the recent past (Sweileh, 2021). As colistin is the last resort for the treatment of infections caused by *Acinetobacter baumannii* and/or *Pseudomonas aeruginosa* in humans, also due to the fast spread of its resistance by horizontal transfer (Sherry & Howden, 2018), the colistin resistance has gained a lot of significance. In the animal sector, the colistin was used not only to treat the infections caused by bacteria of the *Enterobacteriaceae* family but also as a growth promoter (albeit clandestinely) to improve the animal productivity (Davis & Walsh, 2018). The MCR-1 gene was identified in many Gram-negative bacterial species such as *Klebsiella, Salmonella, Shigella*, *E. coli,* and *Enterobacter* in humans and animals (Jeannot et al., 2017). In 2016, the Chinese government banned the use of colistin as a feed additive in livestock and poultry, which was quickly followed by Brazil (Sweileh, 2021). In 2019, the government of India banned the use of colistin in animals. In a study of *E. coli* isolates from humans, animals, foods, and environment, Pormohammad et al. (2019) reported that the prevalence of extended-spectrum beta-lactamase (ESBL)-producing *E. coli* was found to be highest in animals. It is also appropriate to note that Damborg et al. (2009) reported *E. coli* isolates that belong to the same phylogenetic group B2 or D among family members (owners) and dogs (pets).

Restrictions on the use of gentamicin in the animal sector (cattle and poultry) in Europe were found to be directly associated with the lower AMR rates in *E. coli* and *Salmonella* species for gentamicin, whereas significant resistance rates were found for the same in LMICs. In *Campylobacter* species, the highest resistance rate of 60% was found for tetracyclines and quinolones, and moderate resistance rate of $<30\%$ was found for erythromycin in LMICs. In the United States, the highest resistance rate for tetracyclines up to 100% was observed. However, the resistance to quinolones in *E. coli* and *Campylobacter* species is lower in the United States compared to the LMICs and Europe due to the ban on the use of quinolones in poultry since 2005 (Van Boeckel et al., 2019).

E. coli is known for its vulnerability to develop AMR against more than one antibiotic that is in circulation for general therapeutic use in humans and animals. It is also a potential reservoir for many AMR genes (Dolejska et al., 2009). Therefore, to monitor the general level of resistance, *E. coli* is considered an excellent indicator. Furthermore, the detection of AMR in commensal bacteria such as *E. coli* will be valuable as it serves as an early warning signal on the development of possible resistance to antibiotics in the pathogenic bacteria (Abbas et al., 2019). Many reports are available on the multidrug-resistant (MDR) *E. coli* in humans, animals, and environment. The potential for transfer of AMR *E. coli* from food animals to humans

through the food chain or environment is to be considered with great significance (O'Neill, 2015).

Monitoring AMR in commensal bacteria like *E. coli* from food animals provides significant information on the emergence of AMR and its associated risks to humans (WHO, 2017). Manishimwe et al. (2021) assessed the antibiotic resistance profiles among *E. coli* and *Salmonella* isolated from dairy cattle feces in Texas, USA. A protocol for the detection and estimation of the prevalence of AMR, suitable for resource-limited laboratories in developing countries, was adopted in this study. The non-type-specific *E. coli* and *Salmonella* that were isolated on selective media without antibiotic supplements and the *E. coli* isolates that were not susceptible to third-generation cephalosporins and ciprofloxacin (cultured on selective media supplemented with cefotaxime and ciprofloxacin) were tested for antibiotic sensitivity by disk diffusion test. It was reported that the resistance to tetracycline was found to be the highest among non-type-specific *E. coli* isolated on McConkey agar without antibiotics. Resistance to ceftriaxone is 56.8% in *E. coli* recovered from McConkey agar supplemented with cefotaxime. Resistance to nalidixic acid and ciprofloxacin was 77.3% and 54.5%, respectively, in *E. coli* recovered from McConkey agar supplemented with ciprofloxacin. Manishimwe et al. (2021) further performed whole-genome sequencing on selected bacterial isolates of *E. coli* and *Salmonella* and reported that the phenotypic profiles of antibiotic resistance observed were largely substantiated by genotypic profiles.

Song et al. (2022) assessed the AMR profiles and trends in commensal *E. coli* isolated from the feces of healthy cattle, pigs, and chickens in South Korea during the period 2010 and 2020. A panel of 12 antibiotics, viz. amoxicillin/clavulanic acid, ampicillin, cefoxitin, ceftiofur, chloramphenicol, ciprofloxacin, colistin, gentamicin, nalidixic acid, streptomycin, tetracycline, and trimethoprim/sulfamethoxazole, was used to test the resistance/susceptibility profile of *E. coli* isolates and reported that an overall 56% of tested isolates showed multidrug resistance. The proportion of multidrug-resistant (MDR) *E. coli* was high in chickens (87.1%), followed by pigs (73.7%). However, in cattle, the proportion of MDR isolates of *E. coli* was low (17.1%). Song et al. (2022) concluded that due to such a high resistance in commensal *E. coli* against commonly used antibiotics (including the critically important antibiotics listed), these bacteria could become a potential resistance reservoir and there is a very high possibility to spread this resistance to pathogenic bacteria by gene transfer.

It is commonly perceived that in organized farming antibiotics are misused due to intensive farming systems, which has ultimately become a significant driver for AMR. But reports on MDR *E. coli* even from unorganized pig farms in the Mizoram state of India generate a lot of concern about AMR scourge. Significantly higher resistance against the third-generation cephalosporins was reported in *E. coli* isolates from unorganized piggery farming. The same isolates were reported to exhibit more ESBL and non-ESBL AMR genes, with due correlation to phenotypic observations in antibiotic sensitivity tests. A large number of MDR *E. coli* isolates were reported in unorganized pig farming compared to organized pig farming (Mandakini et al., 2020). This scenario is a serious indication of misuse/improper use of antibiotics and contamination of the environment.

Pseudomonas aeruginosa is regarded as the major human pathogen, and also a significant nosocomial infection. *P. aeruginosa* is also responsible for many diseases in both livestock and companion animals. In cattle, it was reported in mastitis; in horses, it was detected in endometritis; and in dogs, it was recovered from otitis and urinary tract infections (Haenni et al., 2015). The MDR *P. aeruginosa* was isolated mainly from certain otitis cases in dogs. The population of *P. aeruginosa* is generally acknowledged as nonclonal with diversified clonal groups and very little or no association between clonal groups. However, very few reports are available on the studies of genetic diversity and AMR mechanisms in *P. aeruginosa* isolated from animals (Haenni et al., 2015).

In a study conducted by Haenni et al. (2017) to assess the carbapenem resistance in *P. aeruginosa* strains, it was found that certain canine and bovine origins *P. aeruginosa* isolates were not susceptible to imipenem and/or meropenem, though these animals were not treated with the carbapenems at any point in time. The decreased susceptibility was found in meropenem compared with imipenem. Due to mutations in genes coding for efflux pumps, these *P. aeruginosa* isolates showed resistance to carbapenems. These results necessitate the importance of taking up studies on *P. aeruginosa* isolates from animals as there is a high probability that animals may represent a reservoir for MDR *P. aeruginosa* strains.

For another important foodborne pathogen *S. aureus,* the resistance rates were higher across all the antimicrobials in Asia than in other regions. Penicillin, with a 40–80% resistance rate, stood out as the antibiotic with the highest rate of resistance, whereas, for erythromycin, tetracycline, and oxacillin, the resistance rate was 20–60% (Van Boeckel et al., 2019). In *S. aureus*, the sublineages carrying different SCC*mec* cassettes specific to particular geographic regions might have influenced the differences in AMR levels (Asadollahi et al., 2018).

Regarding MRSA, it is interesting to note the evolution of MRSA in livestock from methicillin-susceptible *Staphylococcus aureus* in humans (Price et al., 2012). The livestock-associated (LA-MRSA) clonal complex cc398 is found to frequently infect people both inside and outside of the livestock industry. A predominant human-to-animal direction of transmission is presumed in certain MRSA isolated from dogs and cats because most of these isolates belong to MRSA clonal lineages prevalent in human healthcare facilities. This is a typical case of most likely spillover from "owners (humans)" to "pets (animals)" (Nienhoff et al., 2009).

The MRSA isolated from dairy cows is usually found to be resistant to penicillins and cephalosporins. But isolation of multidrug-resistant MRSA is widely reported (Bhattacharya et al., 2016; Mistry et al., 2016). In such a scenario, if MRSA becomes a common bacterial pathogen of mastitis in dairy animals, veterinarians will be left with few or no antibiotics of choice to treat mastitis (Oliver & Murinda, 2012).

Staphylococcus pseudintermedius is an opportunistic pathogen of dogs that is responsible for skin, ear, and wound infections. The emergence and spread of methicillin-resistant *Staphylococcus pseudintermedius* (MRSP) is a classic example of AMR in animal health that has not received the required attention. Pyoderma in dogs due to MRSP was reported for extended treatment periods. However,

apprehensions are raised about deeper infections and certain surgical infections with MRSP becoming life-threatening in dogs (Van Duijkeren et al., 2011).

Vancomycin-resistant enterococci (VRE) such as *Enterococcus faecalis* and *Enterococcus faecium* pose challenges in the clinical settings of humans. The emergence of VRE was profoundly observed in European countries, which was attributed due to the widespread use of the antibiotic avoparcin in the 1990s as a growth promoter in animals. Though enterococci are found as commensal bacteria in the intestines of humans and domestic animals, they are also found in the environment. *Enterococcus faecalis* and *Enterococcus faecium* are the most important species recovered from humans and animals (Hammerum, 2012).

Intrinsically enterococci species are resistant to many first-line antibiotics. Varied levels of resistance to cephalosporins and aminoglycosides were reported; furthermore, enterococci can also acquire resistance against quinolones, macrolides, and glycopeptides (Arias & Murray, 2008). Bates et al. (1993) first reported the animal origin of vancomycin-resistant enterococci. Subsequently, several reports were published on the detection of vancomycin-resistant enterococci from different animal species, viz., cats, dogs, horses, pigs, birds, poultry, and foxes, and foods of animal origin like pork and poultry meat (Hammerum, 2012). Though avoparcin was banned as a feed additive for animals between 1995 and 1997 in many European countries, vancomycin-resistant enterococci clones were detected even after 13 years of its ban. This persistence of vancomycin-resistant enterococci might be due to co-selection with other antibiotics and metals (Hammerum, 2012). Extensive typing studies were conducted with pulse field gel electrophoresis (PFGE), amplified fragment length polymorphism (AFLP), and multilocus sequence typing (MLST) for comparing *E. faecium* isolates of animal origin with those of human origin for determining their clonal complex (CC) and sequence types (ST) (Willems et al., 2000).

Different studies were conducted to establish the transfer of vancomycin resistance A (*van*A) gene from *E. faecium* isolate of animal origin to *E. faecium* isolate of human origin. In an experiment, it was found that the transfer of the *vanA* gene was at a high frequency between animal origin and human origin isolates of *E. faecium* (Moubareck et al., 2003). Though based on molecular typing studies, the enterococci are regarded as host-specific or host-adapted that do not necessarily prevent the transfer of AMR genes between animal and human isolates of enterococci. In fact, the AMR genes seem to spread easily between enterococci from different reservoirs (Heuer et al., 2006). It was further reported by Larsen et al. (2011) that similar antibiotic resistance patterns, virulence gene profiles, and MLST/PFGE types were detected in *E. faecalis* isolates from human patients and pigs, thus indicating the significance of pigs with antibiotic-resistant enterococci probably constituting a threat to human health.

Antibiotics use in animals, AMR in the bacteria, and abundance of antibiotic resistance genes (ARGs) in animal manure need to be thoroughly studied to understand the AMR in the animal sector. A larger proportion of antibiotics administered to animals is excreted in the form of parent compound or active metabolites (Van Epps & Blaney, 2016) and contaminates the environment. Ghirardini et al. (2020)

reviewed the reports of antibiotics and their residues in animal wastes during the last four decades and reported higher concentrations of antibiotics like enrofloxacin, oxytetracycline, and chlortetracycline in both untreated and treated manure. Mobile genetic elements (MGEs) such as plasmids and transposons are responsible for AMR spread from manure to soil, thus manure operates as a hot spot for horizontal gene transfer (HGT) of MGE harboring ARGs (Redondo-Salvo et al., 2020). Therefore, the increase in AMR in bacteria of livestock and poultry production systems will have a direct impact on soil health and environment.

5 Point Prevalence Surveys (PPS) to Map AMR

The PPS used to estimate the AMR rates in the animal sector have some limitations. The prevalence of AMR and AMU can be appreciated with PPS at one point in time for human samples in a hospital. But the PPS for animal samples are different as samples from food animals are taken from the healthy animals at the time of their slaughter. The type of microbes and the magnitude of their resistance to antibiotics are different in sick animals compared to healthy animals (Moore, 2019). Therefore, for accurate appreciation of AMR in the animal sector, a global surveillance network system on AMR in the animal sector on the lines of GLASS is the need of the hour. Otherwise, a component may be introduced in GLASS to accommodate the AMR data from the animal/veterinary sector, which will be helpful for integrated assessment of rates of resistance against antimicrobials across the sectors of human, animal, and environment. In India, with support from the FAO, the Indian Network for Fisheries and Animals Antimicrobial Resistance Network (INFAAR) was launched in 2019 by the Indian Council of Agricultural Research (ICAR) with 11 veterinary and animal science institutes and 8 fishery institutes (Mutua et al., 2020).

6 Antibiotics and Animal Health

Antibiotics are indispensable tools in animal health. In animals, antibiotics are prescribed for therapeutic use in conditions like mastitis, endometritis, pyometra, ear infections, systemic bacterial infections, and during surgical procedures (Page & Gautier, 2012). Most of the studies point out the impact of antibiotic resistance in bacterial pathogens of veterinary importance on public health. But the impact of AMR in bacterial pathogens of veterinary importance on animal health has not received the much-needed attention. AMR seriously affects the health and welfare of livestock, food animals, pet animals, and sports animals, which leads to negative social and economic consequences for the farmers and owners (Bengtsson & Greko, 2014). The exact burden of AMR on animal health is still fully not known.

In countries like India where cattle and buffaloes are meant for dairy purpose, mastitis is the condition where most of the time the misuse of antibiotics is noticed (Mutua et al., 2020). In poultry sector, many times farmers trust the representatives

of certain companies who provide feed mixtures that contain antibiotic additives under the pretext of improved growth and productivity (Davis & Walsh, 2018).

The WHO has listed certain antibiotics as critically important antimicrobials for human medicine (WHO, 2019). Similarly, the WOAH has also listed certain antibiotics as critically important in veterinary medicine (OIE, 2007). Certain classes of antibiotics are listed as critically important in both the human medicine and veterinary medicine. They include fluoroquinolones and third- and fourth-generation cephalosporins. Therefore, the OIE recommends using these antibiotics with the conditions that (a) they should not be used for metaphylaxis, (b) they should not be used as first-line treatment unless justified and should be guided by antibiotic sensitivity testing, (c) off label use should be limited and reserved when no alternatives are available, and (d) they not to be used as growth promoters.

Infections with MRSA and multi-antibiotic-resistant Gram-negative bacteria in dogs compel veterinarians to explore options for glycopeptides, oxazolidinones, and carbapenems (Papich, 2013). In such situations, veterinarians are perplexed to use such antibiotics keeping in view the emergence of antibiotic resistance in the bacteria with a potential spread to humans. This type of situation has more serious social consequences as these animals are prerequisites for physically and mentally challenged persons to cope with their daily activities (Bengtsson & Greko, 2014). Antibiotic resistance in the animal sector can also have an economic impact on the owner of the animal. For good animal health practices, the availability of effective antibiotics is imperative. However, due to the emergence and spread of AMR, the available arsenal of antibiotics is getting depleted, which will have serious consequences on animal health. In livestock, the increased AMR is responsible for financial losses due to higher mortality of animals, reduced productivity, and early culling of breeding and production animals. This will be eventually responsible for increased prices for foods of animal origin, which will be a burden to consumers (Bengtsson & Greko, 2014).

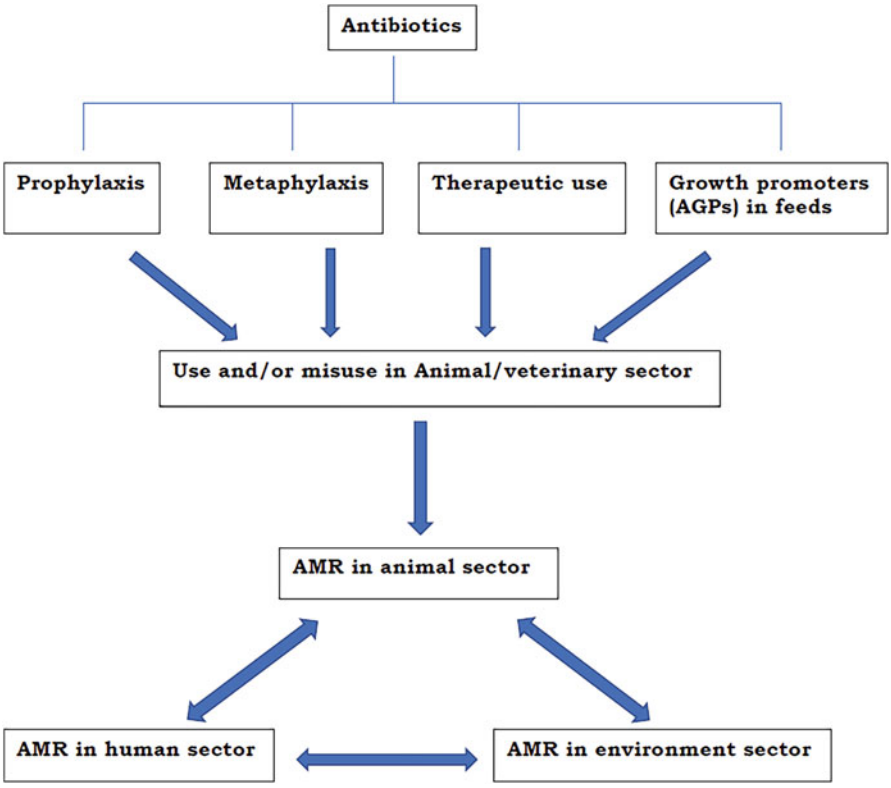
Penicillin was the first choice of antibiotic for mastitis caused by *S. aureus* in dairy animals during the 1950s. However, today penicillin is no longer a first-line therapeutic choice to treat mastitis caused by *S. aureus* (Oliver & Murinda, 2012). Similarly, due to the emergence of resistance against penicillin and tetracycline in *Pasteurella multocida* and *Mannheimia haemolytica,* presently these two antibiotics are not considered as first-line therapeutic choice to treat respiratory infections and pneumonia caused by *P. multocida* and *M. haemolytica* in calves (Portis et al., 2012).

As the older drugs (antibiotics) become obsolete due to the development of resistance, new antibiotics with broad-spectrum activity are introduced for therapeutic use in animal health, which may be responsible for imposing broader selection pressure for developing AMR in the bacteria (Vaarten, 2012).

The World Bank report released in 2017 stated that by 2050 the global livestock population would fall by 3–8% every year due to AMR, with serious consequences on economic and developmental aspects, including sustainable livelihoods. In the event of a high-impact AMR scenario, 11% loss of livestock production is estimated especially in LMICs (Jonas et al., 2017). Therefore, appropriate mitigation measures need to be taken to address the AMR in the animal/veterinary sector.

7 Measures to Mitigate AMR in the Animal Sector

1. Providing effective veterinary services to livestock and poultry sector
2. Medically rational and prudent use of antibiotics by veterinarians
3. Duly implementing the regulatory measures to prevent the sale of antibiotics without prescription
4. Good animal husbandry practices, including improved economical biosecurity settings in livestock and poultry farms
5. Promoting natural products like phytochemicals and essential oils with antimicrobial activity as feed additives to replace antibiotic growth promoters
6. Promoting the use of immunomodulators and synbiotics (probiotics with prebiotics) as feed additives for the overall improvement of animal health and production
7. Vaccination of animals with available vaccines against all the important infectious diseases



Antibiotic Resistance in Animal Sector: An Overview

References

Aarestrup, F. M., Wegener, H. C., & Collignon, P. (2008). Resistance in bacteria of the food chain: Epidemiology and control strategies. *Expert Review of Anti-Infective Therapy, 6*(5), 733–750.

Abbas, G., Khan, I., Mohsin, M., Sajjad-ur-Rahman, Y. T., & Ali, S. (2019). High rates of CTX-M group-1 extended-spectrum β-lactamases producing Escherichia coli from pets and their owners in Faisalabad. *Pakistan, Infection and Drug Resistance, 12*, 571–578.

Allen, H. K., & Stanton, T. B. (2014). Altered egos: Antibiotics effects on food animal microbiomes. *Annual Review of Microbiology, 68*, 297–315.

Arias, C. A., & Murray, B. E. (2008). Emergence and management of drug-resistant enterococcal infections. *Expert Review of Anti-Infective Therapy, 6*, 637–655.

Asadollahi, P., Farahani, N. N., Mirzali, M., Khoramrooz, S. S., Belkum, A. V., Asadollahi, K., Dadashi, M., & Darban-Sarokhalil, D. (2018). Distribution of the most prevalent *spa* types among clinical isolates of methicillin-resistant and susceptible Staphylococcus aureus around the world. *Frontiers in Microbiology, 9*, 163.

Bates, J., Jordens, Z., & Selkon, J. B. (1993). Evidence for an animal origin of vancomycin-resistant enterococci. *Lancet, 342*, 490–491.

Bengtsson, B., & Greko, C. (2014). Antibiotic resistance-consequences for animal health, welfare, and food production. *Upsala Journal of Medical Sciences, 119*, 96–102.

Bhattacharya, D., Banerjee, J., Bandyopadhyay, S., Mondal, B., Nanda, P. K., Samantha, I., Mahanti, A., Das, A. K., Das, G., Dandapat, P., & Bandyopadhyay, S. (2016). First report on vancomycin resistant Staphylococcus aureus in bovine and caprine milk. *Microbial Drug Resistance, 22*(8), 675–681.

Broom, L. J. (2017). The sub-inhibitory theory for antibiotic growth promoters. *Poultry Science, 96*, 3104–3108.

Cerca, N., Martins, S., Sillankorva, S., Jefferson, K. K., Pier, G. B., Oliviera, R., & Azeredo, J. (2005). Effects of growth in the presence of subinhibitory concentrations of dicloxacillin on staphylococcus epidermidis and staphylococcus haemolyticus biofilms. *Applied and Environmental Microbiology, 71*, 8677–8682.

Cromwell, G. L. (2002). Why and how antibiotics are used in swine production. *Animal Biotechnology, 13*, 7–27.

Damborg, P., Nielsen, S. S., & Guardabassi, L. (2009). Escherichia coli shedding patterns in humans and dogs: Insights into-within household transmission of phylotypes associated with urinary tract infections. *Epidemiology and Infection, 137*, 1457–1464.

Davis, M., & Walsh, T. R. (2018). A colistin crisis in India. *The Lancet, 18*(3), 256–257. https://doi.org/10.1016/S1473-3099(18)30072-0

Dibner, J. J., & Richards, J. D. (2005). Antibiotic growth promoters in agriculture: History and mode of action. *Poultry Science, 84*, 634–643.

Dolejska, M., Bierošová, B., Kohoutova, L., Literak, I., & Čížek, A. (2009). Antibiotic-resistant salmonella and Escherichia coli isolates with integrons and extended-spectrum beta-lactamases in surface water and sympatric black-headed gulls. *Journal of Applied Microbiology, 106*, 1941–1950.

Drummond, L. J., Smith, D. G. E., & Poxton, I. R. (2003). Effects of sub-MIC concentrations of antibiotics on growth of and toxin production by Clostridium difficile. *Journal of Medical Microbiology, 52*, 1033–1038.

Friedman, H., & Warren, G. H. (1974). Enhanced susceptibility of penicillin resistant staphylococci to phagocytosis after *in vitro* incubation with low doses of nafcillin (38177). *Proceedings of the Society for Experimental Biology and Medicine, 146*, 707–711.

Gaskins, H. R., Collier, C. T., & Anderson, D. B. (2002). Antibiotics as growth promotants: Mode of action. *Animal Biotechnology, 13*, 29–42.

Ghirardini, A., Grillini, V., & Verlicchi, P. (2020). A review of the occurrence of selected micropollutants and microorganisms in different raw and treated manure–environmental risk due to antibiotics after application to soil. *Science of the Total Environment, 707*, 136118.

Giedraitiene, A., Vitkauskiene, R., Naginiene, R., & Pavilonis, A. (2011). Antibiotic resistance mechanisms of clinically important bacteria. *Medicinia, 47*, 137–146.

Hacker, J., Ott, M., & Hof, H. (1993). Effects of low, sub-inhibitory concentrations of antibiotics on expression of a virulent gene cluster of pathogenic Escherichia coli by using a wild-type gene fusion. *International Journal of Antimicrobial Agents, 2*, 263–270.

Haenni, M., Hocquet, D., Ponsin, C., Cholley, P., Guyeux, C., Madec, J., & Bertrand, X. (2015). Population structure and antimicrobial susceptibility of *Pseudomonas aeruginosa* from animal infections in France. *BMC Veterinary Research, 11*(9), 1–5.

Haenni, M., Bour, M., Châtre, P., Madec, J.-Y., Plésiat, P., & Jeannot, K. (2017). Resistance of animal strains of Pseudomonas aeruginosa to carbapenems. *Frontiers in Microbiology, 8*, 1847. https://doi.org/10.3389/fmicb.2017.01847

Hammerum, A. M. (2012). Enterococci of animal origin and their significance for public health. *Clinical Microbiology and Infection, 18*(7), 619–625.

Heuer, O. E., Hammerum, A. M., Collignon, P., & Wegener, H. C. (2006). Human health hazard from antimicrobial-resistant enterococci in animals and food. *Clinical Infectious Diseases, 43*, 911–916.

Jeannot, K., Bolard, A., & Plesiat, P. (2017). Resistance to polymyxins in gram-negative organisms. *International Journal of Antimicrobial Agents, 49*(5), 526–535.

Jonas, O. B., Irwin, A., Berthe, F. C. J., Le Gall, F. G., & Marquez, P. V. (2017). *Drug resistant infections: A threat to our economic future (Vol. 2), Final report (English)*. HNP/Agriculture Global Antimicrobial Resistance Initiative, World bank Group. https://documents1.worldbank.org/curated/en/323311493396993758/pdf/final-report.pdf

Jones, K. E., Patel, N. G., Levy, M. A., Storeygard, A., Balk, D., Gittleman, J. L., & Daszak, P. (2008). Global trends in emerging infectious diseases. *Nature, 451*, 990–993.

Jukes, T. H., Stokstad, E., Tayloe, R., Cunha, T., Edwards, H., & Meadows, G. (1950). Growth promoting effect of aureomycin on pigs. *Archives of Biochemistry, 26*, 324–325.

Larsen, J., Schønheyder, H. C., Singh, K. V., Lester, C. H., Olsen, S. S., Porsbo, L. J., Garcia-Migura, L., Jensen, L. B., Bisgaard, M., Murray, B. E., & Hammerum, A. M. (2011). Porcine and human community reservoirs of *enterococcus faecalis*, Denmark. *Emerging Infectious Diseases, 17*, 2395–2397.

Liu, Y. Y., Wang, Y., Walsh, T. R., Yi, L. X., Zhang, R., Spencer, J., Doi, Y., Tian, G., Dong, B., Huang, X., Yu, L. F., Gu, D., Ren, H., Chen, X., Lv, L., He, D., Zhou, H., Liang, Z., Liu, J.-H., & Shen, J. (2016). Emergence of plasmid mediated colistin resistance mechanism MCR-1 in animals and human beings in China: A microbiological and molecular biological study. *Lancet Infectious Diseases, 16*(2), 161–168.

Magnusson, U., Moodley, A., & Obsbjer, K. (2021). Antimicrobial resistance at the livestock-human interface: Implications for veterinary science. *Revue Scientifique et Technique Office International des Epizooties, 40*(2), 511–521.

Mandakini, R., Roychoudhury, P., Subudhi, P. K., Kylla, H., Samanta, I., Bandyopadyay, S., & Dutta, T. K. (2020). Higher prevalence of multi-drug resistant- extended spectrum ß lactamases producing Escherichia coli in unorganised pig farms compared to organised pig farms in Mizoram, India. *Veterinary World, 13*(12), 2752–2758.

Manishimwe, R., Moncada, P. M., Bugarel, M., Scott, H. M., & Loneragan, G. H. (2021). Antibiotic resistance among *Escherichia coli* and *salmonella* isolated from dairy cattle feces in Texas. *PLoS One, 16*(5), e0242390.

Mistry, H., Sharma, P., Mahato, S., Saravanan, R., Anand Kumar, P., & Bhandari, V. (2016). Prevalence and characterization of Oxacillin susceptible mec A-positive clinical isolates of Staphylococcus aureus causing bovine mastitis in India. *PLoS One, 11*(9), e0162256.

Moore, C. E. (2019). Changes in antibiotic resistance in animals. *Science, 365*(6459), 1251–1252.

Moore, P., Evenson, A., Luckey, T., McCoy, E., Elvehjem, C., & Har, E. (1946). Use of sulfasuxidine, streptothricin, and streptomycin in nutritional studies with chick. *Journal of Biological Chemistry, 165*(2), 437–441.

Moubareck, C., Bourgeois, N., Courvalin, P., & Doucet-Populaire, F. (2003). Multiple antibiotic resistance gene transfer from animal to human enterococci in the digestive tract of gnotobiotic mice. *Antimicrobial Agents and Chemotherapy, 47*, 2993–2996.

Mutua, F., Sharma, G., Grace, D., Bandyopadhyay, S., Shome, B., & Lindahl, J. (2020). A review of animal health and drug use practices in India, and their possible link to antimicrobial resistance. *Antimicrobial Resistance and Infection Control, 9*(1), 103.

Nanduri, B., Lawrence, M. L., Boyle, C. R., Ramkumar, M., & Burgess, S. C. (2006). Effects of subminimum inhibitory concentrations of antibiotics on the *Pasteurella multocida* proteome. *Journal of Proteome Research, 5*, 572–580.

Nienhoff, U., Kadlec, K., Chaberny, I. F., Verspohl, J., Gerlach, G.-F., Schwarz, S., Simon, D., & Nolte, I. (2009). Transmission of methicillin resistant Staphylococcus aureus strains between humans and dogs: Two case reports. *Journal of Antimicrobial Chemotherapy, 64*(3), 660–662.

Niewold, T. A. (2007). The non-antibiotic anti-inflammatory effect of antimicrobial growth promoters, the real mode of action? *A hypothesis, Poultry Science, 86*, 605–609.

O'Neill, J. (2015). *Antimicrobials in agriculture and the environment: Reducing unnecessary use and waste* (Vol. 40). The Review on Antimicrobial Resistance.

O'Neill, J. (Ed.). (2016). *Review on antimicrobial resistance. Tackling a global health crisis: Initial steps. Tackling drug resistant infections globally: Final report and recommendations*. Welcome Trust – HM Government.

Oliver, S. P., & Murinda, S. E. (2012). Antimicrobial resistance of mastitis pathogens. *The Veterinary Clinics of North America. Food Animal Practice, 28*(2), 165–185.

Page, S. W., & Gautier, P. (2012). Use of antimicrobial agents in livestock. *Revue Scientifique et Technique Office International des Epizooties, 31*, 145–188.

Palma, E., Tilocca, B., & Roncada, P. (2020). Antimicrobial resistance in veterinary medicine: An overview. *International Journal of Molecular Sciences, 21*, 1914. https://doi.org/10.3390/ijms21061914

Papich, M. G. (2013). Antibiotic treatment of resistant infections in small animals. *Veterinary Clinics of North America: Small Animal Practice, 43*(5), 1091–1107.

Perry, J., Waglechner, N., & Wright, G. (2016). The prehistory of antibiotic resistance. *Cold Spring Harbor Perspectives in Medicine, 6*, a025197.

Pomba, C., Rantala, M., Greko, C., Baptiste, K. E., Catry, B., van Duijkeren, E., Mateus, A., Moreno, M. A., Pyörälä, S., Ružauskas, M., Sanders, P., Teale, C., Threlfall, E. J., Kunsagi, Z., Torren-Edo, J., Jukes, H., & Törneke, K. (2017). Public health risk of antimicrobial resistance transfer from companion animals. *The Journal of Antimicrobial Chemotherapy, 72*(4), 957–968.

Pormohammad, A., Nasiri, M. J., & Azimi, T. (2019). Prevalence of antibiotic resistance in Escherichia coli strains simultaneously isolated from humans, animals, foods, and the environment: A systematic review and meta-analysis. *Infection and Drug Resistance, 12*, 1187–1191.

Portis, E., Lindeman, C., Johansen, L., & Stoltman, G. (2012). A ten year (2000-2009) study of antimicrobial susceptibility of bacteria that cause bovine respiratory disease complex-*Mannheimia haemolytica Pasteurella multocida*, and *Histophilus somni* – In the United States and Canada. *Journal of Veterinary Diagnostic Investigation, 24*, 932–944.

Prescott, J. F. (2008). Antimicrobial use in food and companion animals. *Animal Health Research Reviews, 9*(2), 127–133.

Prescott, J. F. (2017). History and current use of antimicrobial drugs in veterinary medicine. *Microbiology Spectrum, 5*(6), ARBA-0002-2017. https://doi.org/10.1128/microbiolspec.ARBA-0002-2017

Price, L. B., Stegger, M., Hasman, H., Aziz, M., Lrse, J., Andersen, P. S., Pearson, T., Waters, A. E., Foster, J. T., Schupp, J., Gillece, J., Driebe, E., Liu, C. M., Springer, B., Zdvoc, I., Battisti, A., Franco, A., Zmudzki, J., Schwarz, S., Butaye, P., Jouy, E., Pomba, C., Porrero, M. C., Ruimy,

R., Smith, T. C., Robinson, D. A., Weese, J. S., Arriola, C. S., Yu, F., Laurent, F., Keim, P., Skov, R., & Aarestrup, F. M. (2012). *Staphylococcus aureus* cc398: Adaption and emergence of methicillin resistance in livestock. *Microbiologica, 3*(1), e00305–e00311.

Raboisson, D., Ferchiou, A., Sans, P., Lhermie, G., & Derville, M. (2020). The economics of antimicrobial resistance in veterinary medicine: Optimising social benefits through meso-economic approaches from public and private perspectives. *One Health, 10*, 100145. https://doi.org/10.1016/j.onehlt.2020.100145

Redondo-Salvo, S., Fernandez-Lopez, R., Ruiz, R., Vielva, L., de Toro, M., Rocha, E. P. C., Garcillan-Barcia, M. P., & de la Cruz, F. (2020). Pathways for horizontal gene transfer in bacteria revealed by a global map of their plasmids. *Nature Communications, 11*, 1–13.

Reeks, B. Y., Champlin, F. R., Paulsen, D. B., Scruggs, D. W., & Lawrence, M. L. (2005). Effects of sub-minimum inhibitory concentration of antibiotic levels and temperature on growth kinetics and outer membrane protein expression in *Mannheimia haemolytica* and *Haemophilus somnus*. *Canadian Journal of Veterinary Research, 69*, 1–10.

Sandberg, T., Stenqvist, K., & Svanborg-Eden, C. (1979). Effects of sub-minimal inhibitory concentrations of ampicillin, chloramphenicol, and nitrofurantoin on the attachment of Escherichia coli to human uroepithelial cells *in vitro*. *Reviews of Infectious Diseases, 1*, 838–844.

Sherry, N., & Howden, B. (2018). Emerging gram-negative resistance to last-line antimicrobial agents Fosfomycin, colistin, and ceftazidime-avibactum – Epidemiology, laboratory detection, and treatment implications. *Expert Review of Anti-Infective Therapy, 16*(4), 289–306.

Song, H.-J., Kim, S.-J., Moon, D. C., Mechesso, A. F., Choi, J.-H., Kang, H. Y., Boby, N., Yoon, S.-S., & Lim, S.-K. (2022). Antimicrobial resistance in *Escherichia coli* isolates from healthy food animals in South Korea, 2010–2020. *Microorganisms, 10*, 524–535.

Starner, T. D., Shrout, J. D., Parsek, M. R., Appelbaum, P. C., & Kim, G. (2008). Subinhibitory concentrations of azithromycin decrease non-typeable Haemophilus influenzae biofilm formation and diminish established biofilms. *Antimicrobial Agents and Chemotherapy, 52*, 137–145.

Swann, N. N. (1969). *Report of the joint committee on the use of antibiotics in animal husbandry and veterinary medicine*. HMSO.

Sweileh, W. M. (2021). Global research activity on antimicrobial resistance in food-producing animals. *Archives of Public Health, 79*, 49.

Tang, K. L., Caffrey, N. P., Nobrega, D. B., Cork, S. C., Ronksley, P. E., Barkema, H. W., Polachek, A., Ganshorn, H., Sharman, N., Kellner, J. D., & Ghali, W. A. (2017). Restricting the use of antibiotics in food-producing animals and its associations with antibiotic resistance in food-producing animals and human beings: A systemic review and meta-analysis. *Lancet Planetary Health, 1*(8), e316–e327.

Teillant, A., & Laxminarayan, R. (2015). Economics of antibiotic use in US swine and poultry production. *Choices, 30*(1), 1–11.

Vaarten, J. (2012). Clinical impact of antimicrobial resistance in animals. *Revue Scientifique et Technique Office International des Epizooties, 31*, 221–229.

Van Boeckel, T. P., Brower, C., Gilbert, M., Grenfell, B. T., Levin, S. A., Robinson, T. P., Teillant, A., & Laxminarayan, R. (2015). Global trends in antimicrobial use in food animals. *Proceedings of the National Academy of Sciences of the USA, 112*(18), 5649–5654.

Van Boeckel, T. P., Glennon, E. E., Che, D., Gilbert, M., Robinson, T. P., Grenfell, B. T., Levin, S. A., Bonhoeffer, S., & Laxminarayan, R. (2017). Reducing antimicrobial use in food animals. *Science, 357*(6358), 1350–1352.

Van Boeckel, T. P., Pires, J., Silvester, R., Zhao, C., Song, J., Criscuolo, N. G., Gilbert, M., Bonhoeffer, S., & Laxminarayan, R. (2019). Global trends in antimicrobial resistance in animals in low-and middle-income countries. *Science, 365*, eaaw1944.

Van Duijkeren, E., Catry, B., Greko, C., Moreno, M. A., Pomba, M. C., Pyorala, S., Ruzauskas, M., Sansders, P., Threlfall, E. J., Torren-Edo, J., & Torneke, K. (2011). Review on methicillin resistant *staphylococcus pseudintermidius*. *Journal of Antimicrobial Chemotherapy, 66*(12), 2705–2714.

Van Epps, A., & Blaney, L. (2016). Antibiotic residues in animal waste: Occurrence and degradation in conventional agricultural waste management practices. *Current Pollution Reports, 2*, 135–155.

Wang, R., van Dorp, L., Shaw, L. P., Bradley, P., Wang, Q., Wang, X., Jin, L., Zhang, Q., Liu, Y., Rieux, A., Dorai-Schneiders, T., Weinert, L. A., Iqbal, Z., Didelot, X., Wang, H., & Balloux, F. (2018). The global distribution and spread of the mobilised colistin resistance gene *mcr*-1. *Nature Communications, 9*, 1179.

Willems, R. J. L., Top, J., van den Braak, N., van Belkum, A., Endtz, H., Mevius, D., Stoberringh, E., van den Bogaard, A., & van Embden, J. D. A. (2000). Host specificity of vancomycin-resistant enterococcus faecium. *The Journal of Infectious Diseases, 182*, 816–823.

World Health Organization (WHO). (2017). *Integrated surveillance of antimicrobial resistance in food borne bacteria: Application of a one health approach*. WHO. Licence: CC BY-NC-SA 3.0 IGO.

World Health Organization (WHO). (2019). *Critically important antimicrobials for human medicine, 6th revision*. WHO. 9789241515528-eng.pdf (who.int)

World Organisation for Animal Health (OIE). (2007). *OIE list of antimicrobials of veterinary importance*. OIE. https://www.oie.int/app/uploads/2021/03/oie-list-antimicrobials.pdf

Xiong, W., Sun, Y., & Zeng, Z. (2018). Antimicrobial use and antimicrobial resistance in food animals. *Environmental Science and Pollution Research, 25*, 18377–18384.

Antimicrobial Resistance in Fisheries

Murugadas Vaiyapuri, Mukteswar Prasad Mothadaka, Madhusudana Rao Badireddy, Chandragiri Nagarajarao Ravishankar, and Joykrushna Jena

Contents

Abstract

Antimicrobial resistance (AMR) is becoming a major health concern for both humans and animals. AMR is increasingly being found in aquatic animal systems, as well as in human and terrestrial animal health systems. Because it deals with the

M. Vaiyapuri (✉)
Microbiology Fermentation and Biotechnology Division of ICAR-Central Institute of Fisheries Technology (ICAR-CIFT), Cochin, Kerala, India
e-mail: Murugadas.V@icar.gov.in

M. P. Mothadaka · M. R. Badireddy
Visakhapatnam Research Centre of ICAR-CIFT, ICAR-Central Institute of Fisheries Technology (ICAR-CIFT), Visakhapatnam, Andhra Pradesh, India
e-mail: prasadmm@hotmail.com; mm.prasad@icar.gov.in; bm.rao@icar.gov.in; bmrcift@gmail.com

C. N. Ravishankar
ICAR-Central Institute of Fisheries Technology (ICAR-CIFT), Kochi, Kerala, India

ICAR-Central Institute of Fisheries Education (ICAR-CIFE), Maharashtra, Mumbai, India
e-mail: ravishankar.cn@icar.gov.in

J. Jena
Division of Fisheries Science, Indian Council of Agricultural Research (ICAR), New Delhi, India
e-mail: jkjena2@gmail.com

M. P. Mothadaka et al. (eds.), *Handbook on Antimicrobial Resistance*,
https://doi.org/10.1007/978-981-19-9279-7_5

aquatic environment, the fisheries industry is critical to the whole health system. The blue economy is a result of this sector, which is thought to be crucial to the global economy. The economic benefits are near equal for both capture and farmed fisheries. With the increasing intensification of culture fisheries, there is a growing fear of AMR spreading throughout the industry. With the premise in the current context, the chapter discusses AMR in the fisheries and systems over the previous three decades, with an emphasis on the future trend for management.

Keywords

Antimicrobial resistance · Fisheries · Aquaculture

1 Introduction

Fish and fishery products continue to be a good source of animal protein, and they are advocated for a multitude of deficiency-related disorders. It satisfies the nutritional needs of a poor man's diet. For hundreds of millions of people, fisheries and aquaculture remain significant sources of food, nutrition, income, and livelihood. To meet necessary global nutritional needs, capture fisheries are not adequate. The aquaculture industry has contributed a great deal to the world economy. In 1950, aquaculture provided only 4% of the fish meant for human consumption, which increased to 9% in 1980, 19% in 1990, 52% in 2018 (FAO, 2020a), and is expected to provide 59% of the fish by 2030. The aquaculture economy benefits from both crustaceans and finfish. Carp and tilapia species, for instance, meet a large share of the national and international demand for aquatic meals. Carp, tilapia, and farmed shrimp are predicted to account for more than half of all farmed animal aquaculture production (FAO, 2020a).

Carp satisfies national demand, whereas tilapia serves global needs as well. With the rising contribution of fisheries to the production of animal protein, per capita consumption of fish food has increased to 20.5 kg in 2018 from 9 kg in 1961 (FAO, 2020a). The live weight of fish produced through captured fisheries and aquaculture activities has surpassed 178.5 million tons, as per 2018 estimates. Also, 54% and 46% of its production came from captured fisheries and aquaculture, respectively. In addition, 156.4 million tons of the 178.5 million tons produced are used for human consumption by the world's 7.6 billion people, with a per capita intake of over 20.5 kg per year. Of these, 37.6% are traded for export for a total value of USD 164.1 billion (FAO, 2020a). The EU, China, Norway, Vietnam, Chile, India, Thailand, the United States, Canada, Ecuador, and Russia are the biggest exporting countries in the world. In terms of export values, the EU and China represent approximately 56 billion dollars, while other countries account for 58 billion dollars. The United States, Japan, China, Spain, Vietnam, France, Italy, Germany, Sweden, and South Korea are major seafood importers. China, Vietnam, and the United States are all involved in both exports and imports. Food security is a key component of the 2030 UN Agenda for Sustainable Development Goals, which aims to eradicate poverty and safeguard the environment, and fisheries and aquaculture play a vital role in the achieving this SDG of UN (UN, 2018).

2 A Perusal of Global Fish and Fisheries Sectors

By 2018, global fish production had reached over 179 mmt, with 87% (156 mmt) of it going to human palatals. The global demand for fish and fisheries products is continually increasing. This is meeting the food and nutritional security needs of hundreds of millions of people around the world, as well as providing a stable source of income for millions of fishermen. The production, trade, and consumption of fisheries and aquaculture sectors have made great improvement in recent decades, peaking in 2018. One key point to note is that aquaculture has grown rapidly since the 1990s (Table 1), whilst inland fisheries production has increased slowly and the capture fisheries sector has remained stable.

Aquaculture's continued viability and competent fisheries management were critical in preserving these trends. In the case of fisheries, long-term growth was associated with sound management practices, and the majority of stocks were recovered. However, overfished stocks in some countries and regions were never replenished. This necessitates more measures to ensure the sustainability of the fisheries and aquaculture sectors. In the absence of such, millions of people's food, nutritional security, and socioeconomic conditions around the world could be jeopardized.

3 Aquaculture Production

Aquaculture can be defined as the "farming of aquatic animals that included crustaceans' finfish, molluscs, etc. and aquatic plants, mostly algae, employing or intra freshwater, sea water, brackish water and inland saline water." By 2018, global aquaculture production (Table 2) had reached a peak of 114.5 mmt on a live weight basis, totaling USD 263.6 billion.

In 2018, inland aquaculture via aquatic animals accounted for 62.5% of total production fish farmed for human consumption, totaling 51.3 million tons. Lacustrine and fish farms are two sources of aquaculture produce. For the past two decades, Asia has been the leading contributor to global aquaculture (89%). China, India, Indonesia, Vietnam, Bangladesh, Egypt, Norway, and Chile are major

Table 1 Growth of world fisheries between 1990 and 2018

World rise in capture fisheries, aquaculture production, and fish intake as food from 1990 to 2018	Growth in percent
Enhancement in production of capture fisheries	+14
Enhancement in production of world aquaculture	+527
Enhancement in fish consumption	+122

Table 2 World aquaculture production

Type of aquatic produce	In million tons
Aquatic animals	82.1
Aquatic algae	32.4
Ornamental seashells and pearls	0.26

aquaculture producers. According to the Organization for Economic Co-operation and Development, there were 27 different types of antimicrobials used in animals, and the animal healthcare market was worth roughly USD 22 billion in 2011. According to industry research, the value of animal health care would reach USD 53.42 billion by 2025, up from USD 32.05 billion in 2016. From 2017 to 2025, it is expected to expand at a compound annual growth rate of 5.6%.

The key motivational factors for food sectors are intensification in occurrence of zoonotic; scientific improvements in healthcare markets of research and development sector; mandatory vaccination of animals by controlling agencies; and constant increase in protein rich food intake, viz., eggs, fish, milk, and meat.

The assessed statistical data revealed that antimicrobial employment in animals in 118 countries increased by 89% between 2015 and 2017.

Antimicrobial residues and AMR pathogens have been found in fish and fisheries products, which is a major source of worry. Antimicrobial resistance in microorganisms discovered in aquaculture products has a challenging base to pin down (Karunasagar et al., 2020). Sewage from human hospital settings and livestock farms carries AMR bacteria into aquatic environments. These AMR bacteria can get into aquaculture systems and end up in the fish that are harvested from them. Given the difficulty in identifying the source of AMR bacteria, caution was advised before drawing any conclusions about the source of AMR bacteria, particularly in the context of aquaculture (Karunasagar et al., 2020).

Aquaculture is expanding and must expand at a breakneck pace to fulfil the increasing population and per capita consumption demands, despite limited resources and space. Infectious disorders connected with noninfectious environmental stress inevitably occur as a result of these production pressures. Farmers employ antibiotics or antimicrobial compounds in an inappropriate way or approach to alleviate the production pressure on aquaculture (Reverter et al., 2020; Schar et al., 2018). As a result, antimicrobial resistance develops throughout the system. The aquatic environment is critical because it serves as the vehicle for all run-off in the sector. The use of antimicrobial medications in aquaculture, as well as contamination from the land, contributes to the selection, development, and spread of drug-resistant bacteria, constituting a serious public health risk (FAO, 2020b). Furthermore, global climate change is causing a massive shift in the microbiota in aquatic environments, leading to the introduction of novel infectious illnesses in aquatic animals. To commemorate the extensive contribution to nutritional, food security, and blue economy globally, FAO celebrates 2022 as the international year of artisanal fisheries and aquaculture as declared in UN general assembly. When there is a substantial change in the blue economy for the production of aquatic products, the notion of disease outbreaks in aquatic animals and the emergence of antibiotic resistance is also emphasized (Wenhai et al., 2019). In addition to that food with an acceptable microbiological quality range can also act as a sink for antibiotic resistance development via bacteria, bacteriophages, bacterial DNA, and mobile genetic elements, some of which may include AMR genes. As a result, the food chain ecosystem may provide favorable environments for AMR bacteria gene transfer, selection, and persistence, and this route should not be neglected. The fish that are harvested have numerous distribution steps in a typical seafood production chain, including

harvest to consumer, harvest to processor, harvest to distributors, and harvest to retailers.

Antibiotics were a key breakthrough in human and animal healthcare in the twentieth century, and they are being used in treatments today (Deng et al., 2015). Antimicrobial resistance is the ability of the microbes to resist the action of antimicrobials on survival or multiplications. However, due to the evolution of multidrug resistance (MDR), pan drug resistance (PDR), and extensive drug resistance (XDR) bacteria, a worrisome trend in the rise of antimicrobial resistance has now been noticed in the last two decades, that is, AMR in bacteria, which is driving humanity toward the pre-antibiotic era (Magiorakos et al., 2012). Secondly, the lack of novel antibiotic classes discovered in the past two or three decades has exacerbated the situation and aided the spread of AMR in the health and animal agriculture sectors. Aquaculture animals, unlike terrestrial species, live in close proximity to their environment, such as water and soil, making individualized therapy regimens practically difficult. Antibiotic use affects the pond's ambient microbiota, inevitably increasing the spread of AMR (Aly & Albutti, 2014; Hashmi, 2020). Antimicrobials existing in subtherapeutic concentrations induce AMR in commensal, pathogenic, or seafood safety bacteria, which can easily spread between one other via horizontal or vertical gene transfer pathways (Chow et al., 2021). After the aquaculture crop time has ended, the water is generally released to a neighboring large aquatic body; thus, the risk of spreading to other aquatic habitats is very high (Bashir et al., 2020). Also, the use of antimicrobial medications across the sectors, viz., land-based contamination of waterways, etc., finally enters aquatic environment and aquaculture productions and contribute to the selection, development, and spread of drug-resistant bacteria, posing a serious public health risk (Fig. 1). This may result in the selection

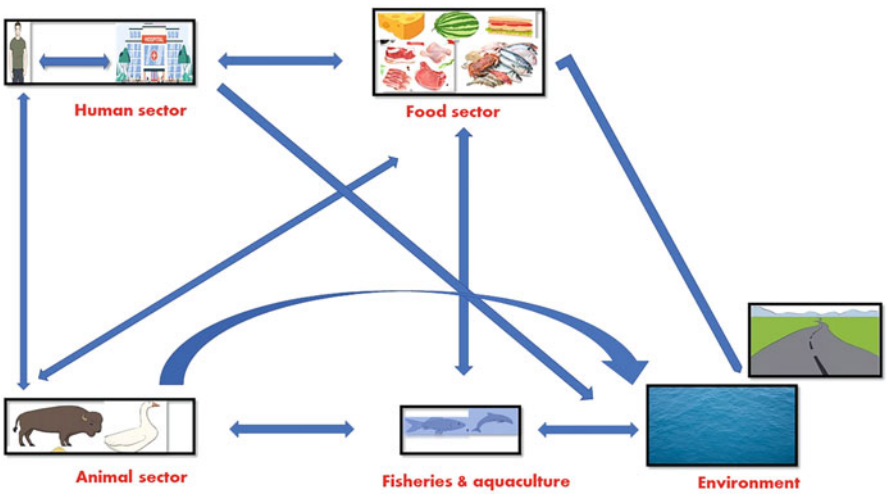


Fig. 1 Flow of antimicrobial usage enters fisheries sector and possible development and spread of antimicrobial resistance in fisheries sector

of more resistant strains in the aquatic environment (Reverter et al., 2020; Van Boeckel et al., 2015). The evolved antimicrobial resistance in the aquatic animal pathogens gets transferred to the pathogens of public health, thereby increasing the complexity of control.

This chapter offers researchers in the aquatic animal's environment and health for the antimicrobial resistance, which is a vital component of one's health, a one-stop destination.

4 Antimicrobial Resistance in Fisheries

Antibiotics reported to be used in fisheries sector include amoxicillin, ampicillin, chloramphenicol, florfenicol, erythromycin, streptomycin, neomycin, furazolidone, nitrofurantoin, oxolinic acid, enrofloxacin, flumequine, oxytetracycline, chlortetracycline, tetracycline, and sulfonamides (Heuer et al., 2009). The trends in the usage of antimicrobials vary between states and countries, and regulatory frameworks are widely different across countries. Hence, the development of antimicrobial resistance also correlates with the antimicrobial usage across the geographically distinct locations with varied temperatures and climatic changes (Karunasagar et al., 2020; Poirel et al., 2012). The global warming also plays a major role in the emergence of new pathogens for aquatic animals and a shift is expected in the usage of antimicrobials across the aquaculture system (MacFadden et al., 2018). The commonly adopted method for control of AMR is the strengthening of the surveillance system across the globe to distinguish the variation in temporal and geographic scales (Schar et al., 2021). The burden of AMR in the fisheries and aquaculture can be ascertained by AMR estimated in the pathogens, commensals associated with aquatic animals obtained through active and passive surveillance, and AMR in public health pathogens from the fish and fishery products. Country-wise antimicrobial resistance reported in the aquatic animals across the bacterial pathogens is depicted in Table 3. AMR is currently a serious threat to public health. Because of globalization of export and import, as well as faster mobility, AMR developed in a country is no longer an issue confined to a certain geographic area. AMR in aquatic animals was observed in the 1970s, but systematic assessment of AMR began in the 2000s (Table 4).

The majority of the research was focused on pathogens in aquatic species such *Vibrio* sp. (*V. parahaemolyticus, V. vulnificus, V. alginolyticus, Vibrio harveyi,* and other *Vibrio* spp.), *Aeromonas* sp., and *A. hydrophila, A. salmonicida, A. veronoii*, and other *Aeromonas* spp. *Streptococcus* sp. (*S. agalactiae, S. uberis,* and *S. iniae*) and *Edwardsiella* sp.. *Lactococcus garvieae, Vibrio anguillarum, Flavobacterium psychrophilum, Flavobacterium columnare, Piscirickettsia salmonis, Renibacterium salmoninarum, Yersinia ruckeri, Tenacibaculum dicentrarchi*, and others that have all been examined for antibiotic resistance (Smith & Egan, 2020). Among the pathogens tested, there were serotype differences in the AMR pattern. Countries with varying levels of resistance were identified; there was no resistance to sulfonamides, tetracycline resistance in a

Table 3 Country-wise antimicrobial resistance detected in finfish and shellfish across pathogens

S. no	Country	Species	Resistance	Bacteria	Reference
1	Australia	Shrimp	Ampicillin, amoxicillin, cephalosporin, erythromycin	*Vibrio* sp.	Akinbowale et al. (2006)
2	Bangladesh	*Oreochromis niloticus* (tilapia), *Labeo rohita* (rohu), and Penaeus monodon (shrimp)	Ampicillin, amoxicillin, cefotaxime, ceftriaxone	*V. parahaemolyticus*	Siddique et al. (2021)
3		Tilapia and koi	Tetracycline, oxytetracycline, chlortetracycline, ciprofloxacin, streptomycin, gentamicin, neomycin	*S. agalactiae*	Rahman et al. (2021)
4	Brazil	Shrimp farms	Ampicillin, tetracycline	*Vibrio* sp.	Rebouças et al. (2011)
5		Shrimp	MDR strains	*Vibrio* sp.	Albuquerque Costa et al. (2015), Rebouças et al. (2011)
6		Shrimp	MDR	*V. parahaemolyticus*	Melo et al. (2011)
7	Chile	Chilean salmon farms	Flumequine, florfenicol and oxytetracycline, GyrB mutations	Commensal (*Pseudomonas* sp.)	Concha et al. (2019)
8		Freshwater salmon farm	Tetracycline, amoxicillin, ampicillin. erythromycin, furazolidone, florfenicol, chloramphenicol, cefotaxime, trimethoprim-sulfamethoxazole	Gram-negative bacteria	Miranda and Zemelman (2002)
9		Chilean freshwater salmon farms	Florfenicol, streptomycin, chloramphenicol, oxytetracycline floR	Florfenicol-resistant bacteria	Fernández-Alarcón et al. (2010)
10		Freshwater salmon farms	Florfenicol, oxytetracycline floR and tet determinants	Fish gut microbiota	Higuera-Llantén et al. (2018)

(continued)

Table 3 (continued)

S. no	Country	Species	Resistance	Bacteria	Reference
11		Chilean salmon farming	Florfenicol, erythromycin, furazolidone, amoxicillin, sulfisoxazole and trimethoprim sul1, sul2, sul3, intI1, int2, dfrA1, dfrA12 and dfrA14	Commensal (*Pseudomonas* sp.)	Domínguez et al. (2019)
12	China	Tilapia	Streptomycin and erythromycin	*S. agalactiae*	Zhang et al., 2018
13		Aquaculture farm	Sulfonamide, tetracycline sul1, sul2, tetM, tetO, tetT, tetW	Commensal and Bacillus	Gao et al. (2012)
14		Aquaculture farms	Sulfonamide, tetracycline and quinolone sul1, sul2, sul3, tetM, tetO, tetQ, tetW, tetX, tetB/P, PMQR- qepA, oqxA, aac-16, qnrS	Sediment DNA analysis	Xiong et al. (2015)
15		Aquaculture farms eels	Aminoglycoside Sulfonamide, tetracycline, quinolone blaTEM, tetC, sulI, aadA, floR, qnrB; int1, int2; Int-1 qacEΔ1/sul1 gene; 14 cassette arrays detected. dfrB4-catB3-blaOXA-10-aadA1, dfrA12-orfF-aadA2	Commensal bacteria	Lin et al. (2016)
16		Tilapia	Penicillin, sulfamethoxydiazine, and sulfadiazine	*S. agalactiae*	Zhang et al. (2018)
17		Ya-Fish	Gentamicin, sinomin (SMZ/TMP), penicillin, tenemycin, fradiomycin, streptomycin	*S. agalactiae*	Schar et al. (2018)
18	China	Aquaculture farms	Florfenicol, sulfonamide, aminoglycoside, tetracyclines. floR, sulII, sulI, strB, strA, aadA, tetS, tetS	Farm sediment	Hong et al. (2018)

19		Salmon fish	Florfenicol, linezolid and chloramphenicol Oxazolidinone/ phenicol-resistant gene optrA	Columnaris diseases	Zeng et al. (2019)
20		Integrated polyculture aquaculture farm	Tetracycline, sulfonamides, quinolones, chloramphenicol, and β-lactamases sul1, sul2, sul3, tetA, tetM, tetT, tetW, aac(6′)-Ib, floR, qnrB, qnrA, qnrS, fexA, fexB, qepA, blaSHV, cmlA, cfr	Fish sediment	Zhou et al. (2019)
21		Shrimp hepatopancreas	Sulfonamide, quinolones, sul1, sul2, floR, strA, gyrA	Aquaculture farm sediment	Zhou et al. (2020)
22		Shrimp	Ampicillin, cefuroxime, amikacin, kanamycin, trimethoprim	*Vibrio* sp.	Li et al. (1999)
23		Shrimp	MDR	*V. vulnificus*	Pan et al. (2013)
24		Shrimp	Chloramphenicol, sulfonamides, trimethoprim, rifamycin, ampicillin, streptomycin, kanamycin	*V. parahaemolyticus*	He et al. (2019)
25	Ecuador	Farmed shrimp	Ampicillin resistance and intermediate resistance to tetracycline and amikacin	*V. parahaemolyticus*	Sperling et al. (2015)
26		Shrimp	Ampicillin, tetracycline, amikacin	*V. parahaemolyticus*	Sperling et al. (2015)
27	Egypt	Tilapia	Penicillin, ampicillin, vancomycin, chloramphenicol, rifampicin, ofloxacin, clindamycin, erythromycin, tetracycline	*S. agalactiae*	Osman et al. (2017)

(continued)

Table 3 (continued)

S. no	Country	Species	Resistance	Bacteria	Reference
28	Finland	Fish farms	Tetracycline, sulfonamide, aminoglycosides, beta-lactam resistance, transposons, efflux pump mechanism tet(32), tetM, tetO, tetS, tetW, tetA, tetE, tetG, tetH, sul2, dfrA1, aadA, aadA1, aadA2, strB, tnpA, qacEΔ1, mexF, oprD, oprJ, pncA, yecL, blaOXY, blaCTX, blaSHV, acrA, vanC, aacC	Aquaculture sediment	Muziasari et al. (2016)
29		Fish farms	Tetracycline, sulfonamide, trimethoprim, chloramphenicol, macrolides, integron, transposons sul1, dfrA1, tet(32), tetM, tetO, tetW, aadA1, aadA2, catA, emrB, matA, mefA, msrAe intI1, qacEΔ, tnpA	Aquaculture sediment	Muziasari et al. (2017)
30	Germany	Fish farm, *Piaractus mesopotamicus*	Macrolide, triclosan, aminoglycoside, aminocoumarin, fluoroquinolone, tetracycline, phenicol 80 different ARGs: rpoB, gyrA, mexQ, mexK, tetQ, tetA, mdtM, acrF, macB, gyrA, acrF, tetQ, TEM-190, QnrS1, tetG, floR, Tn3, IS21, IS91, IS3	Aquaculture farm sediment	Sáenz et al. (2019)
31	India	Shrimp	MDR strains	*Vibrio* sp.	Manjusha and Sarita (2011)
32		Shrimp	MDR strains	*Vibrio* sp.	Manjusha et al. (2005)
33		Shrimp	Ampicillin, colistin	*Vibrio* sp.	Sudha et al. (2014)

34		Shrimp	Ampicillin, cloxacillin, oxacillin, erythromycin, vancomycin, furazolidone	*Vibrio* sp.	Srinivasan and Ramasamy (2009)
35		Shrimp	Ampicillin, chlortetracycline	*Vibrio* sp.	Vaseeharan et al. (2005)
36		Shrimp	Ampicillin	*Vibrio* sp.	Singh et al. (2018)
37		Shrimp	MDR	*V. parahaemolyticus*	Devi et al. (2009)
38		Shrimp	Ampicillin, cefotaxime, cefoxitin, cephalothin, ceftazidime, amoxicillin-clavulanic acid, ciprofloxacin, gentamicin, meropenem	*V. parahaemolyticus*	Narayanan et al. (2020)
39		Shrimp	Ciprofloxacin, ampicillin, vancomycin, rifampicin	*V. harveyi*	Stalin and Srinivasan (2016)
40		Shrimp	Streptomycin, chloramphenicol, cotrimoxazole	*V. harveyi*	Karunasagar et al. (1994)
41	Iran	Shrimp	MDR	*Vibrio* sp.	Ansari and Raissy (2010)
42	Israel	Aquaculture farm silver carp *Hypophthalmichthys molitrix*)	Sulfonamide and tetracycline resistance sul1, tetA, intI1	*Aeromonas* sp.	Patil et al. (2016)
43		Aquaculture farms – Silver silver carp	Tetracycline, sulfonamides β-lactamase	Aquaculture farm sediment	Patil et al. (2020)
44	Italy	Aquaculture (fish, shellfish, crustaceans)	Sulfadiazine-trimethoprim, ampicillin, carbenicillin, kanamycin, cephalothin	*Photobacterium damselae* ssp. piscicida, *Vibrio fluvialis*, *Vibrio alginolyticus*, *Vibrio parahaemolyticus*, *Vibrio metschnikovii*	Laganà et al. (2011)
45		Shrimp	Tetracycline and sulfonamide	*Vibrio* sp.	Labella et al. (2013)
46		Shrimp	MDR	*V. parahaemolyticus*	Ottaviani et al. (2013)

(continued)

Table 3 (continued)

S. no	Country	Species	Resistance	Bacteria	Reference
47		Shrimp	Ampicillin, ceftazidime, cefotetan	*V. alginolyticus*, *V. parahaemolyticus*, *V. vulnificus*	Zanetti et al. (2001)
48		Shrimp	MDR, ampicillin, amoxicillin-clavulanic acid, erythromycin, sulfonamides	*Vibrio* sp.; *V. harveyi*; *V. aestuarianus*	Scarano et al. (2014)
49	Japan	Tilapia	Oxolinic acid, nalidixic acid, quinolones	*Photobacterium damselae* subsp. piscicida	Kim et al. (2005)
50		Finfish farm	Penicillinase	*Aeromonas hydrophila*	Sawai et al. (1976)
51		Finfish farm	Fluroquinolones gyrA, gyrB, parC, parE genes	*V. anguillarum*	Rodkhum et al. (2008)
52		Shrimp	Sulfonamides, streptomycin, chloramphenicol, tetracycline	*V. anguillarum*	Aoki et al. (1974)
53	Korea	Shrimp	Ampicillin, rifampicin, streptomycin, trimethoprim, tetracycline	*V. parahaemolyticus, V. alginolyticus*	Oh et al. (2011)
54	Lithuania	Common carp	Tetracycline	Commensal	Ruzauskas et al. (2021)
55	Malaysia	Shrimp	Ampicillin, tetracycline, and doxycycline	*Vibrio* sp.	Banerjee et al. (2012)
56		Shrimp	Ampicillin, cefoataxime, ciprofloxacin	*V. parahaemolyticus*	Al-Othrubi et al. (2014)
57		Tilapia *Oreochromis* sp.	Sensitive: amoxicillin, ampicillin, erythromycin, chloramphenicol, lincomycin, rifampicin, vancomycin, gentamicin, sulfamethoxazole + trimethoprim and tetracycline Resistant:	*Streptococcus agalactiae*	Abuseliana et al. (2010)

			neomycin, amikacin, kanamycin, streptomycin		
58		Tilapia	Neomycin and gentamicin	*S. agalactiae*	Laith et al. (2017)
59	Mexico	Shrimp	MDR strains	*Vibrio* sp.	Molina-Aja et al. (2002)
60		Shrimp	MDR strains	*Vibrio* sp.	Roque et al. (2001)
61	Nigeria	Shrimp	MDR strains	Vibrio sp.	Igbinosa (2016)
62	Norway	Salmon farm	Fluoroquinolone – gyrA	*Aeromonas salmonicida*	Oppegaard and Sorum (1994)
63		Chilean salmon aquaculture	Tetracycline, sulfonamide, β-lactamase tetA, tetG, dfrA1, dfrA5, dfrA12, sul1, sul2, blaTEM, strA-strB, intl1, aad9, intl2	Commensal bacteria	Shah et al. (2014)
64	Philippines	Shrimp	Oxytetracycline	*Vibrio* sp.	Tendencia and de la Peña (2001), Tendencia (2002)
65	South Africa	Tilapia, trout, and koi aquaculture system	Tetracycline, erythromycin, nalidixic acid resistance dhfr1, oxa2a, pse1	*Aeromonas* sp.	Jacobs and Chenia (2007)
66	South Korea	Finfish farm	Fluoroquinolone gyrA gene	*Edwardsiella tarda*	Shin et al. (2005)
67		Fish farm	Tetracycline, sulfonamide, integron. tetA, tetB, tetD, tetE, tetG, tetH, tetM, tetQ, tetX, tetZ, sul1, IntI1	Fish farm and effluents	Jang et al. (2018)
68	Spain	Tilapia	Tetracycline, oxytetracycline, oxolinic acid, flumequine, enrofloxacin	*Photobacterium damselae* subsp. piscicida	Martínez-Manzanares et al. (2008)
69		Rainbow trout farm	Nalidixic acid, oxolinic acid gyrB gene	*Yersinia ruckeri*	Gibello et al. (2004)
70		Shrimp	Tetracycline, amoxicillin-clavulanic acid, streptomycin	*Vibrio* sp.	Dubert et al. (2015)

(continued)

Table 3 (continued)

S. no	Country	Species	Resistance	Bacteria	Reference
71	Thailand	Tilapia	Oxolinic acid, gentamicin, sulfamethoxazole, trimethoprim	*S. agalactiae*	Dangwetngam et al. (2016)
72		Tilapia	Enrofloxacin	*Streptococcus agalactiae*	Kannika et al. (2017)
73		Shrimp	Amoxicillin-clavulanic acid	*Vibrio* sp.	Maisak et al. (1995)
74		Shrimp	Ampicillin resistance	*V. parahaemolyticus*	Yano et al. (2014)
75	USA	Farmed and wild caught shrimp	Ceftriaxone, tetracycline, ampicillin, ceftriaxone, gentamicin, streptomycin, trimethoprim, nalidixic acid, trimethoprim	Commensal and *Salmonella* spp. and *Vibrios*	Boinapally and Jiang (2007)
76		Shrimp	Ampicillin, apramycin, amoxicillin-clavulanic acid, streptomycin, trimethoprim	*V. parahaemolyticus*	Baker-Austin et al. (2008)
77		Shrimp	Streptomycin, chloramphenicol	*V. vulnificus*	Shaw et al. (2014)

Table 4 Year-wise trend in the detection of AMR

S. no	Resistance pattern observed in fisheries sector	Year
1	Sulfonamides, streptomycin, chloramphenicol, tetracycline	1974
2	Penicillinase	1976
3	Fluoroquinolone	1994
4	Streptomycin, chloramphenicol, cotrimoxazole	1994
5	Amoxicillin-clavulanic acid	1995
6	Ampicillin, cefuroxime, amikacin, kanamycin, trimethoprim.	1999
7	Ampicillin, ceftazidime, cefotetan.	2001
8	Tetracycline, amoxicillin, ampicillin. erythromycin, furazolidone, florfenicol, chloramphenicol, cefotaxime and trimethoprim-sulfamethoxazole	2002
9	Oxytetracycline	2002
10	Nalidixic acid, oxolinic acid	2004
11	Oxolinic acid, nalidixic acid and quinolones	2005
12	Fluoroquinolone	2005
13	Ampicillin, chlortetracycline	2005
14	Ampicillin, amoxicillin, cephalosporin, erythromycin.	2006
15	Tetracycline, erythromycin, nalidixic acid	2007
16	Ceftriaxone, tetracycline, ampicillin, ceftriaxone, gentamicin, streptomycin, trimethoprim, nalidixic acid, trimethoprim	2007
17	Fluoroquinolones	2008
18	Tetracycline, oxytetracycline, oxolinic acid, flumequine, enrofloxacin	2008
19	Ampicillin, apramycin, amoxicillin-clavulanic acid, streptomycin, trimethoprim.	2008
20	Ampicillin, cloxacillin, oxacillin, erythromycin, vancomycin, and furazolidone.	2009
21	Florfenicol, streptomycin, chloramphenicol and oxytetracycline	2010
22	Neomycin, amikacin, kanamycin and streptomycin	2010
23	Ampicillin, tetracycline	2011
24	Sulfadiazine-trimethoprim, ampicillin, carbenicillin, kanamycin, cephalothin	2011
25	Ampicillin, rifampicin, streptomycin, trimethoprim, tetracycline	2011
26	Sulfonamide, tetracycline	2012
27	Ampicillin, tetracycline, doxycycline	2012
28	Tetracycline, sulfonamide	2013
29	Tetracycline, sulfonamide, β-lactamase	2014
30	Ampicillin, colistin	2014
31	Ampicillin, cefotaxime, ciprofloxacin	2014
32	Ampicillin	2014
33	Streptomycin, chloramphenicol	2014
34	Ampicillin, amoxicillin-clavulanic acid, erythromycin, sulfonamides	2014
35	Sulfonamide, tetracycline, quinolone	2015
36	Ampicillin	2015
37	Tetracycline, amoxicillin-clavulanic acid, streptomycin	2015
38	Ampicillin, tetracycline, amikacin	2015
39	Aminoglycoside sulfonamide, tetracycline, quinolone	2016

(continued)

Table 4 (continued)

S. no	Resistance pattern observed in fisheries sector	Year
40	Tetracycline, sulfonamide, aminoglycosides, beta-lactam resistance, transposons, efflux pump mechanism	2016
41	Sulfonamide and tetracycline	2016
42	Oxolinic acid, gentamicin, sulfamethoxazole, trimethoprim	2016
43	Ciprofloxacin, ampicillin, vancomycin, rifampicin	2016
44	Penicillin, ampicillin, vancomycin, chloramphenicol, rifampicin, ofloxacin, clindamycin, erythromycin, tetracycline	2017
45	Tetracycline, sulfonamide, trimethoprim, chloramphenicol, macrolides, integron, transposons	2017
46	Neomycin and gentamicin	2017
47	Enrofloxacin	2017
48	Florfenicol, oxytetracycline	2018
49	Streptomycin, erythromycin	2018
50	Penicillin, sulfamethoxydiazine, sulfadiazine	2018
51	Gentamicin, sulfonamide and trimethoprim, penicillin, tenemycin, fradiomycin, streptomycin	2018
52	Florfenicol, sulfonamide, aminoglycoside, tetracyclines	2018
53	Tetracycline, sulfonamide, integron	2018
54	Ampicillin	2018
55	Flumequine, florfenicol, oxytetracycline	2019
56	Florfenicol, erythromycin, furazolidone, amoxicillin, sulfisoxazole, trimethoprim	2019
57	Florfenicol, linezolid, chloramphenicol	2019
58	Tetracycline, sulfonamides, quinolones, chloramphenicol, β-lactamases	2019
59	Macrolide, triclosan, aminoglycoside, aminocoumarin, fluoroquinolone, tetracycline, phenicol	2019
60	Chloramphenicol, sulfonamides, trimethoprim, rifamycin, ampicillin, streptomycin, kanamycin	2019
61	Sulfonamide, quinolones	2020
62	Tetracycline sulfonamides, β-lactamase	2020
63	Ampicillin, cefotaxime, cefoxitin, cephalothin, ceftazidime, amoxicillin-clavulanic acid, ciprofloxacin, gentamicin, meropenem	2020
64	Ampicillin, amoxicillin, cefotaxime, ceftriaxone	2021
65	Tetracycline, oxytetracycline, chlortetracycline, ciprofloxacin, streptomycin, gentamicin, neomycin	2021
66	Tetracycline	2021

few cases, and fluoroquinolone resistance in others. The identification of fluoroquinolones, phenicols, and tetracycline resistance has been on the rise. Initially, penicillin was used, and tetracycline resistance has been observed up to this point. The presence of florfenicols and tetracycline resistance was the most common (Table 4). Antimicrobial resistance genes in the aquatic environments are presented in Table 5.

Table 5 Resistance commonly noticed in pathogens or bacteria associated with aquatic animals

Bacteria	Resistance	Reference
Aeromonas hydrophila, Aeromonas salmonicida, Aeromonas sp.	Penicillinase	Sawai et al. (1976)
	Fluoroquinolone	Oppegaard and Sorum (1994)
	Sulfonamide and tetracycline	Patil et al. (2016)
	Tetracycline, erythromycin, nalidixic acid	Jacobs and Chenia (2007)
Columnaris disease	Florfenicol, linezolid, chloramphenicol	Zeng et al. (2019)
Commensal bacteria & Bacillus & *Pseudomonas* sp.	Tetracycline	Ruzauskas et al. (2021)
	Sulfonamide, tetracycline	Gao et al. (2012)
	Florfenicol, erythromycin, furazolidone, amoxicillin, sulfisoxazole, and trimethoprim	Domínguez et al. (2019)
	Flumequine, florfenicol, oxytetracycline	Concha et al. (2019)
	Ceftriaxone, tetracycline, ampicillin, ceftriaxone, gentamicin, streptomycin, trimethoprim, nalidixic acid, trimethoprim	Boinapally and Jiang (2007)
	Aminoglycoside, sulfonamide, tetracycline, quinolone.	Lin et al. (2016)
	Tetracycline, sulfonamide, β-lactamase	Shah et al. (2014)
Edwardsiella tarda	Fluoroquinolone	Shin et al. (2005)
Gram negative bacteria	Tetracycline, amoxicillin, ampicillin. erythromycin, furazolidone, florfenicol, chloramphenicol, cefotaxime and trimethoprim-sulfamethoxazole	Miranda and Zemelman (2002)
P*hotobacterium damselae* ssp. piscicida, *Vibrio fluvialis*, *Vibrio alginolyticus*, *Vibrio parahaemolyticus*, *Vibrio metschnikovii*	Sulfadiazine-trimethoprim, ampicillin, carbenicillin, kanamycin, cephalothin	Laganà et al. (2011)
Photobacterium damselae subsp. piscicida	Oxolinic acid, nalidixic acid, quinolones	Kim et al. (2005)
	Tetracycline, oxytetracycline, oxolinic acid, flumequine, enrofloxacin	Martínez-Manzanares et al. (2008)
S. agalactiae	Tetracycline, oxytetracycline, chlortetracycline, ciprofloxacin, streptomycin, gentamicin, neomycin	Rahman et al. (2021)
	Streptomycin and erythromycin	Zhang et al. (2018)
	Gentamicin, sulfonamide and trimethoprim, penicillin, tenemycin, fradiomycin, streptomycin.	Schar et al. (2018)

(continued)

Table 5 (continued)

Bacteria	Resistance	Reference
	Penicillin, ampicillin, vancomycin, chloramphenicol, rifampicin, ofloxacin, clindamycin, erythromycin, tetracycline	Osman et al. (2017)
	Neomycin and gentamicin	Laith et al. (2017)
	Oxolinic acid, gentamicin, sulfamethoxazole, trimethoprim	Dangwetngam et al. (2016)
	Neomycin, amikacin, kanamycin, streptomycin	Abuseliana et al. (2010)
	Enrofloxacin	Kannika et al. (2017)
V. alginolyticus; V. parahaemolyticus; V. vulnificus	Ampicillin, ceftazidime, cefotetan	Zanetti et al. (2001)
V. harveyi	Ciprofloxacin, ampicillin, vancomycin, rifampicin	Stalin and Srinivasan (2016)
	Streptomycin, chloramphenicol, cotrimoxazole	Karunasagar et al. (1994)
V. anguillarum	Fluroquinolones gyrA, gyrB, parC, parE genes	Rodkhum et al. (2008)
	Sulfonamides, streptomycin, chloramphenicol, tetracycline	Aoki et al. (1974)
V. parahaemolyticus	Ampicillin, amoxicillin, cefotaxime, ceftriaxone	Siddique et al. (2021)
	MDR	Melo et al. (2011)
	Ampicillin resistance and intermediate resistance to tetracycline and amikacin	Sperling et al. (2015)
	MDR	Devi et al. (2009)
	Ampicillin, cefotaxime, cefoxitin, cephalothin, ceftazidime, amoxicillin-clavulanic acid, ciprofloxacin, gentamicin, meropenem	Narayanan et al. (2020)
	MDR	Ottaviani et al. (2013)
	Ampicillin, cefotaxime, ciprofloxacin	Al-Othrubi et al. (2014)
	Ampicillin resistance	Yano et al. (2014)
	Ampicillin, apramycin, amoxicillin-clavulanic acid, streptomycin, trimethoprim	Baker-Austin et al. (2008)
	Chloramphenicol, sulfonamides, trimethoprim, rifamycin, ampicillin, streptomycin, kanamycin	He et al. (2019)
	Ampicillin, tetracycline, amikacin	Sperling et al. (2015)

(continued)

Table 5 (continued)

Bacteria	Resistance	Reference
V. parahaemolyticus; V. alginolyticus	Ampicillin, rifampicin, streptomycin, trimethoprim, tetracycline	Oh et al. (2011)
V. vulnificus	MDR	Pan et al. (2013)
	Streptomycin, chloramphenicol	Shaw et al. (2014)
Vibrio sp.	Ampicillin, amoxicillin, cephalosporin, erythromycin	Akinbowale et al. (2006)
	Ampicillin, tetracycline	Rebouças et al. (2011)
	MDR strains	Albuquerque Costa et al. (2015), Rebouças et al. (2011)
	Ampicillin, cefuroxime, amikacin, kanamycin, trimethoprim	Li et al. (1999)
	MDR strains	Manjusha and Sarita (2011)
	Ampicillin and colistin	Sudha et al. (2014)
	Ampicillin, cloxacillin, oxacillin, erythromycin, vancomycin, furazolidone	Srinivasan and Ramasamy (2009)
	Ampicillin	Singh et al. (2018)
	MDR	Ansari and Raissy (2010)
	Tetracycline and sulfonamide	Labella et al. (2013)
	Ampicillin, tetracycline, doxycycline	Banerjee et al. (2012)
	MDR strains	Molina-Aja et al. (2002)
	MDR strains	Roque et al. (2001)
	MDR strains	Igbinosa (2016)
	Tetracycline, amoxicillin-clavulanic acid, streptomycin	Dubert et al. (2015)
	Amoxicillin-clavulanic acid	Maisak et al. (1995)
	MDR strains	Manjusha et al. (2005)
	Ampicillin, chlortetracycline	Vaseeharan et al. (2005)
	Oxytetracycline	Tendencia and de la Peña (2001), Tendencia (2002)
Vibrio sp.; *V. harveyi; V. aestuarianus*	MDR, ampicillin, amoxicillin-clavulanic acid, erythromycin, sulfonamides	Scarano et al. (2014)
Yersinia ruckeri	Nalidixic acid, oxolinic acid gyrB gene	Gibello et al. (2004)

5 Trends in the Detection of Resistance in Fisheries

There is a distinct pattern of resistance detection throughout the country, animal species, and bacterial pathogens. Florfenicol resistance has been reported the most in Chile, but tetracyclines and sulfonamide resistance have been documented in all nations involved in fisheries activities. Resistance to sulfonamide was more common in China. In Japan, tetracycline resistance was uncommon. Resistance to aminoglycosides was more widespread in Malaysia, as can be seen in Table 3. Since the first report of resistance in the fisheries sector in 1976 in salmon fishes, the trend in tetracycline resistance has been recorded annually from 2002 to 2021. Since 1994, quinolone and fluoroquinolone resistance has been documented. Resistance mediated by integrons and efflux pumps was discovered in the early 2010s.

Penicillin, fluoroquinolone, sulfonamide, and tetracycline resistance were found to be more frequent in *Aeromonas* sp. *Edwardsiella tarda* had a higher rate of fluoroquinolone resistance. Oxolinic acid, quinolone, and tetracycline resistance were all widespread in *Photobacterium damselae* subsp. Piscicida. Penicillin, ampicillin, tetracycline, oxytetracycline, chlortetracycline, streptomycin, gentamicin, neomycin, amikacin, kanamycin, enrofloxacin, nalidixic resistance, ciprofloxacin, ofloxacin, sulfonamide, and trimethoprim resistance have all been described in *S. agalactiae*.

Several methods are in practice to detect antimicrobial resistance in the fisheries and aquaculture. The detailed methods available for detection in aquaculture and fisheries are reported in chapter ▶ "Trends in the Determination of Antimicrobial Resistance in Aquaculture and Fisheries." Methods available for characterizing pathogens for understanding the local and global epidemiology are reported in chapter ▶ "Molecular Tools for characterizing AMR Pathogens."

6 AMR Alleviation Measures in Aquaculture

AMR mitigation strategies used in other animals can be used in aquaculture as well. Vaccines were used to treat most major fish infections, and they made a difference, if not totally curing them. The EU's recommendations for avoiding, treating, or removing the effects of diseases including providing optimal circumstances for aquaculture animal growth, such as high-quality water, sufficient water flow, low BOD, and nutritious feed free of all physical, chemical, and biological contaminants; increasing AST's performance where possible; increasing the effectiveness of disease investigation programs in order to avert epidemics; supporting the discovery and use of relevant aquaculture vaccinations; incorporating necessary safety measures, such as stocking density requirements; and biosecurity and hygiene measures, particularly steps to minimize disease importation and transmission, should be prioritized are listed next:

(i) Regular cleaning and/or disinfection of aquaculture ponds between succession of production, and tillage of the ponds between the cycles of production

(ii) Operating an "all-in all-out" system per unit or farm, applying single bay management wherever possible, ensuring
(iii) Stringent enforcement of entry of all personnel to farms, isolation and demarcation of each pond with boots, clothes and equipment
(iv) Hygienic disposal of diseased and deceased fish
(v) Implementation of standard operating procedures for drawing blood or water
(vi) Design of proper transportation system to contain disease transmission during transport through water and restricting contact of diseased aquacultured animals with that of healthy one

The increase in the aquaculture constituent of National Action Plans (NAPs) on AMR was pointed in the right direction (Bondad-Reantaso et al., 2020). There are generic and aquaculture-specific stages in this direction. In generic guidance, the first general step is to review the WHO Global Plan of Action, as well as follow-up of the WOAH and FAO's plans of action. These action plans can help countries construct their national action plans and evaluate which objectives or pillars are appropriate for a country action plan. The relevant health ministry usually leads and coordinates a country's AMR NAP (Bondad-Reantaso et al., 2020). In aquaculture-specific guidance, the basic premise is to understand the aquaculture area and scrutinize diverse aspects and how they may transmit the emergence of AMR in aquaculture (Bondad-Reantaso et al., 2020).

7 Conclusion

Antimicrobial resistance in fisheries will be a future threat as there is a growing demand for aquatic animals to meet global animal protein needs. Antimicrobial usage across the sectors results in the development of resistance in the respective sectors and cross-spread to the other sectors. As a result, adequate rules and surveillance are essential to prevent the misuse of substances that can pose significant health repercussions for consumers. The regulatory framework for the use of antibiotics in aquaculture is inadequate, varies widely between countries, and many of the mainstream aquaculture producers have little or no enforcement. It is highly essential to integrate the direction of the transmission of resistance between aquatic animal pathogens and pathogens of public health importance, along with linking the antimicrobial usage and antimicrobial resistance that is a priority for the studies. Implementation of harmonized standard operating procedures for the confirmation of antimicrobial resistance in the fisheries is necessary.

8 Cross-References

► Molecular Tools for Characterizing AMR Pathogens
► Trends in the Determination of Antimicrobial Resistance in Aquaculture and Fisheries

References

Abuseliana, A., Hassan, D., Saleha, A. A., Siti-Khairani, B., & Milud, A. (2010). *Streptococcus agalactiae* the etiological agent of mass mortality in farmed red tilapia (Oreochromis sp.). *Journal of Animal and Veterinary Advances, 9*, 2640–2646.

Akinbowale, O. L., Peng, H., & Barton, M. D. (2006). Antimicrobial resistance in bacteria isolated from aquaculture sources in Australia. *Journal of Applied Microbiology, 100*(5), 1103–1113.

Albuquerque Costa, R., Araújo, R. L., Souza, O. V., & Vieira, R. H. S. D. F. (2015). Antibiotic-resistant Vibrios in farmed shrimp. *BioMed Research International, 2015*, 505914. https://doi.org/10.1155/2015/505914

Al-Othrubi, S. M. Y., Kqueen, C. Y., Mirhosseini, H., Hadi, Y. A., & Radu, S. (2014). Antibiotic resistance of *Vibrio parahaemolyticus* isolated from cockles and shrimp sea food marketed in Selangor, Malaysia. *Clinical Microbiology, 3*, 148.

Aly, S. M., & Albutti, A. (2014). Antimicrobials use in aquaculture and their public health impact. *Journal of Aquaculture Research & Development, 5*(4), 1.

Ansari, M., & Raissy, M. (2010). In vitro susceptibility of commonly used antibiotics against *Vibrio* spp. isolated from Lobster (*Panulirus homarus*). *African Journal of Microbiology Research, 4*(23), 2629–2631.

Aoki, T., Egusa, S., & Arai, T. (1974). Detection of R factors in naturally occurring *Vibrio anguillarum* strains. *Antimicrobial Agents and Chemotherapy, 6*(5), 534–538.

Baker-Austin, C., McArthur, J. V., Tuckfield, R. C., Najarro, M., Lindell, A. H., Gooch, J., & Stepanauskas, R. (2008). Antibiotic resistance in the shellfish pathogen *Vibrio parahaemolyticus* isolated from the coastal water and sediment of Georgia and South Carolina, USA. *Journal of Food Protection, 71*(12), 2552–2558.

Banerjee, S., Ooi, M. C., Shariff, M., & Khatoon, H. (2012). Antibiotic resistant Salmonella and Vibrio associated with farmed *Litopenaeus vannamei*. *The Scientific World Journal, 2012*, 130136. https://doi.org/10.1100/2012/130136

Bashir, I., Lone, F. A., Bhat, R. A., Mir, S. A., Dar, Z. A., & Dar, S. A. (2020). Concerns and threats of contamination on aquatic ecosystems. *Bioremediation and Biotechnology: Sustainable Approaches to Pollution Degradation*, 1–26. https://doi.org/10.1007/978-3-030-35691-0-1

Boinapally, K., & Jiang, X. (2007). Comparing antibiotic resistance in commensal and pathogenic bacteria isolated from wild-caught South Carolina shrimps vs. farm-raised imported shrimps. *Canadian Journal of Microbiology, 53*(7), 919–924.

Bondad-Reantaso, M. G., Lavilla-Pitogo, C. R., Michelle, M. A., Lopez, L., & Hao, B. (2020). Guidance in development of aquaculture component of a national action plan on antimicrobial resistance. *Asian Fisheries Science, 33*(S1), 119–124. ISSN: 0116-6514, E-ISSN: 2073-3720. https://doi.org/10.33997/j.afs.2020.33.S1.003

Chow, L. K., Ghaly, T. M., & Gillings, M. R. (2021). A survey of sub-inhibitory concentrations of antibiotics in the environment. *Journal of Environmental Sciences, 99*, 21–27.

Concha, C., Miranda, C. D., Hurtado, L., & Romero, J. (2019). Characterization of mechanisms lowering susceptibility to Flumequine among bacteria isolated from Chilean salmonid farms. *Microorganisms, 7*(12), 698.

Dangwetngam, M., Suanyuk, N., Kong, F., & Phromkunthong, W. (2016). Serotype distribution and antimicrobial susceptibilities of *Streptococcus agalactiae* isolated from infected cultured tilapia (*Oreochromis niloticus*) in Thailand: Nine-year perspective. *Journal of Medical Microbiology, 65*(3), 247–254.

Deng, Y., Bao, X., Ji, L., Chen, L., Liu, J., Miao, J., Chen, D., Bian, H., Li, Y., & Yu, G. (2015). Resistance integrons: Class 1, 2 and 3 integrons. *Annals of Clinical Microbiology and Antimicrobials, 14*(1), 1–11.

Devi, R., Surendran, P. K., & Chakraborty, K. (2009). Antibiotic resistance and plasmid profiling of *Vibrio parahaemolyticus* isolated from shrimp farms along the southwest coast of India. *World Journal of Microbiology and Biotechnology, 25*(11), 2005–2012.

Domínguez, M., Miranda, C. D., Fuentes, O., de la Fuente, M., Godoy, F. A., Bello-Toledo, H., & González-Rocha, G. (2019). Occurrence of transferable integrons and sul and dfr genes among

sulfonamide-and/or trimethoprim-resistant bacteria isolated from Chilean salmonid farms. *Frontiers in Microbiology, 10*, 748.

Dubert, J., Fernández-Pardo, A., Nóvoa, S., Barja, J. L., & Prado, S. (2015). Phytoplankton production systems in a shellfish hatchery: Variations of the bacterial load and diversity of vibrios. *Journal of Applied Microbiology, 118*(6), 1264–1275.

FAO. (2020a). *The state of the world fisheries and aquaculture 2020. Sustainability in action*. Food and Agriculture Organization of the United Nations. https://doi.org/10.4060/ca9229en

FAO. (2020b). Understanding antimicrobial resistance in aquaculture 2020. http://www.fao.org/publications/card/en/c/CB2601EN/

Fernández-Alarcón, C., Miranda, C. D., Singer, R. S., López, Y., Rojas, R., Bello, H., … & González-Rocha, G. (2010). Detection of the flo R gene in a diversity of florfenicol resistant Gram-negative bacilli from freshwater salmon farms in Chile. *Zoonoses and Public Health*, *57*(3), 181–188.

Gao, P., Mao, D., Luo, Y., Wang, L., Xu, B., & Xu, L. (2012). Occurrence of sulfonamide and tetracycline-resistant bacteria and resistance genes in aquaculture environment. *Water Research, 46*(7), 2355–2364.

Gibello, A., Porrero, M. C., Blanco, M. M., Vela, A. I., Liébana, P., Moreno, M. A., et al. (2004). Analysis of the gyrA gene of clinical *Yersinia ruckeri*isolates with reduced susceptibility to quinolones. *Applied and Environmental Microbiology, 70*, 599–602.

Hashmi, M. Z. (2020). *Antibiotics and antimicrobial resistance genes: Environmental occurrence and treatment technologies*. Springer Nature.

He, Y., Wang, S., Zhang, J., Zhang, X., Sun, F., & He, B. (2019). Integrative and conjugative elements-positive *Vibrio parahaemolyticus* isolated from aquaculture shrimp in Jiangsu, China. *Frontiers in Microbiology, 10*, 1574.

Heuer, O. E., Kruse, H., Grave, K., Collignon, P., Karunasagar, I., & Angulo, F. J. (2009). Human health consequences of use of antimicrobial agents in aquaculture. *Clinical Infectious Diseases, 49*(8), 1248–1253.

Higuera-Llantén, S., Vásquez-Ponce, F., Barrientos-Espinoza, B., Mardones, F. O., Marshall, S. H., & Olivares-Pacheco, J. (2018). Extended antibiotic treatment in salmon farms select multi-resistant gut bacteria with a high prevalence of antibiotic resistance genes. *PLoS One, 13*(9), e0203641.

Hong, B., Ba, Y., Niu, L., Lou, F., Zhang, Z., Liu, H., … & Zhao, Y. (2018) A comprehensive research on antibiotic resistance genes in microbiota of aquatic animals. *Frontiers in Microbiology*, *9*, 1617.

Igbinosa, E. O. (2016). Detection and antimicrobial resistance of Vibrio isolates in aquaculture environments: Implications for public health. *Microbial Drug Resistance, 22*(3), 238–245.

Jacobs, L., & Chenia, H. Y. (2007). Characterization of integrons and tetracycline resistance determinants in *Aeromonas* spp. isolated from South African aquaculture systems. *International Journal of Food Microbiology, 114*, 295–306.

Jang, H. M., Kim, Y. B., Choi, S., Lee, Y., Shin, S. G., Unno, T., & Kim, Y. M. (2018). Prevalence of antibiotic resistance genes from effluent of coastal aquaculture, South Korea. *Environmental Pollution, 233*, 1049–1057.

Kannika, K., Pisuttharachai, D., Srisapoome, P., Wongtavatchai, J., Kondo, H., Hirono, I., … & Areechon, N. (2017). Molecular serotyping, virulence gene profiling and pathogenicity of *Streptococcus agalactiae* isolated from tilapia farms in Thailand by multiplex PCR. *Journal of Applied Microbiology*, *122*(6), 1497–1507.

Karunasagar, I., Pai, R., Malathi, G. R., & Karunasagar, I. (1994). Mass mortality of *Penaeus monodon* larvae due to antibiotic-resistant *Vibrio harveyi* infection. *Aquaculture, 128*(3–4), 203–209.

Karunasagar, I., Karunasagar, I., & Bondad-Reantaso, M. G. (2020). Complexities involved in source attribution of antimicrobial resistance genes found in aquaculture products. *Asian Fisheries Science, 33*(S1), 16–21.

Kim, M. J., Hirono, I., & Aoki, T. (2005). Detection of quinolone-resistance genes in *Photobacterium damselae* subsp. piscicida strains by targeting-induced local lesions in genomes. *Journal of Fish Diseases, 28*(8), 463–471.

Labella, A., Gennari, M., Ghidini, V., Trento, I., Manfrin, A., Borrego, J. J., & Lleo, M. M. (2013). High incidence of antibiotic multi-resistant bacteria in coastal areas dedicated to fish farming. *Marine Pollution Bulletin, 70*(1–2), 197–203.

Laganà, P., Caruso, G., Minutoli, E., Zaccone, R., & Delia, S. (2011). Susceptibility to antibiotics of Vibrio spp. and *Photobacterium damsela* ssp. piscicida strains isolated from Italian aquaculture farms. *New Microbiologica, 34*(1), 53–63.

Laith, A. A., Ambak, M. A., Hassan, M., Sheriff, S. M., Nadirah, M., Draman, A. S., . . . & Najiah, M. (2017). Molecular identification and histopathological study of natural *Streptococcus agalactiae* infection in hybrid tilapia (*Oreochromis niloticus*). *Veterinary World*, *10*(1), 101.

Li, J., Yie, J., Foo, R. W., Ling, J. M., Xu, H., & Woo, N. Y. (1999). Antibiotic resistance and plasmid profiles of Vibrio isolates from cultured silver sea bream, Sparus sarba. *Marine Pollution Bulletin, 39*(1–12), 245–249.

Lin, M., Wu, X., Yan, Q., Ma, Y., Huang, L., Qin, Y., & Xu, X. (2016). Incidence of antimicrobial-resistance genes and integrons in antibiotic-resistant bacteria isolated from eels and aquaculture ponds. *Diseases of Aquatic Organisms, 120*(2), 115–123.

MacFadden, D. R., McGough, S. F., Fisman, D., Santillana, M., & Brownstein, J. S. (2018). Antibiotic resistance increases with local temperature. *Nature Climate Change, 8*, 510.

Magiorakos, A. P., Srinivasan, A., Carey, R. T., Carmeli, Y., Falagas, M. T., Giske, C. T., Harbarth, S., Hindler, J. T., Kahlmeter, G., Olsson-Liljequist, B., & Paterson, D. T. (2012). Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: An international expert proposal for interim standard definitions for acquired resistance. *Clinical Microbiology and Infection, 18*(3), 268–281.

Maisak, H., Tipmongkolsilp, N., & Wongtavatchai, J. (1995). Minimum inhibitory concentrations of antimicrobials against clinical Vibrio and Streptococcus isolated from aquaculture. In M. G. Bondad-Reantaso, J. B. Jones, F. Corsin, & T. Aoki (Eds.), *Diseases in Asian aquaculture II* (pp. 309–316). Fish Health Section, Asian Fisheries Society.

Manjusha, S., & Sarita, G. B. (2011). Plasmid associated antibiotic resistance in Vibrios isolated from coastal waters of Kerala. *International Food Research Journal, 18*(3), 1171–1181.

Manjusha, S., Sarita, G. B., Elyas, K. K., & Chandrasekaran, M. (2005). Multiple antibiotic resistances of Vibrio isolates from coastal and brackish water areas. *American Journal of Biochemistry and Biotechnology, 1*(4), 201–206.

Martínez-Manzanares, E., Tapia-Paniagua, S. T., Díaz-Rosales, P., Chabrillón, M., & Moriñigo, M. A. (2008). Susceptibility of *Photobacterium damselae* subsp. piscicida strains isolated from Senegalese sole, Solea senegalensis Kaup, and gilthead seabream, *Sparus aurata* L., to several antibacterial agents. *Journal of Fish Diseases, 31*(1), 73–76.

Melo, L. M. R. D., Almeida, D., Hofer, E., Reis, C. M. F. D., Theophilo, G. N. D., Santos, A. F. D. M., & Vieira, R. H. S. D. F. (2011). Antibiotic resistance of *Vibrio parahaemolyticus* isolated from pond-reared *Litopenaeus vannamei* marketed in Natal, Brazil. *Brazilian Journal of Microbiology, 42*(4), 1463–1469.

Miranda, C. D., & Zemelman, R. (2002). Antimicrobial multiresistance in bacteria isolated from freshwater Chilean salmon farms. *Science of the Total Environment, 293*(1–3), 207–218.

Molina-Aja, A., García-Gasca, A., Abreu-Grobois, A., Bolán-Mejía, C., Roque, A., & Gomez-Gil, B. (2002). Plasmid profiling and antibiotic resistance of Vibrio strains isolated from cultured penaeid shrimp. *FEMS Microbiology Letters, 213*(1), 7–12.

Muziasari, W. I., Pärnänen, K., Johnson, T. A., Lyra, C., Karkman, A., Stedtfeld, R. D., . . . & Virta, M. (2016). Aquaculture changes the profile of antibiotic resistance and mobile genetic element associated genes in Baltic Sea sediments. *FEMS Microbiology Ecology*, *92*(4), fiw052.

Muziasari, W. I., Pitkänen, L. K., Sørum, H., Stedtfeld, R. D., Tiedje, J. M., & Virta, M. (2017). The resistome of farmed fish feces contributes to the enrichment of antibiotic resistance genes in sediments below Baltic Sea fish farms. *Frontiers in Microbiology, 7*, 2137. https://doi.org/10.3389/fmicb.2016.02137. Erratum in: Front Microbiol. 2017 Aug 02;8:1491. PMID: 28111573; PMCID: PMC5216021.

Narayanan, S. V., Joseph, T. C., Peeralil, S., Koombankallil, R., Vaiyapuri, M., Mothadaka, M. P., & Lalitha, K. V. (2020). Tropical shrimp aquaculture farms harbour pathogenic *Vibrio parahaemolyticus* with high genetic diversity and Carbapenam resistance. *Marine Pollution Bulletin, 160*, 111551.

Oh, E. G., Son, K. T., Yu, H., Lee, T. S., Lee, H. J., Shin, S., . . . & Kim, J. (2011). Antimicrobial resistance of *Vibrio parahaemolyticus* and *Vibrio alginolyticus* strains isolated from farmed fish in Korea from 2005 through 2007. *Journal of Food Protection*, *74*(3), 380–386.

Oppegaard, H., & Sorum, H. (1994). *gyr*A mutations in quinolone resistant isolates of the fish pathogen *Aeromonas salmonicida*. *Antimicrobial Agents and Chemotherapy, 38*, 2460–2464.

Osman, K. M., Al-Maary, K. S., Mubarak, A. S., et al. (2017). Characterization and susceptibility of streptococci and enterococci isolated from Nile tilapia (*Oreochromis niloticus*) showing septicaemia in aquaculture and wild sites in Egypt. *BMC Veterinary Research, 13*(1), 357. https://doi.org/10.1186/s12917-017-1289-8

Ottaviani, D., Leoni, F., Talevi, G., Masini, L., Santarelli, S., Rocchegiani, E., . . . & Manso, E. (2013). Extensive investigation of antimicrobial resistance in *Vibrio parahaemolyticus* from shellfish and clinical sources, Italy. *International Journal of Antimicrobial Agents, 2*(42), 191–193.

Pan, J., Zhang, Y., Jin, D., Ding, G., Luo, Y., Zhang, J., . . . & Zhu, M. (2013). Molecular characterization and antibiotic susceptibility of *Vibrio vulnificus* in retail shrimps in Hangzhou, People's Republic of China. *Journal of Food Protection*, *76*(12), 2063–2068.

Patil, H. J., Benet-Perelberg, A., Naor, A., Smirnov, M., Ofek, T., Nasser, A., . . . & Cytryn, E. (2016). Evidence of increased antibiotic resistance in phylogenetically-diverse Aeromonas isolates from semi-intensive fish ponds treated with antibiotics. *Frontiers in Microbiology*, *7*, 1875.

Patil, H. J., Gatica, J., Zolti, A., Benet-Perelberg, A., Naor, A., Dror, B., . . . & Cytryn, E. (2020). Temporal resistome and microbial community dynamics in an intensive aquaculture facility with prophylactic antimicrobial treatment. *Microorganisms*, *8*(12), 1984.

Poirel, L., Cattoir, V., & Nordmann, P. (2012). Plasmid-mediated quinolone resistance: Interactions between human, animal and environmental ecologies. *Frontiers in Microbiology, 3*, 24. https://doi.org/10.3389/fmicb.2012.00024

Rahman, M. M., Rahman, M. A., Monir, M. S., Haque, M. E., Siddique, M. P., Khasruzzaman, A. K. M., . . . & Islam, M. A. (2021). Isolation and molecular detection of *Streptococcus agalactiae* from popped eye disease of cultured Tilapia and Vietnamese koi fishes in Bangladesh. *Journal of Advanced Veterinary and Animal Research*, *8*(1), 14.

Rebouças, R. H., de Sousa, O. V., Lima, A. S., Vasconcelos, F. R., de Carvalho, P. B., & dos Fernandes Vieira, R. H. S. (2011). Antimicrobial resistance profile of Vibrio species isolated from marine shrimp farming environments (Litopenaeusvannamei) at Ceará, Brazil. *Environmental Research, 111*(1), 21–24.

Reverter, M., Sarter, S., Caruso, D., Avarre, J. C., Combe, M., Pepey, E., . . . & Gozlan, R. E. (2020). Aquaculture at the crossroads of global warming and antimicrobial resistance. *Nature Communications, 11*(1), 1–8.

Rodkhum, C., Maki, T., Hirono, I., & Aoki, T. (2008). *gyr*A and *par* C associated with quinolone resistance in *Vibrio anguillarum*. *Journal of Fish Diseases, 31*, 395–399.

Roque, A., Molina-Aja, A., Bolán-Mejıa, C., & Gomez-Gil, B. (2001). In vitro susceptibility to 15 antibiotics of vibrios isolated from penaeid shrimps in Northwestern Mexico. *International Journal of Antimicrobial Agents, 17*(5), 383–387.

Ruzauskas, M., Armalytė, J., Lastauskienė, E., Šiugždinienė, R., Klimienė, I., Mockeliūnas, R., & Bartkienė, E. (2021). Microbial and antimicrobial resistance profiles of microbiota in common carps (*Cyprinus carpio*) from aquacultured and wild fish populations. *Animals, 11*(4), 929.

Sáenz, J. S., Marques, T. V., Barone, R. S. C., Cyrino, J. E. P., Kublik, S., Nesme, J., Schloter, M., Rath, S., & Vestergaard, G. (2019). Oral administration of antibiotics increased the potential mobility of bacterial resistance genes in the gut of the fish Piaractusmesopotamicus. *Microbiome, 7*(1), 24.

Sawai, T., Morioka, K., Ogawa, M., & Yamagishi, S. (1976). Inducible oxacillin-hydrolyzing penicillinase in *Aeromonas hydrophila* isolated from fish. *Antimicrobial Agents and Chemotherapy, 10*(2), 191–195. https://doi.org/10.1128/aac.10.2.191

Scarano, C., Spanu, C., Ziino, G., Pedonese, F., Dalmasso, A., Spanu, V., … & De Santis, E. P. (2014). Antibiotic resistance of Vibrio species isolated from *Sparus aurata* reared in Italian mariculture. *The New Microbiologica*, *37*(3), 329–337.

Schar, D., Sommanustweechai, A., Laxminarayan, R., & Tangcharoensathien, V. (2018). Surveillance of antimicrobial consumption in animal production sectors of low- and middle-income countries: Optimizing use and addressing antimicrobial resistance. *PLoS Medicine, 15*, e1002521.

Schar, D., Zhao, C., Wang, Y., Larsson, D. G., Gilbert, M., & Van Boeckel, T. P. (2021). Twenty-year trends in antimicrobial resistance from aquaculture and fisheries in Asia. *Nature Communications, 12*(1), 1–10.

Shah, S. Q., Cabello, F. C., L'Abée-Lund, T. M., Tomova, A., Godfrey, H. P., Buschmann, A. H., & Sørum, H. (2014). Antimicrobial resistance and antimicrobial resistance genes in marine bacteria from salmon aquaculture and non-aquaculture sites. *Environmental Microbiology, 16*(5), 1310–1320.

Shaw, K. S., Goldstein, R. E. R., He, X., Jacobs, J. M., Crump, B. C., & Sapkota, A. R. (2014). Antimicrobial susceptibility of *Vibrio vulnificus* and *Vibrio parahaemolyticus* recovered from recreational and commercial areas of Chesapeake Bay and Maryland Coastal Bays. *PLoS One, 9*(2), e89616.

Shin, S. B., Yoo, M. H., Jeong, J. B., Kim, Y. M., Chung, J. K., Do Huh, M., … & Do Jeong, H. (2005). Molecular cloning of the gyrA gene and characterization of its mutation in clinical isolates of quinolone-resistant *Edwardsiella tarda*. *Diseases of Aquatic Organisms*, *67*(3), 259–266.

Siddique, A. B., Moniruzzaman, M., Ali, S., Dewan, M., Islam, M. R., Islam, M., … & Mahmud, Z. H. (2021). Characterization of Pathogenic *Vibrio parahaemolyticus* Isolated from Fish Aquaculture of the Southwest Coastal Area of Bangladesh. *Frontiers in Microbiology*, *12*, 266.

Singh, B., Tyagi, A., Billekallu Thammegowda, N. K., & Ansal, M. D. (2018). Prevalence and antimicrobial resistance of vibrios of human health significance in inland saline aquaculture areas. *Aquaculture Research, 49*(6), 2166–2174.

Smith, P., & Egan, S. (2020). Critical review of methods used in published studies of susceptibility of Vibrio spp.; lessons to be learnt. *Asian Fisheries Society, 33*(S1), 22–26.

Sperling, L., Alter, T., & Huehn, S. (2015). Prevalence and antimicrobial resistance of Vibrio spp. in retail and farm shrimps in Ecuador. *Journal of Food Protection, 78*(11), 2089–2092.

Srinivasan, P., & Ramasamy, P. (2009). Occurrence, distribution and antibiotic resistance patterns of Vibrio species associated with viral diseased shrimp of South Indian aquaculture environment. *International Journal of Agriculture Sciences, 1*(2), 1.

Stalin, N., & Srinivasan, P. (2016). Characterization of *Vibrio parahaemolyticus* and its specific phage from shrimp pond in Palk Strait, South East coast of India. *Biologicals, 44*(6), 526–533.

Sudha, S., Mridula, C., Silvester, R., & Hatha, A. A. M. (2014). Prevalence and antibiotic resistance of pathogenic Vibrios in shellfishes from Cochin market. *Indian Journal of Geo-Marine Sciences, 43*(5), 815–824.

Tendencia, E. A. (2002). *Vibrio harveyi* isolated from cage-cultured seabass Lates calcarifer Bloch in the Philippines. *Aquaculture Research, 33*(6), 455–458.

Tendencia, E. A., & de la Peña, L. D. (2001). Antibiotic resistance of bacteria from shrimp ponds. *Aquaculture, 195*(3–4), 193–204.

UN. (2018). *The sustainable development goals report*. United Nations.

Van Boeckel, T. P., et al. (2015). Global trends in antimicrobial use in food animals. *PNAS, 112*, 5649–5654.

Vaseeharan, B., Ramasamy, P., Murugan, T., & Chen, J. C. (2005). In vitro susceptibility of antibiotics against Vibrio spp. and Aeromonas spp. isolated from Penaeus monodon hatcheries and ponds. *International Journal of Antimicrobial Agents, 26*(4), 285–291.

Wenhai, L., Cusack, C., Baker, M., Tao, W., Mingbao, C., Paige, K., … & Yufeng, Y. (2019). Successful blue economy examples with an emphasis on international perspectives. *Frontiers in Marine Science, 6*, 261.

Xiong, W., Sun, Y., Zhang, T., Ding, X., Li, Y., Wang, M., & Zeng, Z. (2015). Antibiotics, antibiotic resistance genes, and bacterial community composition in fresh water aquaculture environment in China. *Microbial Ecology, 70*(2), 425–432.

Yano, Y., Hamano, K., Satomi, M., Tsutsui, I., Ban, M., & Aue-Umneoy, D. (2014). Prevalence and antimicrobial susceptibility of Vibrio species related to food safety isolated from shrimp cultured at inland ponds in Thailand. *Food Control, 38*, 30–36.

Zanetti, S., Spanu, T., Deriu, A., Romano, L., Sechi, L. A., & Fadda, G. (2001). In vitro susceptibility of Vibrio spp. isolated from the environment. *International Journal of Antimicrobial Agents, 17*(5), 407–409.

Zeng, Q., Liao, C., Terhune, J., & Wang, L. (2019). Impacts of florfenicol on the microbiota landscape and resistome as revealed by metagenomic analysis. *Microbiome, 7*(1), 1–13.

Zhang, Z., Lan, J., Li, Y., Hu, M., Yu, A., Zhang, J., & Wei, S. (2018). The pathogenic and antimicrobial characteristics of an emerging *Streptococcus agalactiae* serotype IX in Tilapia. *Microbial Pathogenesis, 122*, 39–45.

Zhou, Q., Wang, M., Zhong, X., Liu, P., Xie, X., Wangxiao, J., & Sun, Y. (2019). Dissemination of resistance genes in duck/fish polyculture ponds in Guangdong Province: Correlations between Cu and Zn and antibiotic resistance genes. *Environmental Science and Pollution Research, 26*(8), 8182–8193.

Zhou, R., Zeng, S., Hou, D., Liu, J., Weng, S., He, J., & Huang, Z. (2020). Temporal variation of antibiotic resistance genes carried by culturable bacteria in the shrimp hepatopancreas and shrimp culture pond water. *Ecotoxicology and Environmental Safety, 199*, 110738.

Antimicrobial Resistance in Marine Ecosystem: An Emerging Threat for Public Health

Krupesha Sharma S. R. and Sumithra T. G.

Contents

Abstract

The evolution of pathogenic microorganisms that can resist a wide range of antimicrobial treatments leading to treatment failure and loss of human and animal life represents one of the most thoughtful public health concerns in the world. The marine ecosystem is now increasingly recognized as a potentially significant "hotspot" for the emergence, maintenance, and dispersal of many clinically relevant and potentially novel AMR genes and microbes. The trends on AMR surges in marine life are the reflections of the conditions in humans. AMR in the marine ecosystem is a natural and ancient phenomenon, albeit higher levels are always related to increased human activities. Various marine pollutants and indiscriminate use of chemicals, including antimicrobials, in aquaculture practices contribute to AMR in the marine ecosystem. Antimicrobials used to treat infections in coastal aquaculture practices can get into the marine environment, which could adversely impact the marine biodiversity and terrestrial animal

K. S. S. R. (✉) · S. T. G.
Marine Biotechnology, Fish Nutrition and Health Division, Fish Health Section, ICAR-Central Marine Fisheries Research Institute, Kochi, Kerala, India
e-mail: krupesh.sharma@icar.gov.in; sumithra.G@icar.gov.in

M. P. Mothadaka et al. (eds.), *Handbook on Antimicrobial Resistance*,
https://doi.org/10.1007/978-981-19-9279-7_6

and human health consequent to the selection of AMR bacteria and AMR genes. Persistent pollutants like plastics function as important vectors for the dissemination of AMR bacteria into the marine ecosystem. As there are several ways these AMR bacteria and genes can be conveyed back to terrestrial animals and humans, AMR surges in the marine ecosystem represent a significant zoonotic health risk. The present chapter summarizes the current knowledge on AMR in the marine ecosystem and the major future research perspectives in the area.

Keywords

Marine · Pollutants · Antimicrobials · Aquaculture · Plastics

1 Introduction

The World Health Organization (WHO) contemplates AMR as one of the leading worldwide health concerns that poses a danger not only to humans but also endangers sustainable development goals, ultimately warranting a global response to tackle the problem (WHO, 2020). Since AMR is a multifaceted problem, addressing its rising threat warrants an integrated cross-sectoral "one health" formulation, encompassing humans, animals, food, and the environment. Unfortunately, indiscriminate usage of antimicrobial drugs in human and veterinary medicine focuses on AMR research, while AMR engendered from the environment has mostly remained neglected so far. As the environment plays a critical role in the origin and dissemination of AMR, action plans excluding the environment cannot achieve the desired goals. Filling this critical research gap will ensure and improve the efficacy of existing and future antimicrobials and help design of novel therapeutic and mitigation strategies.

It is now proved that microbes from oceanic and terrestrial environments can share AMR genes (Cattoir et al., 2008). Selection of AMR bacteria in the marine ecosystem resulting from the use of antimicrobials or other means have a harmful bearing on the aquatic and terrestrial animal as well as human health by enabling the movement of AMR genetic determinants from marine microbes to fish, animal, and human pathogens (Burridge et al., 2010). Natural environments recognized as the critical reservoirs of AMR microbes and genes include soil, wild animals, wild birds, and glaciers. The environmental sector that has received little attention in AMR research during the last decade is the marine environment, even though it is the largest aquatic ecosystem making 70% of the surface area on the earth. It was believed that as oceans are dilute systems, all compounds can rapidly diffuse away so that there may be little selection for AMR microbes (Allison, 2005).

Conversely, emerging studies now highlight that the marine ecosystem is the global reservoir of clinically relevant and potentially novel AMR genes and microbes (Chen et al., 2013; Hatosy & Martiny, 2015). Hence, an overview of AMR in the marine ecosystem and possible mechanisms for its emergence and dispersal are presented. It is equally important to note that despite the progress in

maritime research, a thorough knowledge of AMR microbes and AMR genes in the marine ecosystem is still lacking.

2 Evidence on AMR Genes and Microbes in the Marine Ecosystem

A considerable number of research reports have undoubtedly demonstrated the presence of hotspots of AMR in different environments and their functions in the evolution and the spread of AMR genes. Aquatic ecosystems have been considered as a most potential hotspot as it harbors a diversity of AMR bacteria and AMR genes derived from multiple sources. Nevertheless, it is evident that our understanding of AMR bacteria and AMR genes in the natural ecosystem is still insufficient. Regardless of the enormous stretch of world oceans and the continued dependence of man on ocean resources, our understanding of the prevalence, diversity, or types of AMR organisms and AMR genes in the marine environment is meager. AMR genes have been detected in various natural environments, including lakes, oceans, rivers, and the pristine Antarctic ecosystem. AMR genes from clinically relevant bacteria have been found in multiple natural habitats, may be due to anthropogenic activities, including misuse or overuse of antimicrobial drugs in veterinary and human medicine. The mobility of DNA from chromosome to plasmid or between plasmids is promoted by mobile genetic elements, including bacteriophages, transposons, insertion sequences, gene transfer agents, plasmids, and integrative conjugative elements which are found to be responsible for carrying AMR genes. Among these mobile genetic elements, phages are the most abundant biological entities on the earth, which can potentially transfer genetic material between microorganisms. Numerous investigations have demonstrated the presence of AMR microbes and genes in sediments, water, and different species of animals within marine and estuarine environments (De Oliveira et al., 2010; Mudryk et al., 2010; Al-Bahry et al., 2011; Schaefer et al., 2019; Han et al., 2020). Functional metagenomics studies have disclosed the wide diversity of AMR genes, including several novel genes conferring resistance to ampicillin, nitrofurantoin, sulfadimethoxine, and tetracycline in various oceanic environments (Hatosy & Martiny, 2015).

It has been approximated that 90% of microbes in seawater are resistant to one antimicrobial drug, and up to 20% of the microbes are resistant to a minimum of five antimicrobial drugs (Martinez, 2003). Hatosy & Martiny (2015) estimated that the frequency of AMR in the ocean was up to 0.9% of cells.

Marine sediments are considered as the most important reservoirs and vectors for AMR bacteria and AMR genes among different marine ecosystem components due to favorable conditions of nutrients, shelter against sunlight, and protection against protozoan predation. Hence, many of the studies on AMR in the marine environment are focused on marine sediments. Mudryk et al. (2010) found that 20% to 30% of heterotrophic bacteria in a marine sandy beach had multiple drug resistance. Among the sediments from different oceanic zones, the mesopelagic zone harbors maximum AMR genes and AMR microbes (Hao et al., 2018).

Seawater is also known to harbor diverse AMR microbes and AMR genes. In seawater samples of Izmit Gulf, the maximum resistance with 62.5% prevalence was seen against sulbactam-ampicillin combination and trimethoprim-sulfamethoxazole combination and penicillin. Nearly 50% resistance was seen against tetracycline and gentamicin, while the prevalence of chloramphenicol and kanamycin resistance was around 37.5%, followed by resistance against cephoperazone (25%) and amikacin (12.5%) (Karayakar et al., 2004). In one study, Dang et al. (2006a) isolated oxytetracycline-resistant bacteria from mariculture rearing water in China and found that AMR bacteria represented nearly 5.63% and 32.23% cultivable bacteria in sea urchin and sea cucumber rearing waters, respectively. The study also showed that *Vibrio tasmaniensis* and *Vibrio splendidus* were the most prevalent AMR microbes. Matyar et al. (2008) also observed that isolates from the seawater collected from Iskenderun Bay of the Mediterranean Sea harbored a high proportion of streptomycin, cefazolin, ampicillin, and trimethoprim-sulfamethoxazole-resistant bacteria. The study of De Oliveira et al. (2010) revealed an increased prevalence of AMR in marine heterotrophic microbes isolated from sand and seawater in Gonzaguinha, notorious as an organic polluted recreational beach in Southeast Brazil. The results of Matyar (2012) also demonstrated a higher occurrence of AMR phenotypes to ampicillin, cefazolin, and streptomycin with multiple antibiotic resistance index (MARi) of isolates ranging from 0.2 to 0.75. The first evidence on the presence of AMR bacteria belonging to *Enterococcus* spp. was from the marine sediment and water of a Mediterranean mariculture site where there was no history of using any antimicrobials (Di Cesare et al., 2012). Their findings suggested the probability of a marine reservoir for AMR genes that could be potentially transmitted to pathogens in an antimicrobial-independent manner. The study of Al-Sarawi et al. (2017) on *E. coli* isolates from Kuwait's marine environment showed that AMR microbes were widespread in seawater across all the studied sites. In this study, among different antimicrobials tested, ampicillin was the one against which most microbes were resistant both in the summer and winter seasons. Pattern and level of AMR revealed through this study were almost identical to that of *E. coli* isolates from the water near a mariculture farm of China (Wang et al., 2015) and the sewage-polluted beaches of Brazil (da Costa Andrade et al., 2015).

The metagenomic studies had thrown more light on the prevalence of AMR in the marine ecosystem. Chen et al. (2013) and Nathani et al. (2019) have shown that AMR genes encoding resistance to polypeptides, macrolides, and vancomycin were abundant in deep marine sediments. In a study evaluating the AMR genes in marine sediments through metagenomic analyses, Yang et al. (2013) observed that marine sediment is the reservoir of many AMR genes, which included AMR genes providing resistance to 11 classes of antimicrobials, namely, aminoglycosides, bacitracin, β-lactam, chloramphenicol, glycopeptide, fluoroquinolone, macrolides, sulfonamide, streptogramin, tetracycline, and trimethoprim. The same study also detected several shared contigs between marine sediment bacteria and human pathogens, suggesting that marine sediment bacteria acquired AMR genes from human pathogens, and marine sediments act as a significant niche in the exchange of AMR.

Similarly, Tomova et al. (2015) found that sequences of AMR genes encoding resistance to quinolones, namely, *qnrA1*, *qnrB1*, and *qnrS1* among Chilean *E. coli* isolates and Chilean marine microbes were similar, suggesting the horizontal transfer of AMR genes between human pathogens and AMR marine microbes. Out of different AMR genes, one, namely, *bcrA* encoding resistance to bacitracin was reported as the indicator AMR gene for human feces contamination (Li et al., 2018). Metagenomics studies have shown unique marine microbes corresponding to different AMR genes across varied oceanic zones (Li et al., 2018). Nathani et al. (2019) proposed that bacteria belonging to five genera, namely, *Desulfovibrio* sp., *Thermotoga* sp., *Pelobacter* sp., *Nitrosococcus* sp., *Marinobacter* sp., and *Streptomyces* sp. can be used as prospective biomarkers for AMR monitoring in marine environments.

Among different mechanisms of AMR development in microbes, *namely*, efflux pumps, target alteration, target bypass and deactivation of antimicrobials, efflux pumps are the major ones in marine sediments (Chen et al., 2013; Nathani et al., 2019). According to Hatosy & Martiny (2015), previous known AMR genes in marine environments represented only 28% of total identified AMR genes, suggesting that the marine ecosystem is a reservoir of many unknown AMR genes. Transfer of AMR encoding plasmid between several bacterial species in aquatic habitats was also documented in different studies (Baya et al., 1986; Thavasi et al., 2007). Since oceans make >70% of the earth's surface and oceans are the hotspots of considerable commercial and recreational activities, the oceans are presumed to be the reservoirs of diversified AMR genes and bacteria.

3 Evidence on AMR in Marine Biota

Apart from sediments and water, AMR microbes and AMR genes are widely present in varied marine life. These include teleost, elasmobranchs, shellfish, sponges, and marine mammals, proving that AMR exists in both pelagic and demersal marine life (Blackburn et al., 2010; Laport et al., 2016). Most of these studies centered on farmed marine animals than wild species. The studies of Al-Sarawi et al. (2017) revealed that *E. coli* isolates from *Circenita callipyga* inhabiting in Kuwait's oceanic environment are resistant to many antimicrobials. Similarly, Laport et al. (2016) reported the occurrence of AMR genes, namely, e*rmB, mecA, mupA, qnrA, qnrB,* and *tetL* among the microbes of the marine sponge *Petromica citrina*. The occurrence of AMR microbes was also explored to monitor the exposure of green turtles (*Chelonia mydas*) to diverse marine contaminants (Al-Bahry et al., 2011, 2012). This study found that about 60.6% of oviduct fluid isolates were resistant to 15 tested antimicrobials with maximum AMR to ampicillin followed by streptomycin and sulfamethoxazole.

Investigations have revealed the occurrence of AMR bacteria in marine mammals also, from diverse areas of the world including Florida and South Carolina (Schaefer et al., 2009), New England waters (Bogomolni et al., 2008), the waters off England (Blackburn et al., 2010), Pacific coast of California (Johnson et al., 1998), and

Washington State (Lockwood et al., 2006), even though the pattern observed was different in different investigations. In a study on harbor porpoises (*Phoecena phoecena*), Vasquez et al. (2008) showed that all Salmonella isolates from this animal were resistant to clindamycin. Different studies have shown that bacteria isolated from pinnipeds, sharks, redfish, and dolphins (Johnson et al., 1998; Schaefer et al., 2009; Blackburn et al., 2010) exhibited maximum resistance to chloramphenicol except for *Mustelus mento* in Chile (Miranda & Zemelman, 2001). Besides, bacteria isolated from pinnipeds and sharks (Johnson et al., 1998; Blackburn et al., 2010) showed AMR phenotypes of amikacin, enrofloxacin, gentamicin, and sulfamethoxazole. In short, although straight comparisons cannot be made between different investigations, all these studies certified the prevalence of AMR to varied antimicrobials in the marine ecosystem.

Among marine animals, older marine predatory fishes are supposed to be valuable sentinels for AMR surveillance (Blackburn et al., 2010). The possible reason is the long life span and slow growth of these animals, making them susceptible to potentially more prolonged exposure to AMR bacteria in the ocean. The study by Miranda & Zemelman (2001) showed the presence of AMR bacteria on wild-caught fishes. Recently, Schaefer et al. (2019) have examined AMR trends in bottlenose dolphins for the past 13 years, which revealed that the highest AMR prevalence was towards erythromycin, followed by ampicillin and cephalothin. They noticed that resistance to ciprofloxacin among *E. coli* isolates was increased to more than two times between sampling periods. MARi of two zoonotic pathogens, namely, *Pseudomonas aeruginosa* and *Vibrio alginolyticus* significantly surged between 2003–2007 and 2010–2015. In addition, resistance to cefotaxime, ceftazidime, and gentamicin was also raised significantly for all the bacterial isolates. Altogether, the study pointed out that the surges of AMR in marine life reflect the conditions in humans. As there are several routes through which these increasing number of AMR bacteria and AMR genes in the marine environment can be conveyed back to terrestrial animals and humans, such as through seafood, direct contact with seawater, fishing activities etc., these surges of AMR microbes and genes in marine life represent a major zoonotic health risk. An increase of AMR microbes in recreational marine water, coastal sediments, and beach sands can also lead to a high zoonotic risk for people in coastal areas (Erdem-Kimiran et al., 2007).

4 Possible Reasons for the Occurrence of AMR in Marine Environments

Three main possible reasons were proposed for the occurrence of AMR genes and AMR microbes in aquatic environments (Hatosy & Martiny, 2015; Hao et al., 2018). The first reason is the mingling of AMR microbes from terrestrial environments through coastal runoff or during intertidal cycles leads to AMR microbes and genes of bacterial taxa, which are nonnative to marine environments. The second mechanism suggests possession of AMR by marine microbes from the anthropogenic impacts such as antimicrobial runoff, application of antimicrobials in marine cage

farms, etc., leading to a selective pressure on core marine microbes of the affected areas to turn as AMR. The third reason proposes the natural process of AMR selection in response to antimicrobial production by marine microbes as a part of the antagonistic interplay between microbes. Hatosy & Martiny (2015) also proposed a fourth mechanism, which states that specific bacterial proteins may be coopted for AMR phenotypes. Specific bacterial proteins function similar to those of recognized AMR genes, even though their primary functions are not associated with AMR, conferring resistance to the host cell (Hatosy & Martiny, 2015). The same authors observed many non-AMR genes that conferred AMR in marine environments, which led them to formulate this hypothesis. In consonance with this, certain previous research has demonstrated that non-antimicrobial efflux pumps can pump out antimicrobials from cells (Martinez, 2009). It was also shown that *E. coli* cultured at an increased temperature in antibiotic-free media developed resistance against rifampin (Rodríguez-Verdugo et al., 2013), supporting the proposed fourth mechanism. Metagenomics-based studies on the fish intestinal contents sampled from the mariculture farms situated in Baltic Sea revealed that AMR genes found in fish intestines were identical to the AMR genes obtained from farm sediments, indicating that fish feces helps for the selection of AMR genes in the absence of concurrent antimicrobial exposures (Amarasiri et al., 2020). Application of antimicrobials in the hatching and rearing practices of the fish before stocking in cage farms might explain the occurrence of AMR genes in the fish intestines (Muziasari et al., 2017). Among 58 marine microbes screened for the production of antimicrobials by Rosenfeld & Zobell (1947), one *Actinomyces* sp., four *Bacillus* spp., three *Micrococcus* spp., and one *Serratia* sp. showed antagonistic activity against nonmarine microbes. The wide prevalence of such antagonistic interaction among microbes might have led to the origin of AMR genes in pristine marine environments. AMR genes of fluoroquinolones, namely, *qnrA*, *qnrB*, and *qnrS*, are believed to have developed from aquatic bacteria (Takasu et al., 2011), which are then disseminated to other microbes. Thus, certain AMR genes that arose from the aquatic or marine ecosystem have then invaded the human environment (Amarasiri et al., 2020). Baquero et al. (2008) suggested that discharges of fecal matters from the terrestrial ecosystem into the aquatic ecosystem through wastewater treatment discharges by illegal means are one of the possible causes for the estuaries, coastal waters, and beaches getting polluted by fecal matter and for the occurrence of AMR in the marine ecosystem. When the wastewater is used for agriculture activities such as growing vegetables and fruits, and rearing farm animals and poultry, the bacterial contamination would be distributed globally. Agricultural runoff from the farming sector also acts as a key contributor to the contamination of the marine environment by antimicrobial products, AMR genes, and bacteria. Sewage discharges from terrestrial sources also harbor several pollutants of anthropogenic origin that can be co-selected for the AMR phenomenon (Baker-Austin et al., 2006). Aquaculture operations can be a direct path for the entry of antimicrobials or their products into the marine ecosystem (Shah et al., 2014). The absence of regulatory guidelines for using antimicrobials in aquaculture farms in many developing countries that enjoy a significant stake in aquaculture production makes predicting the extent of

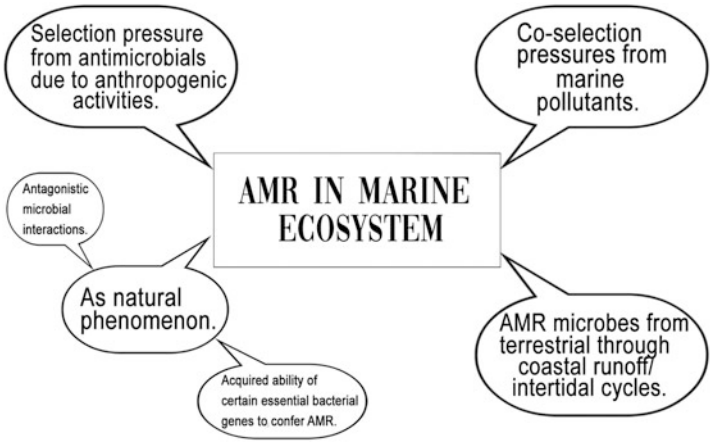


Fig. 1 Contributing factors for the AMR in marine ecosystem

contamination from aquaculture difficult. Once in the marine environment, the AMR bacteria and genes may spread across the ocean through ocean currents. The possible reasons for the occurrence of AMR in the marine ecosystem are represented in Fig. 1.

5 AMR in Pristine Marine Environments

Numerous investigations have revealed the occurrence of AMR microbes and AMR genes in pristine marine sediments and environments (Baya et al., 1986; Zhang et al., 2006; Chen et al., 2013; Hatosy & Martiny, 2015; Nathani et al., 2019). AMR marine microbes are reported to occur as far as 522 km offshore and 8,200 m depths in deep sea (Aminov, 2011). Similarly, AMR microbes and AMR genes have been reported in wild marine fishes unrelated to fish farming operations/polluted sites (Blackburn et al., 2010). A list of AMR genes and different antimicrobials against which microbes were found to be resistant from pristine marine environments are outlined in Table 1.

Zhang et al. (2006) demonstrated multiple AMR nature of all the isolates belonging to *Vibrio* spp. from Hong Kong marine reserve's pristine natural marine water. They also reported that AMR encoding plasmids are largely responsible for AMR phenotypes in these *Vibrio* isolates. AMR genes of macrolides and polypeptides were the most prevalent and predominant ones in the deep, pristine marine sediments of the South China Sea. Efflux pumps were the most abundant AMR mechanism (Chen et al., 2013). Among different macrolide resistance genes, *macB* encoding macrolide efflux pump was the most predominant genotype. It was found that *mexF*, which encodes an efflux protein causing chloramphenicol resistance, was the most abundant AMR gene in all Baltic Sea sediment samples (Muziasari et al., 2017). Certain studies showed that AMR genes confirming resistance to sulfonamide are

Table 1 AMR reported in pristine marine environments

Sl. No	Area under the study	Antimicrobials against which resistance was exhibited	AMR genes detected	Reference
1	Atlantic Ocean (off Beaufort, North Carolina)	Penicillin, Ampicillin, and Novobiocin	Not studied	Baya et al. (1986)
2	Seawater close to Fleves Island and Eretria, Greece	Not studied	*aacC(3)-I* and *ant(2″)-I* confirming resistance to AGs	Heuer et al. (2002)
3	Cape d'Aguilar Marine Reserve of Hong Kong	AMP, CB, CF, CD, CO, COT, E, FC, MET, NA, NIT, NO, P, ST, S, SM, SMX, TM, GM	Not studied	Zhang et al. (2006)
4	Jiaozhou Bay, China	C	*catI* and *cat III*	Dang et al. (2008)
5	Open Pacific Ocean	T	*tetM*	Rahman et al. (2008)
6	South China Sea	Not studied	AMR genes to AGs, BL, C, Q, fosfomycins, fosmidomycins, M, P, T, and sulfonamides, namely, *macB, mexD, acrB, arnA, mexW, mex I, mex F, ceo B, bac A, ros A, fos X, mexB, smeB, tetW* and *sul1*	Chen et al. (2013)
7	Marine sponge *Haliclona simulans* from Kilkieran Bay, Galway, Ireland	Erythromycin	Erythromycin resistance-encoding plasmid	Barbosa et al. (2014)
8	Hawaii Ocean Time-Series	AMP, T, NIT, Sul	Against AMP, T, NIT, Sul	Hatosy & Martiny (2015)
9	Chile	Not studied	Against T, Q and AGs, *namely*, *tet(A)*, *tet(B)*, *tet (K)*, *tet(M)*, *qnrA*, *qnrB*, *qnrS*, and *aac(6′)-Ib-cr*	Tomova et al. (2015)
10	Marine sponge (*Petromica citrina*) from southeastern Brazil (southwestern Atlantic ocean)	Not studied	*nrA, qnrB, ermB, tetL, mecA* and *mupA*	Laport et al. (2016)
11	Baltic Sea sediments	Not studied	Against T, AGs, Q, C, M, BL, V and multidrug resistance-encoding efflux pump genes,	Muziasari et al. (2017)

(continued)

Table 1 (continued)

Sl. No	Area under the study	Antimicrobials against which resistance was exhibited	AMR genes detected	Reference
			namely, *tetG, tetR, aacA4, aacC4, acrR, emrD, mepA, mdtE/yhiU, mtrD, oprD, pncA, tolC-02, qacE1–02, yceL/mdtH-01, acrA, ceoA, floR, mexF, oprJ, yidY/mdtL-01, erm, mphA, oleC, pikR2, vanB, vanC, vanHB, vanYD, ampC, blaCTX-M, blaOXY, blaSFO, blaSHV-01, blaTEM, cphA-01, cphA-02,* and *fox5*	
12	Chile	Not studied	*intI1*	Tomova et al. (2018)
13	Arabian Sea	Not studied	*macB, sav1866, tlrC, srmB, taeA, tetA, oleC and bcrA, vgaB, vgaD, cpxR, tetB, efrB, efrA,* and *vgaE* encoding resistance to bacitracin, macrolides, and glycopeptide	Nathani et al. (2019)

AMP: Ampicillin; CB: Carbenicillin; CF: Cephalothin; C: Chloramphenicol; CD: Clindamycin; CO: Colistin; COT: Cotrimoxazole; E: Erythromycin; FC: Fusidic Acid; GM: Gentamicin; MET: Methicillin; NA: Nalidixic Acid; NIT: Nitrofurantoin; NO: Novobiocin; P: Penicillin G; S: Streptomycin; ST: Sulfonamide-trimethoprim; SM: Sulfamethizole; SMX: Sulfamethoxazole; T: Tetracycline; TM: Trimethoprim; Sul: Sulfadimethoxine; AG: Aminoglycosides; BL: β lactams; Q: Quinolones; M: Macrolides; P: Polypeptides, V: Vancomycin

usually absent in pristine marine sites subject to little anthropogenic influence, even though the same is widely prevalent in polluted marine environments (Chen et al., 2013; Yang et al., 2019).

The absence of anthropogenic impacts in deep marine environments is verified by the absence of common antimicrobials in these environments (Chen et al., 2013). In contrast, the existence of AMR microbes and AMR genes in deep-sea environments supports the hypothesis that AMR is an ancient natural process (Chen et al., 2013). A possible explanation for this type of AMR occurrence might be the antagonistic microbial interactions in the marine environment to preserve nutrient resources, leading to the selection for AMR phenotypes (Hatosy & Martiny, 2015). Another reason could be the effect of certain genes that have functions in the cells, which might have acquired the ability to transport antimicrobials out of cells. For instance, mexF, encoding efflux pump protein that transports out humic acids, a usual

substance in the sediments, has been shown to confer resistance to chloramphenicol highly prevalent in Baltic Sea sediments (Muziasari et al., 2017). The higher levels of AMR observed in the marine ecosystem are certainly a modern phenomenon related to increased human activities (Port et al., 2012; Hatosy & Martiny, 2015). Supporting this argument, Chen et al., (2013) have shown that sediments of human-impacted environments contained at least seven times higher AMR gene-like reads than the pristine environments. A list of antimicrobials against which AMR bacteria were detected, AMR genes reported, and the possible reasons for their occurrence in marine environments are outlined in Table 2.

6 Role of Aquaculture Practices in AMR Development

Feeding a rapidly increasing human population through increased food production without any significant impacts on the environment is the key challenge in the coming years. Switching the human diet towards enhanced consumption of seafood and fish can help meet the increasing demand for animal protein without much impact on human and environmental health. On the other hand, depletion of wild fishery stocks due to overexploitation and climate change necessitate meeting this increasing demand for fish and other seafood through improved aquaculture practices. Accordingly, there has been a substantial increase in aquaculture activities in recent periods. About 70.5 million tons of food fish and 26.1 million tons of aquatic algae were made through aquaculture practices during 2014 only (Watts et al., 2017). The relative share of aquaculture was increased to 49% in 2002 from 5% in 1962 towards the total fish consumption (FAO, 2016). Apart from the enhanced quantity, there has been an increase in the diversity of species being cultivated, reaching a total of >580 species currently, consisting of >362 finfish and > 62 crustaceans (FAO, 2016). The increasing demands for fish and fish products are mainly met by intensifying farming practices as they can yield greater production.

As intensification increases, the fish stocking density and nutrient pollution in the aquaculture environment also increases, which frequently leads to low water quality. The combined effects of increased stocking density and low water quality have increased the incidence of infectious diseases in the aquaculture sector. Climate change has also contributed to the higher incidences of infectious diseases in aquatic environments.

The outcome of such higher incidences of diseases in aquaculture is the higher reliance on antimicrobials and other supplements in farming practices (Watts et al., 2017). As the availability of bacterial vaccines to prevent diseases in fish is minimal, substantial use of antimicrobials has become a routine practice in aquaculture worldwide, both for prophylactic and therapeutic purposes. Generally, the effects of antimicrobials in aquaculture practices are controlled by several factors, namely, the pathogen causing the disease, its antimicrobial sensitivities, treatment periods, host species, disease status, and hydrographical parameters of residing water like salinity, temperature, and photoperiod, as well as by legislation of the corresponding government organization (Watts et al., 2017). More specifically, the application of

Table 2 AMR reported in polluted marine sites

Sl. No	Antimicrobials against which bacteria exhibited resistance	AMR genes detected	Possible reason attributed	Reference
1	Chloramphenicol and Florfenicol	*catII* and *catIV*	Aquaculture	Dang et al. (2006a,b)
		Cat I and *cat III*	Sewage pollution	Dang et al. (2008)
		fexA	Aquaculture	Yang et al. (2013)
		cmlA	Aquaculture	Sousa et al. (2011)
		floR	Aquaculture	Buschmann et al. (2012); Tomova et al. (2015)
2	Tetracycline	T-resistance encoding plasmid	Mercury pollution	Rasmussen and Sorensen (1998)
		tet(M) and *tet(S)*	Aquaculture	Kim et al. (2004)
		tetM	Aquaculture	Nonaka et al. (2007)
		tetM	Aquaculture	Rahman et al. (2008)
		tetA, tetB, tetD, tetE, and *tetM*	Aquaculture	Dang et al. (2006a, b); Dang et al. (2008); Tamminen et al. (2011); Di Cesare et al. (2012)
		tetA	Aquaculture	Rodríguez-Blanco et al. (2012)
		tetC, tet33, tetK, tet41, tetB, tetL, tet35, tet32, and *tetB*	Aquaculture	Yang et al. (2013)
		tetA, teB, and *tetK*	Aquaculture	Buschmann et al. (2012)
		tetA and *tetG*	Aquaculture	Shah et al. (2014)
		tetA, tetB, tetK, and *tetM*	Aquaculture	Tomova et al. (2015)
		tet(32), tetM, tetO, tetS, tetW, tetA, tetE, tetG, and *tetH*	Aquaculture	Muziasari et al. (2017)
3	Aminoglycosides	*aac(3)-I,aac(3)-III/IV, aac(6′)-II/Ib,* and *ant (2″)-I*	Waste water pollution	Heuer et al. (2002)
		strA and *strB*	Aquaculture	Yang et al. (2013); Shah et al. (2014)
		aad1	Aquaculture	Sousa et al. (2011)
		aadA, aadA1, aadA2, and *strB*	Aquaculture	Muziasari et al. (2017)
		aac(3)-I	Plastic pollution	Yang et al. (2019)
4	Penicillin	*bla*TEM	Aquaculture	Shah et al. (2014)
		blaTEM and *blaSHV*	Aquaculture	Sousa et al. (2011)
		blaZ	Aquaculture	Di Cesare et al. (2012)

(continued)

Table 2 (continued)

Sl. No	Antimicrobials against which bacteria exhibited resistance	AMR genes detected	Possible reason attributed	Reference
5	Bacitracin	*bacA*	Plastic pollution	Yang et al. (2019)
6	Fluoroquinolones	*aac(6′)-Ib-cr, intI1,* and plasmid-mediated quinolone resistance (PMQR) genes *qnrA*, *qnrB*, *qnrS,* and *aac(6′)-1b*	Aquaculture	Buschmann et al. (2012)
		qnrA	Aquaculture	Yang et al. (2013)
		aac(6′)-Ib-cr	Aquaculture	Aedo et al. (2014)
		qnrB, qnrA, qnrB74, qnrS, and *aac(6′)- 1b-cr*	Aquaculture	Tomova et al. (2015)
		qnrA, qnrB, and *qnrS*	Aquaculture	Tomova et al. (2018)
7	Macrolides	*mefE* and *mphB*	Aquaculture	Yang et al. (2013)
		Macrolides Trimethoprim *macB*	Plastic pollution	Yang et al. (2019)
		Macrolides Trimethoprim *msr(C)*	Aquaculture	Di Cesare et al. (2012)
8	Trimethoprim	*dfrA1, dfrA5, dfrA12,* and *dfrA13*	Aquaculture	Shah et al. (2014)
		dfrA1	Aquaculture	Muziasari et al. (2017)
9	Sulfamethizole	*sul1*	Aquaculture	Muziasari et al. (2014)
		sul1 and *sul2*	Aquaculture	Shah et al. (2014)
		sul2	Aquaculture	Muziasari et al. (2017)
		sul1, sul2, and sul3	Aquaculture	Sousa et al. (2011)
10	Multidrugs	*mexF and multidrug ABC transporters*	Plastic pollution	Yang et al. (2019)

antimicrobials in aquaculture depends on the specific local regulations that differ widely between countries (Watts et al., 2017). There are rigorous rules on applying antimicrobials in certain developed countries like Europe, North America, and Japan, where only a limited number of antimicrobials are licensed for aquaculture use (Watts et al., 2017). For example, tight regulatory supervision on the application of antimicrobials, enhanced vaccinations, and superior stewardship have resulted in around 99% decline in the usage of antimicrobials during 1987 to 2013 in Norway, even though there was a 20-fold increase in production (O'Neill, 2015). The low consumption of antimicrobials in Norway is mainly attributed to the availability of efficient vaccines against vibriosis and furunculosis, the rapid administration of efficient zoo-sanitary plans, and biosecurity measures like zoning and spatial reallocation of mariculture sites to decrease the horizontal spread of diseases (Midtlyng et al., 2011).

Conversely, 90% of the global aquaculture production is done in developing countries that have no strict rules and enforcement on the use of antimicrobials. For example, the use of antimicrobials in Scotland and Norway varies between ~0.02 and 0.39 g/ton of harvested biomass, while the same reach up to ~660 g/ton in Chile (Rodríguez & Benjamín, 2015). Reports showed that Chilean companies applied nearly 0.53 kg of antimicrobials per ton of harvested salmon, of which 95% were applied in marine farms and 5% in freshwater farms during 2016 (Miranda et al., 2018). The large variations in the amount used between the marine and freshwater sector are caused by the quantity of antimicrobials used to treat *Piscirickettsia salmonis*, a common bacterial pathogen causing systemic infection among salmonids in marine environments (SERNAPESCA, 2017).

The lack of adequate restrictions in developing countries has led to the indiscriminate usage of considerable amounts of antimicrobials during the culturing process, especially in the areas where regulatory limits are not clearly defined or closely monitored. Even though there is no clear evidence for the routine application of antimicrobials in aquaculture in contrast to the livestock industries (Burridge et al., 2010), prophylactic use of antimicrobials has been previously reported in salmon and shrimp farming (Buschmann et al., 2012). As the direct application of antimicrobials to only infected fish is not feasible in aquaculture, metaphylactic treatment (the practice of treating the entire population) is required, so that antimicrobials are given by mixing with feed or, into the water, at proportionally higher doses than those in livestock (Muziasari et al., 2016; Thornber et al., 2020). Exact information on the quantities of antimicrobials applied in aquaculture practices is rare. Conservative estimates showed that around 1500, 950, and 478 metric tons of tetracycline, quinolones, and florfenicol, respectively, were consumed by Chile's salmon aquaculture from 2000 to 2008 alone (Buschmann et al., 2012). As explained, the major reason for the heavy use in Chile salmon aquaculture practices is shown as the higher mortality incidences due to bacterial infections, especially *P. salmonis*, against which no effective and reliable vaccines or antimicrobial drugs are available (Rozas & Enríquez, 2014). The earliest study investigating mariculture impacts on antimicrobial residues of marine environments was conducted in Italy (Lalumera et al., 2004). Afterwards, such research has been generally focused on China, Bangladesh, and South Korea (Kim et al., 2017; Han et al., 2020).

High amounts of antimicrobial residues, AMR genes, and AMR microbes have been demonstrated in different oceanic environments near aquaculture farms (Kim et al., 2004, 2007; Buschmann et al., 2012; Chen et al., 2015; Hatosy & Martiny, 2015; Muziasari et al., 2017). In a recent study assessing AMR risk in mariculture farms around the Yellow Sea, North China, Han et al. (2020) noted that trimethoprim was the most abundant antimicrobial agent in mariculture water samples. They also found that the method of aquaculture showed a significant relation to the levels of the antimicrobials in the mariculture environment. They observed higher concentrations of antimicrobials in greenhouse ponds and outdoor breeding ponds during the wet and dry periods. Another observation was that biofilms in aquaculture environments would act as a sink for heavy metals and antimicrobials in the aquaculture environment. It is proved that unconsumed fish feed containing antimicrobials, unabsorbed

antimicrobials in fish feces, and secreted antimicrobial metabolites through fish excreta, remaining in the water and sediment of aquaculture sites, often retain their antimicrobial activity (Miranda et al., 2018). Of these, leaching from unconsumed fish feed delineates a significant source, as there will be a loss of appetite in diseased animals and lower palatability of medicated feed (Miranda et al., 2018).

Antimicrobials can persist in aquatic environments, particularly in sediments, for different time periods depending on the biodegradability, initial amounts, and different physical and chemical characteristics (Burridge et al., 2010; Buschmann et al., 2012; Chen et al., 2015; Miranda et al., 2018). It is shown that around 70 to 80% of the applied antimicrobials in aquaculture farms are often expelled into the water leading to the alteration in microbial communities. Such persisting antimicrobials cause long-term selection pressure to the indigenous microbial communities of the water and sediments and replace susceptible bacterial and other microbial communities with resistant ones, thus promoting the origin and dispersal of AMR bacteria and AMR genes into surrounding aquatic environments, including the marine ecosystem (Shah et al., 2014; O'Neill, 2015; Tomova et al., 2015; Thornber et al., 2020). In support of this, Nonaka et al. (2007) proved a significant increase in oxytetracycline-resistant microbes within marine sediments surrounding a mariculture site after the application of oxytetracycline. The researchers also identified *tetM* genes among different genera of Gram-positive and Gram-negative bacteria within these marine sediments after the therapy. Similarly, Buschmann et al. (2012) noted significantly higher amounts of AMR bacteria and plasmid-mediated AMR genes to florfenicol, oxytetracycline, and oxolinic acid within sediments of the aquaculture site when compared to the control site. Their research concluded that the use of high quantities of antimicrobials in Chilean aquaculture sites leads to the selection of AMR in marine sediments. Their results also showed a direct correlation between the AMR-impacted aquaculture areas and quantities of antimicrobials used. Buschmann et al. (2012) also proved that the quantity of *tetA*, *tetB*, or *tetK* genes per microbe was significantly higher in tetracycline-resistant selected microbe in sediments from the aquaculture site (1.16 genes per microbe) than that of unselected marine microbe from the same sediments (0.58 genes per microbe). They hypothesized that residual amounts of flumequine that are not easily decayed in the marine environment might select plasmid-mediated quinolone resistance genes, namely, *qnrA*, *qnrB*, *qnrS*, and *aac*(6)-1b in marine microbes (Buschmann et al., 2012). Another evidence was given by Zhu et al. (2017), who described the positive correlations between the amounts of tetracycline and macrolides with the abundance of AMR genes in estuarine sediments. Apart from the quantity of AMR genes, studies have also shown that continuous exposure to antimicrobials increases the complexity of resistome, with higher numbers of AMR genes in mobile genetic elements of the microbes (Rico et al., 2013).

Many AMR genes are shown to persist in the marine ecosystems surrounding the aquaculture sites, even after discontinuing the application of antimicrobials (Tamminen et al., 2011). This is of particular concern to public health since many of the AMR aquaculture pathogens like *Vibrio* sp. and *Aeromonas* sp. can become the dominant strains in the surrounding marine and estuarine environments. More

importantly, certain AMR genes in marine bacteria can be horizontally transferred to human and animal pathogens (Tomova et al., 2015). For instance, horizontal transfer of ribosomal protection protein genes confirming AMR to tetracycline, namely, *tetM*, *tetS*, and *tetW* between marine microbes (gene donors) and *E. coli* was experimentally confirmed by Neela et al. (2009). Additionally, fish can be the reservoirs of zoonotic pathogens that can infect humans through food-borne infections and contact with the aquaculture facility/equipment. The major bacteria in this category include *V. vulnificus*, *Photobacterium damselae*, *Streptococcus iniae*, *Mycobacterium marinum*, and *A. hydrophila*. In addition to directly causing infections in humans, these bacteria are also shown to carry and disseminate AMR genes like extended-spectrum beta-lactamases (ESBL) genes (Dawood & Koshio, 2016).

Similarly, feces from Gilthead seabream (*Sparus aurata*) carry *E. coli* isolates containing AMR genes, namely, *aadA*, *blaSHV*-12, *blaTEM*-52, *cmlA*, *sul*1, *sul*2, *sul*3, and *tetA* (Sousa et al., 2011). Bacteria containing AMR genes were reported from commercial seafood products (Kumar et al., 2017). Muziasari et al. (2017) detected and quantified 71 AMR genes and transposases using a quantitative PCR array with 295 primers within the sediment resistome of aquaculture site in the Northern Baltic Sea, Finland. The authors observed that aquaculture had enriched sediment AMR genes of three antimicrobials used in fish farming, namely, sulfonamide, tetracycline, and trimethoprim in a reproducible mode. At the same time, the same genes were not noticed in pristine sea sediments, suggesting that these AMR genes were introduced to the sea sediments due to aquaculture practices using antimicrobials. Another interesting observation in this study was the co-selection of certain AMR genes like *aadA, aadA1, aadA2*, and *strB* genes conferring aminoglycoside resistance and quaternary ammonium compound resistance gene, *qacEΔ1,* through the use of sulfonamides. This co-selection of AMR genes conferring resistance for the antimicrobials which are not used in aquaculture represents a serious health risk.

Apart from antibiotics, usage of nonantibiotic chemicals such as disinfectants, other pharmaceuticals, metal-containing compounds (copper-containing compounds frequently used for the control of parasites), metal alloys in the cages of aquaculture (Burridge et al., 2010), certain components in fish feeds (high concentrations of cadmium, iron, lead, and mercury have been reported in some commercial finfish feeds), antifouling agents (copper-containing materials frequently employed as antifouling agents for cage farms) in aquaculture have also been shown to increase AMR in marine environments through the co-selection mechanism of AMR phenotypes (Baquero et al., 2008; Seiler & Berendonk, 2012).

In short, the exact reason for of the AMR gene enrichment, including integron and transposon-associated genes within the sediments close to aquaculture sites, remains to be elucidated. The primary reason was supposed to be the selection pressure caused by heavy and prolonged use of antimicrobials. In contrast, the amount of antimicrobials (tetracycline, oxytetracycline, sulfadiazine, sulfamethoxazole, and trimethoprim) was negligible in aquaculture farms sediments of certain studies (Tamminen et al., 2011; Muziasari et al., 2014). Hence, the occurrence of subinhibitory quantities of antimicrobials is pointed out as a critical player in the

enrichment of AMR genes. These subinhibitory quantities might enhance horizontal gene transfer of AMR genes between microbes by activating recombinases such as integrase and transposase, leading to the spread of the AMR phenomenon. It was also shown that antimicrobial treatment could cause AMR gene selection within the intestine of aquatic/marine animals (Giraud et al., 2006). The phenomenon can also occur independently of the usage of antimicrobials as co-selection with heavy metals and other materials.

Altogether, various aquaculture practices act as cardinal drivers in the occurrence and dissemination of AMR microbes and AMR genes in the marine ecosystem (Thornber et al., 2020). The potential link of aquaculture practices to terrestrial and marine resistomes is a primary concern, as several antimicrobials authorized for use in aqua farming practices, namely, oxytetracycline, florfenicol, and amoxicillin represent medically important antimicrobials for human application (O'Neill, 2015). It is reported that among the 60 various antimicrobials currently applied in aquaculture systems, 40 are categorized as critically important by the WHO, necessitating the urgent actions for antimicrobial regulation rules, control, and reporting in aquaculture (Rico et al., 2013; Liu et al., 2017). Even though antimicrobials selected for use in aquaculture are not connected to antimicrobial therapy in humans, cross-resistance between antimicrobials within a class can occur, leading to AMR against all the agents of that particular class, alarming the emergence of AMR microbes to all the currently used antimicrobials. Apart from these indirect threats to human health through the presence of AMR in aquaculture production systems, it would also affect the production by decreasing the drug efficacy, compromising the animal's immune system, and by selecting higher virulent isolates of pathogens (Watts et al., 2017). Therefore, antimicrobial concentrations in any open water aquaculture farm must be monitored regularly, and associated risks must be routinely evaluated to minimize the related impacts in public health and aquatic health perspectives. Since the prohibition of antimicrobials for prophylactic and growth-promoting purposes in aquaculture systems by Europe and other countries, alternative measures are shown to decrease pathogen activity, namely, vaccination, immune stimulation using nutritional factors, hormones, cytokines, and products derived from bacterial or algal sources, phage therapy, quorum sensing disruption of pathogens, and water disinfection with UV or ozone treatment. These alternative schemes, along with a better knowledge of the effects of them on the microbiome, immunome, and transcriptome of the farmed aquatic animals can provide solutions to improve aquaculture function with/without minimal use of antimicrobials, ultimately decreasing the potential for the dissemination of AMR through aquaculture practices (Watts et al., 2017).

7 Marine Pollutants and AMR

Marine pollutants, including human sewage, heavy metals, and pesticides, play an essential role in the acquisition and dispersal of AMR in oceanic environments (Nathani et al., 2019). A direct correlation between the degree of pollution to the frequency and variability of AMR in the marine ecosystem was reported by Erdem-

Kimiran et al. (2007) and de Oliveira et al. (2010). Nathani et al. (2019) showed that AMR genes were much more prevalent and diverse in the human-impacted marine sediments among different open sea pelagic sediment samples and the type of human activities had a significant role in determining the structure of the AMR genes and associated microbes in the deep-sea sediments. Apart from the direct release of antimicrobials, their breakdown products, heavy metals, fertilizers, and pesticides are also frequently discharged into the marine environment through human sewage, animal wastes, aquaculture run-off, or through the manure spread over agricultural farms, driving the co-selection of AMR in marine bacteria (Seiler & Berendonk, 2012).

Studies have shown that heavy metal pollution in marine environments could play a role in the origin and dispersal of AMR (Matyar et al., 2008). For example, the use of cadmium in pesticides and fertilizers is shown to cause AMR co-selection in the marine environment (Seiler & Berendonk, 2012). The occurrence of heavy metals such as Cd^{2+}, Cu^{2+}, Hg^{2+}, and Pb^{2+} induced resistance to multiple antimicrobials had been reported in *Pseudomonas putida* obtained from marine surroundings (Zhang et al., 2009). Many studies have shown that metal-resistant genes (MRGs) and AMR genes are frequently co-selected as the elements upregulating efflux pump gene expression. Thus, MRGs can modulate the expression of AMR genes encoding the efflux pumps (Eckert et al., 2018; Yang et al., 2019). AMR genes and MRGs can also be located in the same mobile genetic elements, such as plasmids and Class 1 integron-integrase gene (*intI1*) (Baker-Austin et al., 2006). Co-transfer of AMR genes and MRGs via the same mobile genetic elements are also reported. In short, metal resistance in microbes can indirectly select the expression of AMR.

In addition to heavy metals, various chemicals, preservatives, and toxic chemicals are also shown to drive the emergence of AMR phenotype. Romero et al. (2017) demonstrated the co-occurrence of resistance to multiple antimicrobials, biocides, preservatives, and metals among the human pathogens isolated from various seafood samples. Baya et al. (1986) noticed that bacterial strains isolated from toxic chemical wastes more often demonstrated AMR phenotypes than those from domestic sewage-polluted waters or pristine open ocean sites. They also reported that streptomycin-resistant isolates were present only in sewage-impacted marine samples. Dang et al. (2008) evaluated the chloramphenicol-resistant microbes and genes within Jiaozhou Bay of China's coastal seawaters and showed that about 0.15 to 6.7% of cultivable microbes were chloramphenicol-resistant. Further they observed that the AMR abundances were concentrated mainly in the sites close to the river mouths or sewage processing plants. Through metagenomics, Bengtsson-Palme et al. (2014) showed that the abundance of AMR genes in a pristine Swedish lake and a polluted Indian lake was 4×10^{-3} and 28.4 copies per 16S rRNA gene. Li et al. (2015) also conducted metagenomic analysis and showed that the quantity of AMR genes in ten different polluted environments (swine wastewater samples, wastewater biofilm, sewage, sludge samples, treated wastewater, drinking water, soils, river water, sediments, and fecal samples) were in the range from 3.2×10^{-3} to 3.1×100 copies per 16S rRNA gene. Gupta et al. (2018) reported 192 subtypes of MRGs encoding resistance to 21 metals in the influent and effluent of one

wastewater treatment facility. Altogether, results of various investigations suggest that the degree of pollution has a significant positive correlation to the prevalence of AMR phenotypes and AMR genes in multiple environments, forecasting that the abundance of AMR genes in the marine environment is likely to increase in the event of increasing marine pollution.

8 Marine Plastics: A Diffusion Route for AMR in Marine Environments

Plastic pollution is of great concern in marine ecosystems, as substantial amounts of annual worldwide plastic production end up as pollutants in the marine environment (Moore et al., 2020). The worldwide plastic production is estimated at 245 million tons per year, which is nearly equivalent to the per capita production of 35 kg plastic in each year, roughly equal to human biomass (Zhang et al., 2017). Even though a large proportion of this production is effectively used, reused, and adequately discarded using accepted waste management methodologies, a proportion of these pollutants enter the marine ecosystem (Moore et al., 2020). Plastic pollution is well-documented in the southwestern Indian Ocean, the Mediterranean Sea, the North Atlantic Ocean, the Bohai Sea, the Baltic Sea, and even the Mariana trench, the deepest part on earth (Zhang et al., 2017). As plastics can resist degradation, they represent persistent and ubiquitous marine pollutants. The North Atlantic Subtropical Gyre is calculated to contain plastic marine debris at a rate of 50,000 plastic pieces per km^2 (Moore et al., 2020).

Direct effects of plastic pollutants in marine animals include choking hazards or blockage of the digestive system. Apart from these direct effects, plastic pollutants are now recognized as carriers for many inorganic and organic contaminants, including antimicrobials, into the oceanic environment (Li et al., 2018; Yang et al., 2019). Diverse microbial communities can also inhabit plastics and microplastics, thus acting as carriers for the dissemination of bacteria and algae, including AMR pathogens into the marine ecosystem (Yang et al., 2019; Moore et al., 2020). There was no significant effect for particle size on the abundance and diversity of microbial AMR genes (Bryant et al., 2016; Yang et al., 2019), showing that microplastics and macroplastics are equally important in AMR dissemination.

Recent research confirmed that microplastics could function as carriers for many harmful chemicals, organic pollutants, antimicrobials, pharmaceuticals, and heavy metals into the marine ecosystem (Li et al., 2018). The weathering of microplastics leads to the leaching of diverse chemicals (aromatic compounds, antioxidants, heat stabilizers, metals, metalloids, slip agents, and plasticizers) and adsorbed pollutants from the surface (Hahladakis et al., 2018), providing an ideal condition for chemical-mediated co-selection of AMR phenotypes and new AMR genes in microbes (Hahladakis et al., 2018; Li et al., 2018). For instance, sophorolipid-stimulated bacterial and phage-mediated AMR gene dispersal in microplastic-tetracycline co-polluted soil (Sun et al., 2018). Arias-Andres et al. (2018) confirmed that horizontal transfer of genes between the microbes present on microplastics occur

at a much faster rate than free-living microbes, displaying that microplastics can function as an ideal platform for metal-driven co-selection of AMR microbes. Furthermore, Eckert et al. (2018) demonstrated that the quantity of integrase 1 (*int1*) genes in plastic-associated microbes were magnified along with the increase in the concentration of microplastic particles. The study of Yang et al. (2019) revealed that multidrug resistance genes, aminoglycoside resistance genes, and uncategorized AMR genes had the greatest average relative abundance among bacterial communities on marine plastics and, *Flavobacteriaceae* are the potential host for many AMR genes. The total abundance of AMR genes among microbes on plastic pollutants of the deep-sea environment was between from 7.7×10^{-4} to 1.2×10^{-2} copies/16SrRNA gene, which was relatively less in amounts than the reports from other environments that have high direct anthropogenic activity (Yang et al., 2019). Recently, Moore et al. (2020) examined the prevalence of AMR among the bacterial communities seen on food-related marine plastic pollutants, which form >90% of macroplastic pollutants seen along the entire coastline of Northern Ireland, through culture-dependent methods. Results showed that food-related marine macroplastic litter could harbor a wide diversity of viable bacteria that showed AMR property to many critically important antimicrobial classes. More specifically, these bacteria were 98% resistant to β-lactam class, namely, ampicillin, cefpodoxime, and ceftazidime and least resistant (16.1%) to minocycline (tetracycline group). Based on these findings, they suggested that the food industry has to act for mitigating the effects of AMR bacterial dispersal/reducing the survival of microbes on colonized food-related plastic pollutants either by masking/removing the negative charge on the surface or by the inclusion of antimicrobials along with the monomers during polymerization step.

Another study by Yang et al. (2019) demonstrated 64 AMR gene subtypes of 11 AMR gene types responsible for resistance to 13 different antimicrobials among microbial communities associated with the macroplastics, while only six AMR gene types were seen in surrounding water samples. This was the first metagenomics study for investigating the quantity and heterogeneity of AMR genes in plastic-associated microbes of a marine environment. Altogether, different studies indicated that plastics could act as important reservoirs for the dispersion of AMR microbes and AMR genes in the marine ecosystem. The plastic pollutants also act as a hotspot for horizontal gene transfer between microbes, leading to the spread of AMR, even between phylogenetically distinct microbes.

9 Future Perspectives

AMR is a widely prevalent phenomenon in the marine ecosystem, but the apparent scarcity of data limits a definite analysis of its extent. Hence, efforts to quantify AMR and evaluate the underlying genetic mechanism of AMR in this ecosystem are urgently needed. More importantly, as there are no standardized methods/strategies currently available for the qualitative and quantitative analysis of AMR genes and AMR from marine environments, optimization of these strategies/methods will be

immediately merited. Earlier studies focused either on culture-dependent methods or a limited number of AMR genes using PCR-based approaches. The use of culture-dependent techniques has resulted in many significant findings. Among these culture-dependent studies, there were often issues with the culture media, lack of adequate control samples, and uniformity in the limit ranges used for finding out the AMR frequencies, and generally without considering the innate resistances of that species (Watts et al., 2017). More importantly, cultivable bacteria represent $<1\%$ of the total microorganisms in natural environments and $< 0.1\%$ in the marine environments, so that cultivation-based methods might underestimate the actual AMR potential (Buschmann et al., 2012). Even though PCR-based methods can find uncultivable methods, these can target only a limited number of AMR genes, and prior sequence information of the target genes is needed. Therefore, there is no adequate AMR profile data in marine environments, which can be resolved in the coming years through NGS sequencing strategies and metagenomic analysis (Port et al., 2012). The advantages and disadvantages of commonly used methods for investigating AMR in environmental samples were recently reviewed by Scott et al. (2020), and the authors have pointed out that the selection of each method should be based on the overall objective of the research. Accordingly, studies focusing on animal or human risk should select methodologies identical to clinical methodologies, namely, including a suitable indicator pathogen, calculation of minimum inhibitory concentration (MIC) against clinically significant antimicrobials, and identifying clinically significant particular AMR gene targets. The research focusing on describing marine ecosystems/inspecting dispersal of AMR genes within the marine environment or defining causes leading to the presence and abundance of AMR genes have to select broad-spectrum methodologies (Scott et al., 2020). Investigations on the spatial and temporal footprint of AMR are also needed in future (Watts et al., 2017).

As discussed earlier, the presence of AMR in the marine ecosystem is an ancient inherent process (Chen et al., 2013). The higher levels and higher prevalence of AMR seen in the marine ecosystem is a modern process that is associated with increased human activities (Port et al., 2012; Chen et al., 2013; Hatosy & Martiny, 2015; Muziasari et al., 2017). Accordingly, future research focusing on anthropogenic activity gradients is needed to better understand the defined function of various activities on the presence and dispersion of AMR microbes and genes in marine environments. Identifying such activities/sources leading to higher levels will help implement suitable management actions to prevent further contamination. Supplementary, there are lacunae about the process involved in the co-selection of AMR by various pollutants and organic enrichment in the marine environment. In the face of increasing marine pollution, the determination of pollutants involved in the co-selection mechanism of AMR will also unravel novel management methodologies. More importantly, the marine ecosystem can function as a platform for the emergence of novel AMR microbes and genes. As the pollutants adsorbed on plastics might be a significant element in the evolution of novel AMR genes in the marine ecosystem (Yang et al., 2019), future research should focus on understanding this link between various pollutants and the prevalence of AMR genes in marine

plastics through both field and microcosm studies. Another potential area to be explored is the determination of potential host microbes of AMR genes in the presence of various pollutants by co-occurrence analysis with different pollutants (Zhu et al., 2017; Yang et al., 2019).

The selection of AMR microbes in the marine ecosystem will be having damaging impacts on marine life as well as on human/terrestrial animal health through the transfer of AMR genetic determinants from marine microbes to fish pathogens and terrestrial microbes, including human and animal pathogens (Burridge et al., 2010; Millanao et al., 2011). It is now proved that many microbes of terrestrial and oceanic environments can share AMR genes. Certain emerging AMR genes in human pathogens were reported to have originated from aquatic bacteria (Cattoir et al., 2008). This type of sharing of AMR genes and movable genetic elements between microbes of diverse ecosystems potentially threatens the treatment of many diseases. These reports strongly propose that marine and terrestrial environments are highly interconnected with regard to the dispersion of AMR microbes and AMR genes (Baquero et al., 2008). Accordingly, there are several paths through which these AMR microbes and genes can be conveyed back from the marine ecosystem to terrestrial animals and humans, either through seafood, direct contact with seawater or fishing activities. Tracking the flow of AMR genes into the marine ecosystem is a challenging task for researchers (Buschmann et al., 2012). Elucidation of the marine ecosystem in origin and dispersal of AMR requires integrated research, including clinical and terrestrial studies.

10 Conclusion

Presently, AMR is not just a clinical issue and emergence, persistence and dissemination of AMR in different environments represent a serious health concern. Among different environments, components of the marine ecosystem are now increasingly recognized as potential "hotspots" for the emergence, persistence, and dispersal of AMR. As marine environments are often subjected to quick horizontal and vertical water movements and are fundamental elements to many human commercial and recreation activities, this environment can function as a crucial global reservoir of AMR genes to clinically significant pathogens. Hence, new studies offering factual data to implement control strategies against the further spread in the marine ecosystem and to mitigate the current health risks will be critical in AMR containment strategies.

References

Aedo, S., Ivanova, L., Tomova, A., & Cabello, F. C. (2014). Plasmid-related quinolone resistance determinants in epidemic *Vibrio parahaemolyticus*, uropathogenic *Escherichia coli*, and marine bacteria from an aquaculture area in Chile. *Microbial Ecology, 68*, 324–328.

Allison, S.D. (2005). Cheaters, diffusion and nutrients constrain decomposition by microbial enzymes in spatially structured environments. *Ecol. Lett. 8*(6), 626–635.

Al-Bahry, S. N., Al-Zadjali, M. A., Mahmoud, I. Y., & Elshafie, A. E. (2012). Biomonitoring marine habitats in reference to antibiotic resistant bacteria and ampicillin resistance determinants from oviductal fluid of the nesting green sea turtle, *Chelonia mydas*. *Chemosphere, 87*(11), 1308–1315.

Al-Bahry, S. N., Mahmoud, I. Y., Al-Zadjali, M., Elshafie, A., Al-Harthy, A., & Al-Alawi, W. (2011). Antibiotic resistant bacteria as bio-indicator of polluted effluent in the green turtles, *Chelonia mydas* in Oman. *Marine Environmental Research, 71*(2), 139–144.

Al-Sarawi, H. A., Jha, A. N., Baker-Austin, C., Al-Sarawi, M. A., & Lyons, B. P. (2017). Baseline screening for the presence of antimicrobial resistance in *E. coli* isolated from Kuwait's marine environment. *Marine Pollution Bulletin, 129*(2), 893–898.

Amarasiri, M., Sano, D., & Suzuki, S. (2020). Understanding human health risks caused by antibiotic resistant bacteria (ARB) and antibiotic resistance genes (ARG) in water environments: Current knowledge and questions to be answered. *Critical Reviews in Environmental Science and Technology, 50*(19), 2016–2059.

Aminov, R. I. (2011). Horizontal gene exchange in environmental microbiota. *Frontiers in Microbiology, 2*, 158.

Arias-Andres, M., Klumper, U., Rojas-Jimenez, K., & Grossart, H. P. (2018). Microplastic pollution increases gene exchange in aquatic ecosystems. *Environmental Pollution, 237*, 253–261.

Baker-Austin, C., Wright, M. S., Stepanauskas, R., & McArthur, J. V. (2006). Co-selection of antibiotic and metal resistance. *Trends in Microbiology, 14*, 176–182.

Baquero, F., Martinez, J. L., & Cantón, R. (2008). Antibiotics and antibiotic resistance in water environments. *Current Opinion in Biotechnology, 19*, 260–265.

Barbosa, T. M., Phelan, R. W., Leong, D., Morrissey, J. P., Adams, C., Dobson, A. D., & O'Gara, F. (2014). A novel erythromycin resistance plasmid from *Bacillus* sp. strain HS24, isolated from the marine sponge *Haliclona simulans*. *PLoS One, 9*, e115583.

Baya, A. M., Brayton, P. R., Brown, V. L., Grimes, D. J., Russek-Cohen, E., & Colwell, R. R. (1986). Coincident plasmids and antimicrobial resistance in marine bacteria isolated from polluted and unpolluted Atlantic Ocean samples. *Applied and Environmental Microbiology, 51*(6), 1285–1292.

Bengtsson-Palme, J., Boulund, F., Fick, J., Kristiansson, E. L., & Joakim Larsson, D. G. (2014). Shotgun metagenomics reveals a wide array of antibiotic resistance genes and mobile elements in a polluted lake in India. *Frontiers in Microbiology, 5*, e648.

Blackburn, J. K., Mitchell, M. A., Blackburn, M. C. H., Curtis, A., & Thompson, B. A. (2010). Evidence of antibiotic resistance in free-swimming, top-level marine predatory fishes. *Journal of Zoo and Wildlife Medicine, 41*(1), 7–16.

Bogomolni, A. L., Gast, R. J., Ellis, J. C., Dennett, M., Pugliares, K. R., Lentell, J., & Moore, M. J. (2008). Victims or vectors: A survey of marine vertebrate zoonoses from coastal waters of the Northwest Atlantic. *Diseases of Aquatic Organisms, 81*, 13–38.

Bryant, J. A., Clemente, T. M., Viviani, D. A., Fong, A. A., Thomas, K. A., Kemp, P., Karl, D. M., White, A. E., & DeLong, E. F. (2016). Diversity and activity of communities inhabiting plastic debris in the North Pacific gyre. *mSystems, 1*, e00024–e00016.

Burridge, L., Weis, J. S., Cabello, F., Pizarro, J., & Bostick, K. (2010). Chemical use in salmon aquaculture: A review of current practices and possible environmental effects. *Aquaculture, 306*, 7–23.

Buschmann, A. H., Tomova, A., López, A., Maldonado, M. A., Henríquez, L. A., Ivanova, L., Moy, F., Godfrey, H. P., & Cabello, F. C. (2012). Salmon aquaculture and antimicrobial resistance in the marine environment. *PLoS One, 7*, e42724.

Cattoir, V., Poirel, L., Aubert, C., Soussy, C. J., & Nordmann, P. (2008). Unexpected occurrence of plasmid-mediated quinolone resistance determinants in environmental *Aeromonas* spp. *Emerging Infectious Diseases, 14*, 231–237.

Chen, B., Yang, Y., Liang, X., Yu, K., Zhang, T., & Li, X. (2013). Metagenomic profiles of antibiotic resistance genes (ARGs) between human impacted estuary and deep ocean sediments. *Environmental Science and Technology, 47*(22), 12753–12760.

Chen, H., Liu, S., Xu, X. R., Liu, S. S., Zhou, G. J., Sun, K. F., Zhao, J. L., & Ying, G. G. (2015). Antibiotics in typical marine aquaculture farms surrounding Hailing Island, South China: Occurrence, bioaccumulation and human dietary exposure. *Marine Pollution Bulletin, 90*(1–2), 181–187.

da Costa Andrade, V., Del Busso Zampieri, B., Ballesteros, E. R., Pinto, A. B., Cardoso, F., & de Oliveira, A. J. (2015). Densities and antimicrobial resistance of *Escherichia coli* isolated from marine waters and beach sands. *Environmental Monitoring and Assessment, 187*, 342.

Dang, H. Y., Ren, J., Song, L. S., Sun, S., & An, L. G. (2008). Dominant chloramphenicol-resistant bacteria and resistance genes in coastal marine waters of Jiaozhou Bay, China. *World Journal of Microbiology and Biotechnology, 24*(2), 209–217.

Dang, H. Y., Song, L., Chen, M., & Chang, Y. (2006a). Concurrence of cat and tet genes in multiple antibiotic-resistant bacteria isolated from a sea cucumber and sea urchin mariculture farm in China. *Microbial Ecology, 52*, 634–643.

Dang, H. Y., Zhang, X. X., Song, L. S., Chang, Y. Q., & Yang, G. P. (2006b). Molecular characterizations of oxytetracycline resistant bacteria and their resistance genes in mariculture waters of China. *Marine Pollution Bulletin, 52*, 1494–1503.

Dawood, M. A. O., & Koshio, S. (2016). Recent advances in the role of probiotics and prebiotics in carp aquaculture: A review. *Aquaculture, 454*, 243–251.

De Oliveira, F. C. A. J., Ranzani de França, P. T., & Pinto, A. B. (2010). Antimicrobial resistance of heterotrophic marine bacteria isolated from seawater and sands of recreational beaches with different organic pollution levels in southeastern Brazil: Evidences of resistance dissemination. *Environmental Monitoring and Assessment, 169*(1–4), 375–384.

Di Cesare, A., Vignaroli, C., Luna, G. M., Pasquaroli, S., & Biavasco, F. (2012). Antibiotic-resistant enterococci in seawater and sediments from a coastal fish farm. *Microbial Drug Resistance, 18*(5), 502–519.

Eckert, E. M., Di Cesare, A., Coci, M., & Corno, G. (2018). Persistence of antibiotic resistance genes in large subalpine lakes: The role of anthropogenic pollution and ecological interactions. *Hydrobiologia, 824*, 93–108.

Erdem-Kimiran, A., Arslan, E. O., Yurudu, N. O. S., Zeybek, Z., Dogruoz, N., & Cotuk, A. (2007). Isolation and identification of enterococci from seawater samples: Assessment of their resistance to antibiotics and heavy metals. *Environmental Monitoring Assessment, 125*, 219–228.

FAO (Food and Agriculture Organization). (2016). *The state of world fisheries and aquaculture*. Rome.

Giraud, E., Douet, D. G., Le Bris, H., Bouju-Albert, A., Donnay-Moreno, C., Thorin, C., & Pouliquen, H. (2006). Survey of antibiotic resistance in an integrated marine aquaculture system under oxolinic acid treatment. *FEMS Microbiology Ecology, 55*, 439–448.

Gupta, S. K., Shin, H., Han, D., Hur, H. G., & Unno, T. (2018). Metagenomic analysis reveals the prevalence and persistence of antibiotic- and heavy metal-resistance genes in wastewater treatment plant. *Journal of Microbiology, 56*, 408–415.

Hahladakis, J. N., Velis, C. A., Weber, R., Iacovidou, E., & Purnell, P. (2018). An overview of chemical additives present in plastics: Migration, release, fate and environmental impact during their use, disposal and recycling. *Journal of Hazardous Materials, 344*, 179–199.

Han, Q. F., Zhao, S., Zhanga, X. R., Wang, X. L., Song, C., & Wang, S. G. (2020). Distribution, combined pollution and risk assessment of antibiotics in typical marine aquaculture farms surrounding the Yellow Sea, North China. *Environment International, 138*, 105551.

Hao, S., Yang, P., Han, M., Xu, J., Yu, S., Chen, C., Chen, W. H., Zhang, H., & Ning, K. (2018). Data-mining of antibiotic resistance genes provides insight into the community structure of ocean microbiome. *BioRxiv,* 246033. https://doi.org/10.1101/246033.

Hatosy, S. M., & Martiny, A. C. (2015). The ocean as a global reservoir of antibiotic resistance genes. *Applied and Environmental Microbiology, 81*(21), 7593–7599.

Heuer, H., Krögerrecklenfort, E., Wellington, E. M. H., Egan, S., van Elsas, J. D., van Overbeek, L., Collard, J. M., Guillaume, G., Karagouni, A. D., Nikolakopoulou, T. L., & Smalla, K. (2002).

Gentamicin resistance genes in environmental bacteria: Prevalence and transfer. *FEMS Microbiology Ecology, 42*, 289–302.

Johnson, S. P., Nolan, S., & Gulland, F. M. D. (1998). Antimicrobial susceptibility of bacteria isolated from pinnipeds stranded in central and northern California. *Journal of Zoo and Wildlife Medicine, 29*, 288–294.

Karayakar, F., Ay, Ö., & Cicik, B. (2004). The identification of plasmid dependent resistancy of *Escherichia coli* against some antibiotics isolated from stations on Mersin shore line. *Ecology, 13*(52), 28–32.

Kim, H. Y., Lee, I. S., & Oh, J. E. (2017). Human and veterinary pharmaceuticals in the marine environment including fish farms in Korea. *Science of the Total Environment, 579*, 940–949.

Kim, S. R., Nonaka, L., & Suzuki, S. (2004). Occurrence of tetracycline resistance genes tet(M) and tet(S) in bacteria from marine aquaculture sites. *FEMS Microbiology Letters, 237*, 147–156.

Kim, Y. H., Jun, L. J., Park, S. H., Yoon, S. H., Chuang, J. K., Kim, J. C., & Jeong, H. D. (2007). Prevalence of tet(B) and tet(M) genes among tetracycline resistant *Vibrio* spp. 002E in the aquatic environments of Korea. *Diseases of Aquatic Organisms, 75*, 209–216.

Kumar, S., Lekshmi, M., Parvathi, A., Nayak, B. B., & Varela, M. F. (2017). Antibiotic resistance in seafood borne pathogens. In O. V. Singh (Ed.), *Food borne pathogens and antibiotic resistance*. Wiley.

Lalumera, G. M., Calamari, D., Galli, P., Castiglioni, S., Crosa, G., & Fanelli, R. (2004). Preliminary investigation on the environmental occurrence and effects of antibiotics used in aquaculture in Italy. *Chemosphere, 54*, 661–668.

Laport, M. S., Pontes, P. V., Dos Santos, D. S., Santos-Gandelman, J., Muricy, G., Bauwens, M., Giambiagi-deMarval, M., & George, I. (2016). Antibiotic resistance genes detected in the marine sponge *Petromica citrina* from Brazilian coast. *Brazilian Journal of Microbiology, 47*(3), 617–620.

Li, B., Yang, Y., Ma, L., Ju, F., Guo, F., Tiedje, J. M., & Zhang, T. (2015). Metagenomic and network analysis reveal wide distribution and co-occurrence of environmental antibiotic resistance genes. *The ISME Journal, 9*, 2490–2502.

Li, L. G., Yin, X., & Zhang, T. (2018). Tracking antibiotic resistance gene pollution from different sources using machine-learning classification. *Microbiome, 6*(1), 93.

Liu, X., Steele, J. C., & Meng, X. Z. (2017). Usage, residue, and human health risk of antibiotics in Chinese aquaculture: A review. *Environmental Pollution, 223*, 161–169.

Lockwood, S. K., Chovan, J. L., & Gaydos, J. K. (2006). Aerobic bacterial isolations from harbor seals (*Phoca vitulina*) stranded in Washington: 1992-2003. *Journal of Zoo and Wildlife Medicine, 37*, 281–291.

Martinez, J. L. (2003). Recent advances on antibiotic resistance genes. In M. Fingerman (Ed.), *Recent advances in marine biotechnology* (Molecular genetics of marine organisms) (Vol. 10, pp. 13–32). London.

Martinez, J. L. (2009). Environmental pollution by antibiotics and by antibiotic resistance determinants. *Environmental Pollution, 157*(11), 2893–2902.

Matyar, F. (2012). Antibiotic and heavy metal resistance in bacteria isolated from the eastern Mediterranean Sea coast. *Bulletin of Environmental Contamination and Toxicology, 89*(3), 551–556.

Matyar, F., Kaya, A., & Dincer, S. (2008). Antibacterial agents and heavy metal resistance in Gram-negative bacteria isolated from seawater, shrimp and sediment in Iskenderun Bay, Turkey. *Science of the Total Environment, 407*(1), 279–285.

Midtlyng, P. J., Grave, K., & Horsberg, T. E. (2011). What has been done to minimize the use of antibacterial and antiparasitic drugs in Norwegian aquaculture? *Aquaculture Research, 42*, 28–34.

Millanao, B. A., Barrientos, H. M., Gomez, C. C., Tomova, A., Buschmann, A., Dölz, H., & Cabello, F. (2011). Injudicious and excessive use of antibiotics: Public health and salmon aquaculture in Chile. *Revista Medica de Chile, 139*, 107–118.

Miranda, C. D., Felix, G. A., & Matthew, L. R. (2018). Current status of the use of antibiotics and the antimicrobial resistance in the Chilean salmon farms. *Frontiers in Microbiology, 9*, 1284.

Miranda, C. D., & Zemelman, R. (2001). Antibiotic resistance in fish from the Concepcio'n Bay, Chile. *Marine Pollution Bulletin, 42*, 1096–1102.

Moore, R. E., Millar, B. C., & Moore, J. E. (2020). Antimicrobial resistance (AMR) and marine plastics: Can food packaging litter act as a dispersal mechanism for AMR in oceanic environments? *Marine Pollution Bulletin, 150*, 110702.

Mudryk, Z., Perliński, P., & Skórczewski, P. (2010). Detection of antibiotic resistant bacteria inhabiting the sand of non-recreational marine beach. *Marine Pollution Bulletin, 60*(2), 207–214.

Muziasari, W. I., Pärnänen, K., Johnson, T. A., Lyra, C., Karkman, A., Stedtfeld, R. D., Tamminen, M., Tiedje, J. M., & Virta, M. (2016). Aquaculture changes the profile of antibiotic resistance and mobile genetic element associated genes in Baltic Sea sediments. *FEMS Microbiology Ecology, 92*(4), fiw052.

Muziasari, W. I., Pärnänen, K. M. S., Karkman, A., Lyra, C., Tamminen, M., Suzuki, S., & Virta, M. (2014). Sulfonamide and trimethoprim resistance genes persist in sediments at Baltic Sea aquaculture farms but are not detected in the surrounding environment. *PLoS One, 9*, e92702.

Muziasari, W. I., Pitkänen, L. K., Sorum, H., Stedtfeld, R. D., Tiedje, J. M., & Virta, M. (2017). The resistome of farmed fish feces contributes to the enrichment of antibiotic resistance genes in sediments below Baltic Sea fish farms. *Frontiers in Microbiology, 7*, 2137.

Nathani, N. M., Mootapally, C., & Dave, B. P. (2019). Antibiotic resistance genes allied to the pelagic sediment microbiome in the Gulf of Khambhat and Arabian Sea. *Science of the Total Environment, 653*, 446–454.

Neela, F. A., Nonaka, L., Rahman, M. H., & Suzuki, S. (2009). Transfer of the chromosomally encoded tetracycline resistance gene tet(M) from marine bacteria to *Escherichia coli* and *enterococcus faecalis*. *World Journal of Microbiology and Biotechnology, 25*, 1095–1110.

Nonaka, L., Ikeno, K., & Suzuki, S. (2007). Distribution of tetracycline resistance gene, tet(M), in Gram-positive and Gram-negative bacteria isolated from sediment and seawater at a coastal aquaculture site in Japan. *Microbes and Environments, 22*, 355–364.

O'Neill, J. (2015). Antimicrobials in Agriculture and the Environment: Reducing Unnecessary Use and Waste. The Review on Antimicrobial Resistance. [Online]. Available at: http://amr-review.org/sites/default/files/Antimicrobials%20in%20agriculture%20and%20the%20environment%20-%20Reducing%20unnecessary%20use%20and%20waste.pdf. Accessed: 25 August 2020.

Port, J. A., Wallace, J. C., Griffith, W. C., & Faustman, E. M. (2012). Metagenomic profiling of microbial composition and antibiotic resistance determinants in Puget Sound. *PLoS One, 7*(10), e48000.

Rahman, M. H., Nonaka, L., Tago, R., & Suzuki, S. (2008). Occurrence of two genotypes of tetracycline (TC) resistance gene tet(M) in the TC resistant bacteria in marine sediments of Japan. *Environmental Science and Technology, 42*, 5055–5061.

Rasmussen, L. D., & Sorensen, S. J. (1998). The effect of long term exposure to mercury on the bacterial community in marine sediment. *Current Microbiology, 36*, 291–297.

Rico, A., Phu, T. M., Satapornvanit, K., Min, J., Shahabuddin, A. M., Henriksson, P. J. G., Murray, F. J., Little, D. C., Dalsgaard, A., & Van den Brink, P. A. (2013). Use of veterinary medicines, feed additives and probiotics in four major internationally traded aquaculture species farmed in Asia. *Aquaculture, 412-413*, 231–243.

Rodríguez, L., & Benjamín, P. (2015). Plan de Contingencia Sernapesca para la Industria del Salmón. [Online]. Available at: http://repositorio.uchile.cl/handle/2250/132847. Accessed: 25 August 2020.

Rodríguez-Blanco, A., Lemos, M. L., & Osorio, C. R. (2012). Integrating conjugative elements as vectors of antibiotic, mercury, and quaternary ammonium compound resistance in marine aquaculture environments. *Antimicrobial Agents and Chemotherapy, 56*(5), 2619–2626.

Rodríguez-Verdugo, A., Gaut, B. S., & Tenaillon, O. (2013). Evolution of *Escherichia coli* rifampicin resistance in an antibiotic-free environment during thermal stress. *BMC Evolutionary Biology, 13*, 50.

Romero, J. L., Grande Burgos, M. J., Pérez-Pulido, R., Gálvez, A., & Lucas, R. (2017). Resistance to antibiotics, biocides, preservatives and metals in bacteria isolated from seafoods: Co-selection of strains resistant or tolerant to different classes of compounds. *Frontiers in Microbiology, 8*, 1650.

Rosenfeld, W. D., & Zobell, C. E. (1947). Antibiotic production by marine microorganisms. *Journal of Bacteriology, 54*(3), 393–398.

Rozas, M., & Enríquez, R. (2014). Piscirickettsiosis and *Piscirickettsia salmonis* in fish: A review. *Journal of Fish Diseases, 37*, 163–188.

Schaefer, A. M., Bossart, G. D., Harrington, T., Fair, P. A., McCarthy, P. J., & Reif, J. S. (2019). Temporal changes in antibiotic resistance among bacteria isolated from common Bottlenose Dolphins (*Tursiops truncatus*) in the Indian River Lagoon, Florida, 2003-2015. *Aquatic Mammals, 45*(5), 533–542.

Schaefer, A. M., Goldstein, J. D., Reif, J. S., Fair, P. A., & Bossart, G. D. (2009). Antibiotic-resistant organisms cultured from Atlantic bottlenose dolphins (*Tursiops truncatus*) inhabiting estuarine waters of Charleston, SC and Indian River Lagoon, FL. *EcoHealth, 6*(1), 33–41.

Scott, L. C., Lee, N., & Gim, A. T. (2020). Antibiotic resistance in minimally human-impacted environments. *International Journal of Environmental Research and Public Health, 17*(11), 3939.

Seiler, C., & Berendonk, T. U. (2012). Heavy metal driven co-selection of antibiotic resistance in soil and water bodies impacted by agriculture and aquaculture. *Frontiers in Microbiology, 3*, 399.

SERNAPESCA. (2017). Informe Sobre Uso de Antimicrobianos en la Salmonicultura Nacional 2016. Valparaíso. [Online]. Available at: http://www.sernapesca.cl. Accessed on 06 May 2020.

Shah, S. Q. A., Cabello, F. C., L'Abée-Lund, T. M., Tomova, A., Godfrey, H. P., Buschmann, A. H., & Sørum, H. (2014). Antimicrobial resistance and salmon aquaculture. *Environmental Microbiology, 16*, 1310–1320.

Sousa, M., Torres, C., Barros, J., Somalo, S., Igrejas, G., & Poeta, P. (2011). Gilthead seabream (*Sparus aurata*) as carriers of SHV-12 and TEM-52 extended–spectrum beta-lactamases-containing *Escherichia coli* isolates. *Foodborne Pathogen Diseases, 8*, 1139–1141.

Sun, M., Ye, M., Jiao, W., Feng, Y., Yu, P., Liu, M., Jiao, J., He, X., Liu, K., Zhao, Y., Wu, J., Jiang, X., & Hu, F. (2018). Changes in tetracycline partitioning and bacteria/phage – Co-mediated ARGs in microplastic-contaminated greenhouse soil facilitated by sophorolipid. *Journal of Hazardous Materials, 345*, 131–139.

Takasu, H., Suzuki, S., Reungsang, A., & Viet, P. H. (2011). Fluoroquinolone (FQ) contamination does not correlate with occurrence of FQ-resistant bacteria in aquatic environments of Vietnam and Thailand. *Microbes and Environments, 26*(2), 135–143.

Tamminen, M., Karkman, A., Lohmus, A., Muziasari, W. I., Takasu, H., Wada, S., Suzuki, S., & Virta, M. (2011). Tetracycline resistance genes persist at aquaculture farms in the absence of selection pressure. *Environmental Science and Technology, 45*(2), 386–391.

Thavasi, R., Aparnadevi, K., Jayalakshmi, S., & Balasubramanian, T. (2007). Plasmid mediated antibiotic resistance in marine bacteria. *Journal of Environmental Biology, 28*(3), 617–621.

Thornber, K., Verner-Jeffreys, D., Hinchliffe, S., Rahman, M. M., Bass, D., & Tyler, C. R. (2020). Evaluating antimicrobial resistance in the global shrimp industry. *Reviews in Aquaculture, 12*, 966–986.

Tomova, A., Ivanova, L., Buschmann, A. H., Godfrey, H. P., & Cabello, F. C. (2018). Plasmid-mediated quinolone resistance (PMQR) genes and class 1 integrons in quinolone-resistant marine bacteria and clinical isolates of *Escherichia coli* from an aquacultural area. *Microbial Ecology, 75*, 104–112.

Tomova, A., Ivanova, L., Buschmann, A. H., Rioseco, M. L., Kalsi, R. K., Godfrey, H. P., & Cabello, F. C. (2015). Antimicrobial resistance genes in marine bacteria and human uropathogenic *Escherichia coli* from a region of intensive aquaculture. *Environmental Microbiology Reports, 7*(5), 803–809.

Vasquez, C. A. V., Macgregor, S. K., Rowcliffe, J. M., & Jepson, P. D. (2008). Occurrence of a monophasic strain of Salmonella group B isolated from cetaceans in England and Wales between 1990 and 2002. *Environmental Microbiology, 10*, 2462–2468.

Wang, R. X., Wang, J. Y., Sun, Y. C., Yang, B. L., & Wang, A. L. (2015). Antibiotic resistance monitoring in *Vibrio* spp. isolated from rearing environment and intestines of abalone *Haliotis diversicolor*. *Marine Pollution Bulletin, 101*(2), 701–706.

Watts, J. E. M., Schreier, H. J., Lanska, L., & Hale, M. S. (2017). The rising tide of antimicrobial resistance in aquaculture: Sources, sinks and solutions. *Marine Drugs, 15*, 158–174.

WHO (World Health Organization). (2020). Antibiotic resistance. [Online]. Available at: https://www.who.int/news-room/fact-sheets/detail/antibiotic-resistance. Accessed: 18 May 2021.

Yang, J., Wang, C., Shu, C., Liu, L., Geng, J., Hu, S., & Feng, J. (2013). Marine sediment bacteria harbor antibiotic resistance genes highly similar to those found in human pathogens. *Microbial Ecology, 65*(4), 975–981.

Yang, Y., Liu, G., Song, W., Ye, C., Lin, H., Li, Z., & Liu, W. (2019). Plastics in the marine environment are reservoirs for antibiotic and metal resistance genes. *Environment International, 123*, 79–86.

Zhang, R., Wang, Y., & Gu, J. D. (2006). Identification of environmental plasmid-bearing *Vibrio* species isolated from polluted and pristine marine reserves of Hong Kong, and resistance to antibiotics and mercury. *Antonie Van Leeuwenhoek, 89*, 307–315.

Zhang, W. W., Zhang, S. F., Wang, J. Y., Wang, Y., Mu, J. L., Wang, P., Lin, X. Z., & Ma, D. Y. (2017). Microplastic pollution in the surface waters of the Bohai Sea, China. *Environmental Pollution, 231*, 541–548.

Zhang, X. X., Zhang, T., Fang, H. H. (2009). Antibiotic resistance genes in water environment. Appl. Microbiol. Biotechnol. *82*, 397–414.

Zhu, Y. G., Zhao, Y., Li, B., Huang, C. L., Zhang, S. Y., Yu, S., Chen, Y. S., Zhang, T., Gillings, M. R., & Su, J. Q. (2017). Continental-scale pollution of estuaries with antibiotic resistance genes. *Nature Microbiology, 2*, 16270.

Status of AMR in Food Sector: Implications for Food Safety and Food Security with Special Reference to Fisheries

Madhusudana Rao Badireddy, Murugadas Vaiyapuri, Mukteswar Prasad Mothadaka, and Chandragiri Nagarajarao Ravishankar

Contents

M. R. Badireddy (✉) · M. P. Mothadaka
Visakhapatnam Research Centre of ICAR-CIFT, ICAR-Central Institute of Fisheries Technology (ICAR-CIFT), Visakhapatnam, Andhra Pradesh, India
e-mail: bm.rao@icar.gov.in; bmrcift@gmail.com; mm.prasad@icar.gov.in; prasadmm@hotmail.com

M. Vaiyapuri
Microbiology Fermentation and Biotechnology Division of ICAR-Central Institute of Fisheries Technology (ICAR-CIFT), Cochin, Kerala, India
e-mail: murugadas.v@icar.gov.in; drmurugadas@gmail.com

C. N. Ravishankar
ICAR-Central Institute of Fisheries Education (ICAR-CIFE), Maharashtra, Mumbai, India
e-mail: ravishankar.cn@icar.gov.in; cnrs2000@gmail.com

M. P. Mothadaka et al. (eds.), *Handbook on Antimicrobial Resistance*,
https://doi.org/10.1007/978-981-19-9279-7_7

Abstract

Worldwide, consumers relish fish as food, owing to its nutritional and health benefits. Bacteria, including antimicrobial-resistant (AMR) strains, gain entry onto fish either during preharvest growth phase or postharvest processing and handling. The domestic and international trade in raw and processed fish makes them inadvertent vehicles for national and transboundary transmission of bacteria. Microorganisms in the aquatic environment adapt to the sublethal concentrations of antibiotic resulting in emergence of resistance that may eventually be disseminated *via* the harvested fish to human pathogens; either in the gut of fish consumers, on food contact surfaces, or in the environment. This chapter gives an overview of the importance of fish in human diet, role of fisheries in addressing food security, antimicrobial use (AMU) in aquaculture, regulations related to AMU in aquaculture, food safety *vis-à-vis* antibiotic residues, Rapid Alert System for Food and Feed (RASFF) notifications of the European Union pertaining antibiotic residues in fish and crustaceans, antimicrobial resistance in preharvest and postharvest fisheries, and suggest measures to mitigate AMR in aquatic animal farming.

Keywords

Fish food · Antimicrobial resistance · AMR · Antimicrobial usage · AMU · Maximum Residue Limit · MRL · Rapid Alert System for Food and Feed · RASFF · Food security

1 Food Security

The term "food security" has its origin in 1974 at the first World Food Conference wherein its definition was in terms of "food supply" but at the World Food Summit in 1996, food security was given a wider definition encompassing "food availability, food access, food use, and stability." Food security exists when there is physical and economic access to sufficient, safe, and nutritious food to all the people at all the times and the accessible food meets the dietary needs and food preferences of all the people for an active and healthy life. The different dimensions of food security include the availability of food both in terms of quantity and quality; access to appropriate food that provides nutritious diet; promotes utilization of food that meets all physiological requirements; and promotes nutritional well-being and access to adequate food at all times. Nutritious food for every living human being is the crux

of food security and in tune with this the United Nations is observing 2016–2025 as the decade of action on nutrition.

All humans on the planet Earth have the basic right to food and currently "the right to food" is enshrined in the constitution of over 40 countries. However, it is disheartening to note that although the world produces enough food, still more than 3 billion people cannot afford a healthy diet. The 17 Global Goals or the Sustainable Development Goals (SDGs) adopted in 2015 by the UN Member states with a target to achieve by 2030 to all people include goals that have important bearing on food security, namely, no hunger (SDG #2), good health and well-being (SDG #3), clean water and sanitation (#SDG 6). In spite of these thrusts, in 2019, nearly 8.9 percent of the global population was undernourished and is expected that by 2030 the number of undernourished people would exceed 840 million. Nearly 340 million children suffer from micronutrient deficiency, and the growth of 144 million children under 5 years of age was stunted. Further, the world is facing continuous threats to food security. The unanticipated COVID-19 pandemic had contracted all the world economies which can seriously undermine the efforts to end global food insecurity. People with low income and low-income countries cannot afford a healthy diet rich in fruits, vegetables, and animal foods but consume starchy staple foods because healthy diets are nearly five times more expensive. Annually, health costs related to diet are projected to exceed USD 1.3 trillion by 2030. It is also projected that COVID-19 pandemic might add 83 to 132 million people to the ranks of undernourished in 2020 due to food supply disruptions and loss of income (FAO, IFAD, UNICEF, WFP and WHO, 2020).

Policy prioritization is needed to address issues threatening food security in the country with more focus on reducing low productivity in agriculture and animal agriculture, reduce postharvest food losses, creating market infrastructure, strengthening transportation networks, creating awareness on the importance of healthy diet, and increasing the purchasing power of people to afford healthy diets.

2 Fish as an Important Component of Nutritious and Healthy Diet

Fish meat is a good source of quality protein, omega-3 fatty acids (eicosapentaenoic acid, EPA; docosohexaenoic acid, DHA), vitamins (A, D, E, B_{12}, folic acid), and minerals (calcium, copper, chromium, iodine, iron, magnesium, selenium, zinc). Health benefits derived from eating fish include reduced coronary diseases, reduced risk of diabetes, improved neurodevelopment in children, and better cognitive function in old age. A positive association was reported between fish intake and performance of school children. Globally, the consumption of fish and fishery products is more than poultry meat, pork, beef, or hens' eggs, and fish constitute the third major source of dietary protein after cereals and milk. Research on production of omega-3 polyunsaturated fatty acid-rich salmon, catfish, tilapia, and shrimp meats through feed manipulation was pursued to make the fish consumption healthier. Increased public

awareness on the nutritional profile and associated health benefits would result in further increase in the consumption of fish and fishery products.

3 Role of Fisheries in Addressing Food Security

Fisheries contribute 7 percent of all protein consumed and 17 percent of animal protein intake of the global population. Fisheries provide 50 percent of animal protein in some countries such as Bangladesh, Cambodia, Gambia, Ghana, Indonesia, Sierra Leone, and Sri Lanka. Nearly, 3.3 billion people consume fish and the estimated annual supply of fish for human consumption is 20.5 kg per capita. The analysis by Golden et al. (2021) demonstrate that an increase in the production of nutrient-rich aquatic animal-source food (AASF) can improve the diets of many nations. The per capita fish consumption in the world increased from 9 kg (1961) to 20.5 kg (2018) and is estimated to reach 21.5 kg per capita by 2030. The increase in per capita fish consumption is attributed to consumers' increased per capita incomes, urbanization, increased fish production, postharvest processing, supply of fish to distant locations, and changes in dietary trends of people towards healthy foods.

The supply of fish from capture fisheries is fast diminishing, and is projected that by 2030, the share of world capture fisheries would fall to 47 percent from the present 54 percent. Aquaculture, that is, the farming of aquatic animals (finfish, crustaceans, and mollusks) is increasingly being seen as a reliable and economic supply of animal protein to meet the national, regional, and global food security. This assumes greater significance as the human population is fast approaching 10 billion. Global aquaculture production in 2018 stood at an impressive 82 million tonnes accounting for 45.8 percent of the total fish production and valued at USD 250 billion (FAO, 2020). Presently 52 percent of fish meant for human consumption originates from aquaculture and this is set to increase to 59 percent by 2030. Aquaculture is dominant in Asia and out of the projected aquaculture fish of 108.5 million tonnes in 2030, Asian countries would contribute 96.35 million tonnes (88.8%). The top five aquaculture-producing countries in the world in 2018 were China (57.93%), India (8.61%), Indonesia (6.61%), Vietnam (5.04%), Bangladesh (2.93%), and the top seven countries in marine capture fisheries were China (15%), Peru (8%), Indonesia (8%), the Russian federation (6%), the United States (6%), India (4%), and Vietnam (4%) (FAO, 2020).

India is one of the largest fish producers in the world with a total fish production of 14.16 million tonnes in 2019–2020, of which inland fisheries contributed 10.43 million tonnes and marine fisheries contributed 3.72 million tonnes. Value wise, the contribution of fisheries to Gross Value Added (GVA) at 2018–2019 prices was Rs 2,12,915 crores (~28.3 billion USD), forming 1.24 percent of total GVA and 7.28 of agricultural GVA (GOI, 2020). India exported 1.29 million tonnes of fish in 2019–2020 valued at USD 6.7 billion (www.mpeda.gov.in). Aquaculture has seen a drastic shift from the traditional farming practices to intensive farming systems in freshwater, brackish water, and marine waters. Unscientific farming with bad aquaculture practices such as higher stocking densities, improper feeding practices, poor

water quality management, weak biosecurity, and irresponsible aquatic animal health management has led to the increase in disease incidence in finfish and shell fish aquaculture. The major bacterial diseases in farmed aquatic animals, in temperate and tropical regions, include *Aeromoniasis*, *Edwardsiellosis*, *Vibriosis*, *Pseudomonasis*, *Flavobacteriosis*, *Mycobacteriosis*, *Streptococcosis*, *Renibacteriosis,* and antibiotics are employed as a means to control these bacterial infections. Sustainability of aquaculture is severely constrained by infectious diseases; directly through production losses and operating costs and indirectly though trade restrictions. Economic losses due to disease in aquaculture is significantly high, and it was estimated that losses in 11 countries during 1987–1994 to be in the order of USD 3 billion (Israngkura and Sae-Hae, 2002). Antibiotics such as oxytetracycline, doxycycline, chlortetracycline, florfenicol, oxolinic acid, amoxicillin, erythromycin, flumequine, sulfadiazine-trimethoprim, sulfadimethoxine-ormetroprim, sulfamethoxazole-trimethoprim, sulfadimethoxine-trimethoprim, sulfadimidine-trimethoprim were approved for use in aquaculture and the approved list of antibiotics varies with the countries. The usage of antibiotics in different countries varies with fish species and under selected conditions. Some countries have an approved list of antibiotics that can be used for therapeutic treatment in aquaculture. The important antimicrobial agents for treatment of fish mentioned in the list of antimicrobial agents of veterinary importance by the World Organization of Animal Health (WOAH) is given in Table 1.

The use of antibiotics such as oxytetracycline, althrocin, ampicillin, sparfloxacin, and enrofloxacin for the control of fish diseases or as prophylactic agents was reported in India (Bharathkumar and Abraham, 2011). A survey of the feed

Table 1 OIE list[a] of antimicrobial agents for use in fish for treatment purpose

Antibiotic class	Antibiotics	Category
Aminoglyocisides	Spectinomycin, Streptomycin, Kanamycin	VCIA#
Bicyclomycin	Bicozamycin	VIA#
Fosfomycin	Fosfomycin	VHIA#
Lincosamides	Lincomycin	VHIA
Macrolides	Erythromycin, Josamycin, Spiramycin	VCIA
Aminocoumarin	Novobiocin	VIA
Aminopenicillins	Amoxicillin, Ampicillin	VCIA
Carboxypenicillins	Tobicillin	VCIA
Phenicols	Florfenicol, Thiamphenicol	VCIA
Quinolones	Enrofloxacin	VCIA
	Flumequine, Miloxacin, Oxolinic acid	VHIA
Sulfonamides	Sulfafurazole, Sulfamethoxine, Sulfamonomethoxine, Sulfadimethoxine, Trimethoprim-Sulfonamide	VCIA
Tetracyclines	Doxycycline, Oxytetracycline, Tetracycline	VCIA

[a]OIE list of antimicrobial agents of veterinary importance, adopted by the OIE International Committee at its 75th General Session in 2007 (Resolution No. XXVIII) and updated in 2013, 2015 and 2018 by the World Assembly of OIE Delegates. # VCIA: Veterinary critically important antimicrobial agents; VHIA: Veterinary highly important antimicrobial agents; VIA: Veterinary important antimicrobial agents

management of 106 fish farmers of Andhra Pradesh, India, revealed that 15% of the farmers used antibiotics for treating bacterial diseases and the typical therapeutants were oxytetracycline, enrofloxacin, sulfamethoxazole, trimethoprim, and chlortetracycline (Ramakrishna et al., 2013). The use, misuse, and inappropriate use of antibiotics in aquaculture potentiates the emergence of antimicrobial resistance (AMR) in bacteria in the aquatic environment and consequently the aquatic system becomes a reservoir of antibiotic resistance genes. Unlike in human health care and terrestrial animal health care, it is generally not possible to treat individual aquatic animals, and application of antibiotics is done through a metaphylactic approach exposing both the infected and healthy aquatic animals to the drug. The antibiotic is usually mixed with feed and broadcast in pond/farm water. The unconsumed feed containing antibiotic and the discharge of pond effluents with antibiotics results in subtherapeutic exposure of bacteria to antibiotics that potentially leads to emergence of antimicrobial resistance.

4 Antimicrobial Use (AMU) in Animal Agriculture

Antibiotics are used in animal agriculture mainly for therapeutic treatment of diseased animals, for growth promotion, prophylactic treatment for disease prevention, and metaphylactic treatment of diseased and healthy animals. Nontherapeutic use of antibiotics for growth promotion in animal husbandry started in the 1940s with the addition of small doses of antibiotics to feed and was widely practiced in Europe and the United States. Although there was no consensus regarding the mechanism of action but it was thought that antibiotics modulate the gut microbiome. In feed, antibiotics have been used in poultry to control coccidiosis and necrotic enteritis. The AMU in animal agriculture is very high. In the United States alone, 13.6 million kg of antibiotics was sold in 2011 for use in farmed animals (FDA, 2015). The antimicrobial consumption was 63,151 tonnes in 2010 and is projected to rise by 67% in 2030 (Laxminarayan et al., 2015; van Boeckel et al., 2015). In the United States, about 80% of all antibiotics consumed is in the animal feeds (CDDEP, 2015). Antibiotics are used for prophylaxis (in the absence of the disease) or for metaphylaxis (both infected and healthy animals). Antibiotics are used in animal agriculture as growth promoters at lower or subtherapeutic doses to increase productivity of food-producing animals. However, the practice of use of antibiotic as growth promoters was prohibited legally (EU directives 2001/82/EC; 1831/2003/EC) or voluntarily (Veterinary Feed Directive of USFDA, 2015). European Union banned the nontherapeutic prophylactic use of antibiotics in 2001 through the EU Veterinary Medicinal Products Directive, 2001/82/EC. The inappropriate use of antibiotics in the animal agriculture was reported in China (Hu and Cheng, 2016) and Vietnam (Kim et al., 2013).

The intensification of animal agriculture in emerging economies is estimated to result in 67% increase in demand for antimicrobials by 2030 (van Boeckel et al., 2015). Generally, the classes of antibiotics used in animal agriculture and aquaculture were different from those used in human health care. The use of tetracyclines,

sulfonamides, trimethoprim, and lincosomides was relatively higher in animal production whereas penicillins, fluroquinolones, and cephalosporins were frequently used in human health (Public Health England, 2015).

5 Antibiotic Use in Aquaculture

Unlike in human and veterinary health care, there are no antibiotics specifically developed for use in aquaculture (FAO, 2017). The greater species diversity (finfish, crustaceans, cephalopods, mollusks) coupled with different farming systems (freshwater, brackish water, marine water) makes antibiotic advocacy relatively difficult in fish health management. However, antibiotics are used in aquaculture, mainly to prevent mass mortalities rather than growth promotion. Sulfonamides potentiated with trimethoprim were the first antibiotics used in fish farming in the 1970s. Globally, the number of antibiotics and the quantity of antibiotics used in aquaculture increased over the years. Major classes of antibiotics used in aquaculture for controlling bacterial infections include sulfonamides (with ormetroprim/trimethoprim), penicillins (amoxicillin), macrolides (erythromycin), quinolones (oxolininc acid, flumequine), phenicols (florfenicol) and tetracyclines (oxytetracycline, doxycycline, chlortetracycline). The antimicrobial consumption in global aquaculture was estimated at 10,259 tonnes in 2017, of which more than 50% consumption was in China (Schar et al., 2020). Fish species wise, the antimicrobial consumption was highest in catfish (157 mg kg^{-1}) followed by trout (103 mg kg^{-1}), tilapia (59 mg kg^{-1}), shrimp (46 mg kg^{-1}), and salmon (27 mg kg^{-1}) farming. The same study projected the global antimicrobial consumption in 2030 at 236,757 tonnes of which the share of aquaculture (5.7%) would be lower than human use (20.5%) and terrestrial animal use (73.7%) (Schar et al., 2020). The emergence of diseases such as Acute Hepatopancreatic Necrosis Disease (AHPND) with known bacterial etiology may incentivize increases in antibiotic use in shrimp aquaculture.

Antibiotic usage in aquaculture varies widely across the countries. The use of antibiotics in different countries is guided by several factors such as the incidence of bacterial infections (diagnostic capability to differentiate bacterial from viral infections), access to antibiotics (free or regulated access), treatment advisories (government/private laboratories), food safety regulations (domestic/export markets). Use of antibiotics had been reported from Chile in salmon farming; Vietnam in pangasius farming; Bangladesh, Thailand, Vietnam, and China in shrimp farming; Thailand and China in tilapia farming. Shrimp farmers in Vietnam used 11 different antibiotics in 2004 (Le and Munekage, 2004). Norway used 0.02–1.3 g of antibiotic/ton of salmon fish, British Columbia (Canada) used 43.7 g of antibiotic /ton of salmon fish while Chile used 660 g–700/ ton of salmon production (Bridson, 2014; Watts et al., 2017).

The antibiotics approved for use in aquaculture in the United States were florfenicol, oxytetracycline, and sulfadimethoxine/ormetoprim and the antibiotics commonly used in aquaculture in Europe were florfenicol (amphenicol), oxolinic acid (quinolone), and flumequine (quinolone). In Asia, the commonly used antibiotics in aquaculture include

amoxicillin, erythromycin, florfenicol, oxytetracycline, chlortetracycline, tetracycline, doxycycline, sulfadimethoxine/ormethoprim, and sulfadimethoxine/ trimethoprim. Veterinarians or authorized aquatic animal health professionals need to prescribe only those medicines authorized for use in food-producing animals. However, when there is no suitable product to treat fish, a suitable product approved in other food animals is prescribed. This prescribing system is known as "cascade system" in Europe or "extra-label use" in the United States. In such cases, it is vital that veterinarians adhere to pharmacovigilance guidelines and strictly follow the prescribed withdrawal times to avoid drug residues in edible tissue.

6 Withdrawal Period of Antibiotics in Fish

Maximum residue limit (MRL) is the maximum level of antibiotic that may be present in the edible animal tissue without causing any harm to the consumer. The time period between the administration of the last medication and slaughter of the fish to ensure that the residues of antibiotics have depleted to level below the MRL is known as the withdrawal period. In terrestrial farm animals, the withdrawal period is generally given in days but for farmed aquatic animals it is given in degree days as the metabolic rate of fish, due to its poikilothermic nature, is determined by environmental temperature. Generally, a 10% increase in metabolic rate is expected for every 1 °C rise in temperature. The standard withdrawal period for fish has been set as 500-degree days. However, withdrawal period for different finfish and shellfish species have not been established. Moreover, the withdrawal period under different water conditions (salinities, pH) has not been worked out. The lack of this information restricts the prescription of antibiotics for farmed aquatic animals intended for human consumption. The Government of India according to the Drugs and Cosmetics Act, 1940 and the Drugs and Cosmetic Rules, 1945 states that if the specific withdrawal period has not been validated, the withdrawal period shall not be less than 500-degree days for fish.

7 Food Safety Concerns of Antibiotics Residues in Fish Meat

World over, fish consumers' demand for farmed fish products raised without antibiotics. Eating fish meat-containing residues of antibiotics or metabolites of antibiotics affects the health of the consumers (Table 2). Harmful effects were associated with the consumption of food containing chloramphenicol, nitrofuran metabolites, β-lactam antibiotics, sulfonamides, gentamicin, tetracyclines. In tune with this, stringent quality requirements were laid by different countries regarding the presence of antibiotics residues/antibiotic metabolites in fish meat, and this has profoundly impacted the application of antibiotics in aquaculture.

Several antibiotics were prohibited for use in aquaculture by many aquafarming nations (Table 3). Antibiotic residue testing has become mandatory for farmed fish destined to export markets. The testing facilities were strengthened in the

Table 2 Human health issues related to antibiotic residues in food

Antibiotic/metabolite	Effect on human health
Chloramphenicol	Aplastic anemia, bone marrow toxicity,
β-lactams (penicillins, cephalosporins)	Allergy, dermatitis, cutaneous eruptions, anaphylaxis, and gastrointestinal symptoms
Tetracycline	Staining of teeth in young children, poor development of fetuses, gastrointestinal disorders, cytotoxic and immune-pathological effects
Sulfonamides	Skin allergies
Gentamicin	Mutagenic, nephropathic, hepatotoxic
Nitrofurans and their metabolites	Genotoxic and carcinogenic

fish-producing countries by way of preharvest testing of fish to process antibiotic-free fish and pre-shipment testing of finished products, while in the importing countries the testing of fish at the port of entry and screening fish products in the retail markets became a practice. In the event of detection of antibiotics or their metabolites (above MRL/ MRPL) in fish products, the faulty products are labeled as unfit for human consumption and rejected or destroyed. MRLs are generally established for approved/permitted antibiotics such as oxytetracycline. Minimum required performance limit (MRPL) is the minimum content of an analyte in a sample, which at least has to be detected and confirmed. MRPLs are generally established for prohibited antibiotics such as nitrofurans, chloramphenicol etc. The European Union established a minimum required performance limit (MRPL) for chloramphenicol (0.3 μg kg^{-1}) and nitrofuran metabolites (1 μg kg^{-1}) in aquaculture products intended for human consumption.

7.1 Antibiotic Residues in Fish Meat Vis-à-Vis Food Safety Regulations

The use, misuse, or inadvertent use of antibiotics in shrimp aquaculture system has led to rejections of farmed shrimp exported by India to the EU. The rejections were attributed mainly to the presence of nitrofuran (metabolites) and chloramphenicol and detection of excess amounts of tetracyclines. The misuse of prohibited antibiotics and inappropriate use of permitted antibiotics in aquaculture leads to twin problems, namely, antibiotic residues in farmed fish meat and emergence of antimicrobial resistance with implications for food safety and health care.

Maximum residue limit (MRL) of antibiotics indicates the maximum level of the residue of the antibiotic that may be present in the edible tissue of food-producing animal without presenting any harm to the fish consumer. MRLs are established based on scientific assessment with an objective to protect public health. In the case of fish, the edible tissue is defined as muscle with adherent skin in natural proportions. A factor of 300 g is taken for MRL calculation as it represents fish meat contribution to the acceptable daily intake (ADI) (Alderman, 2009). *Salmonidae*

Table 3 Antibiotics prohibited for use in aquaculture

European Union (EU)	United States of America (USA)	China	India	Indonesia	Thailand	Vietnam
Chloramphenicol Nitrofurans Dimetridazole Metronidazole Ronidazole	Chloramphenicol Nitrofurans Fluoroquinolones Quinolones	Chloramphenicol Nitrofurans (Furazolidone Nitrofurantoin Nitrofurazone) Bacitracin Zinc Ciprofloxacin Norfloxacin Erythromycin Olaquindox Tylosin	Chloramphenicol Nitrofurans (Furazolidone Furaltadone Nitrofurantoin Nitrofurazone Furylfuramide Nifuroxime Nifurprazine Nifuratel) Neomycin Nalidixic acid Sulfamethoxazole Glycopeptides	Chloramphenicol Nitrofurans (Furazolidone Nitrofurantoin Nifurpirinol Nifurtoinol Nifurtoinol) BacitracinZinc Florfenicol Spiramycin Thiamphenicol Tylosin Virginiamycin	Chloramphenicol Nitrofurans (Furaltadone Furazolidone Nitrofurantoin Nitrofurazone)	Chloramphenicol Nitrofurans (Furazolidone) Fluoroquinolones (Ciprofloxacin Enrofloxacin) Glycopeptides

family of fish is recognized as the major species in the EU and any MRL for *Salmonidae* is extended to all other finfish species. The EU list of MRLs of veterinary medicinal products in food of animal origin including finfish is given in Table 4. MRLs have been fixed by European Union for amoxicillin, ampicillin, benzyl penicillin, chlortetracycline, cloxacillin, colistin, danofloxacin, dicloxacillin, difloxacin, enrofloxacin, erythromycin, florfenicol, flumequine, lincomycin, neomycin, oxacillin, oxolinic acid, oxytetracycline, paromomycin, sarafloxacin, spectinomycin, sulfonamides, tetracycline, thiamphenicol, tilmicosin, trimethoprim, and tylosin in fish and other animal foodstuffs (EU, 2010). The MRLs range between 50 ppm to 1000 ppm, depending on the antibiotic.

8 Antibiotic Use in Aquaculture in India

The role of antimicrobial usage (AMU) on the emergence of AMR in Indian aquaculture is hard to assess as there is acute paucity of precise published data on the antibiotic usage in Indian aquaculture. The data is difficult to obtain due to several reasons, such as over the counter availability of antibiotics, unregulated use of antibiotics, weak diagnostic infrastructure, and lack of structured AMU surveillance. The use of antibiotics such as oxytetracycline, althrocin, ampicillin, sparfloxacin, and enrofloxacin for the control of fish diseases or as prophylactic agents was reported (Bharathkumar and Abraham, 2011). Recently, Schar et al. (2020) estimated that in 2017, India accounted for 11.3% (~1150 tonnes) of global antimicrobial consumption in aquaculture and is projected to remain the same in 2030 (Schar et al., 2020). The routes of administration of antibiotics in fishes is mainly through medicated feed or immersion in antibiotic-laden water and rarely by injection. A survey of the feed management of 106 fish farmers of Andhra Pradesh revealed that 15% of the farmers used antibiotics for treating bacterial diseases and the typical therapeutants were oxytetracycline, enrofloxacin, sulfamethoxazole, trimethoprim, and chlortetracycline (Ramakrishna et al., 2013). India, by the Gazette Notification, has prohibited the use of antibiotics such as chloramphenicol, glycopeptides, nalidixic acid, neomycin, nitrofurans (furaltadone, furazolidone, furylfuramide, nifuratel, nifuroxime, nifurprazine, nitrofurantoin, nitrofurazone), and sulfamethoxazole in aquaculture farms, or in hatcheries, or in feed manufacturing units or in preprocessing and processing units of shrimps, prawns, or finfish (GOI, 2002; FSSAI 2011). Recently, the Indian government based on section 26A of the Drugs and Cosmetics Act, 1940, prohibited the use of colistin in aquaculture and directed the manufacturer to label the container with the words "not to be used in food-producing animals, poultry, aqua farming, and animal feed supplements." However, India does not have a list of approved antibiotics for use in aquaculture but the maximum residue level (MRL) in fish meat was notified for four antibiotics, namely, tetracycline (0.1 mg kg^{-1}), oxytetracycline (0.1 mg kg^{-1}), trimethoprim (0.05 mg kg^{-1}), and oxolinic acid (0.3 mg kg^{-1}) (EIC, 2002; FSSAI, 2011). However, guidelines regarding the use and dosage of these antibiotics for different farmed fish species (finfish, shrimp, crabs), different age groups (fingerlings,

Table 4 European Union's maximum residue limits (MRLs) of veterinary medicinal products in fish and other animal foodstuffs

Antibiotic	Marker residue	Animal species	MRL	Target tissues
Amoxicillin	Amoxicillin	Food-producing animals	50 μg kg^{-1}	Muscle
Ampicillin	Ampicillin	Food-producing animals	50 μg kg^{-1}	Muscle
Benzylpenicillin	Benzylpenicillin	Food-producing animals	50 μg kg^{-1}	Muscle
Chlortetracycline	Sum of parent drug and its 4-epimer	Food-producing animals	100 μg kg^{-1}	Muscle
Cloxacillin	Cloxacillin	Food-producing animals	300 μg kg^{-1}	Muscle
Colistin	Colistin	Food-producing animals	150 μg kg^{-1}	Muscle
Danofloxacin	Danofloxacin	All other food-producing animals[a]	100 μg kg^{-1}	Muscle
Dicloxacillin	Dicloxacillin	Food-producing animals	300 μg kg^{-1}	Muscle
Difloxacin	Difloxacin	All other food-producing animals[a]	300 μg kg^{-1}	Muscle
Enrofloxacin	Sum of enrofloxacin and ciprofloxacin	All other food-producing animals[a]	100 μg kg^{-1}	Muscle
Erythromycin	Erythromycin A	Food-producing animals	200 μg kg^{-1}	Muscle
Florfenicol	Sum of florfenicol and its metabolites measured as florfenicol amine	Finfish	1000 μg kg^{-1}	Muscle and skin in natural proportions
Flumequine	Flumequine	Finfish	600 μg kg^{-1}	Muscle and skin in natural proportions
Lincomycin	Lincomycin	Food-producing animals	100 μg kg^{-1}	Muscle
Neomycin (including framycetin)	Neomycin B	Food-producing animals	500 μg kg^{-1}	Muscle
Oxacillin	Oxacillin	Food-producing animals	300 μg kg^{-1}	Muscle
Oxolinic acid	Oxolinic acid	Food-producing animals	100 μg kg^{-1}	Muscle
Oxytetracycline	Sum of parent drug and its 4-epimer	Food-producing animals	100 μg kg^{-1}	Muscle
Paromomycin	Paromomycin	Food-producing animals	500 μg kg^{-1}	Muscle

(continued)

Table 4 (continued)

Antibiotic	Marker residue	Animal species	MRL	Target tissues
Sarafloxacin	Sarafloxacin	*Salmonidae*	30 μg kg^{-1}	Muscle and skin in natural proportions
Spectinomycin	Spectinomycin	All other food-producing species except ovine	300 μg kg^{-1}	Muscle
Sulfonamides (all substances belonging to the sulfonamide group	Parent drug	Food-producing animals	100 μg kg^{-1}	Muscle
Tetracycline	Sum of parent drug and its 4-epimer	Food-producing animals	100 μg kg^{-1}	Muscle
Thiamphenicol	Thiamphenicol	Food-producing animals	50 μg kg^{-1}	Muscle
Tilmicosin	Tilmicosin	All other food-producing species except poultry	50 μg kg^{-1}	Muscle
Trimethoprim	Trimethoprim	All other food-producing species except *Equidae*	50 μg kg^{-1}	Muscle
Tylosin	Tylosin A	Food-producing animals	100 μg kg^{-1}	Muscle

[a]All animals except bovine, ovine, caprine, and poultry
[b]For fin fish the "muscle" relates to "muscle and skin in natural proportions." MRLs for fat, liver, and kidney do not apply to fin fish

postlarvae, juveniles, adults), different water conditions (freshwater, brackish water, marine water) and withdrawal periods were not specified.

9 Indirect Indicators of Antibiotic Use in Aquaculture in India

Although there is paucity of data on antibiotic consumption in aquaculture in India, there are few indirect means of assessing the use of antibiotics in aquaculture system.

9.1 Antibiotic Use as Indicated by Export Rejections of Fish/Shrimp

The analysis of rejections due to the presence of antibiotics/veterinary medicinal products in consignments of fish and fishery products exported from a particular

country provides insights into the type of antibiotics being used in aquaculture. Presence of antibiotics in farmed shrimp exported from India was reported by the Rapid Alert System for Food and Feed of the European Union, the Food and Drug Administration of the United States, and Ministry of Health, Labour and Welfare of Japan. The Rapid Alert System for Food and Feed (RASFF)of the European Union (EU) is a key tool that ensures the flow of information regarding public health risks detected in the food chain (https://ec.europa.eu/food/safety/rasff_en). We have analyzed the RASFF alerts pertaining to the residues of veterinary medicinal products in finfish and crustaceans exported to the European Union during the last ten years (01/01/2010 to 31/12/2019).

9.1.1 RASFF Alerts Due to Presence of Veterinary Medicinal Products in Finfish

A total of 116 consignments of fish and fishery products and 170 consignments of crustaceans were found to be nonconforming to the food safety requirements of the EU vis-à-vis veterinary medicinal products. Residues of veterinary medicinal products were reported in consignments of finfish exported from 19 countries, namely, Vietnam, China, Chile, Germany, Denmark, France, Czech, Greece, Thailand, Poland, Austria, Belgium, Belarus, Indonesia, Italy, Japan, Lithuania, Slovakia, and Turkey of which maximum rejected consignments were from Vietnam (62%) followed by China (9.5%) and Chile (5.2%). RASFF alerts due to veterinary medicinal products were reported every year, but, maximum alerts were reported in 2014.

Antibiotics reported in finfish exported to EU were amoxicillin, azithromycin, chloramphenicol, doxycycline, oxytetracycline, ofloxacin, norfloxacin, furazolidone (AOZ), furaltadone (AMOZ), nitrofurazone (SEM), trimethoprim, sulfonamide, sulfadiazine, neomycin, and ivermectin, and the dyes reported were leucomalachite green, malachite green, Victoria pure blue, crystal violet, and leucocrystal violet (Fig. 1).

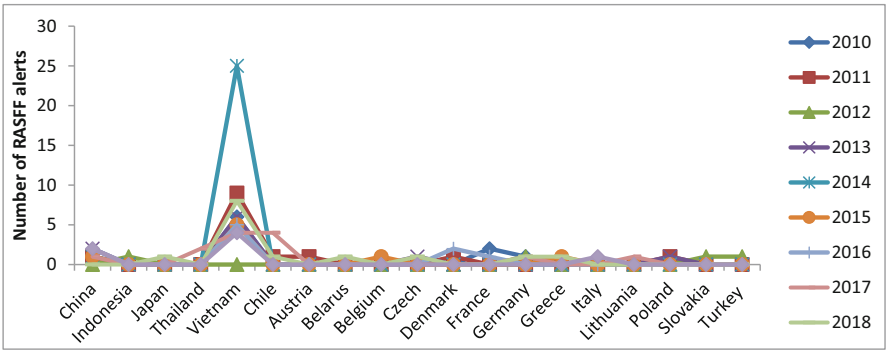


Fig. 1 RASFF alerts due to presence of veterinary medicinal products in finfish exported to EU during 2010–2019

The antibiotic responsible for maximum rejections was nitrofurazone-SEM (30%) followed by oxytetracycline (7%), sulfadiazine (5%), trimethoprim (4%), chloramphenicol (3.5%), and sulfonamide (3%). The other antibiotics reported were ivermectin, neomycin, ofloxacin, norfloxacin, azithromycin, furaltadone (AMOZ), furazolidone (AOZ), doxycycline, and amoxicillin (Fig. 2).

The finfish species in which antibiotic residues reported were pangasius (*Pangasianodon hypophthalmus*), tilapia (*Oreochromis niloticus*, *O. placidus*), rainbow trout (*Oncorhynchus mykiss*), catfish (*Clarias spp., Clarias macrocephalus*), sea bass / barramundi (*Lates spp*.), Atlantic salmon (*Salmo salar*), Atlantic wolffish

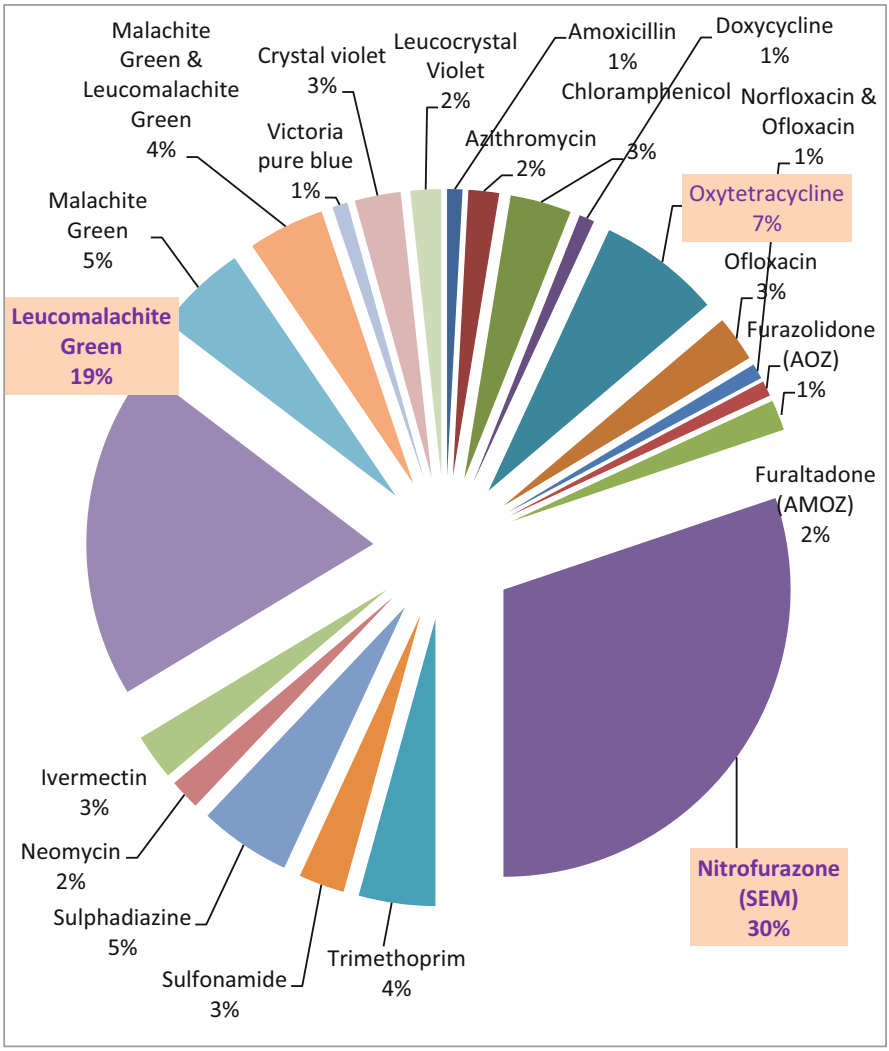


Fig. 2 Antibiotics and dyes reported in finfish and fishery products

(*Anarhichas lupus*), Asian swamp eel (*Monopterus albus*), red tail tin foil barb (*Puntius spp*.), mud goby (*Pseudapocryptes lanceolatus*), red cheek barb (*Puntius orphoides*), climbing perch (*Anabas testudineus*), walking catfish (*Clarias batrachus*), amberjack, red mullet, and carp (Table 5). Antibiotic residues were reported in fishery products such as fish paste, caviar and smoked catfish. The data indirectly suggests the use of different type of antibiotics in the farming of finfish. The use of nitrofurans is indirectly indicated in pangasius farming in Vietnam, oxytetracycline in Atlantic salmon rearing in Chile, and sulfonamide/trimethoprim in tilapia farming in Vietnam and China. However, the use of dyes was more in rainbow trout farming in European countries.

9.1.2 RASFF Notifications due Veterinary Medicinal Products in Crustaceans

During the period of 2010–2019, a total of 172 notifications were reported due to the presence of veterinary medicinal products in crustaceans (mainly shrimp) exported to EU and maximum consignments originated from shrimp farming countries from Asia (Fig. 3) and the major exporting countries were India (50%) and Vietnam (31%) followed by China (8%) and Bangladesh (7.6%). The trend of the RASFF notifications due to the presence of veterinary medicinal products in shrimp consignments showed a gradual decrease during the last few years (2016–2019). The decline in RASFF notifications may be majorly attributed to implementation of Hazard Analysis Critical Control Point (HACCP)-based Food Safety Management Systems (FSMS) during preharvest and postharvest production in the shrimp-farming nations and to lesser extent due to the decrease in the quantities of shrimp exported to the EU compared to previous years from countries such as India.

Furazolidone-AOZ (40%), oxytetracycline (18%), chloramphenicol (14%), and nitrofurazone-SEM (10%) were the most commonly reported antibiotics in farmed shrimp exported to the EU (Fig. 4). Other antibiotics reported were doxycycline, tetracycline, chlortetracycline, ciprofloxacin, cephalexin, sulfadiazine, and sulfamethoxazole.

Furazolidone, nitrofurazone, furaltadone, oxytetracycline, doxycycline, and chloramphenicol were detected in both shrimps and finfish indirectly indicating the use of these antibiotics in both finfish and shrimp aquaculture (Table 6). Sulfa drugs, trimethoprim, ofloxacin, azithromycin, and neomycin were relatively more commonly detected in finfish indicating probable use of these antibiotics in fish farming.

9.1.3 RASFF Notifications Pertaining to Shrimp Exported from India

The antibiotic residues/metabolites were reported in 85 consignments of farmed shrimp exported from India in all the reporting years from 2009 to 2019. Furazolidone-AOZ (72%) was the most commonly reported antibiotic followed distantly by Chloramphenicol (14%), Nitrofurazone-SEM (5%), and oxytetracycline (5%). The annual trend in the total notifications due to antibiotic residues in farmed shrimp was mainly influenced by furazolidone, AOZ (Fig. 5). Similar trend was reported by Rao and Prasad (2015) as they observed that nitrofurans were the reason for RASFF notifications in 100%, 97%, and 90% of *Macrobrachium rosenbergii*

Table 5 Categorization of RASFF alerts in finfish imported by EU (2010–2019)

Fish species/fish product	Antibiotics/dyes	Exporting country	Total RASFF alerts
Pangasius (*Pangasianodon hypophthalmus*)	Nitrofurazone-SEM (32)[a] Ivermectin (2) Neomycin (2) Chloramphenicol (1) Oflaxacin (1) Crystal Violet (2) Malachite green (1)	Vietnam	41
Tilapia (*Oreochromis niloticus*, *O. placidus*)	Sulfadiazine (6) Sulfonamide (3) Trimethoprim (3) Azithromycin (2) Ofloxacin (1) Furazolidone-AOZ (1) Leucomalachite green (1)	Vietnam, China	17
Rainbow trout (*Oncorhynchus mykiss*)	Furaltadone-AMOZ (1) Amoxicillin (1) Malachite green (1), Crystal violet (1) Leucomalachite green(11) Leucocrystal violet (1)	Italy Germany Czech Denmark Greece Poland Turkey Slovakia	16
Catfish (*Clarias* species *Clarias macrocephalus*)	Ofloxacin (2), Leucomalachite green (5), Leucocrystal violet (1) Malachite green + Leuco MG (2)	Vietnam Indonesia	10
Atlantic Salmon (*Salmo salar*)	Oxytetracycline (5) Chloramphenicol (1)	Chile	6
Amberjack	Oxytetracycline (1)	Japan	1
Sea bass/ Barramundi (*Lates* spp.)	Furaltadone-AMOZ (1) Ivermectin (1) Leucomalachite green + malachite green (1)	Vietnam Greece	3
Red Mullet	Chloramphenicol (2)	Vietnam	2
Carp	Leucomalachite green (2) Leucomalachite green + malachite green (1)	Belarus Lithuania Czech	3
Atlantic wolffish (*Anarhichas lupus*)	Oxytetracycline (1)	France	1
Asian swamp eel (*Monopterus albus*)	Nitrofurazone-SEM (1)	Vietnam	1
Red tail tinfoil barb (*Puntius* spp.)	Leucomalachite green + malachite green (1)	Vietnam	1
Mud goby (*Pseudapocryptes lanceolatus*)	Malachite green (1)	Vietnam	1

(continued)

Table 5 (continued)

Fish species/fish product	Antibiotics/dyes	Exporting country	Total RASFF alerts
Red cheek barb (*Puntius orphoides*)	Malachite green (1)	Vietnam	1
Climbing perch (*Anabas testudineus*)	Doxycycline (1) Oxytetracycline (1) Leucomalachite green (1)	Vietnam	3
Walking catfish (*Clarias batrachus*)	Malachite green (1)	Vietnam	1
Fish paste	Norfloxacin + Ofloxacin (1)	Vietnam	1
Caviar	Trimethoprim (2) Leucomalachite green (1)	China France	3
Smoked catfish	Nitrofurazone-SEM (2)	Thailand	2

[a]Value in parenthesis indicates the number of RASFF alerts

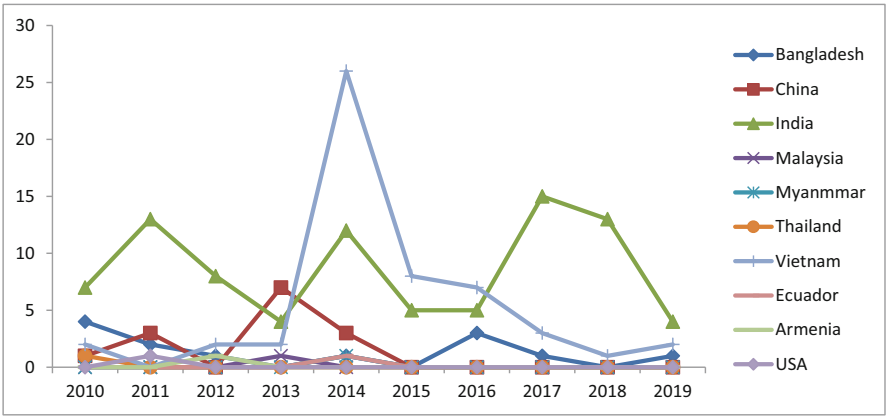


Fig. 3 Trends in the country-wise RASFF notifications of veterinary medicinal products in crustaceans (mainly shrimp) exported to EU

(giant freshwater prawn), *Penaeus monodon* (black tiger shrimp), and *Litopenaeus vannamei* (pacific white shrimp) exported from India to the European Union during 2003–2014.

The RASFF notifications during the 10-year period, 2010–2019, indirectly indicate the use of the furazolidone in the shrimp culture in India. Furazolidone is a nitrofuran with broad antibacterial activity against both Gram-positive and -negative bacteria and was previously used to treat intestinal infections in humans and animals. Nitrofurans are synthetic antibiotics that have a characteristic 5-nitrofuran ring. Nitrofuran "parent" compounds, that is, furazolidone, furaltadone, nitrofurazone, and nitrofurantoin metabolize rapidly after ingestion by the live shrimp. The nitrofuran parent compounds have a short *in vivo* half-life of 7–63 min and hence detecting their presence in harvested shrimp for assessing food safety would be futile. However, the nitrofuran parent compounds metabolize to tissue-bound

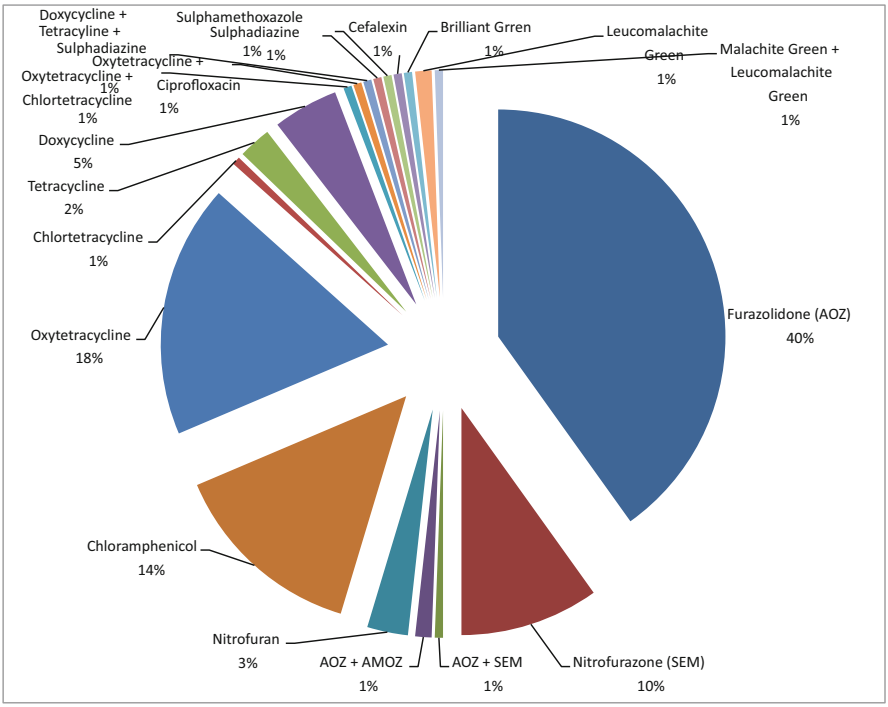


Fig. 4 Antibiotics and dyes reported in crustaceans exported to the EU during 2010–2019

"metabolites" in live shrimp, namely, furazolidone metabolizes to 3-amino-2-oxazolidinone, AOZ; furaltadone metabolizes to 3-amino-5-morpholinomethyl-1,3-oxazolidinone, AMOZ; nitrofurazone metabolizes to semicarbazide (SEM); and nitrofurantoin metabolizes to 1-aminohydantoin, AHD. The nitrofuran metabolites (AOZ, AMOZ, SEM, and AHD) bind tightly to shrimp tissue and remain in the body for many weeks posttreatment. The nitrofuran metabolites are stable even during the postharvest phase, not destroyed by cooking, frying, grilling, and microwaving of meat and hence serve as good markers for assessing food safety.

9.2 Antibiotic Use as Indicated by National Surveillance Program on Antibiotic Residues

The AMU surveillance programs provide crucial information on the use of antibiotics in aquaculture. The national-level antibiotic residue monitoring programs provide valuable clues regarding the use of antibiotics in the aquafarming system. National Residue Control Plan (NRCP) of India is a system for monitoring residues of aquaculture drugs/veterinary medicinal products in farmed shrimp, shrimp seed, and shrimp feed used in Indian aquaculture. NRCP results for 2018 indicates that 0.9% of the farmed shrimp (*Penaeus vannamei*) samples (Table 7) and 32% of

Table 6 Comparison of antibiotic residues in RASFF alerts of finfish and shrimp exported to the EU

Antibiotic	Crustaceans	Finfish
Furazolidone-AOZ	1.1 to 290* (28 ± 61)**	5.5
Nitrofurazone-SEM	1.3 to 170 (19 ± 50)	1.1 to 13.5 (3.5 ± 2.9)
Furaltadone-AMOZ	2.1	1.1 to 2 (1.5 ± 0.4)
Nitrofuran	1.6 to 55 (15.5 ± 26)	–
Oxytetracycline	98 to 2065 (243 ± 311)	113 to 365 (256 ± 109)
Doxycycline	140 to 800 (295 ± 227)	266
Tetracycline	68 to 293 (206 ± 84)	–
Chlortetracycline	67 to 560 (305 ± 247)	–
Sulfadiazine	–	158 to 576 (326 ± 207)
Sulfonamide	–	131 to 500 (273 ± 198)
Sulfamethoxazole	146	–
Trimethoprim	–	76 to 880 (429 ± 294)
Chloramphenicol	0.11 to 125 (5 ± 24)	0.6 to 70 (18 ± 35)
Ivermectin	111 to 300 (178 ± 106)	4 to 98.5 (36.8 ± 54)
Amoxicillin	–	394
Cefalexin	101	–
Ciprofloxacin	114	–
Ofloxacin	–	1.2 to 183 (38 ± 81)
Norfloxacin	–	17
Azithromycin	–	7 to 21 (14 ± 10)
Neomycin	–	656 to 1385 (1062 ± 371)

*Mean ± SD; **Range

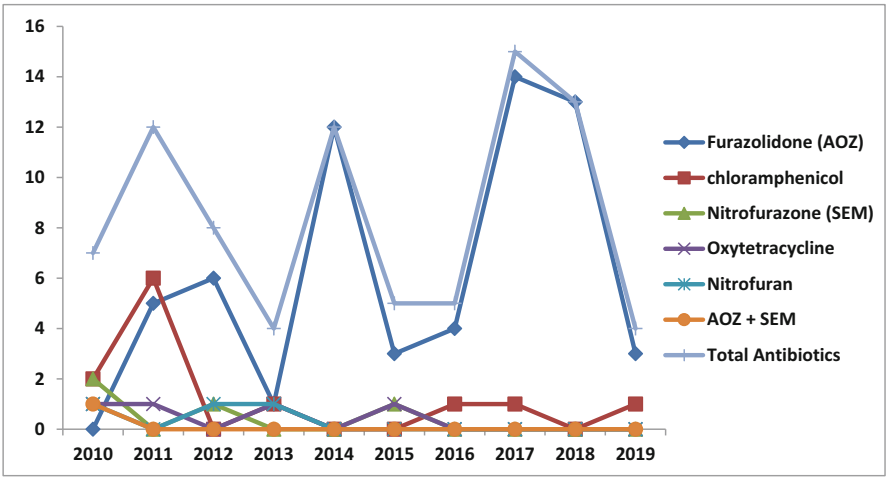


Fig. 5 RASFF notifications pertaining to shrimp exported from India to the EU

Table 7 Results of National Residue Control Plan for antibiotic residues in farmed shrimp in India

Antibiotic (metabolite)	2015 (n = 1109)[a]	2016 (n = 1355)	2017 (n = 1390)	2018 (n = 1720)
Chloramphenicol	169[b]	2	12	8
Furazolidone (AOZ)	6	3	9	5
Nitrofurantoin (AHD)	0	0	0	1
Nitrofurazone (SEM)	0	0	0	2
Antibiotic Positive	16%	0.4%	1.5%	0.9%

[a]Number of farmed shrimp samples tested; [b]Number of samples positive

Table 8 Results of National Residue Control Plan for antibiotic residues in shrimp seed and feed in India

	Shrimp seed (postlarvae)		Shrimp feed	
Year	Number of samples tested	Positive samples	Number of samples tested	Positive samples
2015	134	44%	23	0
2016	129	39%	45	0
2017	209	38%	64	0
2018	133	32%	21	0

shrimp hatchery seed samples (Table 8) were positive for the presence of antibiotic residues. These results indicate usage of antibiotics at the preharvest stages. The usage may be purposefully for disease treatment or unknowingly through feeding inputs leading to residues in farmed shrimp and export rejections.

10 Farming of Aquatic Animals Without Antibiotics

Antibiotic-free aquaculture is possible if aquaculture farmers and hatchery operators adopt scientific practices with stringent biosecurity measures and good aquaculture practices so as to prevent infection in the aquatic animals, thereby preventing the necessity for antibiotics usage. In the fisheries sector, the best lesson can be learnt from the experience of Norway. Norwegian aquaculture used high quantities of antimicrobial drugs to control bacterial diseases affecting farmed Atlantic salmon and rainbow trout during the 1980s and the 1990s. In 1987, Norway used 887 mg antibacterial agents per kg salmon fish produced. Since then, even though there was huge increase in the biomass of fish produced, Norway substantially reduced the use of antimicrobial agents. The consumption reduced to around 1 mg kg^{-1} produced fish, with 0.36 mg kg^{-1} in 2014. Norway could achieve this through the introduction of efficient vaccines, selection of fish-farm locations with good water exchange rates, general improvement of the hygiene, strict biosecurity and regulatory requirements. Moreover, the use of antibiotics in food production in Norway was strictly regulated through

pharmacy sales and prescriptions only by veterinarians and aqua medicine biologists under government monitoring (Norwegian Veterinary Institute, 2016).

11 Antimicrobial Resistance (AMR) in Aquaculture

Waksman (1947) coined the term "antibiotic" and described it as a substance produced by a microorganism that was antagonistic to the growth of other microorganisms. However, now the term antibiotic is more generalized and includes natural, synthetic, and semisynthetic compounds that destroy microorganism by a specific mode of action. Antibiotics cure diseases in terrestrial and aquatic animals but their curative powers are lost due to overuse, misuse, or abuse in human health and animal agriculture. Penicillin and sulfonamide were the first antibiotics discovered, and there was no new class of antibiotics discovered since daptomycin in 1987. The large void in the discovery of antibiotics can be squarely attributed to the lower return of investment for commercial pharmaceutical companies on new antibiotics compared to drugs for lifestyle diseases such as diabetes and hypertension.

AMR organisms include bacteria, viruses, fungi, and parasites that resist killing by antibacterial, antiviral, antifungal, and antiparasitic drugs and chemicals. Though antivirals, antifungals, antiprotozoal along with antibiotics are all considered as antimicrobials but the focus on AMR is largely concentrated on the antibiotics due to their sheer magnitude of use both in human health care and animal agriculture. The consequence of AMR is the inability to treat infections that were previously treatable. The transfer of AMR between closely related and diverse bacterial species is mediated through horizontal gene transfer (HGT) and occurs externally in the environment and internally within the gastrointestinal tracts of humans and animals. Albeit AMR is a natural process, it is the inappropriate use of antimicrobials that accelerates the process of AMR. AMR in human pathogens results in potent disease manifestation, higher morbidities, higher mortalities, treatment failures, poor prognosis, and higher health care costs. The discoverer of antibiotics, Alexander Fleming, has warned of antimicrobial resistance (AMR) as early as in 1945, and of late it is predicted that ten million lives per year would succumb to AMR by 2050 (O'Neill, 2016). All AMR bacteria are either directly or indirectly harmful for human and animal health, and among them those resistant to colistin and carbapenems are the most harmful as they are considered as drugs of the last resort. Colistin is the choice of drug for treating infections caused by drug-resistant strains of *Acinetobacter baumannii*, *Pseudomonas aeruginosa,* and *Enterobacteriaceae,* and carbapenems were the preferred drugs for treating systemic infections caused by multidrug-resistant *Enterobacteriaceae*.

Aquaculture environment is perceived as hotspot for AMR where genetic exchange and recombination leads to emergence, persistence, and transmission of resistance at a relatively higher frequency. Inappropriate use of antibiotics leads to development of resistance and dissemination of resistance in bacteria. The aquatic environments also act as reservoirs of AMR genes. The aquatic environment of the aquaculture farms has high bacterial diversity, and depending upon the culture

practices, the water may contain fish pathogens and human pathogens. Bacteria gain entry into the aquaculture pond during the culture phase through several sources (Fig. 6). Probiotics are defined as live microorganisms which when administered in adequate amounts confer a health benefit on the host (FAO/WHO, 2001). Beneficial bacteria in the form of probiotics are purposefully added to the aquaculture ponds for improving the health status of the aquatic animal (gut probiotics) or improving and making the water (water remediators) and soil conducive (soil remediators) for the growth of the farmed aquatic animals. Bacteria such as *Lactobacillus*, *Bacillus*, *Nitrosomonas*, *Nitrobacter*, *Rhodococcus* were employed in aquaculture farms as probiotics and/or bioremediators. Commercially available probiotics (gut probiotics / water and soil bioremediators) contain more than billion bacteria per gram, and addition of probiotics at weekly intervals adds large populations of bacteria. It was reported that some of the probiotic carry AMR genes and transfer them to other bacteria in the gut or aquatic environment (Verraes et al., 2013).

Resistance to more than four antibiotics was detected in 10 percent of the bacteria isolated from probiotic products used in shrimp aquaculture, and their genomes contained *erm*D (macrolide), *tetL* (tetracycline), *fexA* (phenicol), and *dfrD*, *dfrG,* and *dfrK* (trimethoprim) resistance genes (Noor Uddin et al., 2015). Fish seed (fry/postlarvae) carry bacteria from the hatcheries. The pond soil contains ten times higher bacterial loads compared to the water column. The soil contains bacteria from the previous crop. Moreover, soil particles act as interface for microbial community interactions. Water exchange is common in aquaculture practice. Water meshes used for screening water before pumping into the pond do not generally prevent the entry of bacteria. Manure from poultry and livestock is used in fish farms to promote phytoplankton growth but manure adds huge number of bacteria (Wohlfarth and Schroeder, 1979). Manure contains both AMR bacteria and antibiotic residues from poultry rearing and livestock treatment, domestic farm, and poultry waste along with antibiotic residues from animal husbandry (Checcucci et al., 2020). Integrated fish

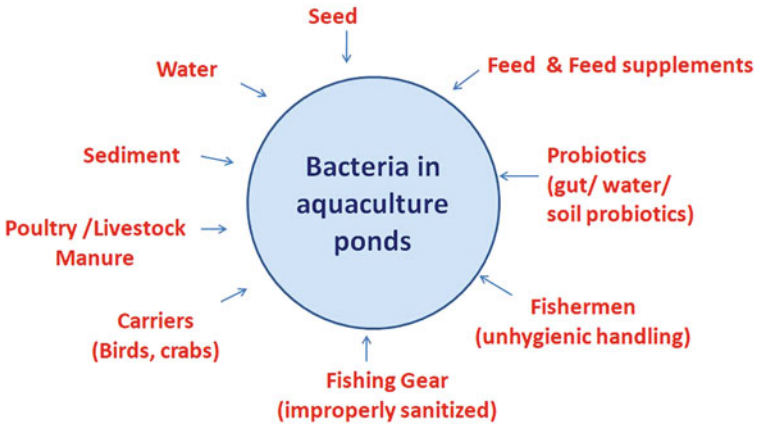


Fig. 6 Sources of entry of bacteria (probably including AMR strains) into aquaculture farms

farming systems continuously add bacteria to the pond water. Feed and feed supplements used regularly during the growing stage of the fish contain relatively low number of bacteria but nevertheless add microorganism into the system. Poor biosecurity measures allow the entry of birds and crabs into the ponds, and these may introduce bacteria into the pond water. Farm personnel/ fishermen enter the pond regularly for feeding and the time of fish harvest. Unhygienic personnel and improperly sanitized fishing gear introduce bacteria into the pond water. Some of the bacteria introduced through these channels may already be resistant to antibiotics (AMR strains) or may develop resistance in the pond environment. Diseased aquatic animal generally shows inappetence leading to diminished intake of feed. Antibiotics and/or metabolites of antibiotics originating from feed left over and fish feces accumulate in aquaculture pond water and sediment wherein they retain their antimicrobial activity and continue to select AMR bacteria and influence microbial diversity. The discharge of untreated water and disposal of pond sediment poses AMR threat to adjoining environment. The absence of AMU but emergence of AMR in aquaculture farms is possible through co-selection. The presence of heavy metals in the aquatic environment co-select AMR bacteria (Wales and Davies, 2015). The introduction of heavy metals such as copper from anti-fouling agents and cadmium from pesticides and fertilizers increase the ability of bacteria to resist antibiotics through co-resistance (Schlenk et al., 1998; Bruins et al., 2000). A large proportion of the antibiotics used in animal agriculture and aquaculture get excreted into the surrounding environment in unmetabolized form by animals. Chlortetracycline and enrofloxacin were detected in the feces of cows and chicken, respectively (Zhao et al., 2010), and use of antibiotic-laden manure as fertilizer inadvertently introduces antibiotics into the new environment.

AMR was reported in Aeromonas, Pseudomonas, Salmonella, and Vibrio from fish farms, shrimp farms, and the aquatic environment of India. Resistance was observed towards ampicillin, amoxicillin, carbenicillin, cephalexin, cephalothin, colistin, erythromycin, gentamicin, kanamycin, novobiocin, penicillin-G, rifampin, sulfadiazine, tetracycline, and vancomycin. However, majority of the AMR strains showed susceptibility towards chloramphenicol and ciprofloxacin.

12 Transmission of AMR Bacteria Through Global Trade

Global food trade is likely to play a role in spreading AMR bacteria between trading countries and probably increase the national burden of AMR via imported foods. AMR bacteria resistant to cephalosporins, colistin, fluroquinolones, and macrolides were detected in 20% of the pangasius fillets and prawn imported by Denmark from Asia (Ellis-Iversen et al., 2019). Similarly, 17% of the imported shrimp harbored bacteria that showed resistance to at least one antibiotic (CBC Marketplace, 2019). It was reported that ornamental fish and their carriage water act as reservoirs of AMR bacteria (Verner-Jeffreys et al., 2009). The probability of emergence of AMR in the aquaculture system is higher given the fact that it is difficult to administer antibiotics to individual fish and the general treatment practice is the metaphylactic approach,

that is, administering the drug to the entire population (including healthy animals) in the aquaculture pond/cage. The occurrence and spread of antimicrobial resistance in bacteria associated with marine and freshwater fish farms has been reported (Alderman and Hastings, 1998). Use of antibiotics in aquaculture may potentially act as reservoir of antimicrobial resistance genes (ARGs) that may eventually be transferred to clinically relevant bacteria and have been designated as genetic hotspots for gene transfer. Bacteria resistant to ampicillin, kanamycin, tetracycline, and oxytetracycline were reported to be higher in ponds with a history of antibiotic usage (McPhearson et al., 1991). Dispersion of large quantities of antibiotics in Salmon aquaculture resulted in significant increase in antimicrobial-resistant population in pond sediments (Buschmann et al., 2012). AMR genes were more frequently found in *E. coli* isolates from Chileans living in salmon aquaculture regions (Tomova et al., 2015). The presence of AMR in the animal food chain is attributed to contamination of the food during production at the farm level or due to cross-contamination during preprocessing, processing, and transport (Verraes et al., 2013).

13 AMR in Postharvest Fisheries in India

The AMR bacteria in food produced from farm animals or aquatic animals is transmitted to humans through the consumption of contaminated food, contact with the animals, and /or environmental contamination. The resistance to antibiotics can be transmitted not only through pathogenic bacteria but also through commensal bacteria. The entry of antibiotics onto the fish after they are harvested from natural waters or aquaculture ponds is generally not seen. However, bacteria including AMR resistance strains gain entry on to the fish through contaminated water, contaminated ice, unhygienic food contact surfaces, utensils, and equipment of processing (Fig. 7). The water used for fish

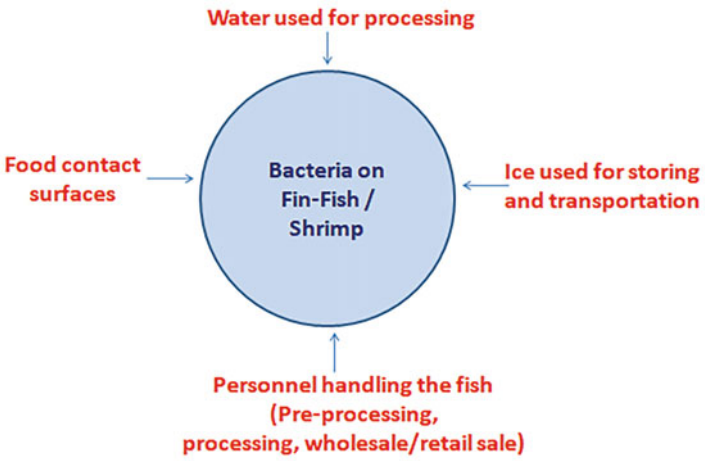


Fig. 7 Sources of entry of bacteria onto finfish during postharvest phase

processing should be potable and meet the quality requirements such as 98/83/EC. Use of contaminated water for cleaning the fish adds more bacteria including pathogens and AMR strains. Ice is extensively used for preserving quality of harvested fish. The ice used for chilling the fish should be prepared from potable water. In tropical conditions, the requirement of ice to bring down the core temperature of fish to less than 4 °C is 1:1, that is, 1 kg ice for every kg of fish. Block ice is crushed in stainless steel ice crushers. Improper cleaning of ice crushers or use of contaminated water for ice making increases bacterial loads in ice. Harvested fish comes in contact with different food contact surfaces such as crates, weighing scales, utensils, preprocessing and processing tables, freezing pans, conveyor belts, etc. Improperly washed food contact surfaces carry high counts of bacteria. The unhygienic practices of food handlers during raw material handling, preprocessing, processing, transport and sale of fish are possible source of entry of AMR bacteria (Verraes et al., 2013).

Antimicrobial resistance was reported in India in *Aeromonas hydrophila*, *Aeromonas* spp., *E. coli*, *Salmonella*, *Staphylococcus aureus*, *Vibrio cholerae*, *Vibrio parahaemolyticus* in postharvest fish. Aeromonas isolated from retail fish were resistant to ampicillin, bacitracin, colistin, rifampicin, and novobiocin. Salmonella isolates were resistant to carbenicillin, sulfamethoxazole, and oxytetracycline. *E. coli* isolated from retail fish were found to be resistant towards ampicillin, amoxicillin, aztreonam, bacitracin, cephalothin, cefotaxime, ceftazidime, cefpodoxime, ciprofloxacin, ertapenem, erythromycin, gentamicin, meropenem, nalidixic acid, nitrofurantoin, norfloxacin, penicillin-G, tetracycline, and vancomycin. *Staphylococcus aureus* isolated from harvested fish were found to be resistant to ampicillin, amoxicillin/clavulanic acid, clarithromycin, erythromycin, linezolid, of loxacin. Methicillin-resistant *Staphylococcus aureus* isolated from retail fish were resistant to ampicillin, amoxicillin/clavulanic acid, azithromycin, clarithromycin, erythromycin, penicillin, and methicillin. *Vibrio cholerae* isolated from postharvest fish were resistant to augmentin, cefpodoxime, colistin, and ticarcillin. *Vibrio parahaemolyticus* isolated from postharvest fish were resistant to ampicillin, amoxicillin, carbenicillin, cefpodoxime, cephalexin, cephalothin, colistin, gentamicin, kanamycin, and streptomycin.

The reported results of AMR in preharvest and postharvest fishes indicate relatively higher incidence of resistance to antibiotics of human health significance in bacteria isolated from postharvest fish. Although AMR exists in the fishery environment, several factors involved in postharvest operations such as water, ice, food contact surfaces and food handlers influence the AMR pattern of bacteria on fish. The preharvest and postharvest phases need separate measures to control the incidence of AMR but considering that fish is a healthy food both areas of preharvest and postharvest fisheries need to be given equal attention.

14 Antibiotic Resistance Genes (ARGs)

Bacteria develop resistance to antibiotics by adopting different strategies such as modifying the antimicrobial molecule, preventing the antibiotic from reaching the target site, changing the antibiotic target sites, and bypassing the antibiotic target

Table 9 Antibiotic resistance genes (ARGs) detected in fish pathogens

Antibiotic	ARGs	Fish pathogens
Quinolones	*gyrA*, *gyrB*, *parC*, *parE, qnrB, qnrS*	*Aeromonas hydrophila, Aeromonas salmonicida,* *Edwardsiella tarda*, *Escherichia coli, Flavobacterium psychrophilum*, *Photobacterium damselae, Vibrionaceae*, *Vibrio anguillarum*, *V. parahaemolyticus, Yersinia ruckeri*
Tetracycline	*tetA, tetB, tetD, tetE, tetG, tetH, tetL, tetM, tetO, tetQ, tetS, tetW. tet34,* and *tet*35	*Edwardsiella tarda, Aeromonas spp., Edwardsiella ictalurid, Escherichia coli, Vibrio spp.*
Florfenicol	*floR*	*Edwardsiella ictaluri*
Sulfonamide and Streptomycin	*sul2*, *strA, strB*	*Aeromonas bestiarum*

sites. The enzymes/proteins involved in the antibiotic resistance are encoded by several antibiotic resistance genes (ARGs). The bacteria in the aquaculture environment receive the ARGs, mainly through horizontal gene transfer from other bacteria that enter into the aquatic environment. Transfer of ARGs is restricted neither by phylogentic differences among bacteria nor by sectoral boundaries such as human sector and terrestrial animals. The ARGs reported in bacteria associated with aquaculture include *gyrA, gyrB, parC, pare, qnrB, qnrS* genes responsible for quinolone resistance; *tetA, tetB, tetD, tetE, tetG, tetH, tetL, tetM, tetO, tetQ, tetS, tetW, tet34*, and *tet35* associated with tetracycline resistance; *sul2* associated with sulfonamide resistance; *strA–strB* associated with streptomycin resistance; and *floR* responsible for florfenicol resistance (Table 9). Data on the prevalence of ARGs in the aquaculture environment is pertinent for quantitative risk assessments and control of fish diseases. Research thrust is needed to decipher the resistome (collection of all ARGs in a bacterial community) of different aquaculture habitats, namely, freshwater aquaculture, brackish water aquaculture to understand the emergence and spread of AMR in the specific habitats.

15 Transfer of Antibiotic Resistance from Bacteria Associated with Aquaculture

The dreaded consequence of AMR in aquatic bacteria is that ultimately they transfer the resistance to human pathogenic bacteria. The transfer of tetracycline resistance and phenicol resistance in the aquatic environment from bacteria associated with aquaculture to other bacteria has been reported (Table 10). Tetracycline resistance was transferred from *Aeromonas* to *E. coli* (Agerso et al., 2007) and *A. salmonicida* to other Aeromonads (Adams et al., 1998) and from *Edwardsiella* to *E. coli* (Dung et al., 2009). Transfer of phenicol resistance from *Aeromonas salmonicida* to *Edwardsiella tarda* and *Aeromonas hydrophila* was reported (McIntosh et al., 2008). Clonal link

Table 10 Transfer of AMR from bacteria from the aquatic environment

Antibiotic resistance transferring bacteria	Antibiotic resistance receiving bacteria	Antibiotic	Mechanism of transfer	Reference
Aeromonas strains	*Escherichia coli*	Tetracycline resistance	Horizontal transfer of large plasmids	Agerso et al. (2007)
Aeromonas salmonicida	Environmental and clinical isolates of *Aeromonas* spp.	OTC-resistant isolates	Horizontal transfer *of tet*A carrying R-plasmids	Adams et al. (1998)
Edwardsiella ictalurid	*Escherichia coli* recipients	Tetracycline resistance	Horizontal transfer of *tet*A carrying plasmids	Dung et al. (2009)
Aeromonas salmonicida	*Aeromonas hydrophila* and *Edwardsiella tarda*	Phenicol resistance	Conjugative transfer of IncA/C plasmid harboring *floR*, *sul2*, and *tetA* genes	McIntosh et al. (2008)

based on virulence gene profiling and pulsed field gel electrophoresis was reported between the animal and human isolates of colistin-resistant *E. coli* (Liu et al., 2016). These findings indicate the potential exchange of antibiotic resistances in the aquatic environment.

16 AMU and AMR Relationship

The linkage between AMU and AMR seems intriguing as some researchers reported significantly positive relationship whereas others did not observe any relationship. Feedlot cattle fed with diets containing chlortetracycline resulted in tetracycline-resistant *C. jejuni* (Inglis et al., 2005) and those fed with tylosin resulted in erythromycin-resistant *Enterococci* (Beukers et al., 2015). Non-usage of antibiotics resulted in lower resistances. *E. coli* isolates from pigs and broilers reared on organic farms showed lower resistance than those reared on conventional farms (Hoogenboom et al., 2008). On the contrary, though the use of carbapenem is not permitted in pig rearing in Germany, Salmonella resistant to carbapenem were isolated from pig farms (Fischer et al., 2013). On similar lines, the use of enrofloxacin was prohibited in the United States (FDA, 2005) but ciprofloxacin-resistant *Campylobacter* were detected in chicken (Nannapaneni et al., 2009). The antibiotic growth promoter, Avoparcin was banned by Denmark in 1995 that led to marked decrease in vancomycin-resistant *Enterococci* in broilers from 72.7% in 1995 to <3% in 2005 (Aarestrup et al., 2001; Hammerum et al., 2007; DANMAP, 2015). Netherlands was one of the highest consumers of antibiotics for animal agriculture in Europe but the consumption of antibiotics decreased from 565 tonnes per year in 2007 to 217 tonnes per year in 2013. The reduction in AMU by 67% was

largely driven by government policies which had also resulted in lowering the levels of AMR. Statistically significant association between the use of fluoroquinolone and tetracycline classes of antibiotics in the production of cattle, pigs, cattle, broilers, turkeys, and the consequent development of resistance in *E. coli, Salmonella, Campylobacter* towards fluoroquinolone, tetracycline in these animals was reported (ECDC/EFSA/EMA, 2015, 2017). Similarly, positive correlation between AMU and resistance in *E. coli* to the corresponding antibiotic was observed in cattle, pigs, and poultry (Chantziaras et al., 2013).

17 AMR and Food Security

AMR has two main direct and contrasting links to food security. On the one hand antibiotics are essential for infection control and treatment in terrestrial and aquatic animals and thereby aids in farm productivity that is vital for providing food security to the people (Wu, 2017). The reduction in use of antibiotics due to the policies governing rational use of antibiotic to mitigate AMR might have some effect on animal productivity where antibiotics are being used as growth promoters (AGP). AGP is defined as the administration of antimicrobial, usually as feed additive, over a period of time to growing animals that results in improving physiological performance (i.e., weight gain, feed conversion). The total AGP use in 2012 was about 10,000 tonnes (Laxminarayan et al., 2015). The antimicrobial use increased from 10–20 g/ton in the early 1950s to 30–110 g/ton in 1970 and part of this increase was attributed to AMR (Teillant and Laxminarayan, 2015). The subtherapeutic use of human medically important antibiotics as growth promoters has been first banned in Sweden in 1986, followed by Denmark in 1992 and Europe Union in 2006 (EC, 2011) and in the United States from 2017 (FDA, 2016).

On the other hand, antibiotic-resistant bacteria make it difficult to control diseases thereby increasing the cost for animal production that adversely impacts food security. AMR results in increased morbidity, fatality rates and decreases productivity in terrestrial and aquatic animals. There is urgent need to develop alternatives to control AMR bacteria, otherwise in an attempt to control persistent infections the farmers end up using more quantities of antibiotics and different classes of antibiotics with further dire consequences of AMR emergence. In the food production and processing sector, AMR increases the cost of health care treatment of farm personnel and processing workers but also decrease work productivity due to prolonged illness (Bennani et al., 2020). Globally, the economic burden due to AMR is estimated to reduce 2–3.5% of GDP or up to 60–100 trillion USD in 2050 (O'Neill, 2014 O'Neill, 2016). Recently, using predictive statistical modelling, the Antimicrobial Resistance Collaborators (2022) made a comprehensive assessment of global burden of AMR covering 204 countries and territories in 2019. The study estimated that in 2019, 4.95 million deaths were associated with bacteria AMR, and 1.7 million deaths were attributed to bacterial AMR. Methicillin-resistant *Staphylococcus aureus* (MRSA) caused more than 100,000 deaths.

In an attempt to address AMR, international attention was drawn for the prudent use of antimicrobials in terrestrial and aquatic animals. Regulations were laid for restricting free access to antimicrobials, minimizing the use of antimicrobials, and developing suitable alternatives to antibiotics. The stable and nonbiodegradable nature of antibiotics and their metabolites makes them remain in processed or raw fish and shellfish muscle for longer periods of time (Cabello, 2006; Santos and Ramos, 2016). Globally, consumers demand for antibiotic-free meat is a strong driver for reducing the use of antibiotics in animal agriculture. The share of antibiotic-free beef, pork, and poultry was 5% and is expected to grow based on consumer demands. The incentive for antibiotic-free food products is that their retail price is fixed at a higher price *vis-à-vis* regular meat. The impact of AMU on food security ultimately drives the AMR policy.

18 Antimicrobial Resistance in Bacteria Linked to Food-Processing Practices

AMR in the absence of any AMU is possible in the food processing sector and is linked to cross resistance. For example, exposure of bacteria to disinfectants (biocides) such as acidified sodium citrate, chlorine dioxide, and peroxyacetic acid resulted in the development of resistance in the exposed bacteria to the disinfectant with simultaneous emergence of resistance to antimicrobials (Alonso-Hernando et al., 2009; Gantzhorn et al., 2014). AMR in *E. coli* isolated from different points in the farmed freshwater fish value chain (aquaculture farms, wholesale market, retail vending) indicated lower resistance at the aquaculture farm level but increase in MDR strains at the retail indicates that bacterial contamination during postharvest handling contributed to AMR in fish meat (Basha and Rao, 2019).

Raw foods or unprocessed animal foods are potential source of bacteria, including AMR strains that enter the foods during farming (preharvest) stage. The AMR may arise due to use of antibiotics for treatment or for growth purpose. The contamination of bacteria during harvesting and handling introduces new bacteria on to the raw foods some of which might possibly be AMR strains. On the other hand, food preservation and processing methods are usually detrimental for the growth and survival of bacteria as they either kill the bacteria (thermal processing, acidification, high-pressure processing, pulse light, pulsed electric field, gamma irradiation) or minimize the growth of bacteria (chilling, freezing, drying, modified atmosphere packaging, vacuum packaging). Generally, food processing, especially thermal processing, has negative effect on the emergence of AMR as bacteria, including those that carry antibiotic resistance determinants, get killed during the process. Sabia et al. (2017) observed resistance in 52% of the *Enterobacteriaceae* isolates from rectal swabs of pigs but only 3% of the isolates from processed minced pork showed resistance. Similarly, 62% of pig nasal swabs harbored MRSA but only 1.2% of retail pork samples harbored MRSA (Narvaez-Bravo et al., 2015). No AMR genes were detected on beef products (Noyes et al., 2016). Animal handlers and processing personnel are a source of introduction of AMR on the farms and in food,

respectively. MRSA present in beef was attributed to human contact (Jackson et al., 2013). Food contact surfaces are another source of transmission of AMR bacteria. Extended spectrum β-lactamase (ESBL) producing *E. coli* were present in 39% of poultry contact surfaces (Aliyu et al., 2016).

19 Action Points for the Control of AMR in Preharvest and Postharvest Fisheries

- Ensure that aquaculture farmers and hatchery operators compulsorily practice scientific farming/good aquaculture practices (SPF/SPR broodstock, disease-free and active seed stocking densities, water management, feed management, better biosecurity measures) for infection prevention.
- Develop reliable, rapid on-site diagnostics and make them available to the aquaculture farmers to prevent inappropriate use of antibiotics (unscientifically using antibiotics for treating viral pathogens).
- Test all the feed and feed supplements used in aquaculture for the presence of antibiotics and clearly label the packs.
- Create awareness on personal hygiene and AMR among the fish harvesters (cleaning body before entering into the farm, using hand dips, disinfecting fishing gear).
- Undertake research on developing natural and safe alternatives to antibiotics for disease control in aquatic animals.
- Developing effective vaccines against fish pathogens for disease prevention in farmed finfish.
- Ensure that only antibiotic resistance free probiotic strains are utilized for remediating the water and sediment and improving the health of animals.
- Advocate antibiotic stewardship among veterinarians and fish health professionals for treating fish diseases (correct diagnosis, antibiotic treatment based on AST results; rotation in use of approved antibiotics).
- Strict enforcement of drug regulation at village level to control the accessibility of antibiotics to the aquaculture farmers and hatchery operators.
- Prevent bacterial contamination during harvesting, processing, marketing, and transport by hygienic handling of produce, using tested potable water for processing of fish, using good quality ice for maintaining chilled condition, and maintaining cleanliness and safety of all food contact surfaces.
- Develop technologies for the removal of antibiotics in aquaculture farm effluents prior to their discharge or disposal into the natural environment.
- Prioritize national surveillance on AMU in aquaculture so as to rapidly generate baseline data on the use of antibiotics in aquaculture.
- Strengthen national AMR surveillance in fisheries to elucidate the current status of AMR in different aquaculture systems (freshwater, brackish water and mariculture) and to provide realistic estimate of the risk that AMR in aquaculture poses to the human population.

- Policies need to be framed for the regional management of AMR to prevent transboundary spread.
- Generate research evidence to unequivocally establish the link between inappropriate use of antibiotics in aquaculture and the development of antibiotic resistance in human pathogens.

20 Conclusion

All the fisheries stakeholders, that is, aquaculture farmers, hatchery operators, feed manufacturers, veterinarians, fisheries health professionals, fish processors, fish harvesters, aquaculture farm personnel, food handlers, fish sellers and transporters should become aware that AMR is a pertinent issue in fisheries and act proactively for its mitigation or else the healthy and nutritious fish food would inadvertently become vehicles for AMR transmission and dissemination of ARGs. This has serious and deleterious consequences not only for human health care but also undermines the global efforts to end food insecurity.

21 Cross-References

► Antimicrobial Resistance in Fisheries

References

Aarestrup, F. M., Seyfarth, A. M., Emborg, H. D., Pedersen, K., Hendriksen, R. S., & Bager, F. E. (2001). Effect of abolishment of the use of antimicrobial agents for growth promotion on occurrence of antimicrobial resistance in fecal Enterococci from food animals in Denmark. *Antimicrobial Agents and Chemotherapy, 45*, 2054–2059.

Adams, C. A., Austin, B., Meaden, P. G., & McIntosh, D. (1998). Molecular characterization of plasmid mediated oxytetracycline resistance in Aeromonas salmonicida. *Applied and Environmental Microbiology, 64*, 4194–4201.

Agerso, Y., Bruun, M. S., Dalsgaard, I. & Larsen, J. L. (2007). The tetracycline resistance gene tet (E) is frequently occurring and present on large horizontally transferable plasmids in Aeromonas spp. from fish farms. *Aquaculture 266*, 47–52.

Alderman, D. J. (2009). Control of the use of veterinary drugs and vaccines in aquaculture in the European Union. In C. Rogers & B. Basurco (Eds.), *The use of veterinary drugs and vaccines in Mediterranean aquaculture* (pp. 13–28). CIHEAM.

Alderman, D. J., & Hastings, T. S. (1998). Antibiotic use in aquaculture: Development of antibiotic resistance – potential for consumer health risks. *International Journal of Food Science and Technology, 33*, 139–155.

Aliyu, A. B., Saleha, A. A., Jalila, A., & Zunita, Z. (2016). Risk factors and spatial distribution of extended spectrum b-lactamase-producing- Escherichia coli at retail poultry meat markets in Malaysia: A cross-sectional study. *BMC Public Health, 16*, 699.

Alonso-Hernando, A., Capita, R., Prieto, M., & Alonso-Calleja, C. (2009). Comparison of antibiotic resistance patterns in Listeria monocytogenes and Salmonella enterica strains pre-exposed and exposed to poultry decontaminants. *Food Control, 20*, 1108–1111.

Antimicrobial Resistance Collaborators. (2022). Global burden of bacterial antimicrobial resistance in 2019: A systematic analysis. *The Lancet, 399*, 629–655.

Basha, K. A., & Rao, B. M. (2019). Multi drug resistant Escherichia coli in the value chain of farmed freshwater fish. In M. M. Prasad, T. C. Joseph, B. Madhusudana Rao, G. K. Sivaraman, & V. Murugadas (Eds.), *Book of Abstracts-National Seminar on 'AMR in Indian Fisheries: Measures of Mitigation'* (p. 81). ICAR-Central Institute of Fisheries Technology (ICAR-CIFT), Society of Fisheries Technologists, India (SOFTI) and Marine products Export Development Authority (MPEDA), 7–8 Nov 2019.

Bennani, H., Mateus, A., Mays, N., Eastmure, E., Stark, D. C. K., & Hasler, B. (2020). Overview of evidence of antimicrobial use and antimicrobial resistance in the food chain. *Antibiotics, 2020*(9), 49.

Beukers, A. G., Zaheer, R., Cook, S. R., Stanford, K., Chaves, A. V., Ward, M. P., et al. (2015). Effect of in-feed administration and withdrawal of tylosin phosphate on antibiotic resistance in enterococci isolated from feedlot steers. *Frontiers in Microbiology, 6*, 483.

Bharathkumar, G., & Abraham, T. J. (2011). Antibiotic susceptibility of Gram-negative bacteria isolated from freshwater fish hatcheries of West Bengal, India. *Indian Journal of Fisheries, 58*(3), 135–138.

Bridson, P. (2014). Monterey Bay Seafood watch four region summary document (Atlantic Salmon, Coho Salmon) for Norway, Chile, Scotland, British Columbia. Monterey Bay Aquarium Seafood Watch Program.

Bruins, M. R., Kapil, S., & Oehme, F. W. (2000). Microbial resistance to metals in the environment. *Ecotoxicology and Environmental Safety, 45*, 198–207.

Buschmann A. H., Tomova, A., Lopez, A., Maldonado, M. A., A. Henriquez, L. A., Ivanova, L., Moy, F., Godfrey, H. P. & Cabello, F. C. (2012). Salmon aquaculture and antimicrobial resistance in the marine environment. *PLoS ONE, 7*, e42724.

Cabello, F. C. (2006). Heavy use of prophylactic antibiotics in aquaculture: A growing problem for human and animal health and for the environment. *Environmental Microbiology, 8*, 1137–1144.

CBC Marketplace. (2019). Shrimp containing antibiotic-resistant bacteria found in Canadian grocery stores. https://www.cbc.ca/news/canada/shrimp-antibiotics-resistance-amr-marketplace-1.5055101

CDDEP. (2015). https://cddep.org/publications/state_worlds_antibiotics_2015/.

Chantziaras, I., Boyen, F., Callens, B., & Dewulf, J. (2013). Correlation between veterinary antimicrobial use and antimicrobial resistance in food-producing animals: A report on seven countries. *The Journal of Antimicrobial Chemotherapy, 69*, 827–834.

Checcucci, A., Trevisi, P., Luise, D., Modesto, M., Blasioli, S., Braschi, I., & Mattarelli, P. (2020). Exploring the animal waste resistome: The spread of antimicrobial resistance genes through the use of livestock manure. *Frontiers in Microbiology, 11*, 1416.

DANMAP. (2015). Use of antimicrobial agents and occurrence of antimicrobial resistance in bacteria from food animals, food and humans in Denmark. https://www.danmap.org/-/media/arkiv/projekt-sites/danmap/danmap-reports/danmap%2D%2D2015/danmap-2015.pdf?la=en

Dung, T. T., Haesebrouck, F., Sorgeloos, P., Tuan, N. A., Pasmans, F., Smet, A., et al. (2009). IncK plasmid mediated tetracycline resistance in Edwarsiella ictaluri isolates from diseased freshwater catfish in Vietnam. *Aquaculture, 295*, 157–159.

EC (2011) European Commission Action plan against the rising threats from Antimicrobial Resistance: Communication from the commission to the European parliament and the council. Brussels.

ECDC/EFSA/EMA. (2015). First joint report on the integrated analysis of the consumption of antimicrobial agents and occurrence of antimicrobial resistance in bacteria from humans and food-producing animals. *EFSA Journal, 13*.

ECDC/EFSA/EMA. (2017). Second joint report on the integrated analysis of the consumption of antimicrobial agents and occurrence of antimicrobial resistance in bacteria from humans and food-producing animals: Joint interagency antimicrobial consumption and resistance analysis (JIACRA) report. *EFSA Journal, 15*.

EIC. (2002). Compendium of Fish and Fishery Products Export Inspection Council, Ministry of Commerce and Industry, Govt. of India, S.O. 729 (E) dated 21st August 1995; Subsequently amended vide No. Order S.O.722 (E) dated 10th July, 2002.

Ellis-Iversen, J., Seyfarth, A. M., Korsgaard, H., Bortolaia, V., Munck, N., & Dalsgaard, A. (2019). Antimicrobial resistant E. coli and enterococci in pangasius fillets and prawns in Danish retail imported from Asia. *Food Control*. https://doi.org/10.1016/j.foodcont.2019.106958

EU. (2010). Commission Regulation (EU) No 37/2010 on pharmacologically active substances and their classification regarding maximum residue limits (MRLs) in foodstuffs of animal origin.

FAO. (2017). Antimicrobial resistance (AMR) in aquaculture, Committee on Fisheries, Sub-committee on aquaculture, Ninth Session, Rome, 24–27 October 2017, COFI:AQ/IX/2017/SBD.11.

FAO. (2020). The state of the world fisheries and aquaculture 2020. Sustainability in action. https://doi.org/10.4060/ca9229en.

FAO, IFAD, UNICEF, WFP and WHO. (2020). *The State of Food Security and Nutrition in the World 2020. Transforming food systems for affordable healthy diets*. FAO. https://doi.org/10.4060/ca9692en

FAO/WHO (2001). *Evaluation of health and nutritional properties of powder milk and live lactic acid bacteria*. Food and Agriculture Organization of the United Nations and World Health Organization; 2001.

FDA. (2005). Withdrawal of approval of the new animal drug application for enrofloxacin in poultry. Docket number 2000N–1571 Department of Health and Human Services, United States Food and Drug Administration. Available at: http://www.fda.gov/oc/antimicrobial/baytril.pdf

FDA. (2015). *Antimicrobials sold or distributed for use in food-producing animals*. US Food and Drug Administration; 2015. http://www.fda.gov/downloads/ForIndustry/UserFees/AnimalDrugUserFeeActADUFA/UCM440584.pdf

FDA. (2016). Drugs transitioning from Over-the-Counter (OTC) to Veterinary Feed Directive (VFD) Status. In: FDA (ed.). http://www.fda.gov/AnimalVeterinary/DevelopmentApprovalProcess/ucm482107.htm.

Fischer, J., Rodríguez, I., Schmoger, S., Friese, A., Roesler, U., Helmuth, R., et al. (2013). Salmonella enterica subsp. enterica producing VIM-1 carbapenemase isolated from livestock farms. *Journal of Antimicrobial Chemotherapy, 68*, 478–480.

FSSAI. (2011). *Food Safety and Standards (Contaminants, toxins and residues) regulations, 2011 (version 3, 31/08/2018)*. Ministry of Health and Family Welfare, Govt. of India.

Gantzhorn, M. R., Pedersen, K., Olsen, J. E., & Thomsen, L. E. (2014). Biocide and antibiotic susceptibility of Salmonella isolates obtained before and after cleaning at six Danish pig slaughterhouses. *International Journal of Food Microbiology, 181*, 53–59.

GOI. (2002). The Gazette of India extraordinary, dated 10th July 2002, vide order No.729 (E) of the Ministry of Commerce and Industry, Government of India.

GOI. (2020). *Handbook on fisheries statistics 2018*. Fisheries Statistics Division, Department of Fisheries, Ministry of Fisheries, Animal Husbandry & Dairying, Government of India.

Golden, C. D., Koehn, J. Z., Shepon, A., et al. (2021). Aquatic foods to nourish nations. *Nature*. https://doi.org/10.1038/s41586-021-03917-1

Hammerum, A. M., Heuer, O. E., Emborg, H. D., Bagger-Skjot, L., Jensen, V. F., Rogues, A. M., Skov, R. L., Agerso, Y., Brandt, C. T., Seyfarth, A. M., et al. (2007). Danish integrated antimicrobial resistance monitoring and research program. *Emerging Infectious Diseases, 13*, 1632–1639.

Hoogenboom, L. A. P., Bokhorst, J. G., Northolt, M. D., van de Vijver, L. P. L., Broex, N. J. G., Mevius, D. J., et al. (2008). Contaminants and microorganisms in Dutch organic food products: A comparison with conventional products. *Food Additives & Contaminants: Part A, 25*, 1195–1207.

Hu, Y., & Cheng, H. (2016). Health risk from veterinary antimicrobial use in China's food animal production and its reduction. *Environmental Pollution, 219*, 993–997.

Inglis, G. D., McAllister, T. A., Busz, H. W., Yanke, L. J., Morck, D. W., Olson, M. E., et al. (2005). Effects of subtherapeutic administration of antimicrobial agents to beef cattle on the prevalence of antimicrobial resistance in Campylobacter jejuni and Campylobacter hyointestinalis. *Applied and Environmental Microbiology, 71*, 3872–3881.

Israngkura, A., & Sae-Hae, S. (2002). A review of the economic impacts of aquatic animal diseases. In J. R. Arthur, M. J. Phillips, R. P. Subasinghe, M. B. Reantaso, & I. H. MacRae (Eds.), *Primary aquatic animal health care in rural, small-scale, aquaculture development* (FAO Fisheries technical paper no. 406) (pp. 253–286). FAO.

Jackson, C. R., Davis, J. A., & Barrett, J. B. (2013). Prevalence and characterization of methicillin-resistant Staphylococcus aureus isolates from retail meat and humans in Georgia. *Journal of Clinical Microbiology, 51*, 1199–1207.

Kim, D. P., Saegerman, C., Douny, C., Dinh, T. V., Xuan, B. H., Dang, B., et al. (2013). First survey on the use of antibiotics in pig and poultry production in the red river delta region of Vietnam. *Food and Public Health, 3*, 247–256.

Laxminarayan, R., van Boeckel, T., & Teillant, A. (2015). *The economic costs of withdrawing antimicrobial growth promoters from the livestock sector*. OECD Food, Agriculture and Fisheries Papers.

Le, T. X., & Munekage, Y. (2004). Residues of selected antibiotics in water and mud from shrimp ponds in mangrove areas in Viet Nam. *Marine Pollution Bulletin, 49*, 922–929.

Liu, Y. Y., Wang, Y., Walsh, T. R., Yi, L. X., Zhang, R., Spencer, J., Doi, Y., Tian, G., Dong, B., Huang, X., et al. (2016). Emergence of plasmid-mediated colistin resistance mechanism mcr-1 in animals and human beings in china: A microbiological and molecular biological study. *The Lancet Infectious Diseases, 16*, 161–168.

McIntosh, D., Cunningham, D., Ji, B., Fekete, F. A., Parry, E. M., Clark, S. E., et al. (2008). Transferable, multiple antibiotic and mercury resistance in Atlantic Canadian isolates of Aeromonas salmonicida subsp. Salmonicida is associated with carriage of an IncA/C plasmid similar to the Salmonella enterica plasmid pSN254. *The Journal of Antimicrobial Chemotherapy, 61*, 1221–1228.

McPhearson, R. M., et al. (1991). Antibiotic-resistance in gram-negative bacteria from cultured catfish and aquaculture ponds. *Aquaculture, 99*(3–4), 203–211.

Nannapaneni, R., Hanning, I., Wiggins, K. C., Story, R. P., Ricke, S. C., & Johnson, M. G. (2009). Ciprofloxacin-resistant Campylobacter persists in raw retail chicken after the fluoroquinolone ban. *Food Additives & Contaminants. Part A, Chemistry, Analysis, Control, Exposure & Risk Assessment, 26*, 1348–1353.

Narvaez-Bravo, C., Toufeer, M., Weese, S. J., Diarra, M. S., Deckert, A. E., Reid-Smith, R., et al. (2015). Prevalence of methicillin-resistant Staphylococcus aureus in Canadian commercial pork processing plants. *Journal of Applied Microbiology, 120*, 770–780.

Noor Uddin, G. M., Larsen, M. H., Christensen, H., Aarestrup, F. M., Phu, T. M., & Dalsgaard, A. (2015). Identification and antimicrobial resistance of bacteria Isolated from probiotic products used in shrimp culture. *PLoS One, 10*(7), e0132338. https://doi.org/10.1371/journal.pone.0132338

Norwegian Veterinary Institute. (2016). *Use of antibiotics in Norwegian Aquaculture*. The Norwegian Veterinary Institute, Report 22, 11 p.

Noyes, N. R., Yang, X., Linke, L. M., Magnuson, R. J., Dettenwanger, A., Cook, S., et al. (2016). Resistome diversity in cattle and the environment decreases during beef production. *eLife, 5*, e13195.

O'Neill, J. (2016). Tackling drug-resistant infections globally: Final report and recommendations. https://amr-review.org/sites/default/files/160525_Final%20paper_with%20cover.pdf

O'Neill, J. (2014). *Antimicrobial resistance: Tackling a crisis for the health and wealth of nations*. HM Government and Wellcome Trust.

Public Health England. (2015). UK One Health report: Joint report on human and animal antibiotics use, sales and resistance 2013.

Ramakrishna, R., Shipton, T. A., & Hasan, M. R. (2013). *Feeding and feed management of Indian major carps in Andhra Pradesh, India. FAO Fisheries and Aquaculture Technical Paper, 578* (p. 90). Food and Agriculture Organization of the United Nations.

Rao, B. M., & Prasad, M. M. (2015). Residues of veterinary medicinal products (antibiotics) in shrimp exported from India to the European Union (EU). *Fishing Chimes, 34*, 6–11.

Sabia, C., Stefani, S., Messi, P., de Niederhausern, S., Bondi, M., Condo, C., Iseppi, R., & Anacarso, I. (2017). Extended-spectrum B-lactamase and plasmid-mediated AMPC genes in Swine and Ground Pork. *Journal of Food Safety, 37*, e12282.

Santos, L., & Ramos, F. (2016). Analytical strategies for the detection and quantification of antibiotic residues in aquaculture fishes: A review. *Trends in Food Science and Technology, 52*, 16–30.

Schar, D., Klien, E. Y., Laxminarayan, R., Gilbert, M., & van Boeckel, T. P. (2020). Global trends in antimicrobial use in aquaculture. *Scientific Reports, 10*, 21878.

Schlenk, D., Gollon, J. L., & Griffin, B. R. (1998). Efficacy of copper sulfate for the treatment of Ichthyophthiriasis in Channel catfish. *Journal of Aquatic Animal Health, 10*, 390–396.

Teillant, A., & Laxminarayan, R. (2015). Economics of antibiotic use in US swine and poultry production. *Choices, 30*, 1–11.

Tomova, A., Ivanova, L., Buschmann, A. H., et al. (2015). Antimicrobial resistance genes in marine bacteria and human uropathogenic Escherichia coli from a region of intensive aquaculture. *Environmental Microbiology Reports, 7*, 803–809.

Van Boeckel, T. P., Brower, C., Gilbert, M., Grenfell, B. T., Levin, S. A., Robinson, T. P., et al. (2015). Global trends in antimicrobial use in food animals. *Proceedings of the National Academy of Sciences, 112*, 5649–5654.

Verner-Jeffreys, D. W., Welch, T. J., Schwarz, T., Pond, M. J., Woodward, M. J., Haig, S. J., et al. (2009). High prevalence of multidrug-tolerant bacteria and associated antimicrobial resistance genes isolated from ornamental fish and their carriage water. *PLoS One, 4*(12), e8388.

Verraes, C., van Boxstael, S., Meervenne, E. V., Coillie, E. V., Butaye, P., Catry, B., de Schaetzen, M. A., Huffel, X. V., Imberechts, H., Dierick, K., Daube, G., Saegerman, C., de Block, J., Dewulf, J., & Herman, L. (2013). Antimicrobial resistance in the food chain: A review. *International Journal of Environmental Research and Public Health, 10*, 2643–2669.

Waksman, S. A. (1947). What is an antibiotic or an antibiotic substance? *Mycologia, 39*, 565–569.

Wales, A. D., & Davies, R. H. (2015). Co-selection of resistance to antibiotics, biocides and heavy metals, and its relevance to foodborne pathogens. *Antibiotics, 4*, 567–604.

Watts, J. E. M., Schreier, H. J., Lanska, L., & Hale, M. S. (2017). The rising tide of antimicrobial resistance in aquaculture: Sources, sinks and solutions. *Marine Drugs, 15*, 158.

Wohlfarth, G. W., & Schroeder, G. L. (1979). Use of manure in fish farming - A review. *Agricultural Wastes, 1*, 279–299.

Wu, Z. (2017). Measuring food security impact of antimicrobial use in food animal production: A review of economic literature. *China Agricultural Economic Review*. https://doi.org/10.1108/CAER-07-2016-0106

Zhao, L., Dong, Y. H., & Wang, H. (2010). Residues of veterinary antibiotics in manures from feedlot livestock in eight provinces of China. *Science of the Total Environment, 408*, 1069–1075.

Antimicrobial Resistance in Brackishwater Aquaculture

Subhendu Kumar Otta and Sudama Swain

Contents

Abstract

Brackishwater aquaculture is a multibillion-dollar food industry that is growing at a rapid pace with an aim to increase the production and productivity, and meet the dietary demands of ever-increasing world population. Disease is the major constraint that potentially hampers the progress of this important sector. Adoption of high-density culture and impact of climate change have resulted in the emergence of diseases, resulting in decreased production, causing severe financial losses to the farmers. To cope with this adverse effect, farmers use several antimicrobials (AMs). There is ample evidence to show that several AMs are used in brackishwater aquaculture, particularly in hatcheries. However, when these AMs are used indiscriminately, it is responsible for the advent of resistance in bacteria that are present in the aquaculture system toward AMs.

S. K. Otta (✉) · S. Swain
ICAR-Central Institute of Brackishwater Aquaculture, Chennai, India
e-mail: Subhendu.otta@icar.gov.in

M. P. Mothadaka et al. (eds.), *Handbook on Antimicrobial Resistance*,
https://doi.org/10.1007/978-981-19-9279-7_8

Investigations carried out in this sector clearly indicate the presence of AMR bacteria in cultured animals, water, and sediment. There are several mechanisms through which these AMR organisms can spread to humans, livestock, and environment. Timely intervention is required before the situation goes out of control. A combined approach between farmers, researchers, and government officials is necessary to solve this important issue. Effective mitigation strategies, along with stringent regulations, are essential to control AMR in this sector.

Keywords

Brackishwater aquaculture · Finfish · Shellfish · Antimicrobials · Antimicrobial resistance

1 Introduction

Aquaculture is evolving at a rapid pace to cope with the population and economy-driven demand for affordable protein. The entire aquaculture production is almost utilized for human consumption (FAO, 2016). Aquaculture also accounted for more biomass than some of the animal sectors (Edwards et al., 2019). Aquaculture has overtaken capture fisheries in terms of total biomass production. As per FAO (2014), the aquaculture needs to expand further by 50% by 2050 to meet the protein demand of the increased population. Brackishwater has been considered a unique ecosystem for the aquaculture practice. This ecosystem is considered a fertile ground for the aquatic organisms as it is situated in the buffering zone between marine and freshwater systems. Because of this, it acts as a habitat for the growth of juvenile aquatic organisms. Brackishwater aquaculture is also considered a zero-water footprint technology as the water is not suitable for other purposes unlike the freshwater and, therefore, provides scope for complete utilization for aquaculture practice. Apart from some fin fishes, molluscans, and seaweeds, particularly shrimp aquaculture has become very popular in brackishwater ecosystem. In the last two decades, continuous increase in production of almost three million tons of shrimp per annum has been recorded (FAO, 2019). In order to achieve higher production, increase in stocking density per unit area and use of high-quality artificial feed, chemicals, and biologicals to avoid disease have become a part of standard high-intensity aquaculture practice. As a result, the ecosystem always remains under tremendous pressure, giving scope for imbalance and disease outbreaks.

Among the major hurdles that the aquaculture has been facing for the past several years, disease occupies the topmost position. Intensification of aquaculture practices has been a major cause of disease outbreaks, which can be responsible for more than 50% of the total estimated losses (Assefa & Abunna, 2018). While a majority of disease-associated losses (about 60%) in aquaculture are because of viral infection, 20% of the losses are accounted to be of bacterial origin (Flegel, 2012), indicating

the importance of bacteria as a pathogen creating the problem. The impact of acute hepatopancreatic necrosis disease (AHPND) on shrimp aquaculture that spread out to different parts of the world can be taken as the best example as far as losses due to bacterial diseases are concerned (Feng et al., 2017). Different species of bacteria are responsible for causing diseases to both fin fishes and shellfishes of brackishwater aquaculture origin. Vibriosis, particularly diseases triggered by *Vibrio harveyi* and *V. parahaemolyticus*, is a key problem in shrimp culture. Similarly, bacterial pathogens like *V. harveyi*, *V. anguillarum*, and *Streptococcus* spp. are important for finfish species.

Antimicrobials (AMs) are substances that are generally used to kill different microorganisms and save valuable life and include antibiotics, antivirals, antifungal, and antiparasitic drugs. It started with the purpose of saving human life from major bacterial diseases (Fair & Tor, 2014) and further applied to livestock in the treatment of diseases and also as promoters of growth (Van Boeckel et al., 2015). Parallel to the utilization in human medicine and livestock, AMs also found their way to aquaculture in dealing with diseases of infection and saving aquatic lives (Romero et al., 2012).

Though evidence regarding the usage of AMs in aquaculture facilities is highly disorganized, the residues of AM have been detected in various amenities in aquaculture (Husevag et al., 1991; Samuelsen et al., 1994; Smith et al., 1994; Capone et al., 1996). Human sewage, livestock, and aquaculture discharges carry AMs and their breakdown products to major aquatic environments, and these may again come back to aquaculture facilities (Robinson et al., 2016). Studies conducted with respect to aquaculture activities indicated that the release from these environments can contribute to antimicrobial resistance (AMR) in aquatic environment, which in turn can pose risk to AMR in human beings (Rico et al., 2017; Xu et al., 2017). Besides, AMs can affect the nonharmful microorganisms and primary producers, creating imbalance in the diversity (Guo et al., 2015). However, based on recent studies, only the use of AMs might not be the sole cause for the development of AMR, and sometimes the environmental parameters such as higher water temperature can be a major factor that has been generally observed in case of terrestrial bacteria, indicating the importance of climate change factors (MacFadden et al., 2018; Reverter et al., 2020).

Use of AMs in the aquaculture environment is strongly discouraged, and, therefore, brackishwater aquaculture system is not an exception. Many of the importing countries are now taking stringent action with respect to AM residues in aquatic animals, particularly shrimp. Rejection of consignments is often faced by a number of countries, which results in huge losses to the farming communities and the respective countries. It is, therefore, necessary to have an idea regarding the AM uses in brackishwater aquaculture system. Brackishwater aquaculture is more familiar due to culture of some economically important species such as shrimps and crabs. These invertebrates do not have an improved immune system like the vertebrates and therefore easily become susceptible to diseases. These species often require preventive and treatment strategies to get rid of the diseases and secure a profitable harvest. This special culture system will use more AMs and pose risk to the aquatic as well as

terrestrial environment through food chain. This chapter aims to provide an overall idea regarding AM usage and its risks.

2 Usages of AMs in Brackishwater Aquaculture System

While attempts have been made to estimate the global use of AMs, the same has not been possible to divide based on the species cultured or production systems adopted, which is mainly due to the lack of any monitoring system (Done et al., 2015). In particular, for aquaculture use no specific rules and regulations have been put forth in many of the developing countries, and, therefore, it has been difficult to estimate the use of exact amount. The diversity of species cultured and different production systems, etc., used (Fig. 1) has again made it more complicated (Lozano et al., 2018; Henriksson et al., 2017). However, centered on the existing and inadequate information, it is known that antimicrobials are used in aquaculture systems and the same has been reported (Cabello et al., 2013; Rico et al., 2013).

Scant information is available on the application of AMs in brackishwater aquaculture. The original application of antibiotic was for *Macrobrachium rosenbergii* larvae in Tahiti, and since then the use of antibiotics in shrimp hatcheries has become a general practice (Brown, 1989). The sporadic information available is again based on the limited data from selected farms and hatcheries. Putting together all the information, it is accepted that all ranges of AMs such as antibiotics, antivirals, and antiparasitics are used in brackishwater aquaculture. Taking shrimps culture into account, which is synonymous with brackishwater aquaculture, it is implicit that AMs were

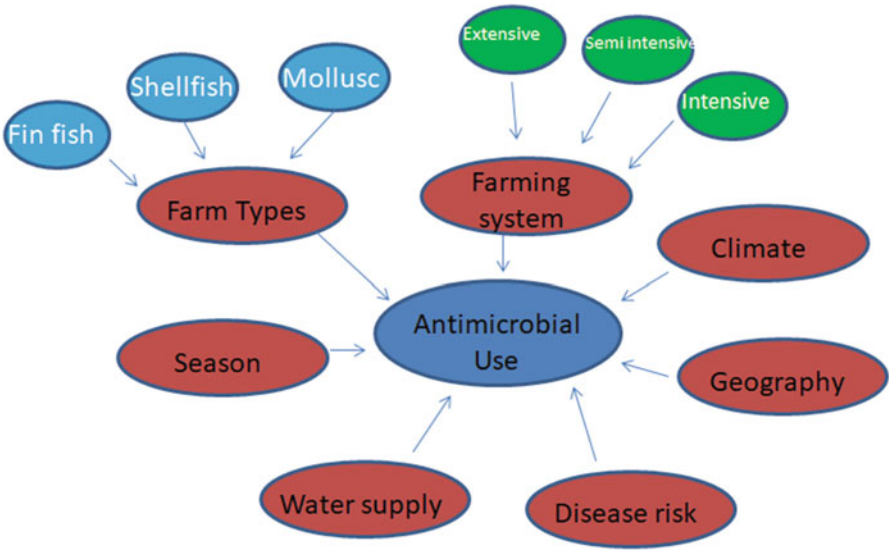


Fig. 1 Factors determining the use and quantification of AMs in brackishwater aquaculture

widely used at least in hatchery system to control several bacterial diseases (Ali et al., 2016; Chi et al., 2017; Hinchliffe et al., 2018; Thornber et al., 2020). Even the report by Hinchliffe et al. (2018) indicated that about 80 kg of antibiotics was used per hatchery per crop. When in larval stages, aquatic animals are more susceptible to different diseases, including bacterial diseases such as vibriosis. Therefore, many of the hatcheries adopt the method of providing AMs as a practice of prophylaxis to dodge the diseases and get better survival (Zhang et al., 2011; Smith, 2012).

Compared with the hatcheries, uses of AMs, particularly that of antibiotics for farmed shrimp, are low. However, many farms use several chemicals for other pathogens, namely, viruses and parasites. Oftentimes, AMs are simply dumped in farms as a preventive measure sans understanding whether it really works or not. Though inconsistent application of AMs in shrimp aquaculture, however, has been reported in many studies (Rico et al., 2013; Ali et al., 2016; Liu et al., 2017; Chi et al., 2017). Chemotherapy was widely practiced in shrimp farms to circumvent diseases associated with bacteria of luminescence (Baticados & Paclibare, 1992), and many farms also used a wide range of antibiotics for the treatment of vibriosis (Baticados et al., 1990; Primavera et al., 1993)

3 Selection and Mode of Application of AMs in Brackishwater Aquaculture

AMs for BW aquaculture use are generally selected randomly based on their observed effects on human beings and livestock. Sometimes, farmers apply the AMs blindly having no cognizance of name and mechanism of their action (Le & Munekage, 2004). Similar to the case of human and livestock where antibiotic use is prescribed based on pathogen isolation and antibiotic sensitivity assay, the same is not implemented in aquaculture. In developing countries, there is absence of specialized authorities to introduce antimicrobials for aquaculture practice. Secondly, once the disease outbreak occurs, mortality starts immediately and very less time is left to carry out the diagnostic test and start the treatment thereafter. Though only a few antibiotics and other antimicrobials have been approved by the FDA for aquaculture use, the same is not followed and even the banned items are used in aquaculture practice (Henriksson et al., 2017).

A general practice of using AMs in brackishwater aquaculture is through feed. In this way, the AMs are either dissolved in water and mixed with feed or directly coated to feed and applied on farms. Sometimes, the AMs are also directly added to water depending upon the pond size or importance of the species cultured, such as brooders where individual animals are treated with certain AMs.

Antibiotics as prophylactic or growth promoter are usually not practiced in shrimp or finfish farms in brackishwater. However, this can be considered metaphylactic, where the entire population is treated with antibiotics though only a part of animals gets affected (Thornber et al., 2020). Therefore, both diseased and normal animals are exposed to the antibiotic added for disease treatment, and generally the dosages are calculated based on the total biomass present in a particular pond.

4 Status of AMR in Brackishwater Aquaculture

4.1 AMR in Cultured Shrimp

AMR in shrimp aquaculture has been extensively reported as shrimp is the most widely cultured species in brackishwater ecosystem. Since antibiotics were extensively used in shrimp hatcheries, the development of AMR in such an environment was also high. *Vibrio harveyi* exhibiting antibiotic resistance has been observed to be the sole reason for total elimination of shrimp larvae in hatchery (Karunasagar et al., 1994). Surprisingly, such AMR status was not observed either in input sea water or shrimp eggs. Therefore, the study directly correlated the development of AMR with the frequent and higher doses of antibiotic applied. Similarly, with studies in a similar line, Otta et al. (2001) observed the presence of resistant vibrio species from a number of hatcheries, and these were mostly from the larval tanks, which further correlated the increase of AMR directly to the application of antibiotics.

Recently, a large number of ARG were identified in sediments of different aquatic environments, and this has global distribution (Chen et al., 2015). In this regard, the different AMR genes associated with sulfonamide groups (*sul1, sul2*), tetracycline group (*tetB, tetC, tetM, tetO, tetW*), quinolone group (*qnrA*), and beta-lactamase groups (*blaTEM, blaSHV, blaCTX-M*, *blaNDM*) were found to be present when sediments of different aquatic environments were analyzed (Pei et al., 2006; Yang et al., 2013; Czekalski et al., 2014; Chen et al., 2015). In particular regarding marine sediments, a huge number of AMR genes concomitant to the tetracycline group were detected. Additionally, a large number of contigs showing high similarity to the transposons or plasmids of pathogens from humans were also reported. This clearly indicated that the bacterial community present in the sediment had acquired this resistance from the genes of human pathogens (Yang et al., 2013).

Likewise, indiscriminate use of antibiotics in shrimp farm and the collapse of culture due to the development of resistant pathogens have been reported in Taiwan (Lin, 1989). The investigations carried out in several other studies also indicate the presence of AMR strains of different bacteria in shrimp farms (Table 1). Many of the reports also indicated the presence of AMR in shrimps collected from the retail markets. However, it is not confirmed whether the source shrimp samples were from the aquacultured ponds or from the wild.

4.2 AMR in Other Species Cultured in Brackishwater

When a comparison was made between two ecosystems, *Aeromonas hydrophila* isolated from finfish of brackishwater ecosystem showed a higher multiple antibiotic resistance index than the freshwater system (Matyar et al., 2007). Among *Vibrio parahaemolyticus* that were isolated from brackishwater, fish, crab, and shrimp, 40% of the isolates exhibited multidrug resistance (Quintoil et al., 2007). Multidrug-resistant bacteria of high incidence were observed in fish farms of the coastal region

Table 1 Incidence of AMR in cultured shrimp ponds

Species cultured	Types of samples collected	Country of origin	Reference
P. monodon	Shrimp and water	India	Abraham et al. (1997)
Penaeus sp.	Shrimp	Mexico	Roque et al. (2001)
P. monodon	Shrimp, water, sediment	Philippines	Tendencia and de la pena (2001)
Penaeids	Shrimp	Mexico	Molina-Aja et al. (2002)
P. monodon	Water and sediment	Vietnam	Le et al. (2005)
P. vannamei	Water and sediment	China	Zhang et al. (2011)
P. monodon and *P. vannamei*	Shrimp	Thailand	Yano et al. (2011)
P. vannamei	Shrimp, water	Brazil	Reboucas et al. (2011)
P. monodon	Shrimp	India	Marhual et al. (2012)
P. vannamei	Shrimp, water	Malaysia	Banerjee et al. (2012)
P. monodon	Shrimp, water, sediment	Malaysia	Hua and Apun (2013)
P. vannamei, P. monodon	Shrimp	Thailand	Yano et al. (2014)
P. monodon	Water, sediment	India	Jana et al. (2014)
P. vannamei	Shrimp	Brazil	Costa et al. (2015)
P. vannamei, P. monodon	Shrimp	Thailand	Yano et al. (2015)
Unknown	Shrimp	Iran	Arfatahery et al. (2016)
P. vannamei	Shrimp	Brazil	Rocha et al. (2016)
P. monodon	Sediment	India	Stalin and Srinivasan (2016)
P. vannamei	Shrimp, water, sediment	China	Su et al. (2017)
P. vannamei	Shrimp, water	Thailand	Rotana et al. (2018)
P. vannamei	Shrimp	India	Navaneeth et al. (2020)

of the Italian Adriatic Sea located in the northern and southern parts (Labella et al., 2013). Significantly higher numbers of *Vibrio harveyi* isolates were reported from finfish farms in Italy from which a large number of strains exhibited multidrug resistance to antibiotics of several types (Scarano et al., 2014). From the different *Pseudomonas* spp. isolated from the gastropods found in brackishwater ecosystem, more than 96% isolates had a MAR index of more than 0.2, indicating the higher use of antibiotics (Sampson et al., 2020). Several strains of *E. coli* collected from the mangrove oyster cultured in estuaries showed cephalothin and amoxicillin resistance (Oliveira et al., 2020). *Aeromonas schubertii* was found to be responsible for mortality of brackishwater wild Nile tilapia, and the isolate showed resistance to at least ten of the antibiotics used (Ren et al., 2019). The resistance to multiple antibiotics was seen in 100% of the *Enterobacter* spp. and *Streptococcus* spp., which were isolated either from the fish or water of a brackishwater ecosystem in Kerala, India (Pokhrel et al., 2018).

5 Regulatory Mechanism for the Antimicrobials

There are no known antibiotics that are used for bacterial infection and specifically developed for aquaculture practice while many such antibiotics have found their way either from veterinary or human medicine. For the aquaculture practice, only a handful of antibiotics have been approved, whereas the situation is completely different for the treatment of other terrestrial animals. Uses of antibiotics in aquaculture are differentially regulated based on the country in which it is practiced. Higher stocking density is one of the major factors for disease emergence (Shoemaker et al., 2000). The application of AMs in aquacultured farms for disease control seems akin to other animal production systems, viz., cattle, poultry, etc., (Alderman & Hastings, 1998; Defoirdt et al., 2011; Done et al., 2015). However, based on the country of origin, the AMs' usage in aquaculture is structured and, for each country, the variety of agents putative for use is quite different (Smith, 2008).

Several products that were used in European countries' aquaculture practice were reported to contain antibiotics such as amoxicillin, florfenicol, oxytetracycline, sulfonamides, and first-generation quinolones (Guichard & Licek, 2006). Wherever reports are available, it is with the assumption that license was provided only for a few of the antibiotics for their use in aquaculture, which again depends upon a particular country. In the real sense and for the use in aquaculture, there is only limited availability of drugs that have been recommended after specifying their maximum residue limits (MRLs) based on the report by the European Commission in 1990 (Rodgers & Furones, 2009).

For aquaculture use, only five drugs were permitted by the USFDA that included four of the antimicrobials such as florfenicol, oxytetracycline, sulfamerazine, and a combination of sulfadimethoxine and ormetoprim (Benbrook, 2002; Romero et al., 2012). Similarly, only six of the antimicrobial drugs (oxytetracycline, amoxicillin, oxolinic acid, erythromycin, flumequine, and florfenicol) were approved in Chile for their use in several aquaculture practices. However, these have been ratified with the conditions that occurrences of infections and prescribed antimicrobials, along with the methods of administration of those products, should be produced by farmers to be capable of application of these antibiotics in field conditions (Burridge et al., 2010). The Ministry of Agriculture, Livestock, and Supply (Ministério da Agricultura, Pecuária e Abastecimento-MAPA) in Brazil has set up the norms for a specific program called the National Programme for the Control of Residues and Contaminants (Plano Nacional de Controle de Resíduos e Contaminantes-PNCRC), and, according to that norm, only two of the antibiotics, namely, oxytetracycline and florfenicol, were permitted by the government for use only when bacterial infections are detected in any of the aquaculture practices.

Many Asian countries like India, Bangladesh, China, Vietnam, Indonesia, the Philippines, and Thailand are considered leaders in aquaculture production and play a significant role in the aquaculture industry. Therefore, scientific publications generated from these countries indicate a wide-ranging application of AMs in these countries. In this context, an assessment by Rico et al. (2012) mentioned the use of about 36 antibiotics for aquaculture practice in different Asian countries. All

these antibiotics were found to be included under different classes, viz., tetracyclines, beta-lactams, aminoglycosides, quinolones, nitrofurans, macrolides, sulfonamides, amphenicol, colistin, and suppressors, namely, trimethoprim, which were commonly used in human medicines. Among the antibiotics, chloramphenicol and oxytetracycline were described to be employed in all the seven Asia countries in which studies were carried out. However, chloramphenicol is a banned antibiotic in most of the Asian countries and is not supposed to be used in aquaculture practice. Due to the overuse of antibiotics in animal production, the incidence of MDR bacteria to other drugs is showing an increasing trend, and, therefore, it is expected to stimulate the growth of bacteria that possess super-resistance (Poirel et al., 2017).

6 Possible Impact of AMR from BW Aquaculture

BW aquaculture is labor–intensive, and many of the workers come in direct contact with the farms and hatcheries environment regularly. The aquatic environment, including shrimp and other organisms present, harbors different bacteria, namely, *V. parahaemolyticus*, *V. vulnificus*, *V. cholerae,* etc., which are considered pathogenic to human beings (Nair et al., 2007; Jones & Oliver, 2009). Therefore, it is possible that those pathogens harboring AMR genes can infect human beings and thus spread of AMR.

As per one of the estimates, 90% of the bacteria from marine environment are reported to be resistant to a minimum of one to more than one of the known antibiotics, and at the same time about 20% of this population showed resistance to minimum five of these antibiotics (Martínez, 2003). In another case, finfish and shellfish were analyzed for 20 frequently employed antibiotics (fluoroquinolones, tetracyclines, beta-lactams, macrolides, sulfonamides, and phenicols) that were obtained from various sites of Shanghai City, and, based on this analysis, the residues of antibiotic were reported in as high as 52% of the samples collected [this included a higher number of finfish samples (40–91%) and comparatively lower number (17%) of shrimp samples] (Wang et al., 2017). As per the impact analysis, AMR-producing bacteria are accountable for 35,000 human mortalities per annum in the United States, nearly 33,000 mortalities in European countries, and approximately 58,000 mortalities in India and possibly much higher in Southeast Asian countries, and these numbers are expected to be much higher in the near future (CDC, 2019; Cassini et al., 2019).

Shrimp is considered a delicacy and is consumed worldwide. Pathogens or other organisms present in shrimp when consumed come in contact with human gut microorganisms. It is possible that through horizontal gene transfer (HGT), the AMR organisms can spread it to other normal gut flora. Some of the processes like mutation or horizontal gene transfer (HGT) and other processes like conjugation, transformation, and transduction can help the bacteria to acquire the necessary genes and transform into AMR bacteria (Reverter et al., 2020), and, subsequently, it becomes resistant to multidrugs or sometimes can be called superbugs, which will be difficult to treat.

Many of the BW aquaculture farms present in developing countries have a practice of discharging the wastewater to the surrounding environment. During the monsoon, the ponds get inundated and overflow into the surroundings. Some of the wild animals like crabs can enter the pond and then go back to their original environment. Nearby wild and domestic animals can drink water from the area where discharge water is released. It is possible that all these activities can spread the AMR bacteria present in aquaculture to the adjoining vicinities and thus further spread of AMR by direct contamination and horizontal gene transfer.

Some of the traditional BW aquaculture farmers have a practice of integrated farming where livestock come in close contact with the farms. Correspondingly, the water and sediment from the farms are used for agriculture practice in pond dyke or neighboring fields. Thus, AMR from BW farms can spread to agriculture and livestock, which subsequently can contaminate human population.

7 Public Health Implications Due to Aquaculture-Associated Food Safety Risks

Different biological and chemical agents can contaminate the aquaculture products and create food safety issues, which ultimately become the cause of health-associated risks. These food safety issues of aquaculture products can vary based on the region and habitat according to the environmental conditions, production methods used, and different practices adopted for management. Infections due to food consumption, particularly when associated with several other factors such as the presence of multidrug-resistance bacterial pathogens, different agrochemical residues, drugs used for veterinary practices, and contamination with different heavy metals were listed as impending threats for the harvests originating from aquaculture practices (Reilly et al., 1997). When AM drugs are used extensively, it poses risks to consumers, which may include drug-related resistance development, several hypersensitivity reactions to other drug use, disturbance in the distribution of normal gut flora, act as a carcinogen, and responsible for mutagenic effects or teratogenic effects (Heuer et al., 2009; Karunasagar, 2012).

Conditions in the aquatic environment can be the major source for the growth of AMR bacteria that will subsequently spread to other organisms such as human beings, animals, or other aquatic animals and cause diseases, which can be very difficult to treat. Many of the microbes, viz., S*almonella*, Shigella spp., or many of the vibrios, like *V. cholerae*, *V. vulnificus,* or *V. parahaemolyticus,* can be directly transmitted to humans from the aquatic environment. Similarly, many of the opportunistic bacteria such as *Plesiomonas shigelloides*, *Streptococcus iniae*, *E. coli, Aeromonas hydrophila, and Edwardsiella tarda* can also spread when conditions become favorable for them and cause diseases. When human beings come in direct contact with the water or the organisms residing in it either through drinking water and consuming aquatic food or handling it, the chances of AMR bacteria spreading are high. Approximately 80% of AMs enter into the environment from aquaculture that was used during the culture period. Acquired resistance from mutation or genes

of multiple resistances is transmitted through mobile genetic elements to other bacteria. These conditions bring in changes to aquatic microbiota and the biodiversity therein, subsequently the common flora of fish and the shellfish. The prevalence of both native and pathogenic microbes of human and animal origin in aquatic environment in addition to incidence of AMs residues, biofilms, and high concentration of bacteriophages can permit exchange of genetic materials between aquatic and terrestrial bacteria. According to WHO (2007) and based on some recently found genetic material and resistance determinants for beta-lactamases, tetracyclines, and quinolones it is expected that genetic materials can be transmitted between fish or shellfish pathogens and human pathogens, which occurred in aquatic bacteria.

The consequences of antimicrobial resistance in bacteria causing human and other animal infections include increased number of infections and frequency of treatment failures; therefore, this will lead to an increase in severity of infection, and, finally, an increase in expenditures to the society. Increased severity of infection includes prolonged duration of illness and increased frequency of bloodstream infections, hospitalization, and mortality (Samuelsen et al., 1992).

8 Possible Ways to Restrict AMR Spread in BrackishWater Aquaculture

Mitigating AMR development and spread in brackishwater aquaculture is a combined approach that requires the involvement of farmers, researchers, governmental organizations, and the general public. A number of approaches can be adopted either to decrease or avoid the use of AMs in brackishwater farming systems (Table 2). A strong coordination between the different partners involved in this industry is required to achieve the goal.

9 Conclusion

Development and spread of AMR are major global concerns. Based on the One Health concept, a combined approach is required to tackle this problem. Brackishwater aquaculture is expanding at present and is likely to continue in the future also. High stocking density through the intensive culture practice will be the future strategy to increase the production, make it profitable, and meet the supply demand. Furthermore, it is predicted to have an impact on climate-associated factors and pollution in aquaculture practice. It is likely that several disease conditions shall emerge in future BW aquaculture practice. Having control over disease occurrence will be the greatest challenge in the future. In such conditions, effective disease control through either alternative approaches or minimal use of AMs will be an appropriate strategy. Stringent regulations and appropriate education are highly essential to control the indiscriminate use of AMs in aquaculture. Efforts from researchers are also required to develop specific products and procedures either to completely avoid the disease or to control it as early as possible without using AMs.

Table 2 Possible ways to restrict AMs in brackishwater aquaculture

Mitigating measures	Possible strategies
Species selection	Select species with higher disease resistance capacity. Adopt species diversity.
Effective disease diagnosis	Stock with disease-free larvae. Continuously monitor the ponds for disease occurrence. Identify the disease and decide on AMs use.
Improve health status of cultured organisms	Use of quality high protein feed. Use of immunostimulants and vaccines.
Alternate to AMs	Adopt biological control such as probiotics and bacteriophages.
Better management practice	Control of inputs in farms. Maintain appropriate stocking density. Biosecurity measures. Adopt better culture practices such as race-way culture and biofloc culture.
Regulation	Stringent rules should be in place regarding sale and use of AMs. All the inputs used in aquaculture should be regulated for the presence of AMs. Farm certification should be in place for constant monitoring.
Awareness program	Farmers and field technicians should be properly educated through training and awareness programs regarding need and effective use of AMs.
Implementation of One Health approach	Understanding the ecology of each emerging disease those have zoonotic importance is essential for risk assessment studies. Based on this, only plans can be developed for any kind of response or control measures. Collective and multidisciplinary steps across all the sections such as human, animal, and environment are essential to achieve this.

In any case, control of AMR in brackishwater aquaculture is also highly essential and the need of the hour, and appropriate early steps need to be taken for complete control.

References

Abraham, T. J., Manley, R., Palaniappan, R., & Dhevendaran, K. (1997). Pathogenicity and antibiotic sensitivity ofluminous *Vibrio harveyi* isolated from diseased penaeid shrimp. *Journal of Aquaculture in the Tropics, 12*, 1–8.

Alderman, D. J., & Hastings, T. S. (1998). Antibiotic use in aquaculture: Development of antibiotic resistance – Potential for consumer health risks. *International Journal of Food Science & Technology, Oxford, 33*(2), 139–155.

Ali, H., Rico, A., Murshed-e-Jahan, K., & Belton, B. (2016). An assessment of chemical and biological product use in aquaculture in Bangladesh. *Aquaculture, 454*, 199–209.

Arfatahery, N., Davoodabadi, A., & Abedimohtasab, T. (2016). Characterization of toxin genes and antimicrobial susceptibility of *Staphylococcus aureus* isolates in fishery products in Iran. *Scientific Reports*, 1–7. https://doi.org/10.1038/srep34216

Assefa, A., & Abunna, F. (2018). Maintenance of fish health in aquaculture: Review of epidemiological approaches for prevention and control of infectious disease of fish. *Veterinary Medicine International, 2018*, 5432497.

Banerjee, S., Ooi, M. C., Shariff, M., & Khatoon, H. (2012). Antibiotic resistant Salmonella and Vibrio associated with farmed *Litopenaeus vannamei*. *The Scientific World Journal*, 130136. https://doi.org/10.1100/2012/130136

Baticados, M. C. L., & Paclibare, J. O. (1992). The use of chemotherapeutic agents in aquaculture in the Philippines. In I. M. Shariff, R. P. Subasinghe, & J. R. Arthur (Eds.), *Diseases in Asian aquaculture* (pp. 531–546). Fish Health Section, Asian Fisheries Society.

Baticados, M. C. L., Lavilla-Pitogo, C. R., Cruz-Lacierda, E. R., de la Peña, L. D., & Sunaz, N. A. (1990). Studies on the chemical control of luminous bacteria *Vibrio harveyi* and *V. splendidus* isolated from diseased *Penaeus monodon* larvae and rearing water. *Diseases of Aquatic Organisms, 9*, 133–139.

Benbrook, C. M. (2002). *Antibiotic drug use in US aquaculture*. Institute for Agriculture and Trade Policy. Report 2. https://www.iatp.org/sites/default/files/421_2_37397.pdf

Brown, J. H. (1989). Antibiotics – Their use and abuse in aquaculture. *World Aquaculture, 20*(2), 34–43.

Burridge, L., Weis, J. S., Cabello, F., Pizarro, J., & Bostick, K. (2010). Chemical use in salmon aquaculture: A review of current practices and possible environmental effects. *Aquaculture, 306*, 7–23.

Cabello, F. C., Godfrey, H. P., Tomova, A., Ivanova, L., Dolz, H., Millanova, A., & Buschmann, A. H. (2013). Antimicrobial use in aquaculture re-examined: Its relevance to antimicrobial resistance and to animal and human health. *Environmental Microbiology, 15*, 1917–1942. Accessed 3 June 2022.

Capone, D. G., Weston, D. P., Miller, V., & Shoemaker, C. (1996). Antibacterial residues in marine sediments and invertebrates following chemotherapy in aquaculture. *Aquaculture, 145*, 55–75.

Cassini, A., Hogberg, L. D., Plachouras, D., Quattrocci, A., Hoxha, A., Simosen, G. S., Kretzschmar, M. E., Devleesschauwer, B., Cecchini, M., Quakrim, D. A., Oliveira, T. C., Struelens, M. J., Suetens, C., & Monnet, D. L. (2019). Attributable deaths and disability-adjusted life-years caused by infections with antibiotic-resistant bacteria in the EU and the European economic area in 2015: A population-level modelling analysis. *The Lancet Infectious Diseases, 19*, 56–66. Accessed 3 June 2022.

CDC. (2019). *Antibiotic resistance threats in the United States*. U.S. Department of Health and Human Services, CDC.

Chen, H., Liu, S., Xu, X. R., Liu, S. S., Zhou, G. J., Sun, K. F., Zhao, J. L., & Ying, G. G. (2015). Antibiotics in typical marine aquaculture farms surrounding Hailing Island, South China: Occurrence, bioaccumulation and human dietary exposure. *Marine Pollution Bulletin, 90*, 181–187.

Chi, T. T. K., Clausen, J. H., Van, P. T., Tersbøl, B., & Dalsgaard, A. (2017). Use practices of antimicrobials and other compounds by shrimp and fish farmers in Northern Vietnam. *Aquaculture Reports, 7*, 40–47.

Costa, A. R., Araujo, R. L., Souza, O. V., & Vieira, R. H. S. F. (2015). Antibiotic-resistant vibrios in farmed shrimp. *BioMed Research International, 2015*, 505914. https://doi.org/10.1155/2015/505914. Accessed 3 June 2022.

Czekalski, N., Gascón Díez, E., & Bürgmann, H. (2014). Wastewater as a point source of antibiotic-resistance genes in the sediment of a freshwater lake. *The ISME Journal, 8*, 1381–1390.

Defoirdt, T., Sorgeloos, P., & Bossier, P. (2011). Alternatives to antibiotics for the control of bacterial disease in aquaculture. *Current Opinion in Microbiology, 14*, 251–258.

Done, H. Y., Venkatesan, A. K., & Halden, R. U. (2015). Does the recent growth of aquaculture create antibiotic resistance threats different from those associated with land animal production in agriculture? *The AAPS Journal, 17*, 513–524. https://doi.org/10.1208/s12248-015-9722-z

Edwards, P., Zhang, W., Belton, B., & Little, D. C. (2019). Misunderstandings, myths, and mantras in aquaculture: Its contribution to world food supplies has been systematically over reported. *Marine Policy, 106*, 103547.

Fair, R. J., & Tor, Y. (2014). Antibiotics and bacterial resistance in the 21st century. *Perspect Medicin Chem*. https://doi.org/10.4137/PMC.S14459

FAO. (2014). *The state of world fisheries and aquaculture*. Food and Agriculture Oraganization of the United Nations. https://doi.org/92-5-105177-1.

FAO. (2016). *The state of world fisheries and aquaculture, contributing to food security and nutrition for all*. Food and Agriculture Organization of the United Nations.

FAO. (2019). GLOBEFISH highlights January 2019 issue, with Jan. – Sep. 2018 Statistics – A quarterly update on world seafood markets. Globefish Highlights 4–68.

Feng, B., Liu, H., Wang, M., Sun, X., Pan, Y., & Zhao, Y. (2017). Diversity analysis of acute hepatopancreatic necrosis disease-positive *Vibrio parahaemolyticus* strains. *Aquaculture and Fisheries, 2*(6), 278–285.

Flegel, T. W. (2012). Historic emergence, impact and current status of shrimp pathogens in Asia. *Journal of Invertebrate Pathology, 110*(110), 166–173.

Guichard, B., & Licek, E. (2006). Antimicrobial resistance in aquaculture. A comparative study of antibiotics registered for use in farmed fish in European countries. Poster presented at the First OIE Global Conference on Aquatic Animal Health, 10 October, Bergen, Norway.

Guo, J., Boxall, A., & Selby, K. (2015). Do pharmaceuticals pose a threat to primary producers? *Critical Reviews in Environmental Science and Technology, l45*, 2565–2610. https://doi.org/10.1080/10643389.2015.1061873

Henriksson, P. J. G., Rico, A., Troell, M., Klinger, D. H., Buschmann, A. H., Saksida, S., Chadag, M. V., & Zhang, W. (2017). Unpacking factors influencing antimicrobial use in global aquaculture and their implication for management: A review from a systems perspective. *Sustainability Science, 13*, 1105–1120. Accessed 3 June 2022.

Heuer, O. E., Kruse, H., Grave, K., Collingnon, P., Karunasagar, I., & Angulo, F. J. (2009). Human health consequences of use of antimicrobial agents in aquaculture. *Clinical Infectious Diseases, 49*, 1248–1253.

Hinchliffe, S., Butcher, A., & Rahman, M. M. (2018). The AMR problem: Demanding economies, biological margins, and co-producing alternative strategies. *Palgrave Communications, 4*, 142.

Hua, L. M., & Apun, K. (2013). Antimicrobial susceptibilities of *Vibrio parahaemolyticus* isolates from tiger shrimps (Penaeus monodon) aquaculture in Kuching, Sarawak. *Research Journal of Microbiology, 8*(1), 55–62. https://doi.org/10.3923/jm.2013.55.62

Husevag, B., Lunestad, B. T., Johannessen, P. J., & Samuelsen, E. O. B. (1991). Simultaneous occurrence of *Vibrio salmonicida* and antibiotic-resistant bacteria in sediments at abandoned aquaculture sites. *Journal of Fish Diseases, 14*, 631–640. https://doi.org/10.1111/j.1365-2761.1991.tb00621. Accessed 3 June 2022.

Jana, T. K., Banerjee, R. D., Lahiri, S., & Jana, B. (2014). Management induced changes of antibiotic resistant strains of heterotrophic bacteria in shrimp farming ponds. *Indian Journal of Animal Sciences, 84*, 323–328.

Jones, M. K., & Oliver, J. D. (2009). *Vibrio vulnificus*: Disease and pathogenesis. *Infection and Immunity, 77*, 1723–1733.

Karunasagar, I. (2012). Public health and trade impact of antimicrobial use in aquaculture. Improving biosecurity through prudent and responsible use of veterinary medicines in aquaculture. In M. G. BondadReantaso, R. Arthur, & R. Subasinghe (Eds). *FAO Fisheriesand Aquaculture Technical Paper* 547.

Karunasagar, I., Pai, R., Malathi, G. R., & Karunasagar, I. (1994). Mass mortality of *Penaeus monodon* larvae due to antibiotic-resistant *Vibrio harveyi* infection. *Aquaculture, 128*, 203–209.

Labella, A., Gennari, M., Ghidini, V., Trento, I., Manfrin, A., Borrego, J. J., & Lleo, M. M. (2013). High incidence of antibiotic multi-resistant bacteria in coastal areas dedicated to fish farming. *Marine Pollution Bulletin, 70*, 197–203.

Le, T. X., & Munekage, Y. (2004). Residues of selected antibiotics in water and mud from shrimp ponds in mangrove areas in Viet Nam. *Marine Pollution Bulletin, 49*, 922–929.

Le, T. X., Munekage, Y., & Kato, S. I. (2005). Antibiotic resistance in bacteria from shrimp farming in mangrove areas. *Science of the Total Environment, 349*(1–3), 95–105. https://doi.org/10.1016/j.scitotenv.2005.01.006

Lin, C. K. (1989). Shrimp culture in Taiwan: What went wrong? *World Aquaculture, 20*, 19–20.

Liu, X., Steele, J. C., & Meng, X. Z. (2017). Usage, residue, and human health risk of antibiotics in Chinese aquaculture: A review. *Environmental Pollution, 223*, 161–169.

Lozano, I., Dıaz, N. F., Muñoz, S., & Riquelme, C. (2018). Antibiotics in Chilean aquaculture: A review. In S. Savic (Ed.), *Antibiotic use in animals*. https://doi.org/10.5772/intechopen.71780

MacFadden, D. R., McGough, S. F., Fisman, D., Santillana, M., & Brownstein, J. S. (2018). Antibiotic resistance increases with local temperature. *Nature Climate Change, 8*, 510.

Marhual, N. P., Das, B. K., & Samal, S. K. (2012). Characterization of *Vibrio alginolyticus* and *Vibrio parahaemolyticus* isolated from *Penaeus monodon*: Antimicrobial resistance, plasmid profile and random amplification of polymorphic DNA analysis. *African Journal of Microbiology, 6*, 4261–4269.

Martínez, J. L. (2003). Recent advances on antibiotic resistance genes. In M. Fingerman (Ed.), *Recent advances in marine biotechnology* (Molecular genetics of marine organisms') (Vol. 10, pp. 13–32). CRC Press.

Matyar, F., Kaya, A., & Dinçer, S. (2007). Distribution and antibacterial drug resistance of *Aeromonas* spp. from fresh and brackish waters in Southern Turkey. *Annales de Microbiologie, 57*, 443.

Molina-Aja, A., Garcia-Gasca, A., Abreu-Grobois, A., Bolan-Mejia, C., Roque, A., & Gomez-Gil, B. (2002). Plasmid profiling and antibiotic resistance of Vibrio strains isolated from cultured penaeid shrimps. *FEMS Microbiology Letters, 213*, 7–12.

Nair, G. B., Ramamurthy, T., Bhattacharya, S. K., Dutta, B., Takeda, Y., & Sack, D. A. (2007). Global dissemination of *Vibrio parahaemolyticus* serotype O3:K6 and its serovariants. *Clinical Microbiology Reviews, 20*(1), 39–48.

Navaneeth, K. A., Bhuvaneswari, T., Rajan, J. J. S., Alavandi, S. V., Vijayan, K. K., & Otta, S. K. (2020). Characterization of *Vibrio parahaemolyticus* isolates from shrimp farms of Southeast coast of India with special reference to acute hepatopancreatic necrosis disease (AHPND) status. *Aquaculture, 518*, 734813.

Oliveira, A. M. S., Barauna, R. A., Marcon, D. J., Lago, L. A. B., Silva, A., Lusio, J., Tavares, R. D. S., Tacao, M., Henriques, I., & Schneider, M. P. C. (2020). Occurrence, antibiotic resistance and virulence of *E. coli* strains isolated from mangrove oysters (*Crassostrea gasar*) farmed in estuaries of Amazonia. *Marine Pollution Bulletin, 157*, 111302.

Otta, S. K., Karunasagar, I., & Karunasagar, I. (2001). Bacteriological study of shrimp, *Penaeus monodon* fabricius, hatcheries in India. *Journal of Applied Ichthyology, 17*, 59–63.

Pei, R., Kim, S. C., Carlson, K. H., & Pruden, A. (2006). Effect of river landscape on the sediment concentrations of antibiotics and corresponding antibiotic resistance genes (ARG). *Water Research, 40*, 2427–2435.

Poirel, L., Jayol, A., & Nordmann, P. (2017). Polymyxins: Antibacterial activity, susceptibility testing, and resistance mechanisms encoded by plasmids or chromosomes. *Clinical Microbiology Reviews, 30*, 557–596.

Pokhrel, H., Baishya, S., Phukan, B., Pillai, D., & Rather, M. A. (2018). Occurrence and distribution of multiple antibiotic resistance bacteria of public health significance in brackish water and aquaculture farm. *International Journal of Current Microbiology and Applied Sciences, 7*, 975–987.

Primavera, J. H., Lavilla-Pitogo, C. R., Ladja, J. M., & dela Peña, M. (1993). A survey of chemical and biological products used in intensive prawn farms in the Philippines. *Marine Pollution Bulletin, 26*, 35–40.

Quintoil, M. N., Porteen, K., & Pramanik, A. K. (2007). Studies on occurrence of *Vibrio parahaemolyticus* in fin fishes and shellfishes from different ecosystem of West Bengal. *Livestock Research for Rural Development, 19*, 1.

Reboucas, R. H., de Sousa, O. V., Lima, A. S., Vasconcelos, F. R., de Carvalho, P. B., & Vieira, R. H. S. F. (2011). Antimicrobial resistance profile of Vibrio species isolated from marine shrimp farming environments (*Litopenaeus vannamei*) at Ceará, Brazil. *Environmental Research, 111*, 21–24. Accessed 3 June 2022.

Reilly, A., Lima, C., & DosSantos, P. M. (1997). Food safety and products from aquaculture. *WHO in the Tech Rep Ser., FAN 17*, 23.

Ren, Z., Cai, Y., Wang, S., Liu, S., Li, A., Xiong, Y., Tang, J., Sun, Y., Guo, W., & Zhou, Y. (2019). First case of *Aeromonas schubertii* infection in brackish water wild Nile tilapia, *Oreochromis niloticus* in China. *Aquaculture, 501*, 247–254.

Reverter, M., Sarter, S., Caruso, D., Avarre, J. C., Combe, M., Pepey, E., Pouyaud, L., Vega-Heredia, S., de Verdal, H., & Gozlan, R. E. (2020). Aquaculture at the crossroads of global warming and antimicrobial resistance. *Nature Communications, 11*, 1870. Accessed 3 June 2022.

Rico, A., Satapornvanil, K., Haque, M. M., Min, J., Nguyen, P. T., Telfer, T. C., & Van Den Brink, P. J. (2012). Use of chemicals and biological products in Asian aquaculture and their potential environmental risks: A critical review. *Review in Aquaculture, 4*, 75–93.

Rico, A., Phu, T. M., Satapornvanit, K., Min, J., Shahabuddin, A. M., Henriksson, P. J. G., Murray, F. J., Dalsgaard, A., Van Den Brink, P. J., & Little, D. C. (2013). Use of veterinary medicines, feed additives and probiotics in four major internationally traded aquaculture species farmed in Asia. *Aquaculture, 412–413*, 231–243. https://doi.org/10.1016/j.aquaculture.2013.07.028. Accessed 3 June 2022.

Rico, A., Jacobs, R., Van den Brink, P. J., & Tello, A. (2017). A probabilistic approach to assess antibiotic resistance development risks in environmental compartments and its application to an intensive aquaculture production scenario. *Environmental Pollution, 231*, 918–928. https://doi.org/10.1016/j.envpol.2017.08.079

Robinson, T. P., Bu, D. P., Carrique-Mas, J., Fevre, E. M., Gilbert, M., Grace, D., Hay, S. I., Jiwakanon, J., Kakkar, M., Kariuki, S., Laxminarayan, M., Lubroth, J., Magnusson, U., Thi Ngoc, P., Van Boeckel, T. P., & Woolhouse, M. E. J. (2016). Antibiotic resistance is the quintessential One Health issue. *Transactions of the Royal Society of Tropical Medicine and Hygiene, 110*, 377–380. https://doi.org/10.1093/trstmh/trw048. Accessed 3 June 2022.

Rocha, R. D. S., de Sousa, O. V., & Vieira, R. H. S. D. F. (2016). Multidrug-resistant Vibrio associated with an estuary affected by shrimp farming in Northeastern Brazil. *Marine Pollution Bulletin, 105*(1), 337–340. https://doi.org/10.1016/j.marpolbul.2016.02.001

Rodgers, C. J., & Furones, M. D. (2009). Antimicrobial agents in aquaculture: Practice, needs and issues. *Options Méditerranéennes, Montpellier, Série A, 86*, 41–59.

Romero, J., Feijoó, C. G., & Navarrete, P. (2012). Antibiotics in aquaculture use, abuse and alternatives. In E. D. Carvalho, G. Silva David, & R. J. Silva (Eds.), *Health and environment in aquaculture* (pp. 160–198). InTechOpen.

Roque, A., Molina-Aja, A., Bolan-Mejia, C., & Gomez-Gil, B. (2001). In vitro susceptibility to 15 antibiotics of vibrios isolated from penaeid shrimps in Northwestern Mexico. *International Journal of Antimicrobial Agents, 17*, 383–387.

Rotana, C., Wajjwalku, W., Boonyawiwat, V., Hrianpreecha, C., Thongratsakul, S., & Amavisit, P. (2018). Antimicrobial resistance and pirAB-like profiles of *Vibrio parahaemolyticus* in Pacific white shrimp. *Agriculture and Natural Resources, 52*, 377–381. Accessed 3 June 2022.

Sampson, T., Akani, N. P., & Hakam, O. (2020). Molecular characterization and detection of antibiotic resistance genes in *Pseudomonas* sp. isolated from *Tympanotonus fuscatus*. *Journal of Advances in Microbiology, 20*, 37–45.

Samuelsen, O. B., Torsvik, V., & Ervik, A. (1992). Long-range changes in oxytetracycline concentration and bacterial resistance towards oxytetracycline in a fish farm sediment after a medication. *Science of the Total Environment, 114*, 25–36.

Samuelsen, O. B., Lunestad, B. T., Ervik, A., & Fjelde, S. (1994). Stability of antibacterial agents in an artificial marine aquaculture sediment studied under laboratory conditions. *Aquaculture, 126*, 283–290. https://doi.org/10.1016/0044-8486(94)90044-2

Scarano, C., Spanu, C., Ziino, G., Pedonese, F., Dalmasso, A., Spanu, V., Virdis, S., & De Santis, E. P. L. (2014). Antibiotic resistance of Vibrio species isolated from *Sparus aurata* reared in Italian mariculture. *New Microbiologica, 37*, 329–337.

Shoemaker, C. A., Evans, J. J., & Klesius, P. H. (2000). Density and dose: Factors affecting mortality of *Streptococcus iniae* infected tilapia (Oreochromis niloticus). *Aquaculture, 188*, 229–235.

Smith, P. (2008). Antimicrobial resistance in aquaculture. *Revue Scientifique et Technique (International Office of Epizootics), Paris, 27*, 243–264.

Smith, P. (2012). Antibiotics in aquaculture; Reducing the use and maintaining the efficacy. In B. Austin (Ed.), *Infectious diseases in aquaculture* (pp. 161–189). Woodhead.

Smith, P., Hiney, M. P., & Samuelsen, O. B. (1994). Bacterial resistance to antimicrobial agents used in fish farming: A critical evaluation of method and meaning. *Annual Review of Fish Diseases, 4*, 273–313. https://doi.org/10.1016/0959-8030(94)90032-9

Stalin, N., & Srinivasan, P. (2016). Molecular characterization of antibiotic resistant *Vibrio harveyi* isolated from shrimp aquaculture environment in the south east coast of India. *Microbial Pathogenesis, 97*, 110–118.

Su, H., Liu, S., Hu, X., Xu, X., Xu, W., Xu, Y., Li, Z., Wen, G., Liu, Y., & Cao, Y. (2017). Occurrence and temporal variation of antibiotic resistance genes (ARGs) in shrimp aquaculture: ARGs dissemination from farming source to reared organisms. *Science of the Total Environment, 607–608*, 357–366. Accessed 3 June 2022.

Tendencia, E. A., & de la Pena, L. D. (2001). Antibiotic resistance bacteria from shrimp ponds. *Aquaculture, 195*, 193–204.

Thornber, K., Verner-Jeffreys, D., Hinchliffe, S., Rahman, M. M., Bass, D., & Tyler, C. R. (2020). Evaluating antimicrobial resistance in the global shrimp industry. *Reviews in Aquaculture, 12*, 966–986.

Van Boeckel, T. P., Brower, C., Gilbert, M., et al. (2015). Global trends in antimicrobial use in food animals. *Proceedings of the National Academy of Sciences, 112*, 5649–5654. https://doi.org/10.1073/pnas.1503141112

Wang, H., Ren, L., Yu, X., Hu, J., Chen, Y., He, G., & Jiang, Q. (2017). Antibiotic residues in meat, milk and aquatic products in Shanghai and human exposure assessment. *Food Control, 80*, 217–225.

WHO. (2007). http://www.who.int/foodsafety/publications/micro/pif_guidelines.pdf?ua=1. Accessed 2 Feb 2017.

Xu, Y., Wang, C., Zhang, G., et al. (2017). ISCR2 is associated with the dissemination of multiple resistance genes among *Vibrio* spp and *Pseudoalteromonas* spp isolated from farmed fish. *Archives of Microbiology, 199*, 891–896. https://doi.org/10.1007/s00203-017-1365-2

Yang, J., Wang, C., Shu, C., Liu, L., Geng, J., Hu, S., & Feng, J. (2013). Marine sediment bacteria harbor antibiotic resistance genes highly similar to those found in human pathogens. *Microbial Ecology, 65*, 975–981.

Yano, Y., Hamano, K., Satomi, M., Tsutsui, I., & Aue-umneoy, D. (2011). Diversity and characterization of oxytetracycline-resistant bacteria associated with non-native species, white-leg shrimp (*Litopenaeus vannamei*), and native species, black tiger shrimp (*Penaeus monodon*), intensively cultured in Thailand. *Journal of Applied Microbiology, 110*, 713–722.

Yano, Y., Hamano, K., Satomi, M., Tsutsui, I., Ban, M., & Aue-umneoy, D. (2014). Prevalence and antimicrobial susceptibility of Vibrio species related to food safety isolated from shrimp cultured at inland ponds in Thailand. *Food Control, 38*, 30–36.

Yano, Y., Hamano, K., Tsutsui, I., Aue-umneoy, D., Ban, M., & Satomi, M. (2015). Occurrence, molecular characterization, and antimicrobial susceptibility of *Aeromonas* spp. in marine species of shrimps cultured at inland low salinity ponds. *Food Microbiology, 47*, 21–27.

Zhang, Y. B., Li, Y., & Sun, X. L. (2011). Antibiotic resistance of bacteria isolated from shrimp hatcheries and cultural ponds on Donghai Island, China. *Marine Pollution Bulletin, 62*, 2299–2307.

Antimicrobial Resistance in Ornamental Fisheries: Causes and Preventive Measures

Lekshmi Narendrakumar, Prasannan Geetha Preena, and Thangaraj Raja Swaminathan

Contents

Abstract

Ornamental fisheries are a multibillion business and livelihood of many, especially in the developing countries. India also plays an important role in ornamental fisheries and stands at 26th position in the international ornamental fish trade. Though the sector is flourishing with advanced hatchery technologies, there have been also anomalies in profit making in the sector due to fish diseases and associated mortality. The incidence of diseases in ornamental fishes, especially bacterial infections, are common and it adversely affects the export. To overcome the economic loss, farmers frequently use antibiotics in aquaculture systems which have been identified as a major driver for antimicrobial resistance (AMR). AMR is now considered a hot topic as there are no strict rules and regulations for the use of antibiotics in ornamental fisheries unlike that in edible fish culture. There have been increasing reports of resistance development and transfer of antibiotic resistance genes (ARGs) in both Gram-negative and Gram-positive bacterial pathogens as well as environmental bacteria that is known to have a colossal impact on the aquatic ecosystem. This chapter summarizes the major drivers of AMR in ornamental fisheries and the challenges, diversity of

L. Narendrakumar · P. Geetha Preena · T. R. Swaminathan (✉)
Peninsular and Marine Fish Genetic Resources Centre, ICAR National Bureau of Fish Genetic Resources, CMFRI Campus, Kochi, Kerala, India
e-mail: t.swaminathan@icar.gov.in

M. P. Mothadaka et al. (eds.), *Handbook on Antimicrobial Resistance*,
https://doi.org/10.1007/978-981-19-9279-7_9

antimicrobial-resistant bacteria, modes of resistance acquisition, and lastly different management strategies and alternative approaches to reduce AMR.

Keywords

Antimicrobial resistance · Aquaculture · *Mycobacterium* species · Ornamental fisheries · Probiotics · Vaccines

1 Introduction

The ornamental fisheries sector has become an indispensable component of international wildlife trade from the past decade. Interestingly, the developing countries account for almost two-thirds of the total ornamental fish trade, and it plays an integral role in their revenue generation and development. Singapore and other East-Asian countries account for 80% of the global trade, and about 500 million US$ worth ornamental fishes are imported to the USA annually (Satam et al., 2018). Ornamental fisheries include culturing and trade of both fresh water and marine ornamental fishes. Southeast Asian countries account for about 68% of marine ornamental fish trade, which is estimated at 200–300 million US$ (Chogale et al., 2017). India is endowed with more than 400 marine and 375 freshwater species of native ornamental fishes and exports 287 native fishes. The Northeastern region of India is the most potential area for ornamental fisheries in India accounting for 85% of ornamental fish export from India. Apart from the Northeastern region, the Western Ghats serves as the traditional center for ornamental fishes from its riverine systems. The Southern states rear Goldfish, Angelfish, Mollies, and Fighter fishes while Andaman and Nicobar islands, Lakshadweep reefs, Gulf of Kutch to Mumbai, Gulf of Mannar, and Palk Bay contribute to India's marine ornamental fisheries. In India, the internal ornamental fish trade is estimated to be about Rs 25 crores (∼4 million USD) while export of ornamental fishes yields approximately 10 crores (∼1.6 million USD) (Krishnakumar, 2017). However, India stands at 26th position in the international ornamental fish trade.

Though ornamental fisheries is an multibillion business, there are various factors that have to be considered with utmost importance to have a profit. One of the major constraints in ornamental fishery is the lack of trained personnel with knowledge in nutrition, breeding, and disease management. Further, there is also lack of information on feeding, reproductive habits, and diseases of indigenous ornamental fishes. There have been various viral, bacterial, and fungal diseases reported in ornamental fishes. The most commonly reported viral disease in ornamental fish is the lymphocystis disease (Cardoso et al., 2019), while the most common fungal disease reported is caused by *Saprolegnia* (Shin et al., 2017). Gram-negative bacterial diseases are more predominant in ornamental fishes than Gram-positive bacterial infections (Preena et al., 2020a). Major Gram-negative bacterial pathogens of ornamental fishes include pathogens of genera: *Aeromonas, Citrobacter, Edwardsiella, Flavobacterium, Mycobacterium, Pseudomonas,* and *Vibrio* (Zanoni et al., 2008; Sreedharan et al., 2012; Walczak et al., 2017; Wahli & Madsen, 2018).

Streptococcus and *Staphylococcus* are the main Gram-positive pathogens identified to cause bacterial infections in ornamental fishes (Ruane et al., 2013; Pękala-Safińska, 2018). Almost all the bacterial pathogens of fish are natural inhabitants of aquatic system itself. It is primarily the external factors like environmental stressors, poor water quality, inadequate nutrition, and overcrowding that act as predisposing factors for bacterial diseases (Lewbart, 2001).

The incidence of bacterial diseases in ornamental fishes adversely affects the export, and to overcome the economic loss, farmers frequently use antibiotics in aquaculture systems. The increasing use of antibiotics and other antimicrobials in aquaculture has also caused a rise in antimicrobial resistance (AMR) development in many bacterial pathogens (Watts et al., 2017). It has been noted that pathogens that are resistant to multiple antibiotics get transferred from aquaculture environment to natural environment, leading to the transfer of AMR genes to natural aquatic bacterial flora which under stress causes infection in wild fishes. This flow of AMR genes from aquaculture systems to humans can be either direct or indirect; however, the consequences remains the same (Watts et al., 2017). Hence, aquaculture systems and fish farms are now considered as "hot spots" of AMR as they can propagate AMR genes to other bacteria in the environment as well. Hot spots of AMR are regions that contain a higher proportion of AMR pathogens and associated antibiotic-resistant genes (ARG). These hot spots also facilitate the spread of resistant bacteria and ARGs. The fact that most of the antibiotics used in aquaculture are also being used to treat human infections aggravates the severity of the spread of AMR. Around 51 antibiotics that are recommended by the Food and drug Administration (FDA) that can be used in aquaculture have been prescribed to humans as well and of them, about six classes have been listed as critically important antimicrobials by the World Health Organization (WHO) (Preena et al., 2020a). This chapter expounds on the major causes and adverse implications of AMR in ornamental fisheries and ways to overcome the crisis.

2 Use of Antibiotics in Ornamental Fisheries and Major Drivers of AMR

Antibiotics have been widely used in ornamental fisheries to keep primarily bacterial diseases at bay. Most antibiotics at its specific dosage do not kill the bacteria, but inhibit the bacterial growth thereby providing the immune system of the fish sufficient time to clear the bacteria. There are specific antibiotics to treat Gram-negative and Gram-positive bacterial pathogens. Selection and proper administration of antibiotics are important factors for controlling bacterial diseases in ornamental fisheries. Further, most of the antibiotics used in ornamental fisheries are sold by different companies and their composition of active ingredients varies, and thus standardization of appropriate dosage is very important. Injection, oral administration, and bath treatments are the three major routes by which antibiotics are administered to fishes (Heuer et al., 2009). The basis for choosing an antibiotic for the treatment of a disease is its efficacy, availability, safety, and cost. Further, it is also

important to know the local resistance pattern of the pathogen for choosing the appropriate antibiotic. Hence, it is highly recommended that the resistance pattern of the causative organism is known. Apart from antibiotics, other predominantly used chemicals include disinfectants, formalin, malachite green, potassium permanganate, and methylene blue. Formalin, malachite green, potassium permanganate, and methylene blue are used to treat the fungal and parasitical diseases.

Officially, there are only a few FAO-approved antibiotics (Oxytetracycline, florfenicol, sulfonamides, erythromycin, and sarafloxacin) to treat ornamental fishes (FAO, 2005). Florfenicol, oxytetracycline, and sulfadimethoxine/ormetoprim are FDA-approved drugs for aquaculture use. Five drugs, namely, Oxytetracycline, Sulfamerazine, Ormetoprim, Formalin, and Tricaine methanesulfonate are the only FDA-legalized drugs used in US aquaculture (FDA, 1998). In India, chloramphenicol followed by oxytetracycline and erythromycin is widely used (Chanda et al., 2011). No antibiotics are approved for aquaculture in countries like Japan, Australia, and South Africa (Schar et al., 2020). Though such regulations are followed by edible fish aquaculture, no strict regulations are followed in ornamental aquaculture. However, in order to maintain the health of ornamental fishes during rearing and shipment, antibiotics are often used in hatcheries and shipping waters (Kleingold et al., 2001) . Previous reports of Kleingold et al., had revealed that majority of the ornamental fishes being exported contained residues of antibiotics and was a major cause of increase in resistance index to enrofloxacin (Kleingold et al., 1996). Few of the antibiotics often used in aquaculture and their spectrum are as follows: Erythromycin and penicillin antibiotics such as ampicillin and amoxicillin have been identified to be most effective to treat Gram-positive bacteria such as *Streptococcus* spp. and *Staphylococcus* spp. Aminoglycosides, including gentamicin, neomycin, kanamycin, and amikacin, have been identified to be effective to treat Gram-negative bacteria. Tetracycline, florfenicol, and quinolone antibiotics are broad-spectrum antibiotics that act upon both Gram-negative and Gram-positive bacteria and have been extensively used in ornamental fisheries (Lulijwa et al., 2020).

In most of the cases, the application of antibiotics is not supervised by any scientific experts and is based on the experience of farmers. However, due to the increased and indiscriminate use of antibiotics, there have been reports of resistance development in major pathogens of ornamental fishes. Tetracycline resistance was the most frequently reported, followed by penicillins and sulfonamides (Sicuro et al., 2020). In cage-cultured fishes, antibiotic use and thus antibiotic resistance was identified to be lesser due to the complexity of antibiotic use (Neela et al., 2014). Whereas, the antibiotic use in pond systems, pools, and tank cultures are extremely high and act as a suitable place for bacterial adaptation to antibiotics (Neela et al., 2014). Further, disposal of untreated water from these culture systems contaminate natural aquatic water bodies, thereby increasing antibiotic adaptation of aquatic bacteria (Kumar et al., 2017). There have been reports of sulfonamide, quinolone, and erythromycin-resistant bacterial pathogens isolated from wild fishes collected from areas near open and close culture systems (Marti et al., 2018). Antibiotic-resistant pathogens from wild fishes are considered to be biological indicators of antibiotic contamination of natural environment.

The major drivers of antibiotic resistance in ornamental fisheries are the poor hygiene and sanitation and unwanted use of antibiotics during culture and shipment. The continuous use of antibiotics as prophylactic and therapeutic agents has been identified to cause selective pressure on microbial community, which is one of the major reasons for AMR (Gao et al., 2012; Narendrakumar et al., 2020). Egested food and fish feces have been identified to retain residues of antibiotics (Burridge et al., 2010). Residues of antibiotics have also been identified from fishery products (Sørum, 2006). Extended uses of antibiotics in veterinary medicine and agriculture practices have also been identified to provoke selection of resistance genes and spread of resistant bacteria into aquaculture (Aarestrup, 2005; Cabello, 2006). Suboptimal rapid diagnostics and suboptimal preventative medicines and vaccines are also other important factors that cause rise in antibiotic usage.

3 Diversity of Antimicrobial-Resistant Pathogens

In ornamental fishes, the most commonly encountered bacterial infections are caused by pathogen of the Aeromonadaceae family. *Aeromonas hydrophila, A. veronii, A. caviae, A. media*, *A.aquariorum, A.jandaei, and A.culicicola* have been associated with septicemia and ulceration in ornamental fishes (Walczak et al., 2017). Sulfathiazole and tetracycline resistance was first reported from *A. salmonicida* (Snieszko and Bullock, 1957). There have been reports of *A. caviae, A. sobria,* and *A. hydrophila* isolated from ornamental fishes such as gold fish, koi carp, red sword tail, Oscar, and sucker to more than 80% of antibiotics used commonly in ornamental fisheries. Reduced resistance of *A. veronii isolated from* cichlid Oscar, *Astronotus ocellatus* was reported by Sreedharan et al. (2011). Moreover, Sreedharan et al. (2012) have reported identification of many transferrable antimicrobial-resistant genes (ARGs) in pathogens of Aeromonadaceae family infecting ornamental fishes (Sreedharan et al., 2012). Additionally, significantly increased resistance towards nalidixic acid, tetracycline, and erythromycin antibiotics have been reported from *A.dhakensis* isolated from ornamental fishes (Jagoda et al., 2014). Further isolation of multidrug-resistant *A. hydrophila* from goldfish co-infected with cyprinid herpesvirus-2 was reported by Sahoo et al. (2016).

Pseudomonas anguilliseptica is another major pathogen of ornamental fishes which has been identified to cause *"Winter Disease Syndrome"* (Sasmal et al., 2004; Magi et al., 2009). Most of the bacteria in the Pseudomonas sp. are members of the natural flora of the fish, but act as opportunistic pathogens under stress conditions. Multidrug-resistant pathogens like *Aeromonas* sp., *Pseudomonas* sp., and *Acinetobacter* sp. associated with guppy fishes and *Edwardsiella tarda*, *Lactococcus, Aeromonas, Comamonas, Pseudomonas* associated with gold fish and koi carp were recently reported by Preena et al. (2019, 2020b). Previously, Dharmaratnam et al. (2018) had reported isolation of multidrug-resistant *Serratia marcescens* from guppy fishes. Multidrug-resistant *Citrobacter freundii, Klebsiella pneumoniae,* and *Proteus hauseri* were identified to be the reason for more than 50% cumulative mortality of moribund koi carp (Kumar et al., 2015). Interestingly, these

pathogens were identified to be phylogenetically very similar to pathogenic bacteria causing urinary tract infections (UTI) in humans (Kumar et al., 2015).

Enterobacter cancerogenus, *E. cloacae*, *E. ludwigii*, *Plesiomonas shigelloides*, *Providencia vermicola*, *Kluyvera cryocrescens,* and *Escherichia coli* possessing reduced susceptibility to most of the antibiotics used to treat Gram-negative pathogens have been previously isolated from infected ornamental fishes (Austin and Austin, 2016). *P. penneri* resistant to more than 15 antibiotics and *P. hauseri* and *P. vermicola* resistant to 14 antibiotics have been reported to be isolated from infected gold fish (Preena et al., 2019). These pathogens were identified to be resistant towards even the fourth-generation cephalosporins. In the same study, *E. cancerogenus* was identified to be resistant towards nine antibiotics. Multidrug-resistant Enterobacteriaceae have been previously isolated from farmed catfish (Sarter et al., 2007). Further, aminoglycoside resistance has been observed from bacterial pathogens isolated from ornamental fishes like goldfish, and most of these pathogens were identified to produce β-lactamases (Liakopoulos et al., 2016). These pathogens were also identified to possess extended spectrum β-lactamases (ESBL) genes that confer the β-lactam resistance. Besides aminoglycoside, tetracycline, and sulfa drug resistance, bacterial pathogens isolated from ornamental fishes were also identified to be resistant to macrolides, first-generation cephalosporins, polymyxin antibiotics, and nitrofurans (Preena et al., 2019). Such reports of emerging and increasing antibiotic-resistant bacterial pathogens associated with ornamental fishes are a major cause of concern among aquarium keepers, fish farmers, and government. Few major pathogens of ornamental fishes, diseases caused, and antibiotics used are enumerated in Table 1.

4 Challenges of Antimicrobial Resistance in Ornamental Fisheries

The major challenge of antimicrobial resistance in ornamental fisheries is the acquisition of zoonotic infections caused by multidrug-resistant pathogens by human. There have been many zoonotic diseases reported in personnel handling ornamental fishes or pet fishes during rearing or aquarium management. Most of the zoonotic diseases associated with ornamental fish contact are primarily bacterial infections. These include infections caused by *Mycobacterium, Erysipelothrix, Campylobacter*, *Aeromonas*, *Vibrio*, *Edwardsiella, Escherichia, Salmonella, Klebsiella,* and *Streptococcus iniae.* Also, *Lactococcus garvieae,* an emerging fish pathogen has also been associated to cause zoonotic infection in humans (Gibello et al., 2016). Sometimes, there will not be any evident symptoms in the fish infected with the MDR pathogen, but it can cause zoonotic infection in humans. Further, immunocompromised persons with specific medical conditions have been identified to be at a higher risk of developing complications from zoonotic disease.

Antibiotic-resistant *Mycobacterium* species including *Mycobacterium marinum, M. fortuitum,* and *M. chelonei* have been associated with acute or chronic atypical mycobacteriosis in humans mostly acquired from fish that are asymptomatic carriers (Hashish et al., 2018). Apart from the multidrug resistance of mycobacterium,

Table 1 List of major pathogens of ornamental fishes, diseases caused, and antibiotics found effective

Organism	Disease	Reported in	Effective antibiotic[a]
Aeromonas veronii	Abdominal dropsy	Oscar *(Astronotus ocellatus)*	Third-generation cephalosporins, carbapenems, ciprofloxacin
Aeromonas hydrophila	Red sore disease, Hemorrhagic septicemia	Goldfish (*Carassius auratus*), golden shiner (*Notemigonus crysoleucas*), walking catfish (*Clarias batrachus*)	Third-generation cephalosporins, carbapenems, ciprofloxacin
Aeromonas sobria	Septicemia	Goldfish (*C. auratus*)	Third-generation cephalosporins, carbapenems, ciprofloxacin
Aeromonas salmonicida	Furunculosis, Carp Erythrodermatitis	Goldfish (*C. auratus*), mirror carp (*Cyprinus carpio*)	Oxytetracycline, sulfadimethoxine, and ormetoprim
Edwardsiella ictaluri	Enteric septicaemia	White catfish (*Ameiurus catus*), brown bullhead (*Ameiurus nebulosus*), ayu (*Plecoglossus altivelis),* green knife fish (*Eigemannia virescens)*	Tetracycline, chloramphenicol, nitrofurantoin, and fosfomycin
Edwardsiella tarda	Edwardsiella septicaemia	Speckled longfin eel (*Anguilla reinhardtii*), Siamese fighting fish (*Betta splendens*)	Gentamycin and ciprofloxacin
Flavobacterium branchiophilum	Bacterial gill disease	Sunfish (*Leopomis* spp.), yellow perch (*Perca flavescens*)	Ciprofloxacin and cotrimoxazole
Flavobacterium columnare	Columnaris disease	Channel catfish (*Ictalurus punctatus*), goldfish (*C. auratus)*	Ciprofloxacin and cotrimoxazole
Flavobacterium psychrophilum	Bacterial cold water disease	Goby (*Chaenogobius urotaenia),*ayu (*P. altivelis),* goldfish (*C. auratus langsdorfii)*	Florfenicol, oxytetracyycline
Francisella spp.	Francisellosis	Three-lined grunt, *Parapristipoma trilinineatum*	Ciprofloxacin, gentamicin
Lactococcus garvieae	Hemorrhagic septicaemia	Lemon damsel (*Pomacentrus moluccensis*), goldfish (*C. auratus*), Koi carp (*C.rubrofuscus*)	Erythromycin, oxytetracycline, amoxiline, and doxycycline

(continued)

Table 1 (continued)

Organism	Disease	Reported in	Effective antibiotic[a]
Mycobacterium spp.	Mycobacteriosis	Tiger barb (Barbus tetrazona), *Siamese fighting fish (B. splendens)*, *goldfish (C. auratus)*, Sailfin molly (*Poecilia latipinna*), angelfish (*Pterophyllum scalare*), Guppy (*P. reticulata*)	Tigecycline, tobramycin, clarithromycin, and amikacin
Piscirickettsia salmonis	Piscirickettsiosis	Blue-eyed plecostomus *(Panaque suttoni*)	Florfenicol and oxytetracycline
Plesiomonas shigelloides	External lesions, impaired swimming, and exophthalmia	*Goldfish (C. auratus)*, algae eaters (*Bristlenose plecos*), *Asian arowana* (*Scleropages formosus*), and cichlids	Norfloxacin, ciprofloxacin, and trimethoprim
Pseudomonas spp.	Pseudomoniasis, fin rot	Goldfish (*C.auratus*), Koi (Anabas testudineus), Guppy (*P. reticulata*)	Tetracycline, penicillin, and naladixic acid
Streptococcus spp.	Hemorrhage, exophthalmia, abdominal distension, ascites, lesions (liver, kidney, spleen, and intestine)	*Rainbow shark* (*Epalzeorhynchos frenatum*), doctor fish, (*Garra rufa)*,red-tailed black shark (*Epalzeorhynchos bicolor*), *rosy barb* (*Pethia conchonius*), Danios, Venustus (*Nimbochromis venustus*)	Gentamicin
Vibrio spp.	Vibriosis	Lemon damsel, caerulean damsel pearl-spot chromis and ocellaris clownfish, mandarin fish	Chloramphenicol, erytromycin, enrofloxacin
Yersinia ruckeri	Enteric redmouth disease	Goldfish (*Carassius auratus)*, emerald shiner (*Notropis atherinoides*)	Isolates resistant to most of the antibiotics

[a]The rationale for choosing an antibiotic to treat the infection is based on the local resistance pattern of the pathogen

biofilm, and thick cell wall of mycobacterium makes atypical mycobacteriosis difficult to treat in humans. *S. iniae* is another multidrug-resistant pathogen that has been associated with cellulitis, arthritis, endocarditis, meningitis, or death in infected persons (Mishra et al., 2018). Further, there has been also a report of antibiotic-resistant *E. rhusiopathiae* isolated from wound infection of person having frequent contact with aquarium fish (Rihana et al., 2018). *Aeromonas* is another major

multidrug-resistant wound-associated pathogen commonly reported in personnel rearing ornamental fishes (Noga, 2010; Dias et al., 2012). Further, there has been report of the spread of Salmonella Java infections from the tropical ornamental fish aquarium to human infants (Threlfall et al., 2005).

The presence of antibiotics or antibiotic-resistant pathogens significantly hampers ornamental fish exports (Sicuro et al., 2020). Analysis of imported ornamental fishes in Norway revealed that they harbored many bacterial pathogens. About 84% of the bacterial pathogens isolated were resistant to tetracycline, 52% isolates were resistant to flumequine, 34% isolates were resistant to neomycin, and 30% isolates to trimethoprim and sulfa drugs (Sicuro et al., 2020). Moreover, 98% of the transport water analyzed revealed to contain one or more antibiotics, predominantly tetracycline and quinolone. Also, 68% contained nitrofurans, 36% contained chloramphenicol, and 14% contained nonlicensed malachite green (Sicuro et al., 2020). Strong correlation of presence of antibiotic-resistant pathogen and antibiotic presence in transport water was identified. In many countries, there are laws that prohibit import of ornamental fishes that tests positive for either antibiotics or fish pathogens.

5 Management Strategies and Alternate Approaches to Mitigate Antimicrobial Resistance

Considering the impact of the growing concern of AMR in ornamental fisheries, it is imperative to develop and pursue alternative strategies that would decrease the global AMR burden. In order to develop novel alternative strategies, the primary objective would be to identify the major pathogens of fishes and understand their resistance profile. Development of novel, rapid, and cost-effective bacterial and resistance gene detection methods that are valid both nationally and internationally is the need of the hour. Awareness on zoonotic pathogens and the ways by which antimicrobial resistance evolve in pathogens have to be given to the general public. Enforcement of strict rules on the indiscriminate use of antibiotics has to be laid. Analysis of transport water for antibiotics and bacterial count has to be made mandatory before exporting, and ornamental fish farms have to be regularly inspected to ensure that it complies with export standards. Active surveillance and monitoring for antibiotic-resistant potential zoonotic bacteria in ornamental fish has to be followed. Educational material has to be made available to ornamental fish farmers and employees to increase awareness on the proper use of antibiotics and potential zoonotic diseases that could be transmitted. Further, training programs on proper diagnosis of ornamental fish infections should be made available to the farmers as there is high incidence of using antibiotics for viral diseases due to ignorance. The ornamental fish farmers have to be made aware of proper pond preparation and liming, use of potassium permanganate in cleaning the ponds, and use of water purification systems to control bacterial diseases (Kent et al., 2009). Additionally, the fish farmers should be made aware of the importance of treating pond/tank water before releasing into the environment. Organizations/ companies to

undertake research and development and impart training and knowledge on the aqua technology have to be set up in every states.

Effective alternatives to antibiotics such as vaccines, probiotics, anti-virulent agents should be used to curb AMR. Oral fish vaccines have been effective in controlling several diseases in fishes and shrimps (Newaj-Fyzul and Austin, 2015). Vaccines against virulent *Aeromonas hydrophila* using the bacterial whole cell (WC), extracellular product (ECP), outer membrane protein (OMP), and biofilm (BF) was developed by Thanga Viji et al. (2013) and its protection ability was demonstrated in goldfish (Thanga Viji et al., 2013). *Edwardsiella ictalurii* and *Flavobacterium columnare* vaccine have been developed to prevent Edwardsiellosis and Columnaris disease in catfish (Sommerset et al., 2005). Further, *Edwardsiella ictaluri* bacterin vaccines were developed to prevent enteric septicemia in Japanese flounder (Dadar et al., 2017). Modified vaccines such as chitosan nanoparticle incorporated DNA vaccine against *V. parahaemolyticus* was developed by Li et al. (2013). However, the response to vaccines depends largely on water temperature and, generally, higher water temperature aids better immune response. Further, for most of the fish vaccines, more than one dose is required and protection duration is short-lived (Vinitnantharat et al., 1999). Additionally, vaccines are not available for all bacterial diseases of ornamental fishes, and thus other alternatives are widely used to prevent infections.

Probiotics that are live microorganisms which provide health benefit to the host is used both as prophylactic as well as therapeutic agents in aquaculture. The health-promoting properties of probiotics include disease control, growth promotion, improved immune system, and improved nutrition. Probiotics are widely used in ornamental fisheries as a dietary supplement (Das et al., 2017). Moreover, water quality is an important criterion to prevent diseases in fishes. Probiotic bacteria that have bioremediation activity can improve water quality and prevent pathogenic bacterial growth in farms as well as during transportation (Iribarren et al., 2012; Ibrahem, 2015). *Bacillus subtilis*, *Bacillus cereus,* and *Bacillus licheniformis* have been identified to be potential probiotics that can improve water quality and reduce heavy metal load (Kim et al., 2005). Similarly, *Rhodopseudomonas palustris*, *Lactobacillus plantarum*, *Lactobacillus casei,* and *Saccharomyces cerevisiae* have been attributed to probiotic potential in the maintenance of water quality (Melgar Valdes et al., 2013). Probiotics that have antibacterial activity against fish pathogens like *A. hydrophila, Edwardsiella tarda, V. harveyi* have been identified and used in many fish farms in Asia (Lin et al., 2017). *Pseudoalteromonas* sp. has been identified to be a potent probiotic that has antibacterial activity against a wide variety of fish pathogens (Sayes et al., 2016). *Lactobacillus plantarum* (LP20), *Bacillus* sp., *Pediococcus* sp. *B.licheniformis, Lactobacillus thuringiensis, B. plantarum, and B. subtilis* (B46) have been identified to improve immune health of various ornamental fishes of economic importance (Batista et al., 2015; Dawood et al., 2015; Bahi et al., 2017).

Many plant extracts such as mango, peppermint, turmeric, jasmine, neem, etc. have showed potential antibacterial activity against major fish pathogens of Aeromonadaceae family (Newaj-Fyzul & Austin, 2015). Bansemir et al. (2006)

had demonstrated potent antibacterial activity of seaweeds such as *Ceramium rubrum*, *Gracilaria cornea,* and *Asparagopsis armata* against *Vibrio anguillarum* and *Pseudomonas anguilliseptica* (Bansemir et al., 2006). Apart from the antibacterial activity, the use of antivirulent natural compounds has recently gained popularity in aquaculture due to its safety profiles and cost-effectiveness. It has been identified that resistance development of pathogens towards these antivirulent compounds are much lower than antibacterial compounds. Many compounds that reduce the virulence gene expression and disrupt the biofilm formation of pathogenic bacteria have been successfully demonstrated both *in vitro* and *in vivo* (Defoirdt et al., 2011).

The actions based on evidence gained through a One Health approach have to be followed. Though the reports of reduction in production, consumption (agriculture and livestock), and pollution by antibiotics are relieving, the presence of already existing active residues in the environmental waters is a cause of concern. Personnel from different sectors such as public health, animal health, plant health, and the environment have to join hands to protect the environment from further antibiotic pollution. The major objective of the One Health approach is to efficiently detect, respond, and prevent outbreaks of zoonotic pathogens and tackle food safety problems. In the One Health approach, information about outbreaks, therapeutics used, epidemiology of the disease, and laboratory results on AMR and new resistance evolved in bacteria should be shared across sectors. Apex-level AMR monitoring working group represented by all the stakeholders should scale up the existing AMR relevant activities and play a prominent role in steering AMR agenda forward in the frontline while strictly regulating the antibiotics for human and animal consumption.

References

Aarestrup, F. M. (2005). Veterinary drug usage and antimicrobial resistance in bacteria of animal origin. *Basic and Clinical Pharmacology and Toxicology, 96*(4), 271–281. https://doi.org/10.1111/j.1742-7843.2005.pto960401.x

Austin, B., & Austin, D. A. (2016). Enterobacteriaceae representatives. In: *Bacterial fish pathogens*. Springer.

Bahi, A., Guardiola, F. A., Messina, C., Mahdhi, A., Cerezuela, R., Santulli, A., & Esteban, M. A. (2017). Effects of dietary administration of fenugreek seeds, alone or in combination with probiotics, on growth performance parameters, humoral immune response and gene expression of gilthead seabream (Sparus aurata L.). *Fish & Shellfish Immunology., 60*, 50–58.

Bansemir, A., Blume, M., Schröder, S., & Lindequist, U. (2006). Screening of cultivated seaweeds for antibacterial activity against fish pathogenic bacteria. *Aquaculture, 252*, 79–84. https://doi.org/10.1016/j.aquaculture.2005.11.051

Batista, S., Ramos, M. A., Cunha, S., Barros, R., Cristóvão, B., Rema, P., & Ozório, R. O. A. (2015). Immune responses and gut morphology of Senegalese sole (Solea senegalensis, Kaup 1858) fed monospecies and multispecies probiotics. *Aquaculture Nutrition, 21*(5), 625–634.

Burridge, L., Weis, J. S., Cabello, F., Pizarro, J., & Bostick K. (2010). Chemical use in salmon aquaculture: A review of current practices and possible environmental effects. *Aquaculture, 306*, 7–23. https://doi.org/10.1016/j.aquaculture.2010.05.020

Buschmann, A. H., Tomova, A., Lopez, A., Maldonado, M. A., Henriquez, L. A., Ivanova, L., Moy, F., Godfrey, H. P., & Cabello, F. C. (2012). Salmon aquaculture and antimicrobial resistance in the marine environment. *PLoS One, 7*, e42724. https://doi.org/10.1371/journal.pone.0042724

Cabello, F. C. (2006). Heavy use of prophylactic antibiotics in aquaculture: a growing problem for human and animal health and for the environment. *Environmental Microbiology, 8*, 1137–1144.

Cardoso, P. H. M., Moreno, A. M., Moreno, L. Z., de Oliveira, C. H., Baroni, F. d. A., Maganha, S. R. d. L., de Souza, R. L. M., & Balian, S. d. C. (2019). Infectious diseases in aquarium ornamental pet fish: prevention and control measures. *Brazilian Journal of Veterinary Research and Animal Science, 56*(2), e151697. https://doi.org/10.11606/issn.1678-4456.bjvras.2019.151697

Chanda, M., Paul, M., Maity, J., Dash, G., & Gupta, S. S. (2011). The use of antibiotics and disinfectants in ornamental fish farms of West Bengal, India. *Journal of Natural Science, Biology and Medicine, 2*(2), 139–140. https://doi.org/10.4103/0976-9668.92311

Chogale, N. D., Satam, S. B., Metar, S. Y., & Singh, H. (2017). Marine Ornamental fishes in Ratnagiri coast, Maharashtra. In V. B. Sakhare, B. Vasantkumar, & C. M. Bharambe (Eds.), *Fish and aquatic resources* (pp. 101–111). Discovery Publishing House Pvt. Ltd..

Dadar, M., Dhama, K., Vakharia, V. N., et al. (2017). Advances in aquaculture vaccines against fish pathogens: global status and current trends. *Reviews in Fisheries Science & Aquaculture, 25*, 184–217.

Das, S., Mondal, K., & Haque, S. A. (2017). Review on application of probiotic, prebiotic and synbiotic for sustainable development of aquaculture. *Journal of Entomology and Zoology Studies, 5*(2), 422–429.

Dawood, M. A., Koshio, S., Ishikawa, M., & Yokoyama, S. (2015). Effects of partial substitution of fish meal by soybean meal with or without heat-killed Lactobacillus plantarum (LP20) on growth performance, digestibility, and immune response of amberjack, Seriola dumerili juveniles. *BioMed Research International, 2015*, 1–11.

Defoirdt, T., Sorgeloos, P., & Bossier, P. (2011). Alternatives to antibiotics for the control of bacterial disease in aquaculture. *Current Opinion in Microbiology, 14*, 251–258. https://doi.org/10.1016/j.mib.2011.03.004

Dharmaratnam, A., Swaminathan, T. R., Kumar, R., Basheer, V. S. (2018). Aeromonas hydrophila associated with mass mortality of adult goldfish Carassius auratus in ornamental farms in India. *Indian Journal of Fisheries, 65*, 116–126. https://doi.org/10.21077/ijf.2018.65.4.72719-14

Dias, C., Mota, V., Martinez-Murcia, A., & Saavedra, M. J. (2012). Antimicrobial resistance patterns of Aeromonas spp. isolated from ornamental fish. *Journal of Aquaculture Research and Development, 3*, 131. https://doi.org/10.4172/2155-9546.1000131

FAO (Food and Agriculture Organization of the United Nations). (2005). Responsible use of antibiotics in Aquaculture. In P. H. Serrano (Eds.). Available: http://www.fao.org/3/a-a0282e.pdf

FDA. (1998). *Chapter 11: Aquaculture Drugs in Fish and Fishery Products Hazards and Controls Guide* (2nd ed., pp. 115–132). Food and Drug Administration.

Gao, P., Mao, D., Luo, Y., Wang, L., Xu, B., & Xu, L. (2012). Occurrence of sulfonamide and tetracycline-resistant bacteria and resistant genes in aquaculture environment. *Water Research, 46*, 2355–2364. https://doi.org/10.1016/j.watres.2012.02.004

Gibello, A., Galán-Sánchez, F., Blanco, M. M., Rodríguez-Iglesias, M., Domínguez, L., & Fernández-Garayzábal, J. F. (2016). The zoonotic potential of *Lactococcus garvieae*: An overview on microbiology, epidemiology, virulence factors and relationship with its presence in foods. *Research in Veterinary Science, 109*, 59–70. https://doi.org/10.1016/j.rvsc.2016.09.010

Hashish, E., Merwad, A., Elgaml, S., Amer, A., Kamal, H., Elsadek, A., Marei, A., & Sitohy, M. (2018). Mycobacterium marinum infection in fish and man: epidemiology, pathophysiology and management; a review. *The Veterinary Quarterly, 38*(1), 35–46. https://doi.org/10.1080/01652176.2018.1447171

Heuer, O. E., Kruse, H., Grave, K., Collignon, P., Karunasagar, I., & Angulo, F. J. (2009). Human health consequences of use of antimicrobial agents in aquaculture. *Clinical Infectious Diseases, 49*, 1248–1253. https://doi.org/10.1086/605667

Ibrahem, M. D. (2015). Evolution of probiotics in aquatic world: Potential effects, the current status in Egypt and recent prospective. *Journal of Advanced Research, 6*, 765–791.

Iribarren, D., Daga, P., Moreira, M. T., & Feijoog. (2012). Potential environmental effects of probiotics used in aquaculture. *Aquaculture International, 20*, 779–789.

Jagoda, S. S., Wijewardana, T. G., Arulkanthan, A., Igarashi, Y., Tan, E., Kinoshita, S., Watabe, S., & Asakawa, S. (2014). Characterization and antimicrobial susceptibility of motile aeromonads isolated from freshwater ornamental fish showing signs of septicaemia. *Diseases of Aquatic Organisms, 109*, 127–137. https://doi.org/10.3354/dao02733

Kent, M. L., Feist, S. W., Harper, C., Hoogstraten-Miller, S., Law, J. M., Sánchez-Morgado, J. M., Tanguay, R. L., Sanders, G. E., Spitsbergen, J. M., & Whipps, C. M. (2009). Recommendations for control of pathogens and infectious diseases in fish research facilities. *Comparative Biochemistry and Physiology C Toxicology and Pharmacology, 149*(2), 240–248. https://doi.org/10.1016/j.cbpc.2008.08.001

Kim, J. K., Park, K. J., Cho, K. S., Nam, S., Park, T., & Bajpai, R. (2005). Aerobic nitrication–Denitrification by heterotrophic bacillus strains. *Bioresource Technology., 96*, 1897–1906.

Kleingold, D. W., et al. (1996). Administration of antibiotics and chemotherapeutants and development of resistance in coldwater ornamentals. *Bulletin of the European Association of Fish Pathologists, 17*, 4–7.

Kleingold, D. W., et al. (2001). Bacterial isolations, resistance development and risk analysis in the ornamental fish trade. *Bulletin de l'Institut Océanographique, 20*, 193–200.

Krishnakumar, P. K. (2017). Ornamental fish industry hit by new regulations. *The Economic Times*, 16th June 2017.

Kumar, R., Swaminathan, T. R., Kumar, R. G., Dharmaratnam, A., Basheer, V. S., & Jena, J. K. (2015). Mass mortality in ornamental fish, Cyprinus carpio koi caused by a bacterial pathogen, Proteus hauseri. *Acta Tropica, 149*, 128–134. https://doi.org/10.1016/j.actatropica.2015.05.022

Kumar, S., Lekshmi, M., Parvathi, A., Nayak, B. B., & Varela, M. F. (2017). Antibiotic resistance in seafood borne pathogens. In O. V. Singh (Ed.), *Food borne pathogens and antibiotic resistance*. Wiley.

Lewbart, G. A. (2001). Bacteria and ornamental fish. *Seminars in Avian and Exotic Pet Medicine, 10*, 48–56. https://doi.org/10.1053/saep.2001.19543

Li, L., Lin, S.-L., Deng, L., & Liu, Z.-G. (2013). Potential use of chitosan microparticles for oral delivery of DNA vaccine in black seabream Acanthopagrus schlegelii Bleeker to protect from Vibrio parahaemolyticus. *Journal of Fish Diseases, 36*, 987–995. https://doi.org/10.1111/jfd.12032

Liakopoulos, A., Mevius, D., & Ceccarelli, D. (2016). A review of SHV extended-spectrum b-lactamases: neglected yet ubiquitous. *Frontiers in Microbiology, 5*, 1374.

Lin, H. L., Shiu, Y. L., Chiu, C. S., Huang, S. L., & Liu, C. H. (2017). Screening probiotic candidates for a mixture of probiotics to enhance the growth performance, immunity, and disease resistance of Asian seabass, Lates calcarifer (Bloch), against Aeromonas hydrophila. *Fish & Shellfish Immunology, 60*, 474–482.

Lulijwa, R., Rupia, E. J., & Alfaro, A. C. (2020). Antibiotic use in aquaculture, policies and regulation, health and environmental risks: a review of the top 15 major producers. *Reviews in Aquaculture, 12*(2), 640–663. https://doi.org/10.1111/raq.12344

Magi, G. E., Lopez-Romalde, S., Magariños, G. E., Lamas, J., Toranzo, A. E., & Romalde, L. (2009). Experimental Pseudomonas anguilliseptica infection in turbot Psetta maxima (L.): a histopathological and immunohistochemical study. *European Journal of Histochemistry, 53*(2), e9. https://doi.org/10.4081/ejh.2009.e9

Marti, E., Huerta, B., Rodríguez-Mozaz, S., Barceló, D., Marcé, R., & Balcázar, J. L. (2018). Abundance of antibiotic resistance genes and bacterial community composition in wild

freshwater fish species. *Chemosphere, 196*, 115–119. https://doi.org/10.1016/j.chemosphere.2017.12.108

Melgar Valdes, C. E., Barba Macías, E., Alvarez-González, C. A., Tovilla Hernández, C., & Sánchez, A. J. (2013). Microorganisms effect with probiotic potential in water quality and growth of the shrimp Litopenaeus vannamei (Decapoda: Penaeidae) in intensive culture. *Revista de Biología Tropical, 61*, 1215–1228.

Mishra, A., Nam, G. H., Gim, J. A., Lee, H. E., Jo, A., & Kim, H. S. (2018). Current challenges of Streptococcus infection and effective molecular, cellular, and environmental control methods in aquaculture. *Molecules and Cells, 41*(6), 495–505. https://doi.org/10.14348/molcells.2018.2154

Narendrakumar, L., Chandrika, S. K., & Thomas, S. (2020). Adaptive resistance evolution of V. cholerae to doxycycline associated with spontaneous mutation. *International Journal of Antimicrobial Agents, 56*(3), 106097.

Neela, F. A., Banu, M. N. A., Rahman, M. A., Rahman, M. H., & Alam, M. F. (2014). Occurrence of antibiotic resistant bacteria in pond water associated with integrated poultry-fish farming in Bangladesh. *Sains Malaysiana, 44*, 371–377. https://doi.org/10.17576/jsm-2015-4403-08

Newaj-Fyzul, A., & Austin, B. (2015). Probiotics, immunostimulants, plant products and oral vaccines, and their role as feed supplements in the control of bacterial fish diseases. *Journal of Fish Diseases, 14*, 937–955. https://doi.org/10.1111/jfd.12313

Noga, E. (2010). Diagnoses made by bacterial culture of kidney or affected organs. In E. Noga (Ed.), *Fish disease, diagnosis and treatment* (2nd ed., pp. 185–190). Iowa State University Press.

Pękala-Safińska, A. (2018). Contemporary threats of bacterial infections in freshwater fish. *Journal of Veterinary Research, 62*(3), 261–267. https://doi.org/10.2478/jvetres-2018-0037

Preena, P. G., Dharmaratnam, A., Raj, N. S., et al. (2019). Antibiotic susceptibility pattern of bacteria isolated from freshwater ornamental fish, guppy showing bacterial disease. *Biologia, 74*, 1055–1062. https://doi.org/10.2478/s11756-019-00261-8

Preena, P. G., Swaminathan, T. R., Kumar, V. J. R., et al. (2020a). Antimicrobial resistance in aquaculture: A crisis for concern. *Biologia, 75*, 1497–1517. https://doi.org/10.2478/s11756-020-00456-4

Preena, P. G., Arathi, D., Raj, N. S., Arun Kumar, T. V., Arun Raja, S., Reshma, R. N., & Raja Swaminathan, T. (2020b). Diversity of antimicrobial-resistant pathogens from a freshwater ornamental fish farm. *Letters in Applied Microbiology, 71*(1), 108–116. https://doi.org/10.1111/lam.13231

Rihana, N., Hemminger, A., & Green, S. (2018). Novel case of penicillin resistant E. rhusiopathiae septicemia: Case report with review of the literature. *IDCases, 11*, 67–69. https://doi.org/10.1016/j.idcr.2018.01.005

Ruane, N. M., Collins, E. M., Geary, M., et al. (2013). Isolation of Streptococcus agalactiae and an aquatic birnavirus from doctor fish Garra rufa L. *Irish Veterinary Journal, 66*, 16. https://doi.org/10.1186/2046-0481-66-16

Sahoo, P. K., Swaminathan, T., Abraham, T. J., Kumar, R., Pattanayaka, S., Mohapatra, A., Ratha, S. S., Patra, A., Adikesavalu, H., Sood, N., Pradhan, P. K., Das, B. K., Jayasankar, P., & Jena, J. K. (2016). Detection of goldfish haematopoietic necrosis herpes virus (cyprinid herpesvirus-2) with multi-drug resistant Aeromonas hydrophila infection in goldfish: first evidence of any viral disease outbreak in ornamental freshwater aquaculture farms in India. *Acta Tropica, 161*, 8–17. https://doi.org/10.1016/j.actatropica.2016.05.004

Sarter, S., Nguyen, H. N., Hung, L. T., Lazard, J., & Montet, D. (2007). Antibiotic 11 resistance in Gram-negative bacteria isolated from farmed catfish. *Food Control, 18*, 1391–1396.

Sasmal, D., Banerjee, T., Bandyopadhyay, S., & Abraham, T. J. (2004). Antibiotic sensitivity of bacterial flora associated with ornamental fish. *Indian Journal of Fisheries, 51*, 245–249.

Satam, S. B., Sawant, N. H., Ghughuskar, M. M., Sahastrabuddhe, V. D., Naik, V. V., & Pagarkar, A. U. (2018). Ornamental fisheries: A new avenue to supplement farm income. *Advanced Agricultural Research and Technology, 2*(2), 193–197.

Sayes, C., Leyton, Y., & Riquelme, C. (2016). Bacterium Pseudoalteromonas sp. potential probiotic for larval fish culture. *Latin American Journal of Aquatic Research, 44*(1), 76–84.

Schar, D., Klein, E. Y., Laxminarayan, R., et al. (2020). Global trends in antimicrobial use in aquaculture. *Scientific Reports, 10*, 21878. https://doi.org/10.1038/s41598-020-78849-3

Shin, S., Kulatunga, D. C. M., Dananjaya, S. H. S., Nikapitiya, C., Lee, J., & De Zoysa, M. (2017). Saprolegnia parasitica Isolated from Rainbow Trout in Korea: characterization, anti-saprolegnia activity and host pathogen interaction in zebrafish disease model. *Mycobiology, 45*(4), 297–311. https://doi.org/10.5941/MYCO.2017.45.4.297

Sicuro, B., Pastorino, P., Barbero, R., Barisone, S., Dellerba, D., Menconi, V., Righetti, M., De Vita, V., & Prearo, M. (2020). Prevalence and antibiotic sensitivity of bacteria isolated from imported ornamental fish in Italy: A translocation of resistant strains? *Preventive Veterinary Medicine, 175*, 104880. https://doi.org/10.1016/j.prevetmed.2019.104880

Snieszko, S. F., & Bullock, G. L. (1957). Treatment of sulfonamide-resistant furunculosis in trout and determination of drug sensitivity. *Fishery Bulletin, 125*, 555–564.

Sommerset, I., Krossøy, B., Biering, E., et al. (2005). Vaccines for fish in aquaculture. *Expert Review of Vaccines, 4*, 89–101.

Sørum, H. (2006). Antimicrobial drug resistance in fish pathogens. In F. M. Aarestrup (Ed.), *Antimicrobial resistance in bacteria of animal origin* (pp. 213–238). ASM Press.

Sreedharan, K., Philip, R., & Singh, I. S. B. (2011). Isolation and characterization of virulent Aeromonas veronii from ascetic fluid of oscar Astronotus ocellatus showing signs of infectious dropsy. *Diseases of Aquatic Organisms, 1*, 29–39. https://doi.org/10.3354/dao02304

Sreedharan, K., Philip, R., & Singh, I. S. B. (2012). Virulence potential and antibiotic susceptibility pattern of motile aeromonads associated with freshwater ornamental fish culture systems: a possible threat to public health. *Brazilian Journal of Microbiology, 43*, 754–765. https://doi.org/10.1590/S1517-83822012000200040

Thanga Viji, V., Deepa, K., Velmurugan, S., Donio, M. B., Adlin Jenifer, J., Babu, M. M., & Citarasu, T. (2013). Vaccination strategies to protect goldfish Carassius auratus against Aeromonas hydrophila infection. *Diseases of Aquatic Organisms, 104*(1), 45–57. https://doi.org/10.3354/dao02581

Threlfall, J., Levent, B., Hopkins, K. L., de Pinna, E., Ward, L. R., & Brown, D. J. (2005). Multidrug-resistant Salmonella Java. *Emerging Infectious Diseases, 11*(1), 170–171. https://doi.org/10.3201/eid1101.031092

Vinitnantharat, S., Gravningen, K., & Greger, E. (1999). Fish vaccines. *Advances in Veterinary Medicine, 41*, 539–550. https://doi.org/10.1016/s0065-3519(99)80040-8

Wahli, T., & Madsen, L. (2018). Flavobacteria, a never ending threat for fish: A review. *Current Clinical Microbiology Reports, 5*, 26–37. https://doi.org/10.1007/s40588-018-0086-x

Walczak, N., Puk, K., & Guz, L. (2017). Bacterial flora associated with diseased freshwater ornamental fish. *Journal of Veterinary Research, 61*(4), 445–449. https://doi.org/10.1515/jvetres-2017-0070

Watts, J. E. M., Schreier, H. J., Lanska, L., & Hale, M. S. (2017). The rising tide of antimicrobial resistance in aquaculture: Sources, sinks and solutions. *Mari Drugs, 15*, e158. https://doi.org/10.3390/md15060158

Zanoni, R. G., Florio, D., Fioravanti, M. L., Rossi, M., & Prearo, M. (2008). Occurrence of Mycobacterium spp. in ornamental fish in Italy. *Journal of Fish Diseases, 31*(6), 433–441. https://doi.org/10.1111/j.1365-2761.2008.00924.x

Environment as Sink for Antimicrobial Resistance and Genes

Devi Sanjeev, Murugadas Vaiyapuri, and
Mukteswar Prasad Mothadaka

Contents

D. Sanjeev
Microbiology, Fermentation and Biotechnology Division, ICAR-Central Institute of Fisheries Technology (ICAR-CIFT), Cochin, Kerala, India
e-mail: devisanjeev95@gmail.com

M. Vaiyapuri
Microbiology Fermentation and Biotechnology Division of ICAR-Central Institute of Fisheries Technology (ICAR-CIFT), Cochin, Kerala, India
e-mail: Murugadas.V@icar.gov.in

M. P. Mothadaka (✉)
Visakhapatnam Research Centre of ICAR-CIFT, ICAR-Central Institute of Fisheries Technology (ICAR-CIFT), Visakhapatnam, Andhra Pradesh, India
e-mail: mm.prasad@icar.gov.in; prasadmm@hotmail.com

M. P. Mothadaka et al. (eds.), *Handbook on Antimicrobial Resistance*,
https://doi.org/10.1007/978-981-19-9279-7_10

Abstract

Environment plays a crucial role in the antimicrobial resistance (AMR). Antimicrobials used across the sectors are directly or indirectly released into the environment. However, minimal focus was given on the "One Health" basis. The assessment of the influence of the environment on advancement and transmission of insusceptibility to drugs is of paramount importance. The status of AMR across the segments of ecosystems, viz., terrestrial and aquatic (rivers, lake), etc., is extremely important. In this chapter, consolidated evidence is provided to understand the AMR across the ecosystems. Studies that estimated the burden of AMR by metagenomics were also given a special criterion for understanding the impact on other sectors.

Keywords

Antimicrobial resistance · Lakes · Wastewaters · Metagenomics

1 Introduction

Antibiotics are low-molecular-weight secondary compounds produced from microorganisms (bacteria or fungi) that are involved either in the inhibition or the elimination of other microbes (Lancini et al., 1995; Stanton et al., 2020). In treating bacterial infections, antibiotics were used as the first resort and were once considered indispensable in modern medicine (Davies & Davies, 2010). Till 2005, several antibiotics were discovered or manufactured since the first antibiotic was discovered in 1928 (Zinner, 2007). Following the release of the antibiotics, they have been used in a variety of sectors (human health, veterinary, agriculture, and aquaculture). These drugs are mass-produced in millions of tons (Wang et al., 2010). Antibiotics that were overproduced or overused were then excreted or discharged into the environment as a result of which low concentration of sublethal antibiotics in the environment puts pressure on the bacterial population in the ecosystem. Because of this, antimicrobial resistance (AMR) has emerged in the bacterial population in the environment. More than ten million people are believed to be at risk as a result of the huge rise in drug-insensitive bacteria (O'Neill, 2016). Antibiotics enter the environment via three main routes: environmental release of antibiotics from production sites, antibiotics used in hospitals, veterinary, and aquaculture systems, and feed additives (Fig. 1). Antimicrobials are expected to be excreted in metabolized or active unmetabolized form from human and animal healthcare and are eventually released into urban wastewater, manure, or biosolids (10–90%). The percentage variance in excretion is caused by the organism's species, chemical structure, or dosage (Zhao et al., 2010). Unused and expired medications are frequently dumped into aquatic sources. Antibiotics used in irrigation and fertilization processes in the water and agroecosystems enter the environment via sludge, biosolids, and manure (Göbel et al., 2004; Yang et al., 2010). Antibiotic residue activity in the environment is impacted by an assortment of ecological dynamics, and water or soil quality

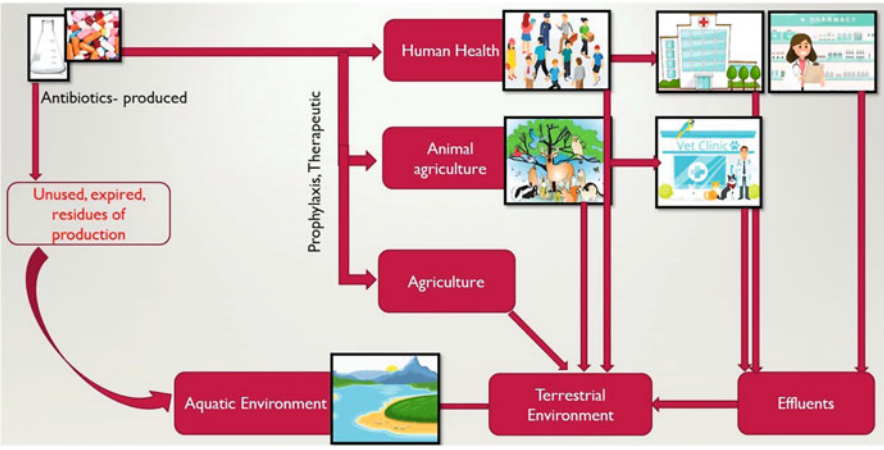


Fig. 1 Pathways of antibiotics used across the sectors entering the environment

parameters (pH, water content, organic carbon content, microbiota), including concentration, and its impact on terrestrial and aquatic ecosystems is not fully understood (Kümmerer, 2009). Similarly, antibiotic residue persistence is influenced by the types of biotic stress (microbes) present in the niche, as well as abiotic stress (pH, temperature, hydrolysis, oxidation, reduction, or photolysis) present in the environment.

The impact of antimicrobial resistance can be a major impediment to reaching most of the SDGs by 2030. They include SD1 and 2 – No poverty and Zero hunger; SDG 3 and 6 – Decent condition and well-being and Unpolluted water and hygiene; SDG 7 and 8 – Inexpensive and clean energy and Decent work and economic growth; SDG 11 and 12 – Viable metropolises and societies & Sustainable intake and making; SDG 14 and 15 – Submarine life and Life on land; and SDG 17: Partnerships for the goals.

2 Major Concerns of AMR in the Environment

The ecological conditions play a crucial role in the development of AMR. The resultant human activity, namely, antibiotics and antimicrobials released into the environments, is an important reason for drug insusceptibility in bacteria, and they, in turn, spread to the microbes in soil, rivers, and seawater. Human consumption of antibiotics increased by 36% in the 2000s. In 2000, the human consumption of antibiotics ranged from 9.2 to 10.5, with an average of 9.8 defined daily doses (DDD). By 2018, it has increased from 37.2 to 43.7, with an average of 40.2 billion DDD. The rate of intensification from 2000 to 2018 was 46%, with an annual mean consumption of 14.3% in 2018 varying from 13.2 to 15.6, and the study reported data at a 95% uncertainty level (Browne et al., 2021). When the antibiotics are taken, 20% are absorbed and the remaining 80% are expelled through exudates. Human consumption of drugs amounts to 30% of the antibiotics. Compost fertilizers are also

a source of antibiotic pollution in surface runoff, groundwater, and drainage networks. It is important to observe that the plants and crops absorb the antibiotics. Of the total antibiotics produced, nearly 70% are used for animal purposes only. The intensification of farming and the pressure to enhance animal growth result in antibiotic use, particularly in developing countries. By 2030, there will be a significant increase in antimicrobial use in livestock to 67%. Major waste flows, including drainage, manures, and agricultural runoff, contain residues of antibiotics and AMR bacteria. It is known that the concentrations of antimicrobials in most sewages are too less to an extent of causing lethality to bacteria, but it is possible that the low concentrations are enough to develop AMR. The occurrence of ARB in fresh source water and water subjected to treatment cannot be ruled out. An enormous collection of pollutants in public and industrial wastewater puts a huge burden on microbes to develop resistance. The landfills and open dumps contain but more than 50% solid wastes from civic sources. Unused drugs and expired drugs are also a part of municipal waste. Reports indicate the occurrence of MDR bacteria in marine waters and sediments, which are in the vicinities of aquaculture, industrial, and civic discharges.

The contaminants of aquatic sources include wildlife: exudes from wild animals; landfills: discharges from landfills; dredging: dredging of the sediments; aquaculture: resultant aquaculture activities; human healthcare: originating from clinical sources in the form of surplus solids and fluid seepages; livestock and farms: application of droppings and stowage; releases: sewage and discharges from effluent treatment plants (ETP); runoffs from agriculture fields; and antibiotics: effluents from the manufacture of antibiotics are also considered the source for antibiotics in the environment.

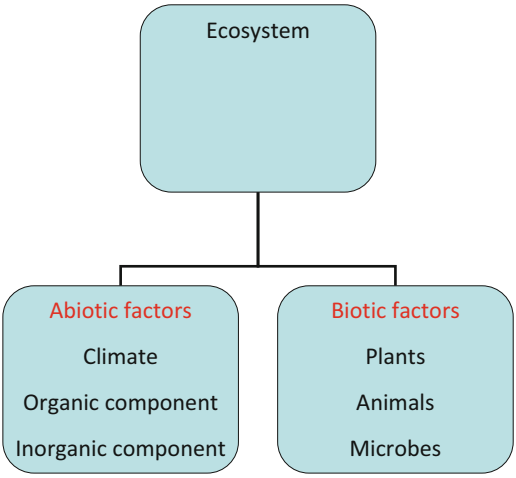
Similarly, air pollution occurs due to emissions from livestock, clinical environments, and human airways. Soil pollution occurs due to excretions from wildlife sources, wastewater treatment plants, sewage: sludge/discharges, livestock, and farms; manure application; and discharges.

Due to various other reasons, humans are impacted by potable and recreational water; domestic animals: direct contact with animals; wildlife: all types of wild animals; livestock: ingestion of livestock products; and polluted air: including fellow human sources. Wildlife is impacted in the same way by ingestion of contaminated foods; direct contact: anthropozoonosis; clinical sources in the form of surplus solids and fluid seepages; and livestock and farms: application of droppings and stowage, releases, and runoffs from agriculture fields.

The thermal stability of antibiotics is also important in their breakdown (Turiel et al., 2005; Dantas et al., 2008). The persistence of drugs in the environs induces an adaptive response to alleviate stress, resulting in the advent of a new-fangled phenotype obdurate to drugs either by mutation in the genome of bacteria or by obtaining genes of insusceptibility by means of horizontal or vertical gene transfer mechanisms (Tenover, 2006). These residues, when present in concentrations lower than the minimal inhibitory concentration, change the signaling cascade, affecting virulence, biofilm, and quorum-sensing mechanisms, as well as non-target bacteria in aquatic and terrestrial ecosystems (Chee-Sanford et al., 2009). This chapter offers researchers sufficient information about what an ecosystem is, how it is classified, and how AMR is reported in ecosystems.

3 Ecosystems and Their Classification

A place with a specific and recognizable landscape (forest, grassland, desert, wetland, or coastal area) and an abiotic component (sunlight, temperature, and rainfall) that supports the circumstances of the biotic components (living organisms) is referred to as an "ecosystem," according to Tansley's definition (Blew, 1996). Various biotic and abiotic factors influence the different ecosystems, according to Odum and Smalley (1959). They include abiotic factors differing substantially between habitats, such as terrestrial (temperature, sunlight, and water) and marine (temperature, sunlight, and water) (salinity and ocean currents). Odum and Smalley categorized these abiotic elements into three categories: climatic, inorganic, and organic components (1959). These biotic components include living species such as plants, animals, and microorganisms that can be divided into three categories: producers, consumers, and decomposers. The majority of bacteria and fungi are classified as decomposers or reducers. Natural ecosystems, which are built based on the interactions between biotic and abiotic components and occur naturally in a cyclic manner, and artificial ecosystems, which are sustained with human intervention, such as farms, are two types of ecosystems. Freshwater and marine environments make up aquatic ecosystems. Flowing water/lotic environment (streams, rivers, etc.) and standing waterbodies/lentic environment (ponds, lakes, swamps, etc.) are two types of freshwater ecosystems that are completely distinct from one another. Natural disasters such as floods and droughts alter the species that live in lotic environments.



4 AMR in Different Ecosystems

4.1 Aquatic System

The aquatic environment not only acts as a maintenance medium for the AMR but also acts as a mixing and mobilizing medium for intra- and inter-species levels because water in the biosphere has a vast cell biomass of $>10^{30}$, which contributes more toward the maintenance, mixing, and mobilization of ARGs. These bacterial or microbial cell biomass act as a pathogen in various living plants and animals, but are also involved in various biogeochemical cycles. There are extreme environments that also harbor extremophiles, and a lot can be understood from these extremophiles. Therefore, these antimicrobials at minimal residuals also derange the bacterial transcription mechanism. These result in co-selection of other population with developing resistance to antibiotics prevalent as residual concentrations. It also facilitates the horizontal or vertical gene transfer of resistance genes through various mechanisms. The microbes with ARGs can be transported via water as a medium to any place easily, and, therefore, it forms a niche for the spread of ARGs to the next population (Taylor et al., 2011).

4.2 River and Lake System

Rivers are any natural flow of water that flows within a definite bank and are well nourished by the precipitation of organic content in water as well as by the runoff from nearby terrestrial areas. Due to human activities, the runoff majorly contains heavy metals and other pollutants, which can be either organic or inorganic. Contamination of river ecosystem can occur mainly from the release of sewage, which contains organic pollutants and pharmaceutical waste, including fecal and pathogenic microorganisms that can coexist and cause transformation with autochthonous microorganism.

Liu and others (2018) studied the genes unresponsive to drugs "*ARGs Antibiotic Resistance Genes*" from all lacustrine sources across China in which the ARGs of surface waters exhibited substantial distance–decay association. The study revealed efflux pump as a vital tool of resistance with predominance of MDR genes. The ARGs' normalized richness was lower in the northern region and higher in the southern and central regions of China. The topographical dissemination of ARGs is impacted by various environmental mechanisms, namely, aligned and nonaligned processes.

Systems such as river–lake are securely linked to land-dwelling bionetworks and appear to be sinks of ARGs and distribution pathways for resistant bacteria for the reason of predisposal by anthropological events sans difficulty. There is currently a scarcity of methodical information on the incidence, transmission hazard, and cause of ARGs in the conformations of river–lakes. Chen et al.'s (2020) study centered on high-throughput summarizing and dynamics of source sink in unraveling drug-unresponsive genes in the deposits of interrelated Fuhe river–lake structure and the resultant unloading brook Baiyang located in the northern region of China. In the

study, 40 outward deposits were acquired and subjected to metagenomic shotgun sequencing. The summary and synchronized incidence of genes unresponsive to drugs in dregs of the river–lake environs, along with the mobile genetic elements (MGEs) that harbor ARGs and their impending dispersal hazard of resistome, were categorized systematically. A novel bacteriophage, namely, CrAssphage, was used to trail the effects of human fluid contamination on ARGs. Fast Expectation-Maximization Microbial Source Tracking (FEAST), an innovative method, combined with Linear Discriminant Analysis Effect Size (LEfSe) scheme was used to assess the impact of river residues on the incidence of ARGs in the receiving lake (Chen et al., 2020). Furthermore, the discovery of nascent ARGs, namely, *mcr*-1, *tetX*, and carbapenemases in the deposits of the river–lake system, is one of the important findings of the study. The ARGs' incidence in the dregs of environs of river–lake is attributed to contamination from human exudes as evidenced by source tracking, and the distribution assessment showed that >80% of ARGs in Lake Baiyang originate from Fuhe River. Grid assessment proposed nonrandom coincidence forms of ARGs intra- and between categories. One more significant observation is that numerous MGE-carrying contigs were recognized with an equal holding of ARGs of one or more, resulting in possible advanced resistome hazard in Lake Baiyang. It is not the same case with most of the lacustrine sources of the world.

In Bangladesh, the research on various components of the environment (wastewater, rivers, ponds, and household waste) revealed the abundance of *Plasmid Mediated Quinolone Resistance* (PMQR) genes, mostly *qnrS* in *E. coli* isolates. This was followed by genes aac (6′)-lb-cr, *oqxAB*, *qnrB*, and *qepA,* which were detected in abundance. Penicillin, cephalosporins, fluoroquinolones, sulfonamides, aminoglycosides, and carbapenems were also detected (Amin et al., 2021). In the Republic of Czechoslovakia, when the Morava River waste and surface water was screened for the presence of 10 antibiotics at predicted no-effect environmental concentrations (PNECs), the wastewater samples exceeded the PNEC and vancomycin-resistant enterococci were identified in both water samples of polluted and surface (Hricová et al., 2021).

The copious and tenacious presence of microbes that are unresponsive to drugs in the environs poses a significant risk to the human well-being. Antimicrobial resistance (AMR)-associated mortalities occur more often than malignancies and traffic accidents combined per annum (O'Neill, 2016). Multiresistant bacteria could spread from humans to aquatic habitats via water purification plants that dump treated contaminated water into other aquatic sources after treatment. Antibiotic resistance genes (ARGs) discovered in gene-transfer units, as well as the growth of MDR, may have ramifications for the well-being of humans and the development in environs (Martinez, 2009). AMR can be caused by chromosomal DNA mutations or mobile element horizontal gene transfer. Integrons are a type of mobile element that are associated with the internment, mobilization, and transmission of genes that are unresponsive to drugs in Gram-negative microbes. Gene cassettes are genomic platforms that allow resistance determinants to be integrated and rearranged (Mazel, 2006).

While the emergence of novel microbes that are insusceptible to different antimicrobials poses new investigative and healing tasks, India continues to battle dreaded diseases, viz., tuberculosis and malaria organisms, which always remain

unaffected by treatment (Chaudhry & Tomar, 2017). Poverty, illiteracy, overcrowding, and hunger are the factors aggravating the problem (Swaminathan et al., 2017). Public's lack of knowledge of infectious diseases, as well as lack of access to healthcare, frequently prevents them from seeking medical advice, which usually leads them to seek antimicrobial drug self-prescription without expert knowledge of the prescription and length of treatment (Laxminarayan et al., 2016).

The growth of the pharmaceutical industry has coincided with an increase in the quantity of trash created by these industries. This garbage reaches waterbodies due to a lack of strong supervisory and legislative procedures, and serves as a perennial cradle of AMR in the environs (Lundborg & Tamhankar, 2017).

ARBs and ARGs have been discovered in a variety of Indian water sources. The discharges of drugs and hospitals into aquatic sources are the two major sources, especially to the adjoining areas of water resources sans proper treatment. The rate of incidence of third-generation cephalosporin-insusceptible *E. coli* was 25% in household water, 70% in domestic and hospital waste, and 95% hospital effluent, respectively (Akiba et al., 2015). The Ganges and Yamuna, India's two major rivers, traverse enormous areas of land and receive several creeks with varied concentrations of bacteria that are immune to drugs. In north Indian rivers, 17.4% of Gram-negative bacteria were ESBL producers (Azam et al., 2016), with the detection of resistance genes such as *blaNDM-1* and *blaOXA48* (Ahammad et al., 2014). The occurrence of third-generation cephalosporin insusceptibility among *E. coli* was 100% in the Cauvery waters of Karnataka (Skariyachan et al., 2015). Water intended for potable and leisure purposes sourced from surface and underground has been shown to harbor *E. coli* insusceptible to third-generation cephalosporin at 17% (central India; Kumar et al., 2013), 7% in Kashmir (northern India; Rather et al., 2013), 50% in Sikkim (eastern India; Poonia et al., 2014), and 100% in Hyderabad (southern India; Lübbert et al., 2017).

The water samples for these studies were procured from various sources, viz., waterways, meres, lochs, springs, manually operated water, and borewells. ESBL, CRE, and quinolone-unresponsive *E. coli* and *Klebsiella* sp. estimation in the Msimbazi River in Tanzania revealed a burden of these resistant isolates of >50% (Kimera et al., 2021). QNL/PEN/SUL, QNL/PEN/TET, and CEP/QNL/PEN were the most common combinations, which might be related to their widespread usage in animals and people, as well as their discharge into the environment populated, and also the disposal of waste is not up to the rate of urbanization (Said et al., 2015). A significant gradation of environments is infected with microbes obdurate to one or more than antimicrobials prevailing in Africa, owing to a high concentration of AMU in fauna and people, undertakings associated with agriculture, and deficiencies in antimicrobial control and disposal (Kimera et al., 2021). The water samples procured from the St. Clair and Detroit river area contained 48% *E. coli* of specified pathotypes and were unresponsive to drugs (Hamelin et al., 2007). The transboundary waters in these locales cater to the needs of millions across Canada and the United States representing a critical shared resource. Many towns depend on these aquatic sources for potability and leisure, and quality is a major concern. Beach closures are now listed as a major water usage limitation in numerous *Areas of*

Concern between the countries, and pathogen contamination and the growth of ARB in the Great Lakes basin environment have also been identified as concerns. The screening revealed *tet(A), tet(B), blaTEM,* and *sulII* genes of drug obduracy are abundant in the study area (Hamelin et al., 2007).

In Germany and Australia, the water samples procured from external areas harbored 24 ARGs active against eight classes of antimicrobials that were assessed using culture-independent techniques (Stoll et al., 2012). The most common ARGs were *sulI*, *sulII* (77–100%), and *dfrA1* (43–55%), which code for sulfonamide and trimethoprim obduracy, respectively. The presence of the gene *ermB* that is responsible for insusceptibility to macrolides was observed in external waters of Germany and was high (68%); however, in Australia, its presence was relatively low (18%). The chloramphenicol resistance gene *catII*, in contrast, was found more commonly in Australia at 64% than in Germany at 9% level. Similarly, the β-lactams resistance gene *ampC* was found to be high in Australian samples (36%) than in German samples (19%). Stoll et al. (2012) emphasized the wide-ranging resistant genes of antibiotics to sulfonamide, trimethoprim, macroline, etc.

4.3 Effluvium and Sewage Sludge

Effluvium is produced in various areas such as industries, hospitals, agriculture, and domestic areas. Effluvium is usually collected in effluvium treatment plants or sometimes directly channeled to some waterbodies. Due to overuse of antimicrobial agents, the effluents from these areas become a hub for antimicrobial residues, antimicrobial-insensitive genes, and microorganisms. This will eventually contaminate the surface parts of groundwater body (Walters et al., 2010; Rizzo et al., 2013). Effluvium produced from hospitals contains antimicrobial obdurate pathogens. Hence, discarding these effluents directly into the waterbody can lead to harboring of AMR pathogen strains in the aquatic environs (Kümmerer, 2001; Carraro et al., 2016).

Sludge removed from sewage and effluvium are used as landfills and manure in the agriculture sector. Since sludge settles most of the antimicrobial residues, AMR genes, and ARB in wastewater and sewage, it contaminates surface water and groundwater. Contamination occurs mainly by leaching process and is also carried along with rainwater as "runoff" (Urbaniak et al., 2017). Application of contaminated sludge as manure can reshape the movements of metabolism and environmental multiplicity of soil (Knapp et al., 2010). Some antibiotics tend to adhere to the soil particles and form stable nonbiodegradable molecules, including fluoroquinolones, sulfonamides, and tetracyclines. The biodegradation process of these antibiotics is very slow than the other antibiotics. Since they are stable in soil, they can leach into groundwater as well as reach surface water (Czekalski et al., 2014).

Likewise, urban effluvium entering the treatment plants is also of major concern in the context of the environment as it is a rich source of cellular biomass and contaminants received from various effluents, including pharmaceutical effluents, hospitals, and domestic. Hence, any lacunae in the treatment process in the sewage sludge result in the accumulation of pathogens, ARBs, ARGs, and antimicrobial

residues, which act as a sink for the pathogens to interact with the antibiotic pressure and thereby facilitate the evolution of newer or novel drug resistance in bacteria (Ferro et al., 2016; Bondarczuk et al., 2016; Waseem et al., 2017).

A study has identified that Tn25 MGE enriches the integron class I in the effluents. In addition, many other transposases and ARGs were also detected and were abundant in the inlets of the treatment (Caucci et al., 2016). There are reports on the correlation of ARGs with the transposable elements in the effluvium environment, along with the prediction of the possible role of heavy metal genes in the spread of resistance (Di Cesare et al., 2016) *sulI* and *int1* were correlated, so were the genes insusceptible to heavy metals (*czcA* and *arsB*) and *tetA*, *ermB*, and *qnrS* genes.

5 Cyanobacterial Blooms and Their Effect on ARGs of Free-Living (FL) and Particulate-Attached (PA) Bacteria

Antibiotic-unresponsive genes and the blooms of cyanobacteria in freshwater structures drew worldwide attention as a public health hazard. The effects of the blooms of cyanobacteria on the taxonomic groups of bacteria are substantial. In contrast, the influence of these blooms on the function of drug obdurate groups was not clearly identified. Based on this, Guo et al. (2018) carried out contemporaneous studies in bloom and bloom-free settings in a subtropical reservoir on ARGs of free-living (FL) and particulate-attached (PA) bacteria using high-throughput methods. The study detected ARGs amounting to 145 and MGEs 9, of which 68.93% were MDR and efflux pump tools being varied and predominant. The bloom-free conditions have shown profusion of ARGs both in FL and PA bacteria than in the bloom condition. The study also revealed that free-living bacteria harbored lesser ARGs in blooming conditions compared with conditions that are free from blooms. Whereas the quantity of ARGs in PA bacteria was consistent. Despite more than 96% ARGs being common in bacteria of free-living and particulate-attached kind or in periods free from blooms or otherwise, the purposeful clusters in particulate-attached bacteria were further intensely affected by blooms of cyanobacteria than the free-living bacteria. The study also assessed the association of the structural conformations between taxonomy and function, and observed that the AR groups were highly adaptable and showed lesser relationship among bloom and non-bloom conditions than that observed in the taxonomic structure, excluding FL bacteria. Guo and others (2018) showed that the blooms of cyanobacteria seem to have a robust suppressive influence on ARG abundance in FL bacteria, and have a significant impact on the structure of AR groups in PA bacteria. The study also proposed that both nonaligned and discerning developments interactively impacted the ARG structural crescendos of the FL and PA bacteria. At the same time, the AR group of FL bacteria demonstrated an advanced progressive sequential conjecturable pattern post bloom period than PA bacteria. Guo et al. (2018) also stressed on the bacterial way of life as a pivotal tool, leading to varied reactions of AR groups to the blooms of cyanobacteria.

The ARG dynamics in potable river reservoirs by high-throughput sum up indicates that complete profusion of ARGs and MGEs was stimulated potentially by periodic precipitation. The dissemination configuration of ARGs and MGEs is dependent mainly on the ecological gradient. In the river as well as reservoir structures in the ARGs' sketches, the MGEs play a dominant role (Chen et al., 2019).

5.1 Agriculture

Exponential growth in population has increased the need for agricultural products such as plant crops and animal products. Since the availability of area is getting reduced due to an increase in the population, animals are reared in congested places because of which there is a high risk of infections and deaths, which leads to less productivity. To enhance the productivity, use of antimicrobials has become a quotidian practice to thwart contagions and associated diseases. Antibiotics are also used as growth stimulators in subtherapeutic dosages (EMA and EFSA, 2017). Commonly used antimicrobial classes such as penicillins, tetracyclines, aminoglycosides, macrolides, quinolones, and sulfonamides are critically important for the treatment of humans. Since there is an uncontrolled usage in the agriculture sector, advancement of antimicrobial resistance is also high. Studies suggest that AMR and ARB can transmit through the food consumed. Hence, the major source of AMR in humans is agriculture (Kümmerer, 2004).

5.2 Airplane Wastewater

International flights can facilitate the dissemination of AMR across the world. The screening of human exudes from flights revealed the incidence of AMR genes of the most commonly used antibiotics. The most commonly identified AMR genes were of tetracycline, macrolide, and beta-lactams (Nordahl Petersen et al., 2015). A study conducted on frequent South Asian travelers revealed that *blaCTX-M* carrying *E. coli* was found with polyclonal acquisition, suggesting that ARGs are acquired during the international travels (Bevan et al., 2018). The detection of resistance genes in these long-distance flights has signaled to tap the genomics to source the track of transmission (Hendriksen et al., 2019, b).

5.3 Travel by Bus, Truck, Waterway, and Air, and Contribution to AMRs

The studies revealed that among the global population India and China contribute to >35% of AMR. In India, 23 million people travel in trains daily in 12,619 trains. In addition, 8395 million people travel per year across the country. By adding the mobility across the world and within the country in trains, buses, trucks, waterways, and airways, the anthropogenic pressure on the global spread of antimicrobial

pressure could be predicted. Similarly, within 24 hours a passenger from one part of the world reaches the other part of the world. This situation asks for the exact role of travel by different means within and between nations in the spread of AMR.

6 Metagenomics for ARGs' Determination in the Environment

Resistance of bacteria in the environment due to the overdosage of antimicrobial reagents is considered a major issue in global health (Giedraitienė et al., 2011). The phenomenon of the emergence of drug obdurate microbes in the environment started in the 1940s with the advent of large-scale production of penicillin. The indiscriminate use of antimicrobials caused a foremost challenge in healthcare settings, agriculture, livestock farming, and aquaculture. The emergence of AMR and ARGs from these fields can sink into the environment.

Resistance is a natural mechanism used by bacteria, which can be induced or noninduced. The widespread use of antibiotics, in contrast, has accelerated the occurrence of extremely antimicrobial-unresponsive microbes. The drug obduracy spreads predominantly among microbes of the identical genus and, to a lesser extent, amidst phyla, resulting in the creation of potentially dangerous bacteria (von Wintersdorff et al., 2016).

Various scientific approaches were developed to identify bacterial virulence, resistance mechanism, and other metabolic activity of bacteria. Schmieder and Edwards (2012) observed that most of the bacteria in the environment are in the VBNC state. In gauging the entire mechanism of resistance in both culturable and nonculturable bacteria, methods such as high-throughput DNA sequencing made the task much easier. Metagenomics facilitated the purpose of identifying the antimicrobial resistance in any samples (Bello-López et al., 2019).

In 1998, Hendelsman coined the term "metagenomics." This method was derived from traditional microbial genomics and reflects the fact that sequencing does not require pristine cultures (Handelsman et al., 2002). Meta-genomic research aids in the discovery of new species and the reconstruction of genomes of creatures that cannot be cultivated *in vitro* (Alves et al., 2018).

The development of metagenomics has aided in the understanding of the microbiome's involvement in a complex environment (Venter et al., 2004). The culture-independent technique gives useful information on the vast range of microbial populations that cannot be propagated in laboratory conditions. The detection of ARGs by collecting gut microflora, sediment, or water, and processing the directly extracted meta-DNA from these samples for the presence of ARGs is a commonly employed culture-independent approach. PCR, PCR-cloning, qPCR, meta-genomic technique using next-generation sequencing, and microarrays can all be used to determine ARGs using a culture-independent method from this meta-DNA.

Next-generation sequencers with better resistome analysis employing meta-genomic data are a recent breakthrough in sequencing methodologies. The multiplicity of ARGs, together with the microbiome/diversity/bacterial community structure, can be determined by sequencing the meta-genome taken from sediment or animal gut

microbiota. In contrast to culture-dependent approaches for microbial community analysis, metagenome analysis employing high-throughput sequencing-based analysis (HTS) provides a thorough community structure that includes both dominating and diverse flora, and the data can be statistically compared between samples.

The key constraint of this strategy is the involvement of millions of dollars (USD) in expenditures for the creation of the infrastructure. This method offers a deeper understanding of the resistome targeted as well as developing unique ARGs. Furthermore, comparison analysis and data storage necessitate both skill and space. However, in recent decades, there has been a significant reduction in the price of analysis, and the creation of multiple commercial laboratories for outsourcing analysis helps tide over the limitations. In aquaculture, a number of investigations have been carried out.

The sequencing-based metagenomics were primarily used to establish resistomes in sediment, animal gut/tissues, or water; determine the intricate processes at work in the host–environment relationship; identify the beneficial flora bearing these transmissible ARGs; and locate resistance materials in elements such as MGEs for mobility assessing the transmission ability. The influence of drugs is specified by feed makeup and metabolic capacity of gut microbiome (Yang et al., 2013; Vaz-Moreira et al., 2014; Kokou et al., 2020).

The research of AMR bacteria and ARGs in the targeted and nontargeted, namely, the culture-based and quantitative PCR and metagenomics methods, is employed (Venter et al., 2004; Sommer et al., 2009; Zhang et al., 2011). Conversely, only 1–10% of bacteria can be cultured depending on the environmental sources (Vaz-Moreira et al., 2014). From this point of view, employing amalgamation of culture-based and culture-free procedures in scrutinizing the environmental resistome is possibly the most effective tool (Hashmi, 2020).

A combination of metagenomics and meta-transcriptomic analysis could reveal the active population in the environment harboring these resistance genes, proving the idea that the existence of antimicrobial drugs at sub-MIC concentrations favors the spread of resistance. There are more studies on efflux pump-mediated resistance and enzymatic breakdown of antibiotics in specific bacteria that are important to human health (Wright, 2005). The total relative abundance of both portable colistin-insusceptible mcr gene variants and tigecycline-insusceptible *tetX* gene variations was more in combined farms than in monoculture farms (Xu et al., 2020). The possibility of ARG transfer from *Acinetobacter baumannii* to *Klebsiella* and *Pseudomonas* was discovered, as well as the need for a paradigm for global epidemiology based on resistome.

Functional metagenomics is a relatively new approach for identifying unknown genes unaffected by antibiotics in the environs, with the ability to disentangle hundreds of resistant genes with a similarity of more than 65% to the existing database. The sensitivity and specificity of resistome analysis are reduced if the rate of ARGs increases due to allelic changes. Researchers are exploring new tools to tackle the complexity of sequence-based analysis. One of these is "target capture platforms," which selectively enrich antimicrobial, heavy metal, and other resistance-related sequences. ResCap is a platform that searches for resistance in >8600 genes (Lanza et al., 2018). Antibiotic Resistance Genes Database (ARDB) is

one of many platforms now accessible for evaluating antibiotic resistance genes utilizing a sequencing-based technique. sraX, ResistoXplorer, MEGARes, SARG, DeepARG, and PRAP: Pan Resistome Analysis Pipeline are some of the other tools and software available for determining AMR from sequencing analysis data (Dhariwal et al., 2017). These tools can analyze the resistome diversity of a range of sequencing data outputs.

The bacterial resistome of untreated sewage that belonged to 79 locations in 60 nations was subjected to metagenomics analysis for categorization (Hendriksen et al., 2019, b). The study also observed systematic variations in the quantity and diversity of AMR genes among Europe/North America/Oceania and Africa/Asia/South America. Furthermore, the study reported a significant correlation of socio-economic conditions, well-being, and dynamics of the environment in the forecast of AMR gene profusions in all nations. The outcome of the study indicated that genetic variations of AMR and its profusion varied with area worldwide and that increasing sanitation and health could help reduce the all-inclusive AMR burden.

7 Conclusion

In the transmission and spread of AMR, the environment plays a critical role in both direct and indirect ways. Among the numerous ecosystems, the aquatic environment plays a very pivotal role in the AMR diffusion as it gets connected to different other ecosystems. However, much emphasis has not been given to the global action on the control of AMR. It is indeed a very much important component of one health and much complex in nature for the control. Even though the antimicrobial residue in the environment is crucial in development, this is not the only factor that drives the AMR in the environment. The transfer of resistance rate has also to be determined holistically in the environment and the way it is carried out with clinical strains. Moreover, the naturally occurring antibiotics may also facilitate the development in the environment that has to be delineated from the evolution that occurs through the application of chemicals. Technologies, viz., biological- and chemical-based available for treating the ARBs containing various ARGs for reducing the risk associated with the environmental persistence of AMR or ARGs, are to be in place for bringing down the burden of AMR in the environmental context. Regarding climate change, there is a huge scope for a shift in the pathogenic flora from the environment to the several hosts and the emergence of newer pathogens with varied patterns of antimicrobial resistance.

References

Ahammad, Z. S., et al. (2014). Increased waterborne bla NDM-1 resistance gene abundances associated with seasonal human pilgrimages to the Upper Ganges River. *Environmental Science & Technology, 48*(5), 3014–3020.

Akiba, M., et al. (2015). Impact of effluvium from different sources on the prevalence of antimicrobial-resistant Escherichia coli in sewage treatment plants in South India. *Ecotoxicology and Environmental Safety, 115*, 203–208.

Alves, L. D. F., Westmann, C. A., Lovate, G. L., de Siqueira, G. M. V., Borelli, T. C., & Guazzaroni, M. E. (2018). Metagenomic approaches for understanding new concepts in microbial science. *International Journal of Genomics, 2018*.

Amin, M. B., Saha, S. R., Islam, M. R., Haider, S., Hossain, M. I., Chowdhury, A., Rousham, E. K., & Islam, M. A. (2021). High prevalence of plasmid-mediated quinolone resistance (PMQR) among E. coli from aquatic environments in Bangladesh. *PLoS One, 16*(12), e0261970. https://doi.org/10.1371/journal.pone.0261970

Azam, M., Jan, A. T., & Haq, Q. M. R. (2016). blaCTX-M-152, a novel variant of CTX-M-group-25, identified in a study performed on the prevalence of multidrug resistance among natural inhabitants of river Yamuna, India. *Frontiers in Microbiology, 7*, 176.

Bello-López, J. M., Cabrero-Martínez, O. A., Ibáñez-Cervantes, G., Hernández-Cortez, C., Pelcastre-Rodríguez, L. I., Gonzalez-Avila, L. U., & Castro-Escarpulli, G. (2019). Horizontal gene transfer and its association with antibiotic resistance in the genus Aeromonas spp. *Microorganisms, 7*(9), 363.

Bevan, E. R., McNally, A., Thomas, C. M., Piddock, L. J., & Hawkey, P. M. (2018). Acquisition and loss of CTX-M-producing and non-producing Escherichia coli in the fecal microbiome of travelers to South Asia. *MBio, 9*(6), e02408–e02418.

Blew, R. D. (1996). On the definition of ecosystem. *Bulletin of the Ecological Society of America, 77*(3), 171–173.

Bondarczuk, K., Markowicz, A., & Piotrowska-Seget, Z. (2016). The urgent need for risk assessment on the antibiotic resistance spread via sewage sludge land application. *Environment International, 87*, 49–55.

Browne, A. J., Chipeta, M. G., Woodhouse, G. H., Kumaran, E. P. A., Hamadani, B. H. K., Zaraa, S., Henry, N. J., Deshpande, A., Reiner, R. C., Jr., Lopez, A. D., Dunachie, S., Moore, C. E., Stergachis, A., Hay, S. I., & Dolecek, C. (2021). Global antibiotic consumption and usage in humans, 2000–18: A spatial modelling study. *The Lancet, 5*(12), E893–E904.

Carraro, E., Bonetta, S., Bertino, C., Lorenzi, E., Bonetta, S., & Gilli, G. (2016). Hospital effluents management: Chemical, physical, microbiological risks and legislation in different countries. *Journal of Environmental Management, 168*, 185–199.

Caucci, S., Karkman, A., Cacace, D., Rybicki, M., Timpel, P., Voolaid, V., et al. (2016). Seasonality of antibiotic prescriptions for outpatients and resistance genes in sewers and effluvium treatment plant outflow. *FEMS Microbiology Ecology, 92*(5), fiw060.

Chaudhry, D., & Tomar, P. (2017). Antimicrobial resistance: The next BIG pandemic. *International Journal of Community Medicine and Public Health, 4*(8), 2632–2636.

Chee-Sanford, J. C., Mackie, R. I., Koike, S., Krapac, I. G., Lin, Y. F., Yannarell, A. C., et al. (2009). Fate and transport of antibiotic residues and antibiotic resistance genes following land application of manure waste. *Journal of Environmental Quality, 38*(3), 1086–1108.

Chen, H., Li, Y., Sun, W., Song, L., Zuo, R., & Yanguo Teng, Y. (2020). Characterization and source identification of antibiotic resistance genes in the sediments of an interconnected river-lake system. *Environment International, 137*, 105538, ISSN 0160-4120. https://doi.org/10.1016/j.envint.2020.105538. https://www.sciencedirect.com/science/article/pii/S0160412019337845

Chen, Y., Su, J. Q., Zhang, J., Li, P., Chen, H., Zhang, B., Gin, K. Y.-H., & He, Y. (2019). High-throughput profiling of antibiotic resistance gene dynamic in a drinking water river-reservoir system. *Water Research, 149*, 179–189.

Czekalski, N., Gascón Díez, E., & Bürgmann, H. (2014). Effluvium as a point source of antibiotic-resistance genes in the sediment of a freshwater lake. *The ISME Journal, 8*(7), 1381–1390.

Dantas, G., Sommer, M. O., Oluwasegun, R. D., & Church, G. M. (2008). Bacteria subsisting on antibiotics. *Science, 320*(5872), 100–103.

Davies, J., & Davies, D. (2010). Origins and evolution of antibiotic resistance. *Microbiology and Molecular Biology Reviews, 74*(3), 417–433.

Dhariwal, A., Chong, J., Habib, S., King, I. L., Agellon, L. B., & Xia, J. (2017). MicrobiomeAnalyst: A web-based tool for comprehensive statistical, visual and meta-analysis of microbiome data. *Nucleic Acids Research, 45*(W1), W180–W188.

Di Cesare, A., Eckert, E. M., D'Urso, S., Bertoni, R., Gillan, D. C., Wattiez, R., & Corno, G. (2016). Co-occurrence of integrase 1, antibiotic and heavy metal resistance genes in municipal effluvium treatment plants. *Water Research, 94*, 208–214.

EMA Committee for Medicinal Products for Veterinary Use (CVMP) and EFSA Panel on Biological Hazards (BIOHAZ). (2017). EMA and EFSA Joint Scientific Opinion on measures to reduce the need to use antimicrobial agents in animal husbandry in the European Union, and the resulting impacts on food safety (RONAFA). *EFSA Journal, 15*(1). https://doi.org/10.2903/j.efsa.2017.4666. Accessed 2 June 2017.

Ferro, G., Guarino, F., Castiglione, S., & Rizzo, L. (2016). Antibiotic resistance spread potential in urban effluvium effluents disinfected by UV/H2O2 process. *Science of the Total Environment*, 560–561, 29–35.

Giedraitienė, A., Vitkauskienė, A., Naginienė, R., & Pavilonis, A. (2011). Antibiotic resistance mechanisms of clinically important bacteria. *Medicina, 47*(3), 19.

Göbel, A., McArdell, C. S., Suter, M. J. F., & Giger, W. (2004). Trace determination of macrolide and sulfonamide antimicrobials, a human sulfonamide metabolite, and trimethoprim in effluvium using liquid chromatography coupled to electrospray tandem mass spectrometry. *Analytical Chemistry, 76*(16), 4756–4764.

Guo, Y., Liu, M., Liu, L., Liu, X., Chen, H., & Yang, J. (2018). The antibiotic resistome of free-living and particle-attached bacteria under a reservoir cyanobacterial bloom. *Environment International, 117*, 107–115. https://doi.org/10.1016/j.envint.2018.04.045

Hamelin, K., Bruant, G., El-Shaarawi, A., Hill, S., Edge, T. A., Fairbrother, J., Harel, J., Maynard, C., Masson, L., & Brousseau, R. (2007). Occurrence of virulence and antimicrobial resistance genes in Escherichia coli isolates from different aquatic ecosystems within the St. Clair River and Detroit River areas. *Applied and Environmental Microbiology, 73*(2), 477–484. https://doi.org/10.1128/AEM.01445-06. (Accessed 31August, 2022 google Scholar).

Handelsman, J., Liles, M., Mann, D., Riesenfeld, C., & Goodman, R. M. (2002). Cloning the metagenome: Culture-independent access to the diversity and functions of the uncultivated microbial world. *Methods in Microbiology, 33*, 241–255.

Hashmi, M. Z. (Ed.). (2020). *Antibiotics and antimicrobial resistance genes: Environmental occurrence and treatment technologies*. Springer Nature.

Hendriksen, R. S., Bortolaia, V., Tate, H., Tyson, G. H., Aarestrup, F. M., & McDermott, P. F. (2019). Using genomics to track global antimicrobial resistance. *Frontiers in Public Health, 7*, 242.

Hendriksen, R. S., Munk, P., Njage, P., Van Bunnik, B., McNally, L., Lukjancenko, O., et al. (2019). Global monitoring of antimicrobial resistance based on metagenomics analyses of urban sewage. *Nature Communications, 10*(1), 1–12.

Hricová, K., Röderová, M., Fryčák, P., Pauk, V., Kurka, O., Mezerová, K., Štosová, T., Bardoň, J., Milde, D., Kučová, P., & Kolář, M. (2021). Prevalence of vancomycin-resistant enterococci and antimicrobial residues in effluvium and surface water. *Life (Basel, Switzerland), 11*(12), 1403. https://doi.org/10.3390/life11121403

Kathi, S. (2017). Antibiotic resistance gene due to manure application. In *Antibiotics and antibiotics resistance genes in soils* (pp. 141–150). Springer.

Kimera, Z. I., et al. (2021). Occurrence of Extended Spectrum Beta Lactamase (ESBL) Producers, Quinolone and Carbapenem Resistant Enterobacteriaceae Isolated from Environmental Samples along Msimbazi River Basin Ecosystem in Tanzania. *International Journal of Environmental Research and Public Health, 18*(16), 8264.

Knapp, C. W., Zhang, W., Sturm, B. S., & Graham, D. W. (2010). Differential fate of erythromycin and beta-lactam resistance genes from swine lagoon waste under different aquatic conditions. *Environmental Pollution, 158*(5), 1506–1512.

Kokou, F., Sasson, G., Mizrahi, I., & Cnaani, A. (2020). Antibiotic effect and microbiome persistence vary 963 along the European seabass gut. *Scientific Reports, 10*(1), 10003.

Kumar, S., Tripathi, V. R., & Garg, S. K. (2013). Antibiotic resistance and genetic diversity in water-borne Enterobacteriaceae isolates from recreational and drinking water sources. *International journal of Environmental Science and Technology, 10*(4), 789–798.

Kümmerer, K. (2001). Drugs in the environment: Emission of drugs, diagnostic aids and disinfectants into effluvium by hospitals in relation to other sources–A review. *Chemosphere, 45*(6–7), 957–969.
Kümmerer, K. (2004). Resistance in the environment. *Journal of Antimicrobial Chemotherapy, 54*(2), 311–320.
Kümmerer, K. (2009). Antibiotics in the aquatic environment–A review–Part II. *Chemosphere, 75*(4), 435–441.
Lancini, G., Parenti, F., & Gallo, G. G. (1995). The antibiotics. *Antibiotics*, 1–14.
Lanza, V. F., Baquero, F., Martínez, J. L., Ramos-Ruíz, R., González-Zorn, B., Andremont, A., et al. (2018). In-depth resistome analysis by targeted metagenomics. *Microbiome, 6*(1), 1–14.
Laxminarayan, R., et al. (2016). Access to effective antimicrobials: A worldwide challenge. *Lancet (London, England), 387*(10014), 168–175. https://doi.org/10.1016/S0140-6736(15)00474-2
Liu, L., Su, J. Q., Guo, Y., Wilkinson, D. M., Liu, Z., Zhu, Y. G., & Yang, J. (2018). Large-scale biogeographical patterns of bacterial antibiotic resistome in the waterbodies of China. *Environment International, 117*, 292–299.
Lübbert, C., et al. (2017). Environmental pollution with antimicrobial agents from bulk drug manufacturing industries in Hyderabad, South India, is associated with dissemination of extended-spectrum beta-lactamase and carbapenemase-producing pathogens. *Infection, 45*(4), 479–491.
Lundborg, C. S., & Tamhankar, A. J. (2017). Antibiotic residues in the environment of South East Asia. *BMJ, 358*.
Martinez, J. L. (2009). The role of natural environments in the evolution of resistance traits in pathogenic bacteria. *Proceedings of the Royal Society B: Biological Sciences, 276*(1667), 2521–2530.
Mazel, D. (2006). Integrons: Agents of bacterial evolution. *Nature Reviews Microbiology, 4*(8), 608–620.
Nordahl Petersen, T., Rasmussen, S., Hasman, H., Carøe, C., Bælum, J., Charlotte Schultz, A., et al. (2015). Meta-genomic analysis of toilet waste from long distance flights; a step towards global surveillance of infectious diseases and antimicrobial resistance. *Scientific Reports, 5*(1), 1–9.
O'Neill, J. (2016). Tackling drug-resistant infections globally: Final report and recommendations. *Review on Antimicrobial Resistance.*. Accessed on April 15, 2017.
Odum, E. P., & Smalley, A. E. (1959). Comparison of population energy flow of a herbivorous and a deposit-feeding invertebrate in a salt marsh ecosystem. *Proceedings of the National Academy of Sciences, 45*(4), 617–622.
Poonia, S., Shantikumar Singh, T., & Tsering, D. C. (2014). Antibiotic susceptibility profile of bacteria isolated from natural sources of water from rural areas of East Sikkim. *Indian Journal of Community Medicine, 39*(3), 156.
Rather, T. A., et al. (2013). Antibiotic sensitivity of E. coli and Salmonella isolated from different water sources in Kashmir, India. *Comparative Clinical Pathology, 22*(4), 729–731.
Rizzo, L., Manaia, C., Merlin, C., Schwartz, T., Dagot, C., Ploy, M. C., et al. (2013). Urban effluvium treatment plants as hotspots for antibiotic resistant bacteria and genes spread into the environment: A review. *Science of the Total Environment, 447*, 345–360.
Said, L. B., et al. (2015). Detection of extended-spectrum beta-lactamase (ESBL)-producing Enterobacteriaceae in vegetables, soil and water of the farm environment in Tunisia. *International Journal of Food Microbiology, 203*, 86–92.
Schmieder, R., & Edwards, R. (2012). Insights into antibiotic resistance through metagenomic approaches. *Future Microbiology, 7*(1), 73–89.
Skariyachan, S., et al. (2015). Environmental monitoring of bacterial contamination and antibiotic resistance patterns of the fecal coliforms isolated from Cauvery River, a major drinking water source in Karnataka, India. *Environmental Monitoring and Assessment, 187*(5), 1–13.
Sommer, M. O., Dantas, G., & Church, G. M. (2009). Functional characterization of the antibiotic resistance reservoir in the human microflora. *Science, 325*(5944), 1128–1131.
Stanton, I. C., Murray, A. K., Zhang, L., Snape, J., & Gaze, W. H. (2020). Evolution of antibiotic resistance at low antibiotic concentrations including selection below the minimal selective concentration. *Communications Biology, 3*(1), 1–11.

Stoll, C., et al. (2012). Prevalence of clinically relevant antibiotic resistance genes in surface water samples collected from Germany and Australia. *Environmental Science & Technology, 46*(17), 9716–9726.

Swaminathan, S., et al. (2017). Strengthening infection prevention and control and systematic surveillance of healthcare associated infections in India. *BMJ (Clinical Research ed.), 358*, j3768. https://doi.org/10.1136/bmj.j3768

Taylor, N. G., Verner-Jeffreys, D. W., & Baker-Austin, C. (2011). Aquatic systems: Maintaining, mixing and mobilising antimicrobial resistance? *Trends in Ecology & Evolution, 26*(6), 278–284.

Tenover, F. C. (2006). Mechanisms of antimicrobial resistance in bacteria. *The American Journal of Medicine, 119*(6), S3–S10.

Turiel, E., Bordin, G., & Rodríguez, A. R. (2005). Study of the evolution and degradation products of ciprofloxacin and oxolinic acid in river water samples by HPLC-UV/MS/MS-MS. *Journal of Environmental Monitoring, 7*(3), 189–195.

Urbaniak, M., Kiedrzyńska, E., & Grochowalski, A. (2017). The variability of PCDD/F concentrations in the effluent of effluvium treatment plants with regard to their hydrological environment. *Environmental Monitoring and Assessment, 189*(2), 1–13.

Vaz-Moreira, I., Nunes, O. C., & Manaia, C. M. (2014). Bacterial diversity and antibiotic resistance in water habitats: Searching the links with the human microbiome. *FEMS Microbiology Reviews, 38*(4), 761–778.

Venter, J. C., Remington, K., Heidelberg, J. F., Halpern, A. L., Rusch, D., Eisen, J. A., et al. (2004). Environmental genome shotgun sequencing of the Sargasso Sea. *Science, 304*, 66–74.

Von Wintersdorff, C. J., Penders, J., Van Niekerk, J. M., Mills, N. D., Majumder, S., Van Alphen, L. B., et al. (2016). Dissemination of antimicrobial resistance in microbial ecosystems through horizontal gene transfer. *Frontiers in Microbiology, 7*, 173.

Walters, E., McClellan, K., & Halden, R. U. (2010). Occurrence and loss over three years of 72 pharmaceuticals and personal care products from biosolids–soil mixtures in outdoor mesocosms. *Water Research, 44*(20), 6011–6020.

Wang, C. J., Li, Z., Jiang, W. T., Jean, J. S., & Liu, C. C. (2010). Cation exchange interaction between antibiotic ciprofloxacin and montmorillonite. *Journal of Hazardous Materials, 183*(1–3), 309–314.

Waseem, H., Williams, M. R., Stedtfeld, R. D., & Hashsham, S. A. (2017). Antimicrobial resistance in the environment. *Water Environment Research, 89*(10), 921–941.

Wright, G. D. (2005). Bacterial resistance to antibiotics: Enzymatic degradation and modification. *Advanced Drug Delivery Reviews, 57*(10), 1451–1470.

Xu, C., Lv, Z., Shen, Y., Liu, D., Fu, Y., Zhou, L., et al. (2020). Metagenomic insights into differences in environmental resistome profiles between integrated and monoculture aquaculture farms in China. *Environment International, 144*, 106005.

Yang, J., Wang, C., Shu, C., Liu, L., Geng, J., Hu, S., & Feng, J. (2013). Marine sediment bacteria harbor antibiotic resistance genes highly similar to those found in human pathogens. *Microbial Ecology, 65*(4), 975–981.

Yang, J. F., Ying, G. G., Zhao, J. L., Tao, R., Su, H. C., & Chen, F. (2010). Simultaneous determination of four classes of antibiotics in sediments of the Pearl Rivers using RRLC–MS/MS. *Science of the Total Environment, 408*(16), 3424–3432.

Zhang, T., Zhang, X. X., & Ye, L. (2011). Plasmid metagenome reveals high levels of antibiotic resistance genes and mobile genetic elements in activated sludge. *PLoS One, 6*(10), e26041.

Zhao, L., Dong, Y. H., & Wang, H. (2010). Residues of veterinary antibiotics in manures from feedlot livestock in eight provinces of China. *Science of the Total Environment, 408*(5), 1069–1075.

Zinner, S. H. (2007). Antibiotic use: Present and future. *New Microbiologica, 30*(3), 321.

Diversity and Antibiotic Resistance of *E. coli* and *Vibrio* from Coastal Waters Across the Globe

A. A. Mohamed Hatha, Reshma Silvester, and P. S. Divya

Contents

Abstract

The current estimates indicate that 40% of the world's population is living in the coastal regions. Enormous quantities of untreated sewage from thickly populated and urbanized coastal regions are being discharged into the natural water bodies. Many coastal cities and towns across the world do not have the infrastructure to manage the ever-increasing loads of sewage, resulting in the entry of enormous quantities of wastewater into the coastal water bodies, which is often raw and laden with several pathogenic bacteria and viruses. The entry of wastewater, along with its diverse array of bacteria of public health significance, *viz*., diarrheagenic *E. coli*, *Salmonella*, *Shigella* spp., pathogenic vibrios, *etc*., is causing serious public health issues either directly by affecting the people who depend on these water bodies for livelihood activities and recreation or by contaminating the fish and shellfish stocks harvested from it. Several of the pathogens entering the coastal waters carry antibiotic resistance determinants and virulence-related genes, which they transfer freely among them through

A. A. Mohamed Hatha (✉) · P. S. Divya
Department of Marine Biology, Microbiology and Biochemistry, School of Marine Sciences, Cochin University of Science and Technology, Cochin, Kerala, India
e-mail: mohamedhatha@cusat.ac.in

R. Silvester
School of Industrial Fisheries, Cochin University of Science and Technology, Cochin, Kerala, India

M. P. Mothadaka et al. (eds.), *Handbook on Antimicrobial Resistance*,
https://doi.org/10.1007/978-981-19-9279-7_11

horizontal gene transfer (HGT). The organically polluted coastal waters provide an ideal platform for HGT and further compounding this issue. This chapter deals with the distribution and antibiotic resistance among these pathogenic bacteria in various coastal environments worldwide.

Keywords

Pathogens · Coastal waters · Diarrheagenic *E. coli* · Vibrio · *E. coli* · Antibiotics · Multidrug resistance

1 Introduction

Worldwide, the marine and estuarine ecosystems are being threatened by pollution. Industrialization, urbanization, improper sewage treatment, and lack of good hygiene practices universally increased the loads of harmful microorganisms in aquatic ecosystems. Besides urban surface runoff, sewers are recognized as the primary source of coastal/debouched water contamination. More than 80% of wastewater is directly disposed into rivers or the ocean waters untreated, causing detrimental effects on the water quality along the coast (Manini et al., 2022). The quantum of wastewater discharged into these water bodies is greatly increased during the rainstorm events.

Besides the above, point and nonpoint discharges also contribute to pollution of the coastal water bodies. The major point discharge sources such as processed and unprocessed sewage and nonpoint sources such as land run-off and stormwater run-off from urban dwellings and commercial areas end in the release of large quantum of pathogenic bacteria into coastal water bodies (Howe et al., 2002). The organic-rich nutrients influx in the coastal and estuarine waters from point and nonpoint sources has a direct influence on the microbial population of the system. Moreover, the coastal water sediment acts as potential reservoirs for survival of harmful bacteria, namely, *E. coli* and *Vibrio* (Chandran et al., 2008; Silvester et al., 2021). Bacterial contamination indicates the sanitary conditions and health status of sewage disposal near the coastal waters. For instance, the level of Shiga Toxin-producing *E. coli* (STEC) present in coastal waters in southeast coast of India revealed the extent of microbial contamination (Kamala et al., 2022). In another study from Chennai coastal waters, it was revealed that beaches located closer to the river mouth were comparatively more contaminated with the fecal coliforms than those located away (Begum et al., 2021). The study also marked the coastal waters of India as a "concoction of sewage indicator bacteria." Entry of fecal pathogens to near-shore waters is also facilitated through on-site septic systems. In a study by the Central Pollution Control Board (CPCB, 2013) of India, it is estimated that only 13% of the discards generated are treated properly and the leftover (87%) is let loose into the untreated natural waters. The ballast water discharged from the ships is also identified as the main vector of international transport of pathogenic bacteria, and many invasive marine species to the coastal waters and harbors (Meena et al., 2022).

For instance, the increase in international shipping activities causes a major concern for transmission of *V. cholerae* across geographic borders. Nearly 72 strains of *V. cholerae* bacteria were isolated from the ballast water of ships from the Andaman and Nicobar Islands in India (Meena et al., 2022). In another study by David et al. (2007), fecal coliforms were discovered in the Mediterranean ballast waters. *E. coli* was detected in the ballast waters of ships that entered Bushehr coastal waters in the Gulf of Persia (Soleimani et al., 2021).

The presence of indicator bacteria such as fecal coliforms in water does not mean the water contains pathogenic microorganisms; however, it suggests the possibility of the presence of pathogens as both of them share the same habitat. Estimation of fecal coliforms in the water bodies gives a brief idea about the extent of fecal pollution in water bodies (Mok et al., 2016). Among the various fecal indicator bacteria, *Escherichia coli* is considered an ideal indicator for fecal pollution as they are common inhabitants in the intestine of homoeothermic organisms and are the major representatives of the fecal coliforms. Although *E. coli* were widely considered commensal, there are several well-established pathogenic serotypes and phylotypes of *E. coli* that are reported across the globe (Divya & Hatha, 2019). The species contains both intestinal and extraintestinal pathogens. Although many of the *E. coli* are nonvirulent, certain strains transmit pathogenic traits, conferring them the ability to give rise to gastrointestinal diseases like diarrhea, besides other infections such as meningitis, septicemia, and urinary tract infections, which are extraintestinal in nature (Divya & Hatha, 2019). Intestinal pathogenic groups of *E. coli* comprise enteropathogenic (EPEC), enterohemorrhagic (EHEC), enterotoxigenic (ETEC), and enteroinvasive and enteroaggregative (EAEC).

Vibrio bacterium is another major pathogen commonly found in aquatic systems. They are widely distributed in marine and coastal waters because of their halophilic nature (Thompson et al., 2004). To date, there are 115 species of Vibrios, of which 12 are recognized human pathogens: *V. alginolyticus, V. cholerae, V. cincinnatiensis, V. damselae, V. harveyi, V. hollisae, V. fluvialis, V. furnissii, V. metschnikovii, V. mimicus, V. parahaemolyticus,* and *V. vulnificus* (Lin et al., 2018). Many of these *Vibrio* species are responsible for caused outbreaks of gastrointestinal diseases in humans (Eiler et al., 2006). Many of them are also found to be pathogenic to marine animals like fish, shellfish, and corals. There is a marked increase in *Vibrio*-related infections across the world, which is related to the overall increase in the sea surface temperature of the world's oceans (Vezzulli et al., 2015). Eutrophication of coastal water bodies and resultant algal blooms, as well as the association of pathogenic vibrios with blooming algal species, is also of great concern among the researchers worldwide. On many occasions, these algae-associated pathogenic vibrios get into filter-feeding bivalves and reach the consumers at a higher load, which often can result in health issues. Accordingly, there is a renewed interest in the study of pathogenic vibrios in the coastal waters both in terms of ecology and epidemiology.

Another major issue that is plaguing the world is the frequent emergence of antimicrobial unresponsive bacteria that are posing a serious threat to public health. The World Health Organization (WHO) has recently declared antimicrobial

resistance (AMR) as a significant threat to human well-being. Although AMR was regarded mainly as a clinical problem until recently, the importance of widespread transmission through food and environmental routes is being highlighted in recent studies. Accordingly, the concept of "One Health" has come to the fore and is currently being recognized as the most important approach to tackle the spread of AMR among bacteria. Overuse and imprudent use of antibiotics are rampant, especially in developing nations, where a chunk of the population is not aware of the negative consequences. Creating awareness about the ill effects of improper and frequent use of antibiotics is of paramount importance, and there are concerted efforts that are taking place in this regard across the globe.

Sewage effluents from the wastewater treatment plants (WWTPs) are believed to be hotspots for the transmission of superbugs and antibiotic resistance genes (ARGs) to the natural waters (Chen et al., 2020). It was discovered that ARGs encoding resistance toward many groups of antibiotics were found in the WWTP effluents in coastal waters in China (Chen et al., 2020). The aquaculture waters in Dongshan Bay, China, were identified as a reservoir of pathogens carrying ARGs (Cui et al., 2022). Polluted natural waters present a suitable habitat for the horizontal gene transfer (HGT) of ARGs between bacteria from various sources. Such water bodies have become a hotspot for diverse antibiotic resistance determinants and multidrug-resistant pathogens, which gets into the humans through the environmental exposure or through food. Animal production systems and aquaculture practices are also contributing to the expansion of drug resistant mutants in an immense way. It is reported that the resistance to antimicrobials and presence of underlying genes among the bacterial strains isolated from the population of Chileans living close to the aquaculture regions was significantly higher than those isolated from non-aquaculture regions (Tomova et al., 2015). Drug-resistant microbes and associated ARGs could be employed as bioindicators to assess the environmental quality of coastal water bodies. The emergence of AMR in natural water bodies has not been given enough attention yet. The release of sewage is reported to be the prime reason for the entry of drug-resistant bacteria from various sources into the natural waters (Silvester, 2017). Antibiotics used in the humans will mostly end up in sewerage system either as degraded or trapped in the sewage sludge, which will be finally released into the rivers. The antibiotic residues reaching the natural waters through wastewater are providing a perfect habitat for the collection and emergence of drug-resistant mutants. Usage of sludge as fertilizer and irrigation of crops with wastewater and contaminated surface waters will also result in the entry of antibiotics into agricultural fields. Contamination of aquatic environments with antibiotics is a serious issue as it will lead to the entry of drug-resistant microbes into humans either through direct contact or *via* the food cycle (Henriques et al., 2006). Various pathways through which antibiotics enter natural water systems are depicted in Fig. 1.

At present, the concept of "One Health" is targeting reduction in antibiotic usage in both human and animal production systems (including aquaculture) as the drug-resistant mutants emerging in any one of the systems could reach the other without

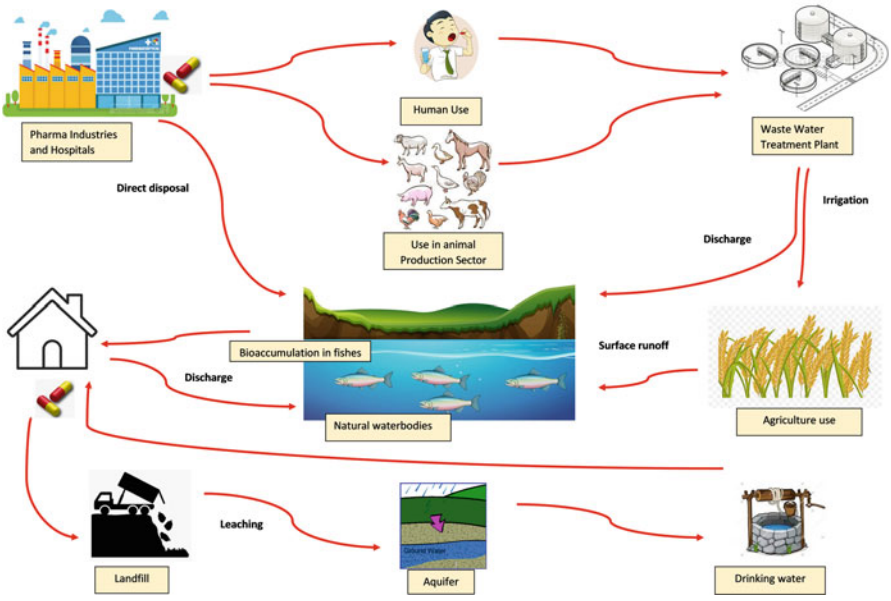


Fig. 1 Pathways of antibiotic contamination in natural water bodies

any difficulty. Rapidly increasing presence of pathogens, specifically MDR, is predicted to severely threaten global health.

While increasing prevalence and multiresistant organisms (MRO) of various waterborne and foodborne pathogens are of equal importance, in this chapter two major organisms were dealt with, namely, *Escherichia coli*, which is fast changing its status from commensal to pathogen, and *Vibrio* spp., whose prevalence and disease-causing potential in the global warming scenario are increasing across the world.

2 Incidence and Drug-resistant Pathogenic Vibrios in the Coastal Waters of Different Parts of the World

World over vibrios inhabit natural waters abundantly. While they can be isolated in culture without much difficulty, the injured cells failed to develop on selective media. However, studies revealed the existence of *Vibrio* bacteria in natural waters. Natural environment is a highly dynamic system, and, hence, the survival of pathogens is greatly affected by various physicochemical and biotic factors. Concentration of the nutrients, ambient temperature, and salinity are the key parameters that affect survival kinetics of *Vibrio* spp. in aquatic systems (Frolicher et al., 2018). Although the species are adaptable enough to thrive in diverse environmental conditions, most species have been shown to grow best above temperature of 17 °C. *Vibrio* communities were identified in a marine saltern hypersaline

environment in Italy (Gorrasi et al., 2020). Few *Vibrio* species "hibernate" in deposits or associate with aquatic life, which enables these microbes to accumulate proteins and form biofilms and ensures that they can adapt effectively to the ongoing changes in ecosystems (Thompson et al., 2004). A positive relationship was reported between the *Vibrio* diversity and chlorophyll in a study conducted from Rosada lagoon in Yucatan Peninsula, and the bacteria were observed to get attached to phytoplankton and zooplanktons present in water (Ortiz-Carrillo et al., 2015).

With the increase in sea surface temperature (SST) due to global warming, the impact of vibrios is predicted to increase further (Frolicher et al., 2018). An increase in SST may proliferate the bacterial growth, leading to more incidences of disease by this bacterium. In temperate countries, temperature is a major driver for prevalence of vibrios, with higher loads in warmer months and greatly reduced counts in colder months (Frolicher et al., 2018). In a study from Japan, an increase in growth of certain vibrios was observed when the seawater temperature increased from 21 to 24.3 °C (Fukui et al., 2010). In tropical countries with a relatively stable temperature, seasonal variation was observed in *Vibrio* spp. distribution (Vijayan & Lee, 2014). Salinity above 25 ppt is known to have a negative impact on the distribution of *V. vulnificus* (Thompson et al., 2004), and for the same reason this organism is commonly found in waters of river mouth that are lesser saline than coastal marine waters. The studies of Barbieri et al. (1999) revealed that temperature and salinity are the two primary aspects influencing the distribution of *V. cholerae* in the natural water bodies.

Several studies have been conducted on the predominance of pathogenic vibrios in coastal waters across the globe. Among infections originating from consumption of seafoods in Asia, *V. parahaemolyticus* is the major responsible organism. It was previously reported to occur in the Vellar estuary and adjoining shrimp ponds in India (Alagappan et al., 2013). The incidence of *V. vulnificus* is reported in coastal waters in India (Jayasree et al., 2006). The inshore coastal waters of Kerala are replenished with indigenous *Vibrio* spp. (Prashanthan et al., 2011). In a study along the Palk Bay, five pathogenic *Vibrio* species, namely, *V. cholerae*, *V. hollisae*, *V. furnissii*, *V. alginolyticus,* and *V. aestuarianus,* were detected, among which *V. cholerae* was predominant (Sneha et al., 2016). *V. fluvialis* were isolated from Uriganga and Turag rivers in Dhaka, Bangladesh (Jainab et al., 2021). A low incidence of *V. parahaemolyticus* was reported in Tunisian coastal waters (Gdoura et al., 2016; Zaafrane et al., 2022). *V. alginolyticus* survived better in seawater compared to *V. parahaemolyticus* and *V. vulnificus* (Eiler et al., 2007). Tarh et al. (2022) reported the presence of *V. parahaemolyticus, V. vulnificus, V. mimicus,* and *V. fluvialis* from seawater in Cross River State, Nigeria. A recent study on the diversity of *Vibrio* in seawaters in Ishigaki, Japan, revealed the prevalence of *V. hyugaensis*, *V. owensii,* and *V. harveyi* (Amin et al., 2016). Out of the 15 *Vibrio* spp. detected in Malaysia, the predominant were *V. campbellii, V. parahaemolyticus, V. harveyi,* and *V. tubiashii* (You et al., 2016). In another study in Malaysia, 27 species of *Vibrio* were identified from the Port Klang estuary and Port Dickson waters, among which the frequently encountered were *V. owensii* and *V. rotiferianus* (Wong et al., 2019). Human pathogenic *V. vulnificus* was also isolated in the study.

V. alginolyticus followed by *V. parahaemolyticus*, non-O1 *V. cholerae,* and *V. vulnificus* preponderated in two estuaries along the Adriatic coast located in Italy (Barbieri et al., 1999). Mansergh and Zehr (2014) explored the diversity and distribution of pathogenic vibrios in Monterey Bay, CA, USA. Pathogenic vibrios belonging to *V. campbellii*, *V. cholerae*, *V. vulnificus*, and *V. parahaemolyticus* were detected in offshore waters and in sediments of southern Gulf of Mexico during two oceanographic cruises (Bernáldez-Sarabia et al., 2021*)*. *Vibrio* species were detected in lagoons of ecological importance from southern Caribbean Sea (Fernández-Delgado et al., 2017). In Europe, though vibriosis has been reported in Denmark, Italy, and France, no major outbreaks have yet been reported. Possible pathogenic *Vibrio* spp. were reported in French marine and delta area environments (Hervio-Heath et al., 2002) and sporadic vibriosis has been recorded in France. There are numerous reports on *V. parahaemolyticus* from marine environments in the United Kingdom (Ford et al., 2020; Martinez-Urtaza et al., 2018). *V. harveyi* clade were recently detected in nearshore ecosystems in the United Kingdom (Harrison et al., 2022). The study also presented the first report of aquaculture pathogens such as *V. jasicida* and *V. rotiferianus* in aquatic sources of the United Kingdom. Pathogenic microbes, namely, *V. cholerae*, *V. vulnificus,* and *V. parahaemolyticus,* were detected in seawater collected from Zanzibar region, Tanzania (Kheir et al., 2022).

Wound infections affected by *V. vulnificus* are frequently reported in the areas of Baltic Sea during summer (Frank et al., 2006). A low-salt condition as observed in the Baltic Sea is ideal for its growth. A case study reported that an 80-year-old man with a minor trauma in left leg was infected with *V. vulnificus* while swimming in the Baltic Sea waters (Meyer et al., 2022). The pathogen enters the body through the open wounds while swimming or bathing in contaminated waters. Wound infections often become lethal, and the affected usually have to be amputated. Immunocompromised patients or those with open wounds are particularly the vulnerable groups. Wound infections due to *V. fluvialis* were also recently reported in the Baltic Sea (Hecht et al., 2022). Occurrence of *V. parahaemolyticus* and *V. alginolyticus* was noted in the waters of the Baltic Sea. There were recent reports highlighting the prevalence of virulent *Vibrio* species in the Baltic Sea of Lithuania and the Curonian Lagoon (Gyraite et al., 2019). These studies clearly give an indication of the public health risks posed by natural bathing waters in the Baltic Sea. Prevalence of *V. fluvialis* in marine waters was reported in Asia, America, Africa, and the Mediterranean Sea (Ramamurthy et al., 2014). Reports from Europe, the United States, and India clearly give an indication that human infections do occur in areas where the pathogen has been recently isolated.

In a study on the diversity of pathogenic *Vibrio* from Cochin estuary along Kerala coast, a total of 16 *Vibrio* species (*V. agarivorans, V. aestuarinus, V. coralliilyticus, V. damselae, V. parahaemolyticus, V. proteolyticus, V. litoralis, V. rumoiensis, V. calviensis, V. natriegens, V. fischeri, V. furnisii, V. mytilii, V. pelagius, V. mimicus, V. pacinii,* and *V. superstes*) were isolated, among which *V. parahaemolyticus* was the dominant species (Silvester, 2017). The estuary waters contained many pathogens such as *V. parahaemolyticus, V. mimicus, Photobacterium damselae* (earlier *V. damselae*), and *V. furnissii*, which are often

implicated in human infections (Silvester, 2017). Thus, regular long-term monitoring programs need to be taken into consideration for assessing human pathogenic *Vibrio* spp. in estuarine or coastal areas.

In general, vibrios are mostly susceptible to clinically used antibiotics. However, recent studies report a higher frequency of drug-resistant vibrios worldwide (Silvester et al., 2015; Sneha et al., 2016; You et al., 2016; Lee et al., 2018; Parthasarathy et al., 2021). Multidrug-resistant pathogenic vibrios from polluted marine and estuarine environments pose a serious threat to consumers of seafood harvested from these water bodies as well as in those who use the system for recreational purpose (Shaw et al., 2014). Multidrug-resistant *V. cholerae* exhibiting resistance to various antibiotics, namely, the β-lactams, azithromycin, chloramphenicol, gentamicin, nitrofurantoin, oxytetracycline, tetracycline, and vancomycin, were reported in Palk Bay, India (Sneha et al., 2016). In a recent study from Southern Andaman Islands, India, *V. cholerae* isolated from ballast waters revealed the presence of multidrug resistance (MDR). Diverse patterns of resistance were encountered, and the prevalent resistance phenotype was streptomycin, chloramphenicol, and erythromycin (Meena et al., 2022). The *Vibrio* isolates from tropical waters in Peninsular Malaysia exhibited relatively higher insensitivity to ampicillin, erythromycin, and mecillinam (You et al., 2016). All the *V. parahaemolyticus* recently isolated from Tunisian coastal waters were resistant toward amikacin, colistin, penicillin, and cefotaxime (Zaafrane et al., 2022). In another study from Tyrrhenian coast, Italy, *Vibrio* strains harboring ARGs that are the source of obduracy to β-lactams and sulfonamide have been isolated (Gambino et al., 2022). MDR strains of *V. cholerae* non-O1/non-O139 isolates collected from shallow water samples in Russia during 2019–2020 were susceptible to gentamicin and doxycycline, and none of the strains were pathogenic (Trishina et al., 2022). This survey was done as part of the annual cholera monitoring program. In a study from the eastern coast of Saudi Arabia, a greater proportion of *V. parahaemolyticus* strains isolated from seawater were resistant to ampicillin, carbenicillin, and cephalothin (Ghenem & Elhadi, 2018). The *Vibrio* strains from Chesapeake Bay were sensitive to most of the antibiotics commonly used to treat them (Shaw et al., 2014). The antibiotic sensitivity studies among the vibrio isolates of surface waters in the Uganda revealed the prevalence of a particular AMR pattern, that is, colistin, cefotaxime, azithromycin, and fluoroquinolone (Onohuean et al., 2022). Antibiotic-resistant strains of *V. alginolyticus* were also reported in the Jeju coast in Korea (Choi et al., 2021). *V. vulnificus* resistant to antibiotics such as cefepime, cefoxitin, and erythromycin was observed in seawaters on Gadeok Island, Korea (Oh et al., 2021). The majority of the *Vibrio* strains from Cochin estuary, India, showed resistance toward life-saving antibiotics such as amikacin, ceftazidime, cephalothin, doxycycline, enrofloxacin, nalidixic acid, nitrofurantoin, sulfamethoxazole, streptomycin, and trimethoprim (Silvester et al., 2015), and all were sensitive to netillin. Plasmid-mediated resistance was observed toward 13 antibiotics, among which the most commonly encountered antibiotic was carbenicillin (Silvester et al., 2019). The presence of plasmid-mediated ARGs may lead to a rapid dissemination of antibiotic resistance between pathogens in these environments.

Antibiotic resistance among pathogenic vibrios in fish and shellfish collected from these contaminated estuarine and marine environments also poses a serious health risk. Antibiotic-resistant luminous *V. harveyi* were isolated from unhealthy shrimp from aquaculture ponds of coastal Andhra Pradesh, India (Jayasree et al., 2006). Lee et al. (2018) reported the presence of carbapenemase-producing *V. parahaemolyticus* in fish collected from marine and freshwater of Selangor. Similarly, multidrug-resistant *V. parahaemolyticus* were isolated from oysters in coastal parts of West Bengal, India (Parthasarathy et al., 2021). Thus, fish or shellfish may also act as a potential carrier for dissemination of MDR strains of *Vibrio* from environmental compartment to humans through the food web.

3 Occurrence and Drug resistance of Diarrheagenic *E. coli* in the Coastal Waters of Different Regions of the World

The incidence of diarrheagenic *Escherichia coli* in aquatic systems poses a huge threat to the public health. Its presence in water bodies indicates fecal pollution of the aquatic system. The strains of enterotoxigenic *E. coli* are responsible for diarrhea in children in developing nations and also traveler's diarrhea. Enteroaggregative *E. coli* is a causative agent of persistent diarrhea in nations belonging to low- and middle-income groups. *E. coli* is classified into different phylogroups A, B1, B2, and D. The A and B1 phylogroups are frequently encountered in environmental samples and considered emerging pathogens that cause intestinal diseases (Escobar-Páramo et al., 2004). Hamelin et al. (2007) assessed the presence of *E. coli* from different aquatic sources and noted spatial variations in the prevalence of phylogenetic group of *E. coli* strains. A and B1 were the most prevalent phylogroups in Kelantan River, deltas, and its adjacent coastal waters in Malaysia (Bong et al., 2020). Nursyirwani and Moestomo (2002) investigated pollution indicators in the water samples from the Bengkalis coast and Bantan Tengah River and observed large concentration of *E. coli* in coastal waters of Bengkalis. Water samples collected from Ennore coastal waters of India were evaluated for fecal pollution indicator bacteria (Bharathi et al., 2018), and a high prevalence of fecal coliforms such as *E. coli* and *S. faecalis* was recorded. In an interesting study from India, a group of researchers attempted to compare the difference in microbial contamination in coastal waters collected from the southeast coast of India during the COVID-19 pre-lockdown and post-lockdown periods (Vashi et al., 2022). The fecal coliform counts during the pre-lockdown were comparatively very high (approximately seven times higher) than the post-lockdown. This clearly indicated the anthropogenic influence on the microbial load in the coastal waters. In the natural water habitats, algae and aquatic plants also have a significant impact on the occurrence of *E. coli* (Bong et al., 2020). A significant correlation was observed among the environmental factors and distribution of *E. coli* in the seashores located in Toronto and Niagara regions, Canada (Sanchez et al., 2021). The association between temperature and turbidity was positively correlated to the *E. coli* levels in both regions. However, certain environmental factors were also found to vary according to the region. For instance, rainfall

was completely related to *E. coli* concentrations in the Toronto region, whereas it was negatively associated in the Niagara region. A higher density of *E. coli* bacteria was reported in coastal lagoon located in Brazil after heavy rainfall events (Neves & Santos, 2021).

A 3-year study on the diversity of *E. coli* bacterium in the estuary of Kochi revealed 58 different serotypes in this polluted water body. Phylogenetic analysis revealed that *E. coli* strains of A and B2 groups were the most abundant in estuarine waters, followed by group B1 and D. The prevalence of fecal coliforms such as *E. coli* in estuarine and coastal water is of great concern to the public health. Insufficient infrastructure to treat the ever-increasing load of wastewater has resulted in the release of partially treated/untreated sewage that has contributed significantly to the deterioration of estuarine water quality.

The *E. coli* is a common inhabitant in humans and other homoeothermic animals and is an ideal choice of microorganism for the study of emergence and transmission of antimicrobial resistance as they are major carriers of ARGs to other bacteria. Of late, the scientific findings indicate the significant role of *E. coli* in the spread of antimicrobial resistance in the environment (Henriques et al., 2006). Water is considered a major vector for transmission of Gram-negative bacteria insusceptible to antimicrobials, namely, that harbor, extended-spectrum beta-lactamases and carbapenemases. Many studies reported the incidence AMR in *E. coli* from aquatic waters across the world (Hatha et al., 2005; Henriques et al., 2006; Chandran et al., 2008; Olaniran et al., 2009). A latest report by Rahimi et al. (2022) screened 120 *E. coli* from nearshore waters of Bushehr, Iran, for their resistance toward numerous antibiotics. About 60% of the isolates were multidrug resistant. Nearly 69 *E. coli* isolated from coastal waters collected from Fujiazhuang beach, which is a bathing beach in China, were screened for antibiotic sensitivity, of which 58% were found to be multidrug resistant (Su et al., 2022). Furthermore, the study concluded that the municipal waste discharge may be the potential source of AMR strains on the beach. Alves et al. (2014) analyzed the incidence of *E. coli* AMR dissemination in Berlenga coastal water, Portugal. Elevated levels of resistance were observed in amoxicillin, cephalothin, streptomycin, and tetracycline. Prevalence of MDR *E. coli* was reported in the coastal recreational waters of southeastern Louisiana, USA (Cameron & Raj, 2018). Similarly, water and sediment samples drawn from various aquatic sources from northern Germany were analyzed, and the presence of *E. coli* harboring $bla_{CTX\text{–}M\text{–}1}$ and mobile colistin resistance gene *mcr1* was identified (Falgenhauer et al., 2019). In another study, ESBL *E. coli* ST-949 clone was found in shallow waters in Germany (Falgenhauer et al., 2021). In a study from Brazil, *mcr*-carrying *E. coli* was isolated from the coastal waters (Cordeiro-Moura et al., 2022). Drug-resistant phenotypes of *E. coli* were found in the shallow waters of Wyoming, located in the Western United States (Kaur, 2021). Nearly 56% of *E. coli* was isolated in nearshore waters of Veraval, India, that were multidrug resistant (Maloo et al., 2017). Drug-resistant *E. coli* in natural waters pose a considerable threat to surfers and other people who use these water bodies for recreation. In a study by Leonard et al. (2018), CTX-M-producing *E. coli* were isolated from UK coastal waters used for surfing activities. Dutch recreational waters have been noted as the major source of *E. coli* harboring extended-spectrum beta-lactamase (ESBL) (Blaak et al., 2014).

Release of wastewater from the treatment plants was found to be the major cause of the transmission of pathogenic bacteria into nearshore and surface waters. In a study during 2019–2020, Victoria et al. (2022) analyzed the nearshore waters along Kanyakumari, India, which are affected by the sewage discharge. And, drug-resistant *E. coli* was isolated from these samples along with other pollution indicator bacterial species such as *Klebsiella* spp., *Enterococcus faecalis*, *Salmonella typhi*, *Vibrio cholerae*, and *Shigella* spp. This study highlighted that untreated sewage discharged into the ocean might have a major negative impact on coastal wildlife and public health. Though *E. coli* is generally regarded as harmless outside the clinical settings, the spread of commensal flora that produce ESBL may pose serious hazards to public health. For instance, such bacteria may transfer the genes conferring resistance to ESBL to intestinal pathogens by means of horizontal gene transfer mechanism or exposure to pathogenic *E. coli* strains that produce ESBL and can result in a difficult-to-treat illness even among healthy people. Most beta-lactam class of antibiotics, including third and fourth generation cephalosporins, are ineffective against ESBL-producing bacteria. In a study from the southwest coast of India, genetically diverse ESBL-producing *E. coli* were isolated from Vembanad lake, which is a fresh-cum-brackish water lake and one of the major tourist hotspots of the region (Vaiyapuri et al., 2021). Mukherjee et al. (2021) analyzed the survival of *E. coli* harboring bla_{NDM1} (New Delhi Metallo beta-lactamase gene) in tropical coastal waters from Versova, off Mumbai coast. A stable survival was exhibited in tropical waters for 2 months, and the strain also maintained the resistance plasmid carrying bla_{NDM1} gene. Estuaries provide a conducive environment for the horizontal transfer of ARGs and dissemination of AMR (Na et al., 2018). In a study from Cochin estuary conducted during the 2001–2002 period, more than 95% of *E. coli* isolates were MDR (Chandran et al., 2008). In a report from the same estuary during 2009–2013, a predominance of *E. coli*-carrying ARGs was observed and 37.6% of the isolates were found to be multidrug resistant. The estuarine isolates exhibiting resistance toward critically and extremely relevant classes of antibiotics were reported (WHO, 2017). *E. coli* isolates from Cochin estuary exhibited a high level of resistance to ampicillin, tetracycline, and nalidixic acid. Comparable results were reported in *E. coli* incidence in aquatic sources from South Africa and Bangladesh (Talukdar et al., 2013; Olaniran et al., 2009).

4 Conclusions

It is well established that the presence of multidrug-resistant bacterial pathogens is likely to increase across the world both as a function of changes in climate and ecology as well as a result of irresponsible human behavior, especially in terms of the usage of antibiotics. We are heading toward an era where antibiotics might lose their status as magic bullets and the discovery of new bioactive molecules to fight infections is hard to come by. The intricate connections between the human and animal production systems, especially in Southeast Asia, could further compound the peril. Strict adherence to the concept of "One Health" and continuous surveillance of coastal and estuarine environments is urged to prevent future disease

outbreaks by microbes that are resistant to various antibiotics, namely, pathogenic *Vibrios* and *E. coli,* that cause diarrhea. It is necessary to regularly monitor the microbial pollution of coastal and estuarine waters as these waters are used for many recreational and commercial purposes. Stringent measures need also be taken to prevent the unregulated discharge of untreated sewage carrying antibiotic residues and multidrug-resistant pathogens into the natural waters.

References

Alagappan, K. M., Deivasigamani, B., & Balamurugan, S. (2013). Occurrences of pathogenic *Vibrio parahaemolyticus* from Vellar estuary and shrimp ponds. *African Journal of Biotechnology, 12*(14), 1624–1629.

Alves, M. S., Pereira, A., Araújo, S. M., Castro, B. B., Correia, A. C. M., & Henriques, I. (2014). Seawater is a reservoir of multi-resistant *Escherichia coli*, including strains hosting plasmid-mediated quinolones resistance and extended-spectrum beta-lactamases genes. *Frontiers in Microbiology, 5*, 426.

Amin, A. K. M. R., Feng, G., & Al-saari, N. (2016). The first temporal and spatial assessment of *Vibrio* diversity of the surrounding seawater of coral reefs in Ishigaki, Japan. *Frontiers in Microbiology, 7*, 1–12.

Barbieri, E., Falzano, L., Fiorentini, C., Pianitti, A., Baffone, W., Fabbri, A., Matarrese, P., Caisere, A., Katouli, M., Kühn, I., Mölby, F., Bruscolini, F., & Donelli, G. (1999). Occurrence, diversity and pathogenicity of halophilic *Vibrio* spp. and Non O1 *Vibriocholerae* from estuarine waters along the Italian Adriatic Coast. *Applied and Environmental Microbiology, 65*, 2748–2753.

Begum, M., Kumar, C. S., Naik, S., Pradhan, U., Panda, U. S., & Mishra, P. (2021). Indian coastal waters: A concoction of sewage indicator bacteria! An assessment on recreational beaches. *Environmental Monitoring and Assessment, 193*(7), 1–21.

Bernáldez-Sarabia, J., Lizárraga-Partida, M. L., Hernández-López, E. L., Gasperin-Bulbarela, J., Licea-Navarro, A. F., Guerrero, A., Sánchez-Castrejón, E., & Franco-Moreno, A. (2021). Distribution of pathogenic vibrios and Vibrio spp. in the water column and sediment samples from the southern Gulf of Mexico. *Marine Pollution Bulletin, 173*, 113116.

Bharathi, M. D., Sundaramoorthy, S., Patra, S., Perumal, M., & Sundaramanickam, A. (2018). Seasonal and spatial distribution of heterotrophic bacteria in relation to physico-chemical properties along Ennore coastal waters. *Indian Journal of Geo-Marine Sciences, 47*(3), 587–597.

Blaak, H., de Kruijf, P., Hamidjaja, R. A., van Hoek, A. H., de Roda Husman, A. M., & Schets, F. M. (2014). Prevalence and characteristics of ESBL-producing E. coli in Dutch recreational waters influenced by wastewater treatment plants. *Veterinary Microbiology, 171*(3–4), 448–459.

Bong, C. W., Chai, S. K., Chai, L. C., & Wang, AJ. And Lee, C. W. (2020). Prevalence and characterization of *Escherichia coli* in the Kelantan River and its adjacent coastal waters. *Water Supply, 20*(3), 930–942.

Cameron, B., & Raj, B. (2018). Presence of antibiotic-resistant bacteria and antibiotic resistance genes in coastal recreational waters of southeast Louisiana, USA. *Journal of Water Supply: Research and Technology-Aqua 1, 67*(8), 800–809.

Chandran, A., Hatha, A. A. M., Varghese, S., & Sheeja, K. M. (2008). Prevalence of multiple drug resistant *Escherichia coli* serotypes in a tropical estuary, India. *Microbes and Environment, 23*, 153–158.

Chen, J., Zhang, Z., Lei, Z., Shimizu, K., Yao, P., Su, Z., & Wen, D. (2020). Occurrence and distribution of antibiotic resistance genes in the coastal sediments of effluent-receiving areas of WWTPs, China. *Bioresource Technology Reports, 11*, 100511.

Choi, W. S., Moon, C. Y., & Heo, M. S. (2021). Distribution of Vibrio alginolyticus inhabiting the Jeju coast. *Journal of Marine Bioscience and Biotechnology, 13*(1), 48–57.

Cordeiro-Moura, J. R., Kraychete, G. B., de Araújo Longo, L. G., Corrêa, L. L., da Silva, N. M. V., Campana, E. H., et al. (2022). Description and comparative genomic analysis of a mcr-1-carrying Escherichia coli ST683/CC155 recovered from touristic coastal water in Northeastern Brazil. *Infection, Genetics and Evolution, 97*, 105196.

CPCB. (2013). *Performance evaluation of sewage treatment plants under NRCD*. Central Pollution Control Board. Accessed on 22 Sept 2018. http://cpcb.nic.in/openpdffile.php

Cui, G., Liu, Z., Xu, W., Gao, Y., Yang, S., Grossart, H. P., et al. (2022). Metagenomic exploration of antibiotic resistance genes and their hosts in aquaculture waters of the semi-closed Dongshan Bay (China). *Science of the Total Environment, 838*, 155784.

David, M., Gollasch, S., Cabrini, M., Perkovič, M., Bošnjak, D., & Virgilio, D. (2007). Results from the first ballast water sampling study in the Mediterranean Sea–the Port of Koper study. *Marine Pollution Bulletin, 54*(1), 53–65.

Divya, S. P., & Hatha, A. A. M. (2019). Screening of tropical estuarine water in south-west coast of India reveals emergence of ARGs-harboring hypervirulent *Escherichia coli* of global significance. *International Journal of Hygiene and Environmental Health, 222*(2), 235–248.

Eiler, A., Johansson, M., & Bertilsson, S. (2006). Environmental influences on *Vibrio* populations in northern temperate and Boreal coastal waters (Baltic and Skagerrak Seas). *Applied and Environmental Microbiology, 72*, 6004–6011.

Eiler, A., Gonzalez-Rey, C., Allen, S., & Bertilsson, S. (2007). Growth response of Vibrio cholerae and other Vibrio spp. to cyanobacterial dissolved organic matter and temperature in brackish water. *FEMS Microbiology Ecology, 60*(3), 411–418.

Escobar-Páramo, P., Grenet, K., Le Menac'h, A., Rode, L., Salgado, E., Amorin, C., Gouriou, S., Picard, B., Rahimy, M. C., Andremont, A., & Denamur, E. (2004). Large-scale population structure of human commensal *Escherichia coli* isolates. *Applied and Environmental Microbiology, 70*(9), 5698–5700.

Falgenhauer, L., Schwengers, O., Schmiedel, J., Baars, C., Lambrecht, O., Heß, S., et al. (2019). Multidrug-resistant and clinically relevant Gram-negative bacteria are present in German surface waters. *Frontiers in Microbiology, 10*, 2779.

Falgenhauer, L., Zur Nieden, A., Harpel, S., Falgenhauer, J., & Domann, E. (2021). Clonal CTX-M-15-producing Escherichia coli ST-949 are present in German surface water. *Frontiers in Microbiology, 12*, 617349.

Fernández-Delgado, M., Suárez, P., Giner, S., Sanz, V., Peña, J., Sánchez, D., & García-Amado, M. A. (2017). Occurrence and virulence properties of Vibrio and Salinivibrio isolates from tropical lagoons of the southern Caribbean Sea. *Antonie Van Leeuwenhoek, 110*(6), 833–841.

Ford, C. L., Powell, A., Lau, D. Y. L., Turner, A. D., Dhanji-Rapkova, M., Martinez-Urtaza, J., & Baker-Austin, C. (2020). Isolation and characterization of potentially pathogenic Vibrio species in a temperate, higher latitude hotspot. *Environ Microbiology Reports, 12*(4), 424–434.

Frank, C., Littman, M., Alpers, K., & Hallauer, J. (2006). Vibrio vulnificus wound infections after contact with the Baltic Sea, Germany. *Euro surveillance: bulletin européen sur les maladies transmissibles European communicable disease bulletin, 11*, E060817.1. https://doi.org/10.2807/esw.11.33.03024-en

Frolicher, T. L., Fischer, E. M., & Gruber, N. (2018). Marine heatwaves under global warming. *Nature, 560*, 360–364.

Fukui, Y., Saitoh, S. I., & Sawabe, T. (2010). Environmental determinants correlated to Vibrio harveyi-mediated death of marine gastropods. *Environmental Microbiology*, *12*(1), 124–133. https://doi.org/10.1111/j.1462-2920.2009.02052.x

Gambino, D., Savoca, D., Sucato, A., Gargano, V., Gentile, A., Pantano, L., Vicari, D., & Alduina, R. (2022). Occurrence of antibiotic resistance in the Mediterranean Sea. *Antibiotics, 11*(3), 332. (Editor access on 25 July 2022 Google Scholar).

Gdoura, M., Sellami, H., Nasfi, H., Trabelsi, R., Mansour, S., Attia, T., Nsaibia, S., Vallaeys, T., Gdoura, R., & Siala, M. (2016). Molecular detection of the three major pathogenic Vibrio species from seafood products and sediments in Tunisia using real-time PCR. *Journal of Food Protection, 79*(12), 2086–2094. (Editor access on 25 July 2022 Google Scholar).

Ghenem, L., & Elhadi, N. (2018). Isolation, molecular characterization, and antibiotic resistance patterns of *Vibrio parahaemolyticus* isolated from coastal water in the Eastern Province of Saudi Arabia. *Journal of Water Health, 16*(1), 57–69.

Gorrasi, S., Pasqualetti, M., Franzetti, A., Pittino, F., & Fenice, M. (2020). Vibrio communities along a salinity gradient within a marine saltern hypersaline environment (Saline di Tarquinia, Italy). *Environmental Microbiology, 22*(10), 4356–4366.

Gyraite, G., Katarzyte, M., & Schernewski, G. (2019). First findings of potentially human pathogenic bacteria *Vibrio* in the south-eastern Baltic Sea coastal and transitional bathing waters. *Marine Pollution Bulletin, 149*, 110546.

Hamelin, K., Bruant, G., El-Shaarawi, A., Hill, S., Edge, T. A., & Fairbrother, J. (2007). Occurrence of virulence and antimicrobial resistance genes in *Escherichia coli* isolates from different aquatic ecosystems within the St. Clair River and Detroitriver areas. *Applied Environmental Microbiology, 73*(2), 477–484.

Harrison, J., Nelson, K., Morcrette, H., Morcrette, C., Preston, J., Helmer, L., Titball, R. W., Butler, C. S., & Wagley, S. (2022). The increased prevalence of Vibrio species and the first reporting of *Vibrio jasicida* and *Vibrio rotiferianus* at UK shellfish sites. *Water Research, 211*, 117942. (Editor access on 25 July 2022 Google Scholar).

Hatha, M., Vivekanandhan, A. A., Joice, G. J., & Christol. (2005). Antibiotic resistance pattern of motile aeromonads from farm raised fresh water fish. *International Journal of Food Microbiology, 98*(2), 131–134.

Hervio-Heath, D., Colwell, R. R., Derrien, A., Robert-Pillot, A., Fournier, J. M., & Pommepuy, M. (2002). Occurrence of pathogenic vibrios in coastal areas of France. *Journal of applied microbiology*, *92*(6), 1123–1135. https://doi.org/10.1046/j.1365-2672.2002.01663.x

Hecht, J., Borowiak, M., Fortmeier, B., Dikou, S., Gierer, W., Klempien, I., Nekat, J., Schaefer, S., & Strauch, E. (2022). Case report: *Vibrio fluvialis* isolated from a wound infection after a piercing trauma in the Baltic Sea. *Access Microbiology, 4*(1). https://doi.org/10.1099/acmi.0.000312. (Editor access on 25 July 2022 Google Scholar).

Henriques, I. S., Fonseca, F., Alves, A., Saavedra, M. J., & Correia, A. (2006). Occurrence and diversity of integrons and beta-lactamase genes among ampicillin-resistant isolates from estuarine waters. *Research in Microbiology, 157*, 938–947.

Howe, A. D., Forster, S., Morton, S., Marshall, R., Osborn, K. S., Wright, P., & Hunter, P. R. (2002). Cryptosporidium oocysts in a water supply associated with a cryptosporidiosis disease, as estimated from studies published between 1992 and 2000. *Bulletin of World Health Organization, 81*, 197–204.

Jainab, T., Hasan, S., Manchur, M. A., Yasmin, M., Nessa, J., & Ahsan, C. R. (2021). Comparison of toxicities between Vibrio fluvialis and Vibrio furnissii strains isolated from environmental samples. *Bangladesh Journal of Microbiology, 38*(2), 73–77.

Jayasree, L., Janakiram, P., & Madhavi, R. (2006). Characterization of *Vibrio* spp. associated with diseased shrimp from culture ponds of Andhra Pradesh (India). *Journal of the World Aquaculture Society, 37*, 523–532.

Kamala, K., Rajeshkumar, S., & Sivaperumal, P. (2022). The predominance of Shiga toxin-producing E. coli in the Southeast Coast of India. *Marine Pollution Bulletin, 174*, 113188. https://doi.org/10.1016/j.marpolbul.2021.113188. (Editor access on 25 July 2022 Google Scholar).

Kaur, H. (2021). *Identification and characterization of antimicrobial resistance in indicator bacteria (E. coli and Enterococcus spp.) from surface waters of Wyoming*. University of Wyoming.

Kheir, K., Mbwele, B., Omar, K., Damas, M., & Namkinga, L. A. (2022). Epidemiological linkages of diarrheagenic Vibrio species from sea water, seafood and patients and their antimicrobial susceptibility patterns in Zanzibar, Tanzania. *Research Square*. https://doi.org/10.21203/rs.3.rs-1612443/v1. (Editor access on 25 July 2022 Google Scholar).

Lee, L.-H., Ab Mutalib, N.-S., Law, J. W.-F., Wong, S. H., & Letchumanan, V. (2018). Discovery on antibiotic resistance patterns of *Vibrio parahaemolyticus* in Selangor reveals carbapenemase

producing *Vibrio parahaemolyticus* in marine and freshwater fish. *Frontiers of Microbiology, 9*, 2513.

Leonard, A. F. C., Zhang, L., Balfour, A. J., Garside, R., Hawkey, P. M., Murray, A. K., Ukoumunne, O. C., & Gaze, W. H. (2018). Exposure to and characterization by antibiotic-resistant *E. coli* in UK coastal water users: Environmental surveillance, exposure assessment, and epidemiological study (Beach Bum Survey). *Environment International, 114*, 326–333.

Lin, H., Yu, M., Wang, X., & Zhang, X.-H. (2018). Comparative genomic analysis reveals the evolution and environmental adaptation strategies of vibrios. *BMC Genomics, 19*, 135.

Maloo, A., Fulke, A. B., Mulani, N., Sukumaran, S., & Ram, A. (2017). Pathogenic multiple antimicrobial resistant *Escherichia coli* serotypes in recreational waters of Mumbai, India: A potential public health rise. *Environmental Science Pollution Research International, 24*(12), 11504–11517.

Manini, E., Baldrighi, E., Ricci, F., Grilli, F., Giovannelli, D., Intoccia, M., Casabianca, S., Capellacci, S., Marinchel, N., Penna, P., Moro, F., Campanelli, A., Cordone, A., Correggia, M., Bastoni, D., Bolognini, L., Marini, M., & Penna, A. (2022). Assessment of spatio-temporal variability of faecal pollution along coastal waters during and after rainfall events. *Water, 14*(3), 502. https://doi.org/10.3390/w14030502. (Editor access on 25 July 2022 Google Scholar).

Mansergh, S., & Zehr, J. P. (2014). Vibrio diversity and dynamics in the Monterey Bay upwelling region. *Frontiers of Microbiology, 5*, 48.

Martinez-Urtaza, J., Trinanes, J., Abanto, M., Lozano-Leon, A., Llovo-Taboada, J., Garcia-Campello, M., Pousa, A., Powell, A., Baker-Austin, C., & Gonzalez-Escalona, N. (2018). Epidemic dynamics of *Vibrio parahaemolyticus* illness in a hotspot of disease emergence, Galicia, Spain. *Emerging Infectious Diseases, 24*(5), 852–859.

Meena, B., Anburajan, L., Sini, E. S., Vinithkumar, N. V., & Dharani, G. (2022). Diversity of *Vibrio cholerae* and prevalence of biomarker genes in the ballast water, Port Blair, South Andaman, India. *Ecological Genetics and Genomics, 23*, 100112. https://doi.org/10.1016/j.egg.2022.100112. (Editor access on 25 July 2022 Google Scholar).

Meyer, H. L., Polan, C., Burggraf, M., Podleska, L., Beck, P., Steinau, H. U., Dudda, M., et al. (2022). The Baltic Sea germ: A case report of necrotizing fasciitis following Vibrio vulnificus infection. *Case Reports in Orthopedics, 2022*, 5908666. https://doi.org/10.1155/2022/5908666. (Editor Access on 25 July 2022 Google Scholar).

Mok, J. S., Lee, T. S., Kim, P. H., Lee, H. J., Ha, K. S., Shim, K. B., Lee, K. J., Jung, Y. J., & Kim, J. H. (2016). Bacteriological quality evaluation of seawater and oysters from the Hansan-Geojeman area in Korea, 2011–2013: Impact of inland pollution sources. *Springer Plus, 5*(1), 1412. https://doi.org/10.1186/s40064-016-3049-9. (Editor Access on 25 July 2022 Google Scholar).

Mukherjee, S., Lekshmi, M., Ammini, P., Nayak, B. B., & Kumar, S. H. (2021). Survival of the blaNDM-harbouring Escherichia coli in tropical seawater and conjugative transfer of resistance markers. *Archives of Microbiology, 203*(7), 4273–4279.

Na, G., Lu, Z., Gao, H., Zhang, L., Li, Q., Li, R., Yang, F., Huo, C., & Yao, Z. (2018). The effect of environmental factors and migration dynamics on the prevalence of antibiotic resistant *Escherichia coli* in estuary environments. *Scientific Reports, 8*, 1663.

Neves, R. A., & Santos, L. N. (2021). Short-term effects of very heavy rainfall events on the water quality of a shallow coastal lagoon. *Hydrobiologia, 849*(17), 3947–3961.

Nursyirwani, N., & Moestomo, B. A. (2002). Comparison of *Escherichia Coli* concentration between Bengkalis coastal waters and estuary Bantan Tengah River. *Journal of Coastal Development, 6*(1), 1–7.

Oh, H. K., Jeong, H. J., Jeong, G. J., Shin, H. Y., Shin, J. H., Jung, W. K., ... & Kim, Y. M. (2021). Detection Characteristics and antimicrobial resistance of vibrio vulnificus isolated from sea water along the gadeok island coast. *Korean Journal of Fisheries and Aquatic Sciences*, *54*(6), 912–917. https://doi.org/10.5657/KFAS.2021.0912

Olaniran, A. O., Naicker, K., & Pillay, B. (2009). Antibiotic resistance profiles of *Escherichia coli* isolates from river sources in Durban, South Africa. *World Journal of Microbiology and Biotechnology, 25*, 1743–1749.

Onohuean, H., Okoh, A. I., & Nwodo, U. U. (2022). Antibiogram signatures of Vibrio species recovered from surface waters in South Western districts of Uganda: Implications for environmental pollution and infection control. *Science of the Total Environment, 807*, 150706. https://doi.org/10.1016/j.scitotenv.2021.150706. (Editor access on 25 July 2022 Google Scholar).

Ortiz-Carrillo, I., Estrella-Gómez, N. E., Zamudio-Maya, M., & Rojas-Herrera, R. (2015). Diversity of Vibrio spp., in karstic coastal marshes in the Yucatan Peninsula. *PLoS One, 10*(8), e0134953.

Parthasarathy, S., Das, S. C., Kumar, A., Chowdhury, G., Miyoshi, S. I., Dutta, S., & Mukhopadhyay, A. K. (2021). Molecular characterization and antibiotic resistance of *Vibrio parahaemolyticus* from Indian oyster and their probable implication in food chain. *World Journal of Microbiology and Biotechnology, 37*(8), 145.

Prashanthan, V., Udayakumar, P., Sarathkumar, & Ouseph, P. P. (2011). Influence of abiotic factors on abundance and distribution of *Vibrio* species in coastal waters of Kerala, India. *Indian Journal of Geo-Marine Sciences, 40*, 4587–4592.

Rahimi, Z., Malekzadegan, Y., Bahador, A., Azimzadeh, M., & Haghighi, M. A. (2022). Phylogenetic study, distribution of virulence genes and antibiotic resistance profiles of *Escherichia coli* isolated from Bushehr coastal water. *Gene Reports, 26*, 101473. ISSN 2452-0144 (Editor access on 25 July 2022 Google Scholar).

Ramamurthy, T., Chowdhury, G., Pazhani, G. P., & Shinoda, S. (2014). *Vibrio fluvialis*: An emerging human pathogen. *Frontiers in Microbiology, 5*, 91.

Sanchez, J., Tustin, J., Heasley, C., Patel, M., Kelly, J., Habjan, A., Waterhouse, R., & Young, I. (2021). Region-specific associations between environmental factors and Escherichia coli in freshwater beaches in Toronto and Niagara Region, Canada. *International Journal of Environmental Research and Public Health, 18*(23), 12841. https://doi.org/10.3390/ijerph182312841. (Editor access on 25 July 2022 Google Scholar).

Shaw, K. S., Rosenberg Goldstein, R. E., He, X., Jacobs, J. M., Crump, B. C., & Sapkota, A. R. (2014). Antimicrobial susceptibility of *Vibrio vulnificus* and *Vibrio parahaemolyticus* recovered from recreational and commercial areas of Chesapeake Bay and Maryland Coastal Bays. *PLoS One, 9*(2), e89616. https://doi.org/10.1371/journal.pone.0089616. (Editor Access on 25 July 2022 Google Scholar).

Silvester, R. (2017). *Genotyping, virulence characterization and survival kinetics of Vibrio spp. from food and environmental sources along the south west coast of India*. Unpublished PhD thesis, Cochin University of Science and Technology, India, available from https://dyuthi.cusat.ac.in

Silvester, R., Alexander, D., & Hatha, A. A. M. (2015). Prevalence, antibiotic resistance, virulence and plasmid profiles of *Vibrio parahaemolyticus* from a tropical estuary and adjoining traditional prawn farm along the South west coast of India. *Annals of Microbiology, 65*, 2141–2149.

Silvester, R., Pires, J., Van Boeckel, P. V., Madhavan, A., Adarsh, B. M., & Hatha, M. (2019). Occurrence of β-lactam resistance genes and plasmid mediated-resistance among *Vibrio* isolated from south west coast of India. *Microbial Drug Resistance, 25*(9), 1306–1315.

Silvester, R., Harikrishnan, M., & Hatha, M. (2021). Survival kinetics of *Vibrio* species in a tropical estuary along the Southwest coast of India- as a function of selected environmental factors. *Fishery Technology, 58*, 40–47.

Sneha, K. G., Anas, A., Jayalakshmy, K. V., Jasmin, C., Vipin Das, P. V., Pai, S. S., Pappu, S., Nair, M., Muraleedharan, K. R., Sudheesh, K., & Nair, S. (2016). Distribution of multiple antibiotic resistant *Vibrio* spp across Palk Bay. *Regional Studies in Marine Sciences, 3*, 242–250.

Soleimani, F., Taherkhani, R., Dobaradaran, S., Spitz, J., & Saeedi, R. (2021). Molecular detection of E. coli and Vibrio cholerae in ballast water of commercial ships: A primary study along the Persian Gulf. *Journal of Environmental Health Science and Engineering, 19*(1), 457–463.

Su, J., Fan, J., Ming, H., Guo, G., Fu, Y., Zhao, X., Zhao, S., Chen, Q., Guan, D., Jin, Y., & Shi, T. (2022). The municipal sewage discharge may impact the dissemination of antibiotic-resistant Escherichia coli in an urban coastal beach. *Water, 14*(10), 1639. https://doi.org/10.3390/w14101639. (Editor access on 25 July 2022 Google Scholar).

Talukdar, P. K., Rahman, M., Rahman, M., Nabi, A., Islam, Z., et al. (2013). Antimicrobial resistance, virulence factors and genetic diversity of *Escherichia coli* isolates from household water supply in Dhaka, Bangladesh. *PLoS One, 8*(4), 61090.

Tarh, J. E., Asikong, B. E. E., & Ukwuoma, C. I. C. (2022). Prevalence of Vibrio species in sea foods and water sources in Cross River State. *Annual Research & Review in Biology*, 63–78. https://doi.org/10.9734/arrb/2022/v37i230488. (Editor access on 25 July 2022 Google Scholar).

Thompson, F., Lida, T., & Swings, J. (2004). Biodiversity of Vibrios. *Microbiology and Molecular Biology Reviews, 68*, 403–422.

Tomova, A., Ivanova, L., Buschmann, A. H., Rioseco, M. L., Kalsi, R. R., Godfrey, H. P., & Cabello, F. C. (2015). Antimicrobial resistance genes in marine bacteria and human uropathogenic *Escherichia coli* from a region of intensive aquaculture. *Environmental Microbiology Reports, 7*, 803–809. https://doi.org/10.1111/1758-2229.12327. (Editor Access on 25 July 2022 Google Scholar).

Trishina, A. V., Bereznyak, E. A., Ezhova, M. I., Bereznyak, Y. L., & Chemisova, O. S. (2022). Antibiotic resistance of environmental isolates of *Vibrio cholerae* nonO1/nonO139. *Public Health and Habitat-ZNISO*, (3), 66–71. https://doi.org/10.35627/2219-5238/2022-30-3-66-71. (Editor access on 25 July 2022 Google Scholar).

Vaiyapuri, M., Sebastian, A. S., George, I., Variem, S. S., Vasudevan, R. N., George, J. C., Badireddy, M. R., Sivam, V., Peeralil, S., Sanjeev, D., Thandapani, M., Moses, S. A., Nagarajarao, R. C., & Mothadaka, M. P. (2021). Predominance of genetically diverse ESBL *Escherichia coli* identified in resistance mapping of Vembanad Lake, the largest fresh-cum-brackish water lake of India. *Environmental Science and Pollution Research, 28*(46), 66206–66222. https://doi.org/10.1007/s11356-021-15110-y. (Editor access on 25 July 2022 Google Scholar).

Vashi, A., Bandopadhyay, D., Begum, M., Naik, S., Panda, U. S., & Mishra, P. (2022). Microbial contamination in the coastal waters of the southeast coast of India: A comparison of pre and post COVID-19 lockdown scenario. In *OCEANS 2022-Chennai* (pp. 1–5). IEEE.

Vezzulli, L., Pezzati, E., Brettar, I., Hofle, M., & Pruzzo, C. (2015). Effect of global warming on *Vibrio* ecology. *Microbiology Spectrum, 3*(3), 1–9.

Victoria, N. S., Kumari, T. S. D., & Lazarus, B. (2022). Assessment on impact of sewage in coastal pollution and distribution of fecal pathogenic bacteria with reference to antibiotic resistance in the coastal area of Cape Comorin, India. *Marine Pollution Bulletin, 175*, 113123. https://doi.org/10.1016/j.marpolbul.2021.113123. (Editor access on 25 July 2022 Google Scholar).

Vijayan, M., & Lee, C. W. (2014). Seasonality and diversity of culturable Vibrios in tropical coastal waters. *Bulletin of Marine Science, 90*, 599–610.

WHO. (2017). *Critically important antimicrobials for human medicine 5th revision*. World Health Organization. Accessed 17 Sept 2018. http://who.int/foodsafety/publications/antimicrobials-fifth/en/

Wong, Y. Y., Lee, C. W., Bong, C. W., Lim, J. H., Narayanan, K., & Sim, E. U. H. (2019). Environmental control of *Vibrio* spp. abundance and community structure in tropical waters. *FEMS Microbiology Ecology, 95*, 176.

You, K. G., Bong, C. W., & Lee, C. W. (2016). Antibiotic resistance and plasmid profiling of *Vibrio* spp. in tropical waters of Peninsular Malaysia. *Environmental Monitoring and Assessment, 188*(3), 171.

Zaafrane, S., Maatouk, K., Alibi, S., & Ben Mansour, H. (2022). Occurrence and antibiotic resistance of Vibrio parahaemolyticus isolated from the Tunisian coastal seawater. *Journal of Water and Health, 20*(2), 369–384. https://doi.org/10.2166/wh.2022.243. (Editor access on 25 July 2022 Google Scholar).

Amphenicols: Dilemma of Use and Abuse in Poultry

Sandeep Kumar Sharma, Vikas Galav, and Pushpendra Singh Rathore

Contents

S. K. Sharma (✉)
Department of Veterinary Microbiology and Biotechnology, Post Graduate Institute of Veterinary Education and Research (PGIVER-RAJUVAS), Jaipur, India
e-mail: drsharmask01@hotmail.com

V. Galav
Department of Veterinary Pathology, Post Graduate Institute of Veterinary Education and Research (PGIVER-RAJUVAS), Jaipur, India

P. S. Rathore
Department of Veterinary Microbiology and Biotechnology, Post Graduate Institute of Veterinary Education and Research (PGIVER-RAJUVAS), Jaipur, India

M. P. Mothadaka et al. (eds.), *Handbook on Antimicrobial Resistance*,
https://doi.org/10.1007/978-981-19-9279-7_12

Abstract

In the past decade, an exemplary transference had taken place in poultry production system across the world that ranges from marginal animal husbandry practices used by small poultry farmers to intensive poultry production system, including routine antimicrobial usage both for therapeutic purposes and enhancing productivity in the poultry and meat industry. This shift has not only resulted in increased antimicrobials that are available for use in poultry but has also increased the chances of antimicrobial resistance in lower resources focused on intensive production system. It is well established that bacterial resistance diminishes the antimicrobials that are available for poultry and meat production, and this may have a serious impact on human medicine as it will reduce the choices and efficacy of antibiotics that are going to be used for clinical settings. Antibiotic resistance has far-reaching consequences in the form of high disease incidences consequently putting economic burden on the meat industry, people, and nations. The poultry industry across the world contributes to the chunk of antimicrobials used in the animal husbandry sector. Imprudent use of antimicrobials, especially amphenicols, in viable small-scale poultry has serious repercussions on poultry and human health besides exacerbating poverty and food insecurity. Although amphenicols are known to be effective antimicrobials, but these are also known to cause bone marrow suppression and have also been reportedly causing liver damage, resulting in abnormal liver functions and jaundice and should be used with great caution in birds, as they may have serious repercussions on human health also. In humans, amphenicols can cause dangerous anemia and can have neurological manifestations resulting in mental disorders, sometimes resulting in allergic reactions and gastrointestinal symptoms, along with nausea, poor appetite, and vomiting.

Keywords

Amphenicols · Antimicrobial Resistance · Poultry

1 Salient Global Figures

- Among the per capita meats, poultry meat became the fastest developing meat at the global level (Castanon, 2007), hence, becoming one of the important sources of animal protein.
- As per FAO assessments (www.fao.org/poultry), the demand for poultry is going to enhance by 271%, 116%, 97%, and 9% for South Asian Eastern, Europe, and

Central Asia, Middle East, and North Africa, East Asia, and the Pacific during the decade from 2000 to 2030.

- In 2020, the world's poultry meat production stood at 133.3 million tons and egg production at 87 million tons.
- In the poultry meat production, the United States, ranks at the top, with 18% contribution, followed by China, Brazil, and Russia.
- In case of egg production, China ranks the top in the world, with 42%, followed by the United States (7%) and India (6%).
- Asia contributes 60% of the world's egg production as the leading egg-producing continent in the world.
- Poultry is considered the backbone of small and marginal classes in developing countries as it is a viable source of income as well as for the availability of food/animal protein and approximately 80% of rural households are involved in small-scale or backyard poultry rearing.

2 Indian Poultry Scenario

In India, raising poultry has traditionally been a crucial part of the livestock production system not only providing with additional income source to both unorganized and organized animal husbandry sectors but also playing a part in the form of food/animal protein source. Poultry production in India has transformed switching from utterly chaotic and irrational agricultural methods to a commercial/intensive production system, which strengthens the whole rural economy. The poultry business has seen rapid expansion both nationally and internationally owing to the increased demand for animal protein. In terms of its contribution to poultry production, India is presently the fourth largest poultry producer in the world. India's share in the world's egg production is 6%, and poultry meat contributes to 45% of total meat production in India, thus establishing the poultry sector as a significant player in the meat industry. According to evaluations, the nation consumed nearly 3.8 million tons of chicken meat in 2019 and egg production was at 109 billion with an estimated value of 7 billion USD (Jaganmohan, 2020). As per the 20th livestock census of India, the growth in poultry population between 2012 and 2019 is shown in the following Table 1:

Table 1 Poultry production scenario

Class	2012 (in million)	2019 (in million)	% Change
Commercial poultry population	511.72	534.74	4.50
Backyard poultry population	217.49	317.07	45.48
Total poultry population	729.21	851.81	16.81

Source: 20th Livestock Census (2019), Department of Animal Husbandry, Dairying and Fisheries, Ministry of Agriculture and Farmers Welfare, Govt. of India

3 Antibiotics Usage in Poultry

A large variety of antimicrobials such as aminoglycosides, beta-lactams, ionophores, lincosamides, macrolides, quinolones, streptogramins, and sulfonamides are used in rearing and raising poultry worldwide. These are used with assumption to promote growth and prevent or therapeutics of various bacterial, viral, and fungal infections (Boamah et al., 2016), but many of its adverse effects such as overweight broilers, skeletal issues, joint issues in poultry, and also increased antibiotic unresponsiveness have been overlooked for a significant period of time. Hence, it is important to regulate the use and consumption of antimicrobials to limit their impact on poultry population as well as on human health. In the global scenario, the use of antibiotics as a growth promoter is a major concern; hence, in 2006, these were banned in European Union countries and subsequently banned in 2017 in the United States but continued to be practiced in Brazil and China. The therapeutic use of antibiotics in the treatment of most important colonic diseases due to *Salmonella* infections, *E. coli*, or *Clostridium* spp. is permitted in all major poultry-producing nations (Access Science Editors, 2017).

4 Impact on Consumer Health and Environment

Indiscreet application of antimicrobials in the veterinary sector accompanied with unscientific and indiscriminate animal farm management practices resulted in sub-therapeutic accretion of drug deposits in animal foods. Apart from the multidrug resistance problem, imprudent use of antibiotics as a consequence increased medication deposits in the environment and in faunal products that are being consumed by humans, thus adversely affecting well-being (Gonzalez Ronquillo & Angeles Hernandez, 2017). Traces or residues of many antibiotics, such as amphenicol, tetracyclines, penicillins, aminoglycoside, and macrolides, were identified and well documented from faunal-originated foods (Diarra & Malouin, 2014). In the same way, tetracyclines are well recognized to interfere with young children's teeth development and antibiotic residues in cattle production observed to exhibit adverse influence on the well-being of humans (Kummerer, 2009). Similarly, many harmful effects have been reported with the use of beta-agonists, such as clenbuterol, that possibly are responsible for food poisoning, tachycardia, trembling of muscles, and palpitations (Chan, 1999). The metabolites of chloramphenicol were reported in poultry meat and meat products and possible link between the incidence of these drug deposits in meat and human cases of aplastic anemia (Gassner & Wuethrich, 1994) demonstrating the hazardous consequences of antibiotic residue on human health.

In the past, antibiotics were originally used on poultry in 1946 (Moore et al., 1946). The antibiotic usage for both human and animal purposes is anticipated to be consumed globally to be between 0.1 and 0.2 million tons (Manzetti & Ghisi, 2014). Furthermore, increasing awareness and impact of ARB have also led to a consequential increase in the use of different antibiotics. This clearly indicates that the

volume of different antibiotics accumulating in the bioenvironment is continuously enhancing at alarming levels, and these are ultimately getting imbibed into the human food chain. Unregulated and unrestrained usage of antimicrobials in animal farming is a prominent reason causing antimicrobial resistance in pathogens and organisms found in both humans and animals that are commensal. This ultimately resulted in the increased incidence of treatment failures in animals, resulting in economic losses to the farmers and also an impending cradle of diffusion of drug unresponsiveness to humans by the ingestion of this animal food. AMR bacteria may be transferred from animals to people through various routes, that is, consumption of contaminated food (Van Boeckel et al., 2015), or via the polluted environment, including via direct contact with animals and contaminated air, water, and soil (Graham et al., 2009; Smith et al., 2013).

The phenomenon of antibiotic resistance may only be evaluated adequately when environmental reservoirs are taken into consideration. Fowls are raised on commercial farms and domestic ones that are known to harbor drug obdurate bacteria (Braykov et al., 2016). It is a well-established fact that poultry harbors a large proportion of enterobacters that are known to be strongly obdurate to aminosides class of antibiotics and tetracycline in gastric tract and in meat, respectively (Yulistiani et al., 2017).

5 Antibiotic Obduracy

As per the WHO, resistance occurs when a microorganism becomes nonsusceptible to a therapeutic agent that was previously susceptible such as antiviral, antibacterial, and antiparasitic. The unresponsiveness to antimicrobials is the capacity of microbes to survive, multiply, and grow in the presence of antibiotics that are predominantly recognized to impede or eliminate microbes of a similar kind of species (RUMA, 2016). The jeopardy of ARB can be attributed to natural occurrences and also can be ascribed to undiscerning use of antibiotics by humans. The selection of ARB strains and the propagation of ARG are mostly caused by the uncontrolled employment of antimicrobial drug, which has grown to a monstrous scale of world well-being; in the present-day conditions, this problem has grown into a major global health challenge.

6 Common Mechanisms of Antibiotic Resistance Gene Transfer

Generally, when an antibiotic is given, it destroys the susceptible population but a small proportion of resistant organisms is left behind. These resistant ones over a period of time multiply, propagate, and also horizontally as well as vertically transfer their resistance character through genes to other bacteria as well. It is widely debated that there is a clear relation between the application of antimicrobial drugs in faunal farming and the advent of AMR, and the most significant reason for this has been the

genetic transfer or exchange of AMR genes among bacteria from different taxa that are distantly related (Musovic et al., 2006).

The Following Common Mechanisms Have Been Reported in Bacteria:

- Lower outer membrane permeability for the antibiotics reduces the amount of antibiotic present in the bacterial cell, causing the bacteria to develop inherent resistance.
- A large number of genes encoding various forms of multidrug resistance exhaust pumps.
- Antibiotic modification by β-lactamase enzymes that includes extended-spectrum and metallo β-lactamases and aminoglycoside-modifying enzymes.
- Addition of antibiotic resistance genes that are either chromosomal or plasmid encoded.
- Chromosomal mutations impacting biochemical "pumps" or enzyme pathways also develop tolerance to antibiotics (e.g., transformations in DNA gyrase and topoisomerase IV genes is the reason behind unresponsiveness to quinolone).

7 Reservoir for Resistant Genes in Poultry Manure

Poultry litter also contain traces of antibiotic residues and act as a reservoir of different pathogenic bacteria that spread to the environment through soil, and this could be a potential reason for the rise in the occurrence of horizontal gene transfer of microbial resistance in the soil environment (Heuer et al., 2009).

8 Resistant Pathogens and Poultry Association

There are many routes through which drug-unresponsive bacteria can get entry into the chicken flock, which includes air, water, feed, insects, and other pests (Mouttotou et al., 2017), by virtue of "*Horizontal Gene Transfer (HGT)*" (Krauland et al., 2009), or through vertical transmission (Pearson et al., 1996). Once a resistant bacterium is introduced into a flock, these are likely to persist in the litter and be discharged in the droppings (Chen & Jiang, 2014).

9 Some of these Resistant Pathogens Are as Follows

9.1 *Pseudomonas* Species

The family Pseudomonadaceae contains the genus *Pseudomonas*, an opportunistic, Gram-negative, aerobic pathogen mainly found on plants, in water, and in soil. The genus constitutes an important member, that is, *Pseudomonas aeruginosa,* along with other species such as *P. stutzeri*, *P. fluorescens, P. pertucinogena, P. putida, P. chlororaphis,* and *P. syringae*. Pseudomonads have been widely reported and

isolated from poultry farms worldwide (Sams, 2001). It produces both systemic and localized diseases, affecting multiple organs (triggering infection of lungs, sinusitis, septicemia, keratoconjunctivitis, pyogenic infections, endocarditis, lameness, etc.). In poultry birds, externally damaged infraorbital sinuses result in inflammatory conditions of the head, joints, sinuses, and wattles. Additionally, this microbe is known to include plasmids, integrons, and transposons that are capable of transferring the genes for antibiotic unresponsiveness to other bacterial species. Studies carried out in Ghana reported class 1 integron that carries multiple ARGs in pseudomonads (Odoi, 2016). The presence of chromosomally encoded β–lactamases, together with efflux pumps and most *P. aeruginosa* strains, is innately resistant to a variety of substances owing to an exterior biofilm matrix of antimicrobials such as cephalosporins, carbapenems, penicillins, quinolones, monobactam, and aminoglycoside.

9.2 *Staphylococcus* Species

This microorganism causes various types of disease manifestations in poultry, of which pododermatitis or bumblefoot and bacterial septicemia are important ones affecting chicken and turkeys. *Staphylococcus* species has become resistant against β-lactams, methicillins [methicillin-resistant *S. aureus* (MRSA); superbugs], and vancomycin [vancomycin-resistant *Staphylococcus aureus* (VRSA)], and the significance of *mecA*-resistant gene has also been reported (Stapleton & Taylor, 2007; Bhedi et al., 2018).

9.3 *Escherichia* Species

Escherichia coli is an established commensal harbored as a gut microbe of all animals, human, and even in birds. This microbe, isolated from birds, exhibited the highest rates of obduracy to amoxicillin, oxytetracycline, streptomycin trimethoprim, and tetracycline (Van den Bogaard et al., 2001).

9.4 *Salmonella* Species

A significant prevalence of *Salmonellae* is observed in poultry farms. Fecal shedding and contaminated litter are the important sources of transmission among bird congregates. Transmission of *Salmonella* infections such as Pullorum caused by *S. pullorum* can either be vertical (transovarian) or via a respiratory pathway, feces, contaminated feed, water, or litter, or indirectly through direct or indirect contact with diseased birds. Antimicrobials used in treating *Salmonella* infections include furazolidone, gentamycin, and sulfonamides group (Msoffe et al., 2009). *Salmonella* strains have also been reported to be resistant against streptomycin, sulfonamides, florfenicol, and ampicillin (Medeiros et al., 2011).

9.5 *Campylobacter* Species

Campylobacteriosis occurs when poultry meat is eaten uncooked or undercooked (Altekruse et al., 1999). Increased unresponsiveness among these microorganisms is comparatively related to extensive application of drugs in food-producing animals, especially in birds (Wilson, 2003). Resistance to antibiotics like tetracycline, erythromycin, and fluoroquinolones has been widely reported.

9.6 *Clostridium* Species

C. perfringens infection causes necrotic enteritis in poultry while *C. colinum* is associated with ulcerative enteritis. Resistance of *Clostridium* to a range of antibiotics, viz., colistin, doxycycline, sulfamethoxazole-trimethoprim, neomycin, and perfloxacin, has been extensively reported (Osman & Elhariri, 2013).

9.7 *Klebsiella* Species

The common pathogenic *Klebsiella* in humans and animals include *K. oxytoca, K. pneumoniae,* and *K. variicola* (Fielding et al., 2012). *Klebsiella*-originated infections included diarrhea, meningitis, pneumonia, septicemia, and "*urinary tract infections (UTI)*" (Podschun & Ullmann, 1998). Aminoglycosides, carbapenem, "third-generation cephalosporins," and quinolones are extensively employed in treating infections associated with *Klebsiella*. *Klebsiella* exuded significant levels of unresponsiveness to amoxicillin, augmentin, cotrimoxazole, and tetracycline (Ajayi & Egbebi, 2011).

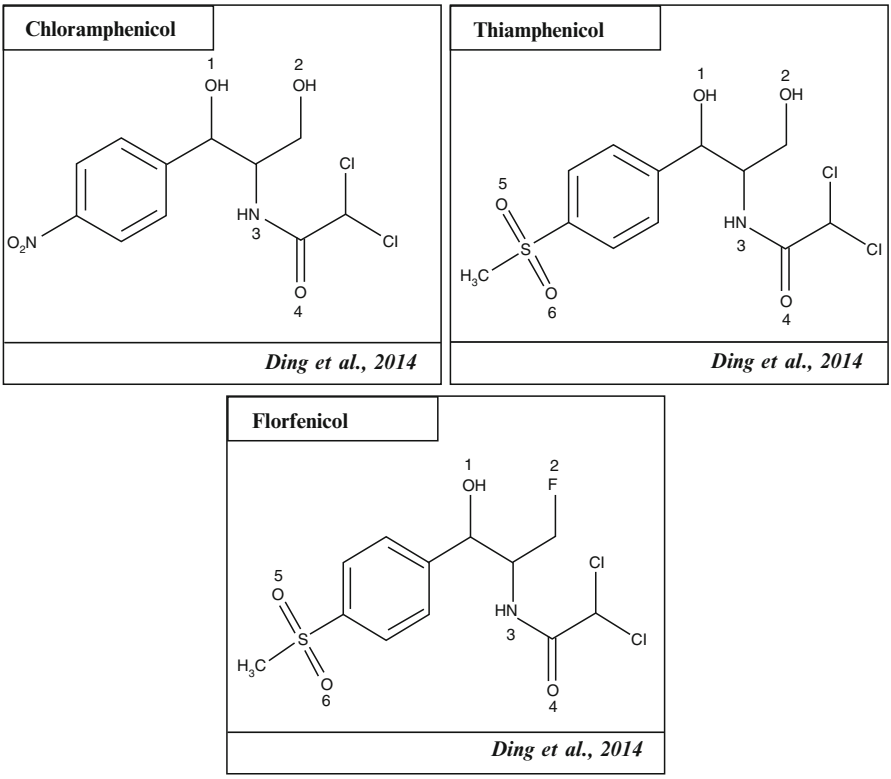
10 Amphenicols

A group of phenylpropanoid antibiotics is known as amphenicols. They have the simplest chemical structure of all antibiotics and mode of action involves blocking peptidyl transferase enzyme on the bacterial 50S ribosome subunit. Chloramphenicol, introduced in 1948, thiamphenicol, azidamfenicol, and florfenicol are all examples of amphenicol antibiotics (Lewis, 2013).

The broad-spectrum antimicrobials such as phenicols inhibit the growth of both Gram-positive and Gram-negative that included aerobes, anaerobes, chlamydiae, mycoplasma rickettsia, and spirochetes. They act as bacteriostatic since it prevents the creation of microbial proteins by fastening to the ribosome's 50S subunit. Chloramphenicol was initially acquired from *Streptomyces venezuelae*, presently being manufactured synthetically, and thiamphenicol is a manmade derivative of chloramphenicol (Bishop, 2001; Papich & Riviere, 2001). Due to its association with side effects, namely, bone marrow suppression, chloramphenicols have been limited or forbidden in numerous nations. In the United States and the European

Union, for usage in food animals, nitroimidazoles, nitrofurans, and chloramphenicol are all forbidden (Davis et al., 2009).

11 Structure of Amphenicols



12 Use of Amphenicols in Poultry

Chloramphenicol, a broad-spectrum antibiotic, is effective against a wide range of microbes, but having potential toxic side effects is being used only as a last resort medication in treating serious bacterial infections in poultry. Amphenicols are widely used in poultry by giving them food or water to drink orally (Botsoglou & Fletouris, 2001). The medicine is delivered throughout the body through the oral route, which is quick but insufficient (Anadon et al., 1994, 2008). The rate of elimination is also associated with the route of administration, and orally administered drug holds for a longer time in the body (Anadon et al., 1994). In layer birds,

amphenicol residues have been reported during many days after oral delivery both from yolk and albumen (Akhtar et al., 1996).

13 Potential Risks of Using Amphenicols in Birds and Humans

Florfenicol has been reported to be carrying some harmful effects and was found to be associated with interference in embryogenesis of the developing egg. It can cause a drop in egg hatchability in breeders (https://www.poultrymed.com/Amphenicols). Due to proven toxicity in humans, use of chloramphenicol is prohibited in many countries (Settepani, 1984). Chloramphenicol leads to bone marrow depression, associated aplastic anemia, and nervous disorders, namely, Gray's syndrome (Feder et al., 1981).

14 Acquired Resistance Against Amphenicols

Unresponsiveness to amphenicols may develop as a result of target alteration by the *cfr* gene that encodes methylase that methylates the C8 position of the A2503 of the 23S rRNA (Kehrenberg et al., 2005). Chloramphenicol resistance is also acquired through the transfer of R-factor, indicating a major role of plasmids. Bacteria-acquiring R-factor produces chloramphenicol acetyl transferase enzyme that inactivates chloramphenicol and leads to resistance (Murray & Shaw, 1997). Efflux of amphenicols is also common (Arcangioli et al., 1999).

15 Conclusions

The antimicrobial use these days is both for therapeutics and for enhancing the food animal productivity. Currently, poultry is the fastest growing food animal industry and, due to rampant use to stimulate growth, this has become the most important source of bacteria that are "multidrug resistant (MDR)." While there is still no viable alternative to the need for antimicrobials in providing sustainable public health, animal production, and agrarian livelihoods, yet the availability and effectiveness of antimicrobials in raising animals for food are progressively declining. Planned and regulated use of amphenicol antibiotics in intensive poultry as well as small-scale development is the only way forward to slow down the impact of drug resistance.

16 Recommendations

- Efforts need to be made for making provisions of providing incentives to farmers for discouraging unnecessary antibiotic use in food animals and promoting organic farming so that the practice of using minimum necessary use of different antimicrobials can be promoted.

- General awareness needs to be created by providing education and training for livestock farmers on antibiotic resistance and responsible use of antibiotics.
- Stringent rules and regulations need to be promulgated, and application of antibiotics in animal feed and feed supplement as growth promoters should not be promoted. Less intensive and more organic is the way forward, and this would need a regulatory approach to overall animal production systems.
- Animal usage of antibiotics that are essential for human treatment should be prohibited.
- Development of alternative systems of growth promoters, such as organic plant-based or herbal supplements, must be promoted as this likely helps in protecting environment, consumer health, and reducing bioaccumulation of different antibiotics in the food chain.
- Research must be done to fine-tune prevention and control strategies toward the overall reduction in the need for antibiotics. For example, vaccinations against bacterial diseases, environmental sanitation for improvement in pasture lands, and disease containment strategies must be implemented at all farms.
- Proper scientific surveillance of antibiotic resistance is also an effective strategy to monitor the magnitude and trends of spreading antibiotic resistance both at national and international levels.

References

Access Science Editors. (2017). *U.S. bans antibiotics use for enhancing growth in livestock*. McGraw-Hill Education. https://doi.org/10.1036/1097-8542.BR0125171. Accessed May 2018.

Ajayi, A. O., & Egbebi, A. O. (2011). Antibiotic susceptibility of Salmonella typhi and Klebsiella pneumoniae from poultry and local birds in Ado-Ekiti, Ekiti-state. Nigeria. *Annals of Biological Research, 2*(3), 431–437.

Akhtar, M. H., ElSooud, K. A., Shehata, A. M., & Anwar-ul-Haq. (1996). Fate and residues of C-14-chloramphenicol in laying chickens. *Journal of Environmental Science and Health Part B-Pesticides Food Contaminants and Agricultural Wastes, 31*, 1061–1084.

Altekruse, S. F., Stern, N. J., Fields, P. I., & Swerdlow, D. L. (1999). Campylobacter jejuni–an emerging food borne pathogen. *Emerging Infectious Diseases, 5*, 28–35.

Anadon, A., Bringas, P., Martı'nez-Larrañaga, M. R., & Diaz, M. J. (1994). Bioavailability, pharmacokinetics and residues of chloramphenicol in the chicken. *Journal of Veterinary Pharmacology and Therapeutics, 17*, 52–58.

Anadon, A., Martinez, M. A., Martinez, M., Rios, A., Caballero, V., Ares, I., & Martı'nez-Larrañaga, M. R. (2008). Plasma and tissue depletion of florfenicol and florfenicol-amine in chickens. *Journal of Agricultural and Food Chemistry, 56*, 11049–11056.

Arcangioli, M. A., Leroy-Setrin, S., Martel, J. L., & Chaslus-Dancla, E. (1999). A new chloramphenicol and florfenicol resistance gene flanked by two integron structures in Salmonella typhimurium DT104. *FEMS Microbiology Letter, 174*(2), 327–332.

Bhedi, K. R., Nayak, J. B., Brahmbhatt, M. N., & Roy, A. (2018). Detection and molecular characterization of methicillin-resistant Staphylococcus aureus obtained from poultry and poultry house environment of Anand district, Gujarat, India. *International Journal Current Microbiology and Applied Sciences, 7*, 867–872.

Bishop, Y. (2001). *The veterinary formulary* (5th ed., p. 692). Pharmaceutical Press.

Boamah, V. E., Agyare, C., Odoi, H., & Dalsgaard, A. (2016). Antibiotic practices and factors influencing the use of antibiotics in selected poultry farms in Ghana. *Journal of Antimicrobial Agents, 2*, 120. https://doi.org/10.4172/2472-1212.1000120

Botsoglou, N. A., & Fletouris, D. J. (2001). *Drug residues in food*. Marcel Dekker, Inc.

Braykov, N. P., Eisenberg, J. N., Grossman, M., Zhang, L., Vasco, K., Cevallos, W., & Levy, K. (2016). Antibiotic resistance in animal and environmental samples associated with small-scale poultry farming in northwestern Ecuador. *mSphere, 1*(1). https://doi.org/10.1128/mSphere.00021-15

Castanon, J. I. R. (2007). History of the use of antibiotic as growth promoters in European poultry feeds. *Poultry Science, 86*, 2466–2471.

Chan, T. Y. (1999). Health hazards due to clenbuterol residues in food. *Journal of Toxicology: Clinical Toxicology, 37*, 517e9.

Chen, Z., & Jiang, X. (2014). Microbiological safety of chicken litter or chicken litter-based organic fertilizers. A review. *Agriculture, 4*, 1–29.

Davis, J. L., Smith, G. W., Baynes, R. E., Tell, L. A., Webb, A. I., & Riviere, J. E. (2009). Update on drugs prohibited from extralabel use in food animals. *Journal of the American Veterinary Medical Association, 235*, 528–534.

Diarra, M. S., & Malouin, F. (2014). Antibiotics in Canadian poultry productions and anticipated alternatives. *Frontier Microbiology, 5*, 282.

Ding, F., Peng, W., Peng, Y. K., & Jiangd, Y. T. (2014). Renal protein reactivity and stability of antibiotic amphenicols: Structure and affinity. *Molecular BioSystems*. https://doi.org/10.1039/c4mb00220b

Feder, H. M., Osier, C., & Maderazo, E. G. (1981). Chloramphenicol: A review of its use in clinical-practice. *Reviews of Infectious Diseases, 3*, 479–491.

Fielding, B. C., Mnabisa, A., Gouws, P. A., & Morris, T. (2012). Antimicrobial-resistant Klebsiella species isolated from free-range chicken samples in an informal settlement. *Archives of Medical Science, 8*(1), 39–42.

Gassner, B., & Wuethrich, A. (1994). Pharmacokinetic and toxicological aspects of the medication of beef-type calves with an oral formulation of chloramphenicol palmitate. *Journal of Veterinary Pharmacology and Therapeutics, 17*, 279e83.

Gonzalez Ronquillo, M., & Angeles Hernandez, J. C. (2017). Antibiotic and synthetic growth promoters in animal diets: Review of impact and analytical methods. *Food Control, 72*, 255e67. Part B.

Graham, J. P., Evans, S. L., Price, L. B., & Silbergeld, E. K. (2009). Fate of antimicrobial-resistant enterococci and staphylococci and resistance determinants in stored poultry litter. *Environmental Research, 109*(6), 682–689.

Heuer, H., Kopmann, C., Binh, C. T., Top, E. M., & Smalla, K. (2009). Spreading antibiotic resistance through spread manure: Characteristics of a novel plasmid type with low %G+C content. *Environental Microbiology, 11*, 937–949.

Jaganmohan, N. (2020). Consumption volume of poultry meat in India from 2013 to 2019. *Statista*. Retrieved July 12, 2020, from https://www.statista.com/statistics/826711/india-poultry-meat-consumption

Kehrenberg, C., Schwarz, S., Jacobsen, L., Hansen, L. H., & Vester, B. (2005). A new mechanism for chloramphenicol, florfenicol and clindamycin resistance: Methylation of 23S ribosomal RNA at A2503. *Molecular Microbiology, 57*(4), 1064–1073.

Krauland, M. G., Marsh, J. W., Paterson, D. L., & Harrison, L. H. (2009). Integron-mediated multidrug resistance in a global collection of nontyphoidal salmonella enterica isolates. *Emerging Infectious Diseases, 15*(3), 388–396.

Kummerer, K. (2009). 'Antibiotics in the aquatic environment – A review part I. *Chemosphere, 75*, 417–34.

Lewis, K. (2013). Platforms for antibiotic discovery. *Nature Review Drug Discovery, 12*(5), 371–387.

Manzetti, S., & Ghisi, R. (2014). The environmental release and fate of antibiotics. *Marine Pollution Bulletin, 79*, 7–15.

Medeiros, M. A. N., de Oliveira, D. C. N., Rodrigues, D. P., & de Freitas, D. R. C. (2011). Prevalence and antimicrobial resistance of salmonella in chicken carcasses at retail in 15 Brazilian cities. *Pan American Journal of Public Health, 30*, 555–560.

Moore, P. R., Evenson, A., Luckey, T. D., McCoy, E., Elvehjem, C. A., & Hart, E. B. (1946). Use of sulfasuxidine, streptothricin, and streptomycin in nutritional studies with the chick. *Journal of Biological Chemistry, 165*, 437–441.

Mouttotou, N., Ahmad, S., Kamran, Z., & Koutoulis, K. C. (2017). Prevalence, risks and antibiotic resistance of salmonella in poultry production chain. In M. Mares (Ed.) *Current topics in salmonella and salmonellosis* (Chap. 12, pp. 215–234). InTech.

Msoffe, P. L., Aning, K. G., Byarugaba, D. K., Mbuthia, P. G., Sourou, S., Cardona, C., Bunn, D. A., Nyaga, P. N., Njagi, L. W., Maina, A. N., & Kiama, S. G. (2009). *Handbook of poultry diseases important in Africa* (p. 83). CRSP: A Project of the Global Livestock.

Murray, I. A., & Shaw, W. V. (1997). O-acetyltransferases for chloramphenicol and other natural products. *Antimicrobial Agents Chemotherapy, 41*(1), 1–6.

Musovic, S., Oregaard, G., Kroer, N., & Sorensen, S. J. (2006). Cultivation-independent examination of horizontal transfer and host range of an IncP-1 plasmid among gram-positive and gram-negative bacteria indigenous to the barley rhizosphere. *Applied Environmental Microbiology, 72*, 6687–6692.

Odoi, H. (2016). Isolation and characterization of multi-drug resistant *Pseudomonas aeruginosa* from clinical, environmental and poultry litter sources in Ashanti region of Ghana (MPhil thesis). Kumasi: Kwame Nkrumah University of Science and Technology.

Osman, K. M., & Elhariri, M. (2013). Antibiotic resistance of Clostridium perfringens isolates from broiler chickens in Egypt. *Review of Science and Technology, 32*(2), 841–850.

Papich, M. G., & Riviere, J. E. (2001). Chloramphenicols and derivatives, macrolides, lincosamides, and miscellaneous antimicrobials. In H. R. Adams (Ed.), *Veterinary phamacology and therapeutics* (8th ed., pp. 868–917). Iowa State University Press.

Pearson, A. D., Greenwood, M. H., Feltham, R. K., Healing, T. D., Donalsdon, J., & Jones, D. M. (1996). Microbial ecology of campylobacter jejuni in a United Kingdom chicken supply chain: Intermittent common source, vertical transmission, and amplification by flock propagation. *Applied Environmental Microbiology, 62*, 4614–4620.

Podschun, R., & Ullmann, U. (1998). Klebsiella spp. as nosocomial pathogens: Epidemiology, taxonomy, typing methods, and pathogenicity factors. *Journal of Clinical Microbiology, 11*(4), 589–603.

RUMA. (2016). *Responsible use of medicines in agriculture alliance (Ruma) information on antibiotic resistance*. Ruma.org.UK/about/position-papers/ruma-informationnote- antibiotics-responsible-use-antibiotics-farm-animals/

Sams, A. R. (2001). *Poultry meat processing* (p. 345). CRC Press.

Settepani, J. A. (1984). The hazard of using chloramphenicol in food animals. *Journal of the American Veterinary Medical Association, 184*, 930–931.

Smith, T. C., Gebreyes, W. A., Abley, M. J., Harper, A. L., Forshey, B. M., Male, M. J., & Davies, P. R. (2013). Methicillin-resistant Staphylococcus aureus in pigs and farm workers on conventional and antibiotic-free swine farms in the USA. *PLoS One, 8*(5).

Stapleton, P. D., & Taylor, P. W. (2007). Methicillin resistance in Staphylococcus aureus. *Science Progress, 85*, 57–72.

Van Boeckel, T. P., Brower, C., Gilbert, M., Grenfell, B. T., Levin, S. A., Robinson, T. P., & Laxminarayan, R. (2015). Global trends in antimicrobial use in food animals. *Proceedings. National Academy of Sciences. United States of America, 112*(18), 5649–5654.

Van den Bogaard, A. E., London, N., Driessen, C., & Stobberingh, E. E. (2001). Antibiotic resistance of faecal Escherichia coli in poultry, poultry farmers and poultry slaughterers. *Journal of Antimicrobial Chemotherapy, 47*(6), 763–771.

Wilson, I. G. (2003). Antibiotic resistance of campylobacter in raw retail chickens and imported chicken portions. *Epidemiology and Infection, 131*, 1181–1186.
www.fao.org/poultry
www.poultrymed.com/Amphenicols
Yulistiani, R., Praseptiangga, D., Raharjo, D., & Shirakawa, T. (2017). Prevalence of antibioticresistance enterobacteriaceae strains isolated from chicken meat at traditional markets in Surabaya, Indonesia. In *IOP Conference Series: Materials Science and Engineering* (Vol. 193, p. 012007). IOP Publishing.

Part II

Antimicrobial Usage (AMU)

Antimicrobial Use in Humans

Renu Gupta and Sangeeta Sharma

Contents

Abstract

Antimicrobial agents, as a major milestone in the history of medicine and human health, have saved millions of lives. Antimicrobial use is the key driver for the development of resistance. Misuse and overuse of antimicrobials accelerate this problem further. As the resistance increases, prescribers are forced to use a higher-generation, broad-spectrum antimicrobials resulting in the development of resistance to these drugs as well.

Considerable variation exists between countries in the volumes of antimicrobial use depending upon socioeconomic factors, cultural differences, and remuneration incentives. Important factors influencing antimicrobial use are disease burden, access to antimicrobials, prevalence of resistance, and local healthcare

R. Gupta (✉)
Microbiology, Institute of Human Behaviour and Allied Sciences, New Delhi, India
e-mail: renugupta2929@gmail.com

S. Sharma
Neuropsychopharmacology, Institute of Human Behaviour and Allied Sciences, Delhi, India
e-mail: sharmasangeeta2003@gmail.com

M. P. Mothadaka et al. (eds.), *Handbook on Antimicrobial Resistance*,
https://doi.org/10.1007/978-981-19-9279-7_13

service issues such as availability of medicines, pricing, affordability, infrastructure, and human resource for health. In order to rationalize antimicrobial use, their consumption needs to be measured and compared over time within and across other settings and countries. Surveillance data is also essential to establish epidemiological association between use of antimicrobials and emergence of resistance over time. The data must be collected using standard methodology and expressed in the comparable units of measurement. Besides assessing the quantum of antimicrobials used, there is a need to study the drivers of use, i.e., reasons for inappropriate prescribing. This chapter aims to provide a broad overview of the relationship between antimicrobial use and resistance and surveillance methodologies for antimicrobial consumption.

Keywords

Antimicrobial use · Antimicrobial consumption · Defined daily dose · Surveillance · Qualitative data

1 Introduction

Antimicrobials are the most important discovery of the past century. They have contributed immensely to reduce morbidity and mortality due to infectious diseases. The serendipitous discovery of penicillin, in 1928 by Alexander Fleming, and subsequent purification, mass production, and distribution in the 1940s for clinical use were a triumph for medical sciences in the war against infectious diseases (Aminov, 2010). However, Fleming, at that time itself, recognized the phenomenon of resistance and its associated dangers. He cautioned that unresponsiveness to penicillin was imminent, if penicillin was not used optimally. This warning has been largely ignored till date.

The discovery of penicillin established a prototype for research, development, and discovery of a large number of antimicrobial agents. Many novel antimicrobial classes were discovered and licensed from the 1940s to the 1960s, and this period came to be known as the "golden era" for discovery of new classes (Gould, 2016). Figure 1 depicts the timeline of antimicrobials finding their way to clinics (Hutchings et al., 2019; Durand et al., 2019; Taneja et al., 2019).

The discovery of new antimicrobial agents, along with improved sanitation, vaccinations, and access to safe water changed the practice of medicine and significantly reduced morbidity and mortality from infectious diseases, thereby doubling lifespan with substantial cost saving by early cure and reduced hospitalization days. Most achievements in medicine such as organ transplants, cancer treatment, and complex surgeries are, in fact, attributed to the use of antimicrobials, but unfortunately misuse came along with their use. Use of antimicrobials gradually extended beyond prophylactic and therapeutic application in human and animal health to unjustified overuse in mild self-limited trivial illnesses along with use for growth promotion in the animal sector to increase the yield for animal protein. The

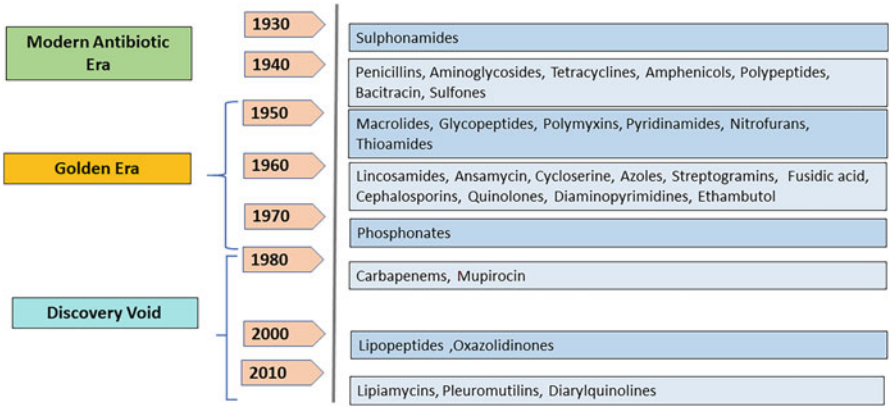


Fig. 1 Brief history of introduction of antimicrobials for clinical use

inappropriate use of antimicrobials resulted in the emergence of antimicrobial resistance (AMR) among the pathogens; the organisms which were being killed earlier started finding mechanisms to thwart the action of antimicrobials in their quest for survival and propagation.

Penicillin-resistant *Staphylococcus aureus* was observed as early as the 1940s, and to combat this, the first penicillinase-resistant β-lactam antimicrobial (methicillin) was developed in 1959 followed by ampicillin in 1961 and other derivatives with improved spectrum of activity and pharmacokinetics (Cunha et al., 2019). Within few years of use of methicillin, methicillin-resistant *Staphylococcus aureus* (MRSA) emerged to destroy methicillin.

The antimicrobial discovery slowed down considerably after the 1970s with very few new antimicrobial classes passing approval along with simultaneous increase in AMR (Durand et al., 2019; Taneja et al., 2019). The period after the 1990s is considered as "discovery void" as no major antimicrobials entered the market during this period (Hutchings et al., 2019). The overall rate of antibacterial approval has become extremely slow with one or two drugs entering the market every year from 2004 onwards which are largely optimization, modification, or combination of already known molecules (WHO, 2019a).

The pipeline for the discovery, and the development of new antimicrobials, has virtually dried out, and pharmaceutical industry is not interested in the development of novel antimicrobials as it is a resource-intensive exercise with cost of development of new drugs being very high (~$1.5–2 billion). Besides, it takes 10–12 years for market approval with no guarantee of return on capital invested in their development since resistance to new agent emerges in a short time frame (Cunha et al., 2019; WHO, 2019a). Antimicrobials are used for shorter durations, compared to drugs for chronic lifestyle diseases such as hypertension, diabetes, etc. In addition, even if new antimicrobials are developed, there is insistence to conserve the newly discovered antimicrobials for seriously ill patients, thereby further shrinking profit margins for the companies (Cunha et al., 2019).

With the dwindling antimicrobial discovery coupled with expanding magnitude of AMR, the "post-antimicrobial era" is imminent when easily curable illness will become incurable, necessitating safe and effective use of antimicrobials (Reardon, 2014; Draenert et al., 2015). The growing issue of AMR is directly linked with antimicrobial use (AMU). The administration (overuse or misuse) of antimicrobials for prophylactic, therapeutic, and non-therapeutic purposes in all sectors including humans, animals, horticulture, fisheries, and agriculture results in survival pressure on microbes, and hence they become resistant to antimicrobials used. Preserving the power of existing antimicrobials by rationalizing their use with investments in finding new innovative antimicrobials/solutions is the need of the hour.

2 Magnitude of Consumption/Use

The global human antimicrobial consumption has soared in the last two decades, mainly due to improved access and affordability in lower middle-income countries (LMICs) as a result of economic development (Van Boeckel et al., 2014). Consumption in developing countries is rapidly converging with high-income countries (HICs). A recent report from the Center for Disease Dynamics, Economics & Policy (CDDEP, 2021) has summarized antimicrobial consumption across nations and shown a 65% rise in overall global antimicrobial consumption between 2000 and 2015, in humans. Consumption in LMICs has increased two- to threefold from 2000 to 2015 with simultaneous increase in defined daily dose (DDDs) per 1,000 inhabitants, with Brazil, China, Africa, Saudi Arabia, and India being the main contributors (CDDEP, 2021). The rate of antimicrobial consumption increased from 11.3 to 15.7 DDDs per 1,000 people (39% increase) in the same period (Klein et al., 2018; CDDEP, 2021). There was a wide variation in consumption rates between LMICs ranging from 4 to 64 DDD per 1000 inhabitants per day probably due to access vs. excess paradox (some countries do not have sufficient access, whereas others are overusing) (WHO, 2018). The total consumption has also increased in high-income countries between 2000 and 2015, but DDDs per 1,000 inhabitants has increased marginally or even declined. However, per capita antimicrobial consumption in LMICs is still lower than HICs (CDDEP, 2021).

India was reported to be the highest consumer of antimicrobials in 2010, and the total consumption increased by 47.4% from 2010 (5411 million DDD) to 2020 (7976 million DDD) (CDDEP, 2021). India alone contributed to 75% of the global average of percentage change in total use from 2010 to 2020 (Klein et al., 2020). The per capita DDD has increased by 1.35 per person from 2010 to 2020 in India (CDDEP, 2021).

The World Health Organization (WHO) is advocating the Access, Watch, Reserve ("AWaRe") tool to streamline the consumption of antibiotics with relaxation to use the *Access* group of antibiotics over *Watch* and *Reserve* group in order to reduce AMR ("AWaRe" discussed later in targeted antibiotics). The global per capita consumption of *Watch* group of antibiotics has risen by 91% from 2000 to 2015 and is largely driven by increased consumption in LMICs (165% increase from 2.0

to 5.3 DDDs per 1,000 people) compared to HICs (27.9% increase from 6.1 to 7.8 DDDs per 1,000 people) (Klein et al., 2020). However, use of *Access* antibiotics has only marginally increased by 26.2% globally during the same period. The use of critically important antimicrobials like oxazolidinones, glycylcyclines, carbapenems, and polymyxins has rapidly increased in all countries (Klein et al., 2020). At the existing rate of consumption, the global antimicrobial consumption could double by 2030 (CDDEP, 2021).

3 Factors Driving Consumption in Humans

Globally antimicrobials are used inappropriately, and nearly half of those used in human healthcare is inappropriate with variation across regions (Laxminarayan et al., 2016). AMU is governed by several patient-related, prescriber-related, system-related, regulatory, supply chain factors, and rationality of their use depends on the context (Cockburn et al., 2005; Castro-Sánchez et al., 2016; Laxminarayan et al., 2016). The major factors for irresponsible antimicrobial use are summarized in Table 1.

In LMICs, enhanced access to antimicrobials, distinct national disease burden, seasonal patterns, and misuse of antimicrobials are largely responsible for increased consumption (Ayukekbong et al., 2017). Antimicrobial prescribing, a complex process, is seen in all clinical settings by all prescribers. A large variation in the rigor of training and knowledge of AMR combined with high workload, poor or limited accessibility to the infectious disease specialists, and nonavailability/non-utilization of point-of-care diagnostic tests further contributes to misuse of

Table 1 The major factors for irresponsible antimicrobial use

Patient related factors	Prescriber related factors	Drug related	Health system related factors
• Anxiety to get well soon • Misconceptions about magic power of antimicrobials • Social, economic and behavioral factors • Self-medication • Non compliance • Not completing prescribed course of antimicrobials • Poor adherence of dosage regimen • Saving antimicrobials for later use	• Informal prescribers • Economic concern due to patient loss • Lack of knowledge and training • Diagnostic uncertainty • Lack of opportunity for patient follow-up • Cognitive dissonance (i.e., knowledge but failure to act on it) • Pressure from pharmaceutical companies • Misleading or erroneous advertising	• Non adherence to regulatory requirements • Over the counter availability • Irrational fixed dose combination • Wrong compounds • Counterfeit and substandard drug • Sub-optimum storage conditions	• Governance and leadership • Overcrowding • Inadequately equipped diagnostic laboratories • Cost-saving pressure to substitute therapy for diagnostic tests • Sub optimum insertion devices • Poor infection prevention and control • Inadequate vaccination

antimicrobials. Very often the nature and severity of the illness, diagnostic uncertainty, high workload, difficulty in follow-up, availability, number of choices, defensive practice, and economic considerations are the deciding factors in real-life setting. A higher antimicrobial use is observed in winter months coinciding with influenza season both for appropriate (e.g., to treat secondary bacterial infections) and inappropriate (e.g., to treat viral infections caused by influenza or other viruses) indications. During COVID-19 pandemic, overuse of antimicrobials has been reported, partly because of concerns regarding bacterial co-infection and misinformation about benefits of antimicrobials for treating COVID-19 patients (Miranda et al., 2020; Beović et al., 2020). Empirical use of broad-spectrum and last-resort antimicrobials also increased in order to improve prognosis in serious illness. A low threshold for prescribing; selection of wrong choice, dose, route of administration, and duration for empirical use; delayed initiation of treatment when indicated; failure to de-escalate after 48–72 hours once the patient stabilizes to narrower-spectrum antimicrobials; and switch from parenteral to oral route due to a lack of awareness of the standard treatment guidelines are among the major prescribing errors (Ayukekbong et al., 2017). Often inappropriate, AMU has been reported in the peri-surgical prophylaxis for prevention of surgical site infections in the form of wrong timing of the first antimicrobial dose, choice, route of administration, and excessive duration despite clear established peri-surgical prophylaxis guidelines (Miliani et al., 2009).

Surveillance data on AMU is limited worldwide, but emerging evidence suggests that overuse and misuse are higher in certain clinical settings, clinical indications, patient demographics, and LMICs (Farooqui et al., 2018; CDDEP, 2021; CDDEP et al., 2021). Despite antimicrobials being prescription drugs, they can be easily accessed over the counter without a valid prescription because of poor enforcement of the laws (Morgan et al., 2011). As a result, patients bypass clinicians and self-medicate by directly purchasing it from the pharmacy and often do not take in adequate doses or complete the entire antimicrobial course. Moreover, private sector healthcare providers, pharmacies, and informal prescribers may advocate prolonged or shorter regimen for economic, rather than clinical, reasons. A higher AMU and misuse are also reported in the primary care setting and acute care wards and for clinicians treating neonatal and pediatric patients or specific infections or syndromes (CDDEP, 2021; CDDEP et al., 2021).

4 Relationship Between Use and Resistance

The development of AMR is a natural biological event but is expedited by the selection pressure exerted by excessive use of antimicrobials (Barboss & Levy, 2000; WHO, 2012; Holmes et al., 2015). Both excessive use and underuse (even when these are indicated) are responsible for the emergence of AMR. Quantum of antimicrobials used and the prescribing practices contribute to the selection of AMR strains.

Several studies conducted across many HICs and LMICs at individual level, healthcare facility level, community level, and country level have found a direct correlation with the amount of antimicrobial use and the development of AMR across spatial and temporal scales (Bronzwaer et al., 2002; Goossens et al., 2005, Goossens, 2009; Costelloe et al., 2010; Bell et al., 2014; Olesen et al., 2018).

Inadequate treatment resulting from limited access, substandard, or falsified agents with poor affordability to complete the full course of treatment also contributes to the emergence of drug-resistant pathogens (Cockburn et al., 2005). In LMICs, treatable infectious diseases currently cause five million deaths due to the lack of access to antimicrobials (CDDEP, 2021).

India and China are among the highest AMR prevalence countries in the world with alarming rates of resistance to almost all the microbes and also to the newer and more expensive drugs (Van Boeckel et al., 2014). Understanding the factors driving AMR in countries with *access* vs. *excess* paradox is highly challenging. Also, deciphering the complex interplay between a myriad of pathogens and antimicrobials in itself is extremely challenging as one microorganism may be resistant to one antimicrobial and susceptible to another and vice versa. To overcome this, the Drug Resistance Index (DRI) has been proposed to measure the average effectiveness of a group of antimicrobials used to treat a given bacterial infection (Klein et al., 2019). The DRI is a composite measure that combines the ability of antibiotics to treat infections with the extent of their use in clinical practice (CDDEP, 2021). It provides a better insight into the complex relationship between antimicrobial use and underuse with AMR in the context of geographical variation and underlying factors (Klein et al., 2020). Some studies identified that HICs like Sweden, Canada, Norway, Finland, and Denmark had the lowest DRIs (despite high use) vs. LMICs which had the highest DRI, reflecting the very low effectiveness of antimicrobial therapy in these countries (Klein et al., 2020).

5 Surveillance of Consumption/Use

Antimicrobial stewardship (AMS) to optimize AMU (reduce the unnecessary consumption with appropriate usage when indicated) can bring back susceptibility among microorganisms over several years (WHO, 2019b). It is imperative that before initiation of any AMS activities, the magnitude of antimicrobial use is measured and analyzed to understand the causes of irrational prescribing practices followed by designing of interventions to rationalize and reduce the AMU. Thus, surveillance for monitoring consumption/use is critical for implementation of a sustainable AMS program.

The data on consumption/use allows knowing the extent of the AMU in countries, regions, healthcare facilities, and departments within facilities to understand the amount and trends of antimicrobial use to guide interventions to regulate the use of antimicrobials and save cost. The AMU data can serve as a benchmark for risk-adjusted inter- and intra-facility use and to understand the quality of use and determinants leading to antimicrobial misuse/overuse at the population/patient

level; to identify the targets for developing strategies/interventions to stop its misuse; to motivate healthcare providers; and to monitor the effect of interventions. Besides, surveillance data is essential to establish epidemiological relationships between antimicrobial use and resistance (WHO, 2018).

The importance of data collection on antimicrobial consumption and analysis was realized by the European Union much before the rollout of the WHO Global Action Plan to combat AMR in 2015 (European Centre for Disease Prevention and Control, 2013). A regional surveillance system, namely, European Surveillance of Antimicrobial Consumption Network (ESAC-Net), was established in 2001 in the European Union/European Economic Area (EU/EEA), which was subsequently expanded to the rest of Europe. Currently, the WHO is providing ongoing support to improve and expand the network with facilitation in analysis and data sharing (ECDC, 2014; WHO, 2017a).

To capture standardized data for consumption, the WHO initiated the global program on surveillance of antimicrobial consumption for LMICs. A common methodology for the measurement of antimicrobial consumption was developed in 2016, based on existing international monitoring systems as reference, such as ESAC-Net and the WHO (ECDC, 2014; WHO, 2017a). The WHO started data collection on consumption for 2014–2016 in selected countries, followed by other countries across the world (WHO, 2017b).

6 Consumption and Use

Antimicrobial consumption (AMC) refers to aggregated data of antimicrobials procured/used at population level (country/hospital/clinical area), whereas antimicrobial use data is patient-level data which is based on indication, treatment regimen, and patient characteristics. AMC data provides the total quantum of antimicrobials consumed and trends for comparison between countries, state, facilities, and wards, whereas AMU data allows assessment of appropriateness of therapy in the context of diagnosis, suspected pathogens, and patient outcomes (WHO, 2017b), though AMC and AMU are two interrelated entities with subtle differences but are often used interchangeably (WHO, 2017b). AMC data is relatively easily accessible and can be collected quickly in comparison with patient-level data which is quite laborious and time-consuming especially in the absence of computerized databases to allow data retrieval.

Both these data are important and serve specific purposes and complement each other. Capturing consumption can be a starting point for resource-limited settings. This consumption data can be used as a proxy for AMU at the patient level.

7 AMU Surveillance Methodologies

Several methods have been employed to measure the magnitude of AMU, but none of the methods is a complete package to garner all the information required (Morris, 2014). The choice of the methodology depends upon the purpose of data collection,

specific objectives of the study, availability of manpower, technical expertise, and infrastructure. Similarly, strategy for data collection can be retrospective (backward), prospective (forward), or concurrent (during treatment) depending on the objective, feasibility in terms of available resources, and time to collect data. It is important to collect data randomly irrespective of the method used in order to draw valid conclusions and generalize results (Sharma, 2017).

The data for consumption/use can be collected quantitatively or qualitatively. Countries or hospitals should define their problems and find methods which are best suited to describe the antimicrobial consumption and link to resistance data (Morris, 2014). Quantitative methods allow estimation of the total volume of antimicrobials consumed/used in particular settings and measurement of any particular antimicrobial used and their trends. These include aggregate data methods, indicator studies, prescription audits, and point prevalence surveys and are useful to give an overall picture of the problem areas.

Qualitative methods allow investigation into the cause of the problem of inappropriate antimicrobial use and include focused discussions, detailed interview, structured observation, and surveys. The different methods to capture quantitative and qualitative data highlighting basic principles, advantages, and disadvantages are discussed below.

7.1 Quantitative Methods

7.1.1 Aggregate Data Methods

Aggregate data gives an overview of the consumption and can be collected relatively with ease from records (WHO, 2017b). Aggregate data on consumption is useful as it provides a broad picture on the quantities of antimicrobials used, the most frequently and infrequently used antimicrobials, and per capita use of specific products at the national, regional, facility, clinical area, or unit level (WHO, 2017b). The consumption can also be matched with the expected consumption based on the morbidity records and is also useful to manage hospital formulary by identifying the most expensive antimicrobials, utilization of Watch or Reserve antimicrobials, etc. Some of the aggregate data methods commonly used for capturing AMU are briefly discussed below.

ABC Analysis

ABC analysis is a selective inventory management tool in which items are classified on the basis of the healthcare cost consumed based on Pareto's 80/20 rule. *A* items are those which consume the maximum budget (either as they are high-cost or high-volume items), and control of these items has a great potential of cost saving with reduced irrational use. *B* items incur a moderate cost, and their use needs to be carefully watched. *C* items are low-cost items and constitute the majority of inventory and do not warrant tight controlling. ABC tool helps in identifying the costliest medicines, those consuming a major proportion of the budget, and designing strategies to rationalize their use (Sharma, et al., 2020). Since antimicrobials usually

consume considerable budget, the ABC principle can be used to identify as to which antimicrobial needs greater attention for control (Anand et al., 2013). Access group antimicrobials are usually low-cost items whose supply must be uninterrupted, whereas tighter control is required for purchase and use of high-cost/high-volume Watch and Reserve group antimicrobials. Similarly, newer-generation broad-spectrum antimicrobials are expensive, and regulating their access can limit their inappropriate use as well as significant cost saving. However, ABC analysis has its limitations. It cannot be used for benchmarking between countries or hospitals due to variability in costs. Also, it does not allow comparison of efficacy between antimicrobials.

Vital, Essential, and Nonessential/Desirable (VEN/D) Analysis

Vital, Essential, and Nonessential/Desirable (VEN (D)) analysis allows for prioritizing selection, procurement, and use of antimicrobials based on their necessity as vital (life-saving or crucial), essential (required for certain significant number of diseases), and nonessential or desirable categories (minor or self-limited illnesses). The VEN analysis must be based on the level of healthcare keeping in view the epidemiology and infectious disease morbidity statistics. A vital for a super-specialty hospital may be nonessential for a primary healthcare center and vice versa.

Using ABC analysis alone may leave out some low-cost and high-consumption antimicrobials which may be essential or lifesaving as they do not appear in category A. Similarly, VEN analysis alone also carries a risk of some nonessential but expensive drugs to get included as category A (Anand et al., 2013). Therefore, a combination matrix of ABC and VEN could be used to control the supply or usage of Group AD items requiring stringent control of critically important antimicrobials or by finding alternatives (such as replacing *Reserve* antimicrobials among A category with *Watch* antimicrobials; switching from broad-spectrum to narrow-spectrum antimicrobials; switching from *Watch* group antimicrobials to *Access* group antimicrobials) while ensuring availability of V and E items (such as *Access* antimicrobials) (Mathew et al., 2016).

Defined Daily Dose

Defined daily dose (DDD) is a WHO standardized reference methodology for measuring the consumption to allow benchmarking across countries, hospitals, and wards (WHO, 2017b). DDD is the assumed average maintenance dose per day for an antimicrobial used for its main indication in adult patients. DDD is defined globally for each medicine by the WHO Collaborating Centre for Drug Statistics in Oslo, Norway, and is regularly updated. The DDD reflects global dosage, and a single DDD is assigned per ATC code irrespective of genetic variations and administration routes (oral/parenteral).

DDD is most frequently expressed as DDD per 1,000 inhabitants per day for total antimicrobial consumption and gives general utilization for the total population (country, hospital). For hospital in-patient use, the WHO recommends DDDs per 100 bed-days for measuring antimicrobial use, and the difference in number of beds between hospitals is adjusted by using the occupancy rate (WHO, 2021). The DDD

utilizes consideration of the WHO Anatomical Therapeutic Chemical (ATC) classification system. User-friendly tools/software are available for calculation of DDD by entering the name of antimicrobial, pack size, and strength in grams (WHO, 2021). However, manual calculation using Microsoft Excel can also be done for calculating DDD.

DDD allows for comparing trends in the utilization of antimicrobials between countries and population groups as it does not take into consideration price, package size, and formulations (Muller et al., 2006). The DDD method elucidates the quantitative and ecological relationship between AMU and resistance (Goosen, 2009). However, it does not give an idea of the intensity of AMU in a particular patient and cannot differentiate between few patients prescribed with many antimicrobials and many patients getting few antimicrobials nor between antimicrobials used in some long-stay patients vs. many short-stay patients (Berrington, 2010). Also, this method has not been standardized to measure antimicrobial use in the pediatric population (Morris, 2014).

7.1.2 Prescribed Daily Dose

The prescribed daily dose (PDD) is the average dose prescribed as determined by a random sample of prescriptions and medical or pharmacy records. The PDD gives the average daily dose prescribed for a particular disease. The PDD varies according to the disease, clinical spectrum, patient demographics, pharmacokinetic/pharmacodynamic considerations, and national treatment guidelines. The PDDs may vary from one setting to another depending upon demography and ethnic differences and thus are not suitable for making national and international comparisons. DDD and PDD do not correspond frequently, and PDD is generally higher than DDD for most of the antimicrobial treatments (Muller et al., 2006).

7.1.3 Days of Therapy

Days of therapy (DOT) are the total number of days any antimicrobial agent is administered to individual patients irrespective of the dose or formulation (CDC, 2021). DOT is more clinically relevant compared to DDDs as it tells the actual treatment received vis-a-vis hypothetical consumption measured by DDD. DOT/patient days can also be used to benchmark consumption within and between institutions and can be used for the pediatric population (including neonates) unlike DDD.

The disadvantages with the use of DOT as a metric is that it only reflects antimicrobial use and cannot distinguish between single dose, multiple dose, or continuous infusion. For example, use of single antimicrobial for 14 days and use of two broad-spectrum antimicrobials in any dosage for 7 days both contribute to 14 DOTs (Morris, 2014).

7.1.4 Targeted Antibiotics (Access, Watch, Reserve Tool)

Antimicrobial stewardship (AMS) is much more required for some antibiotics which are more expensive, more toxic, broad spectrum, and critically important for human use. Reserving these targeted antibiotics for use for correct indications can bring

reduction in AMU. The WHO advocates "AWaRe" to promote the usage of narrow-spectrum antibiotics while reserving broad-spectrum antibiotics for "hardest to treat" infections (WHO, 2019c).

"AWaRe" tool classifies antibiotics into three groups:

1. The Access group consisting of narrow-spectrum antibiotics for common infections/specified infectious syndrome which should be easily accessible. It includes 48 antibiotics such as amoxicillin, cloxacillin, amoxicillin-clavulanic acid, etc. (WHO, 2019c).
2. The Watch group comprises broader-spectrum antibiotics which have potential for development of resistance. It includes 110 antibiotics such as ciprofloxacin, azithromycin, vancomycin, teicoplanin, etc. (WHO, 2019c).
3. The Reserve group consists of last-resort antimicrobials for targeted use in multidrug-resistant infections. It includes 22 antibiotics, viz., oxazolidinones, glycylcyclines, carbapenems, polymyxins, etc. (WHO, 2019c).

Absolute antimicrobial use can be measured by using total consumption data, and then relative use can be determined according to "AWaRe" categories. Patterns of antimicrobial use can be further studied by drug utilization percentage (i.e., number of antimicrobials that constitute 90% of the total use); proportion of antibiotic use (DDDs per 1000 admissions) in each "AWaRe" category over time; the ratio of *Access to Watch* antibiotics; etc. This may allow for designing AMS interventions to reduce the use of *Watch* or *Reserve* group of antibiotics as appropriate to the facility.

Adopting the "AWaRe" categorization also leads to improving availability and accessibility to antibiotics on the *Access* list and reducing the use of those on Watch and Reserve lists. The "AWaRe" classification provides an opportunity to set targets for measuring and reporting progress but may lead to shifting of selection pressure to cheaper agents. The countries should strive to reach a 60% target for antibiotic consumption from the essential category (WHO, 2019c).

One of the limitations of "AWaRe" classification is that some of the antibiotics have not been classified into this category as these are not listed on the WHO Essential Medicine List. There is a need to develop and evaluate pediatric AMS programs based on the "AWaRe" index (Hsia et al., 2019).

7.1.5 Indicator Study Methods

Indicator study methods allow us to explore the factors which drive AMU decisions. The WHO has developed "core drug use indicators" to measure performance or assess drug use practices in various settings over time in three related areas of prescribing practices, patient care, and facility-specific factors (WHO, 1993). The core drug use indicators are objective measures to describe the drug use situation in a country, region, or individual health facility. The core drug use indicators and other commonly used complimentary indicators are listed in Table 2.

These drugs use indicators that are highly specific, consistent, reliable, and representative and can be easily measured without the requirement of specially trained data collectors. Percent encounters with antimicrobial data provide

Table 2 Drug use indicators

Core drug use indicators	Complimentary indicators
Prescribing indicators: • Average number of drugs per encounter • Percentage of drugs prescribed by generic name • Percentage of encounters with an antimicrobial prescribed • Percentage of encounters with an injection prescribed • Percentage of drugs prescribed from Essential Medicines List or formulary	***Complementary drug use indicators*:** • Percentage of patients treated without drugs • Average drug cost per encounter • Percentage of drug cost spent on antimicrobials • Percentage of drug cost spent on injections • Percentage of prescriptions in accordance with treatment guidelines • Percentage of surgical patients who receive appropriate surgical prophylaxis • Number of antimicrobial sensitivity tests reported per hospital admission • Percentage of cases of malaria treated with recommended antimicrobials • Percentage of cases of diarrhea treated with oral rehydration therapy • Percentage of patients receiving medicines without prescription
Patient care indicators: • Average consultation time • Average dispensing time • Percentage of drugs actually dispensed • Percentage of drugs adequately labeled • Patients' knowledge of correct doses	
Facility indicators: • Availability of Essential Medicines List or formulary to practitioners • Availability of standard treatment guidelines • Availability of key drugs	-

Adapted from: World Health Organization (1993). How to investigate drug use in health facilities Selected drug use indicators. WHO/DAP/93.1

information about problem areas in medicine use at facility level and prescriber level and to evaluate the impact of interventions for corrective actions. These indicators are indicative of drug use problem only and are influenced by prescriber type and the disease pattern. These are most useful at primary healthcare facilities and to monitor trends over time. Encounters with antimicrobials indicate the extent of use problem only as reference value or the yardstick for antimicrobial use for a facility type or prescriber type and may vary depending on the clinical case mix presenting with infectious diseases.

These indicators do not provide sufficient information about drug appropriateness or the exact nature of the drug use problem as diagnosis is not considered. Further, drug use is influenced by complex interplay of factors. Core drug use indicators along with the morbidity pattern in a given setting can be used for developing and testing implementation of therapeutic guidelines for treatment of the various disease entities.

7.1.6 Prescription Audit

Prescription audit aids in analyzing the adequacy of the clinical prescription based on specific diagnosis as the number and type of antimicrobials prescribed, their dose, route of administration, timing of administration, etc. (Zhen et al., 2018). These audits allow to study the determinants of prescription like influence of patient

demand, industry interference (pressure from medical representatives), publicity campaigns, prevalent infectious diseases, adherence to standard treatment guidelines (such as use of ORS in diarrhea), and prescriptions for contraindicated or banned drugs.

7.1.7 Point Prevalence Survey

The WHO point prevalence survey (PPS) methodology is an adaptation of the EU and the US CDC protocol for healthcare-associated infections and AMU (WHO, 2019d). The PPS is a practical surveillance tool that reflects on the quality of antimicrobials prescribed. This is cross-sectional data collection method gathering information from patients' chart review in a short span of time (preferably 1 day, a few days up to weeks, may be a month for large national surveys) across the whole hospital (Versporten et al., 2015). The PPS can be repeated after a time interval to monitor trends and assess the impact of interventions. It is a practical alternative to continuous data collection which may not be possible due to the high workload and resource limitations.

The PPS allows for selection of variables (core and optional) at the country level, hospital level, and patient level. Selection of same variables across countries and hospitals allows for better comparability and interpretation of results (WHO, 2019d). Hospitals and countries may also include additional variables (e.g., microbiology results) to improve the understanding of antimicrobial use in hospitals.

By applying the PPS, it is possible to:

- Identify differences among prescribing rates between hospitals, hospital departments, regions, and countries in hospitalized patients.
- Determine variation in antimicrobials, dose, and indication across locations.
- Understand the quality of antimicrobial use, and identify targets for improvement.
- Assess the implementation of treatment guidelines (if indication for treatment available).
- Plan on interventions to promote stewardship.
- Evaluate the effectiveness of interventions through repeated surveys.

The major limitation of the PPS is that information on antimicrobial prescribing is collected from cross-section of all patients hospitalized for infectious disease management irrespective of their being on antimicrobial treatment at the time of data collection.

7.2 Qualitative Methods

Qualitative data is useful to determine the appropriateness of AMU and links antimicrobial usage to reasons (indications) for prescribing at a particular patient or community level. Several cultural and social determinants have been described as barriers to appropriate use such as patient-doctor relationship, perception of the

problem (consulting time, counseling), time constraint due to high workload (explaining antimicrobials prescribed is not necessary as it is time-consuming and unrewarding, decision fatigue, want to appease patient), treatment characteristics (frequency of drug administration), attitudes (perceived risk, fear, anxiety, patient demand or pressure, expecting antimicrobials especially in ambulatory setting and pediatrics), access to treatment (nonavailability of a medicine and patients' direct costs), characteristics and severity of the illness for which the antimicrobial was prescribed (severity at presentation and duration of symptoms), and knowledge (regarding illness and its treatment) (Krockow et al., 2019). Also, when developing any quality intervention, it is important to understand the attitudes, motivations, and intentions of those behaviors determining antimicrobial use as well as local social and environmental context. Qualitative studies in the community provide an understanding of the underlying issues and context of antimicrobial misuse as patient is an important decision-maker for use of antimicrobials. These qualitative methods help to describe the extent and variability in usage and to identify problems deserving more detailed studies. If therapy is identified to be inappropriate, interventions are designed to optimize antimicrobial therapy. Understanding of the social dynamics also helps in characterizing the optimal way of doing stewardship.

8 Conclusions

There is a general trend of rising AMC globally. AMC is directly related to the emergence of AMR. The variability in AMR within and across countries depends on the magnitude and patterns of AMU which is influenced by several socioeconomic, prescriber-related, regulatory, and system-related factors. Controlling global AMC is essential to reduce the menace of rising AMR. To rationalize AMU, it is essential to measure and compare consumption over time and understand drivers for excessive use, particularly the last-resort and *Watch* group antimicrobials.

Trends in AMU and AMR are being monitored in several HICs in humans and livestock, but data are scarce from LMICs. Time series analysis of AMC patterns across all settings and countries could aid in decisions to optimize antimicrobial prescribing and minimizing AMR. For this to be possible, the data must be collected using appropriate indicators, standardized methodologies, and measuring units using adequate sample size.

The choice of indicators to quantify AMU must take into consideration specific objectives of study, level of healthcare and resources, time available, etc. Stepwise approach to capture AMU data is generally recommended as no single indicator is adequate to address all aspects of AMU (Patel et al., 2019). One possible way is to start with capturing aggregate data of consumption at a population/facility level to identify broad issues followed by individual-level data collection and by detailed investigations using "qualitative methods." The impact of interventions can also be evaluated by using these tools.

9 Glossary

AMC	Antimicrobial consumption
AMR	Antimicrobial resistance
AMU	Antimicrobial use
AWaRe	Access, Watch, Reserve
DDD	Defined daily dose
DoT	Days of therapy
HICs	High-income countries
LMICs	Low- to middle-income countries
PDD	Prescribed daily dose
PPS	Point Prevalence Survey
VEN (D)	Vital, Essential, Nonessential (Desirable)
WHO	World Health Organization

References

Aminov, R. I. (2010). A brief history of the antibiotic era: Lessons learned and challenges for the future. *Frontiers in Microbiology, 1*, 134. https://doi.org/10.3389/fmicb.2010.00134. [Online]. Accessed 3 January 2021.

Anand, T., Ingle, G. K., Kishore, J., & Kumar, R. (2013). ABC-VED analysis of a drug store in the Department of Community Medicine of a Medical College in Delhi. *Indian Journal of Pharmaceutical Sciences, 75*(1), 113–117. https://doi.org/10.4103/0250-474X.113543. [Online]. Accessed 7 January 2021.

Ayukekbong, J. A., Ntemgwa, M., & Atabe, A. N. (2017). The threat of antimicrobial resistance in developing countries: Causes and control strategies. *Antimicrobial Resistance and Infection Control, 6*, 47. https://doi.org/10.1186/s13756-017-0208-x. Accessed 3 January 2021.

Barbosa, T. M., & Levy, S. B. (2000). The impact of antibiotic use on resistance development and persistence. *Drug Resist Update, 3*(5), 303–311. https://doi.org/10.1054/drup.2000.0167. [Online]. Accessed 5 January 2021.

Bell, B. G., Schellevis, F., Stobberingh, E., Goossens, H., & Pringle, M. (2014). A systematic review and meta-analysis of the effects of antibiotic consumption on antibiotic resistance. *BMC Infectious Diseases, 14*, 13. https://doi.org/10.1186/1471-2334-14-13. [Online]. Accessed 5 January 2021.

Beović, B., Dousak, M., Ferreira-Cambra, J., et al. (2020). Antibiotic use in patients with COVID-19: A 'snapshot', infectious diseases international research initiative (ID-IRI) survey. *Journal of Antimicrobial Chemotherapy, 75*, 3386–3390.

Berrington, A. (2010). Antimicrobial prescribing in hospitals: Be careful what you measure. *Journal of Antimicrobial Chemotherapy, 65*(1), 163–168. https://doi.org/10.1093/jac/dkp399. [Online]. Accessed 3 January 2021.

Bronzwaer, S., Cars, O., Buchholz, U., et al. (2002). The relationship between antimicrobial use and antimicrobial resistance in Europe. *Emerging Infectious Diseases, 8*(3), 278–282. https://doi.org/10.3201/eid0803.010192. [Online]. Accessed 3 January 2021.

Castro-Sánchez, E., Moore, L., Husson, F., & Holmes, A. (2016). What are the factors driving antimicrobial resistance? Perspectives from a public event in London, England. *BMC Infectious Diseases, 16*(1). https://doi.org/10.1186/s12879-016-1810-x. [Online]. Accessed 3 January 2021.

Center for Disease Control. (2021). CDC National health safety network, Antimicrobial Use and Resistance (AUR) module. Available from https://www.cdc.gov/nhsn/pdfs/pscmanual/11pscaurcurrent.pdf. Accessed on 13 May 2021.

Center for Disease Dynamics Economics and Policy. (2021). Resistance map. Available at: https://resistancemap.cddep.org/. Accessed 25th January 2021.

Center for Disease Dynamics, Economics and Policy, Sriram, A., Kalanxhi, E., Kapoor, G., et al. (2021). State of the world's antibiotics 2021: A global analysis of antimicrobial resistance and its drivers. Accessed 18 May 2021.

Cockburn, R., Newton, P. N., Agyarko, E. K., Akunyili, D., & White, N. J. (2005). The global threat of counterfeit drugs: Why industry and governments must communicate the dangers. *PLoS Medicine, 2*, e100.

Costelloe, C., Metcalfe, C., Lovering, A., Mantm, D., & Hay, A. D. (2010). Effect of antibiotic prescribing in primary care on antimicrobial resistance in individual patients: Systematic review and meta-analysis. *British Medical Journal, 14*(7756), c2096.

Cunha, R. D., Fonseca, L. P., & Calado, R. C. (2019). Antibiotic discovery: Where have we come from, where do we go? *Antibiotics, 8*(2), 45. https://doi.org/10.3390/antibiotics8020045

Draenert, R., Seybold, U., Grützner, E., & Bogner, J. R. (2015). Novel antibiotics: Are we still in the pre-post-antibiotic era? *Infection, 43*(2), 145–151. https://doi.org/10.1007/s15010-015-0749-y. [Online]. Accessed 18 May 2021.

Durand, G., Raoult, D., & Dubourg, G. (2019). Antibiotic discovery: History, methods and perspectives. *International Journal of Antimicrobial Agents, 53*(4), 371–382. https://doi.org/10.1016/j.ijantimicag.2018.11.010. Accessed 1 January 2021.

ECDC European Centre for Disease Prevention and Control. (2014). Surveillance of antimicrobial consumption in Europe, 2012 Stockholm. Available from: https://www.ecdc.europa.eu/en/publications-data/surveillance-antimicrobial-consumption-europe-2012. Accessed 8 October 2021.

European Centre for Disease Prevention and Control (2013) Antimicrobial resistance surveillance in Europe 2012. Annual Report of the European Antimicrobial Resistance Surveillance Network (EARS-Net). Stockholm: ECDC. Available at: https://www.ecdc.europa.eu/en/publications-data/antimicrobial-resistance-surveillance-europe-2012. Accessed 3 January 2021.

Farooqui, H., Selvaraj, S., Mehta, A., & Heymann, D. (2018). Community level antibiotic utilization in India and its comparison vis-à-vis European countries: Evidence from pharmaceutical sales data. *PLoS One, 13*(10), e0204805. https://doi.org/10.1371/journal.pone.0204805. Accessed 1 January 2021.

Goossens, H. (2009). Antibiotic consumption and link to resistance. *Clinical Microbiology Infections, 15*(3), 12–15.

Goossens, H., Ferech, M., Vander Stichele, R., & Elseviers, M. (2005). Outpatient antibiotic use in Europe and association with resistance: A cross-national database study. *The Lancet, 14*(9459), 579–587.

Gould, K. (2016). Antibiotics: From prehistory to the present day. *Journal of Antimicrobial Chemotherapy, 71*(3), 572–575. https://doi.org/10.1093/jac/dkv484. Accessed 3 January 2021.

Holmes, A. H., Moore, L. S., Sundsfjord, A., et al. (2015). Understanding the mechanisms and drivers of antimicrobial resistance. *Lancet*. https://doi.org/10.1016/S0140-6736(15)00473-0. [Online]. Accessed 1 January 2021.

Hsia, Y., Lee, B., Versporten, A., Yang, Y., et al. (2019). Use of the WHO access, watch, and reserve classification to define patterns of hospital antibiotic use (AWaRe): An analysis of paediatric survey data from 56 countries. *The Lancet Global Health, 7*(7), e861–e871. https://doi.org/10.1016/s2214-109x(19)30071-3. [Online]. Accessed 1 January 2021.

Hutchings, M., Truman, A., & Wilkinson, B. (2019). Antibiotics: Past, present and future. *Current Opinion in Microbiology, 51*, 72–80.

Klein, E. Y., Milkowska-Shibata, M., Tseng, K. K., et al. (2020). Assessment of WHO antibiotic consumption and access targets in 76 countries, 2000–15: An analysis of pharmaceutical sales data. *Lancet Infectious Diseases, 21*, 107–115.

Klein, E. Y., Tseng, K. K., Pant, S., et al. (2019). Tracking global trends in the effectiveness of antibiotic therapy using the drug resistance index. *BMJ Global Health, 4*, e001315.

Klein, E. Y., Van Boeckel, T., Martinez, E., et al. (2018). Global increase and geographic convergence in antibiotic consumption between 2000 and 2015. *Proceedings of The National Academy*

of Medical Sciences, 115(15), E3463–E3470. https://doi.org/10.1073/pnas.1717295115. [Online]. Accessed 22 February 2021.

Krockow, E. M., Colman, A. M., Chattoe-Brown, E., et al. (2019). Balancing the risks to individual and society: A systematic review and synthesis of qualitative research on antibiotic prescribing behaviour in hospitals. *Journal of Hospital Infection, 101*(4), 428–439. https://doi.org/10.1016/j.jhin.2018.08.007. [Online]. Accessed 20 May 2021.

Laxminarayan, R., & Chaudhury, R. (2016). Antibiotic resistance in India: Drivers and opportunities for action. *PLoS Medicine, 13*(3). [Online]. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4775002/. Accessed 22 February 2021.

Laxminarayan, R., Matsoso, P., Pant, S., et al. (2016). Access to effective antimicrobials: A worldwide challenge. *Lancet, 387*(10014), 168–175. https://doi.org/10.1016/S0140-6736(15)00474-2. [Online]. Accessed 22 February 2021.

Mathew, B., Panavila, L., Sindhu, K., Rajaneesh, P., Bharath, V., & Doddayya, H. (2016). A study on inventory management by ABC, VED and ABC-VED matrix analysis in pharmacy department of a tertiary care teaching hospital. *Asian Journal of Pharmaceutical and Health Sciences, 6*(4), 1563–1568.

Miliani, K., L'Hériteau, F., & Astagneau, P. J. (2009). Non-compliance with recommendations for the practice of antibiotic prophylaxis and risk of surgical site infection: Results of a multilevel analysis from the INCISO Surveillance Network. *Journal of Antimicrobial Chemotherapy, 64*, 1307–1315.

Miranda, C., Silva, V., Capita, R., Alonso-Calleja, C., Igrejas, G., & Poeta, P. (2020). Implications of antibiotics use during the COVID-19 pandemic: Present and future. *Journal of Antimicrobial Chemotherapy, 75*(12), 3413–3416. https://doi.org/10.1093/jac/dkaa350. [Online]. Accessed 22 February 2021.

Morgan, D. J., Okeke, I. N., Laxminarayan, R., Perencevich, E. N., & Weisenberg, S. (2011). Non prescription antimicrobial use worldwide: A systematic review. *The Lancet Infectious Diseases, 11*, 692–701.

Morris, A. M. (2014). Antimicrobial stewardship programs: Appropriate measures and metrics to study their impact. *Current Treatment Options in Infectious Diseases, 6*(2), 101–112. https://doi.org/10.1007/s40506-014-0015-3. [Online]. Accessed 22 February 2021.

Muller, A., Monnet, D., Talon, D., Henon, T., & Bertrand, X. (2006). Discrepancies between prescribed daily doses and WHO defined daily doses of antibacterials at a university hospital. *British Journal of Clinical Pharmacology, 61*(5), 585–591. https://doi.org/10.1111/j.1365-2125.2006.02605.x. [Online]. Accessed 22 February 2021.

Olesen, S., Barnett, M., MacFadden, D., Brownstein, J., Hernández-Díaz, S., Lipsitch, M., & Grad, Y. (2018). The distribution of antibiotic use and its association with antibiotic resistance. *eLife, 7*. https://doi.org/10.7554/elife.39435. [Online]. Accessed 22 February 2021.

Patel, S., Jhass, A., Hopkins, S., & Shallcross, L. (2019). Informing the development of a standardised approach to measure antibiotic use in secondary care: A systematic review protocol. *British Medical Journal Open, 9*(5), e026792. https://doi.org/10.1136/bmjopen-2018-026792. [Online].

Reardon, S. (2014). WHO warns against 'post-antibiotic' era. *Nature*. https://doi.org/10.1038/nature.2014.15135. [Online]. Accessed 22 February 2021.

Sharma, S. (2017). Tools for assessing and monitoring medicine use. In D. Vohora & G. Singh (Eds.), *Pharmaceutical medicine and translational clinical research (ISBN: 978-0-12-802103-3)* (1st ed., pp. 445–463). Elsevier.

Sharma, S., Tandlich, R., Docrat, M., & Srinivas, S. (2020). Antibiotic procurement and ABC analysis for a comprehensive primary health care clinic in the Eastern Cape province, South Africa. *South African Journal of Infectious Diseases, 35*(1), a134. https://doi.org/10.4102/sajid.v35i1.134. [Online].

Taneja, N., Sethi, S., Tahlan, A. K., & Kumar, Y. (2019). Introductory chapter- stepping into the post-antibiotic era-challenges and solutions. In Y. Kumar (Ed.), *Antimicrobial resistance – A*

global threat. [Online]. Available at: https://www.intechopen.com/books/antimicrobial-resistance-a-global-threat. Accessed 22 February 2021.

Van Boeckel, T. P., Gandra, S., Ashok, A., et al. (2014). Global antibiotic consumption 2000 to 2010: An analysis of national pharmaceutical sales data. *Lancet Infectious Diseases, 14*(8), 742–750. https://doi.org/10.1016/S1473-3099(14)70780-7. [Online]. Accessed 22 February 2021.

Versporten, A., Drapier, N., Zarb, P., et al. (2015). The global point prevalence survey of antimicrobial consumption and resistance (Global-PPS): A worldwide antimicrobial Web-based point prevalence survey. *Open Forum Infectious Diseases, 2(suppl_1)*. https://doi.org/10.1093/ofid/ofv133.25. [Online]. Accessed 22 February 2021.

World Health Organization. (1993). How to investigate drug use in health facilities selected drug use indicators. WHO/DAP/93.1.

World Health Organization. (2012). *The evolving threat of antimicrobial resistance, options for action*. WHO Library Cataloguing-in-Publication Data. Available at: https://apps.who.int/iris/bitstream/handle/10665/44812/9789241503181_eng.pd. Accessed 22 February 2021.

World Health Organization. (2017a). *Antimicrobial medicines consumption (AMC) network, AMC data 2011–2014*. Available at: http://www.euro.who.int/__data/assets/pdf_file/0007/337984/51020-WHO-AMCreport-web.pdf. Accessed 25 September 2020.

World Health Organization. (2017b). *WHO methodology for a global programme on surveillance of antimicrobial consumption*. Available at: http://www.who.int/medicines/areas/rational_use/WHO_AMCsurveillance_1.0.pdf?ua=1. Accessed 25 September 2018.

World Health Organization. (2018). *WHO report on surveillance of antibiotic consumption: 2016-2018 early implementation.* License: CC BY-NC-SA 3.0 IGO Available at: https://apps.who.int/iris/bitstream/handle/10665/277359/9789241514880-eng.pdf. Accessed 23 February 2021.

World Health Organization. (2019a). *Antibacterial agents in clinical development: An analysis of the antibacterial clinical development pipeline.* Available at: https://apps.who.int/iris/bitstream/handle/10665/330420/9789240000193-eng.pdf. Accessed 9 August 2020.

World Health Organization. (2019b). *Antimicrobial stewardship programmes in health-care facilities in low- and middle-income countries. A practical toolkit.* License: CC BY-NC-SA 3.0 IGO. Available at: https://apps.who.int/iris/bitstream/handle/10665/329404/9789241515481-eng.pdf. Accessed 23 February 2021.

World Health Organization. (2019c). 2019 *WHO aware classification database of antibiotics for evaluation and monitoring of use*. Available at: https://www.who.int/publications/i/item/WHOEMPIAU2019.11. Accessed 21 May 2021.

World Health Organization. (2019d). *WHO methodology for point prevalence survey on antibiotic use in hospitals,* 11, WHO reference number: WHO/EMP/IAU/2018.01.

World Health Organization. (2021). *WHO collaborating centre for drug statistics methodology, ATC classification index with DDDs*. Available at: https://www.whocc.no/atc_ddd_index/. Accessed 20 May 2021.

Zhen, L., Jin, C., & Xu, H. N. (2018). The impact of prescriptions audit and feedback for antibiotic use in rural clinics: Interrupted time series with segmented regression analysis. *BMC Health Services Research, 18*(1), 777. https://doi.org/10.1186/s12913-018-3602-z

Antimicrobial Usage in Animal Production Systems

Ayyasamy Manimaran, Arumugam Kumaresan, and Veerasamy Sejian

Contents

Abstract

Antimicrobial usage (AMU) in human and veterinary medicine is the single-most important factor for the development of antimicrobial resistance (AMR), a global public health threat. Although AMR development is a natural process, misuse or overuse of antimicrobials can speed up the process. Surveillance of AMU in animal and agricultural system is the basis for understanding and combating AMR, as nonhuman AMU leads to the development of resistant bacteria in the case of drugs used by humans. Among various indications, mastitis is one of the most common reasons for AMU in dairy animals. However, the pattern of AMU and their influencing factors varies among the countries, species, breeds, production systems, drugs, and other factors. Therefore, it is very important to understand the influencing factors for better implementation of policies to regulate AMU animal production systems. Several methods have been explored in animal production systems for the collection of AMU data. However, the lack of

A. Manimaran (✉) · A. Kumaresan
Southern Regional Station, ICAR-National Dairy Research Institute, Adugodi, Bengaluru, Karnataka, India
e-mail: a.manimaran@icar.gov.in

V. Sejian
ICAR-National Institute of Animal Nutrition and Physiology, Bengaluru, Karnataka, India

M. P. Mothadaka et al. (eds.), *Handbook on Antimicrobial Resistance*,
https://doi.org/10.1007/978-981-19-9279-7_14

harmonized quantification methods is the major limiting factor encountered at present. Under the changing livestock production conditions from small holder, less intensive to highly intensive farming systems, identification of critical factors, and suitable metrics are important to make an evidence-based policy decision for regulation of antimicrobial usage. Several countries have taken measures to reduce AMU in food animal production system. The European Union has done it through regulations on veterinary medicines and medicated feed. Reduction and replacement of antimicrobials, along with redefined animal husbandry practices through preventive approaches, are important measures to reduce AMU in animal production systems.

Keywords

Antimicrobial use · Antimicrobial resistance · Antibiotic residue · Dairy animals · Poultry · India

Abbreviations

AGPs	Antimicrobial growth promoters
AMR	Antimicrobial resistance
AMU	Antimicrobial use
ATI	Antimicrobial treatment incidence
BIS	Bureau of Indian Standards
CAC	Codex Alimentarius Commission
CCC	Cow calculated courses
CDC	Centers for Disease Control and Prevention
CDDEP	Center for Disease Dynamics, Economics and Policy
CIPARS	Canadian Integrated Programme for Antimicrobial Resistance Surveillance
DAHDF	Department of Animal Husbandry, Dairying and Fisheries
DANMAP	Danish Integrated Antimicrobial Resistance Monitoring and Research Programme
DCDvet	Defined course dose for animals
DCGI	Drug Controller General of India
DDDvet	Define daily dose for animals
EFSA	European Food Safety Authority
EMA	European Medicines Agency
ESVAC	European Surveillance of Veterinary Antimicrobial Consumption
EU	European Union
FDA	Food and Drug Administration
FINRESVET	The Finnish Veterinary Antimicrobial Resistance Monitoring and Consumption of Antimicrobial Agents
FSSAI	Food Safety and Standards Authority of India
GERMVET	German National Antibiotic Resistance Monitoring
ICAR	Indian Council of Agricultural Research

ICMR	Indian Council of Medical Research
IPC	Infection prevention control
ITAVARM	Italian Veterinary Antimicrobial Resistance Monitoring
JVARM	The Japanese Veterinary Antimicrobial Resistance Monitoring System
MoHFW	Ministry of Health and Family Welfare
MRL	Maximum residue limit
NARMS	National Antimicrobial Resistance Monitoring System
NethMapMARAN	Monitoring of Antimicrobial Resistance and Antibiotic Usage in Animals in the Netherlands
NMDRD	National Milk Drug Residue Data Base
NORMVET	Norwegian Surveillance System for Antimicrobial Drug Resistance
NSAIDs	Nonsteroidal anti-inflammatory drugs
OIE	Office International des Epizooties
ONERBA	National Observatory of the Epidemiology of Bacterial Resistance to Antibiotics
PCU	Population corrected unit
RESAPATH	French surveillance network for antimicrobial resistance in pathogenic bacteria of animal origin
SVARM	Swedish Veterinary Antimicrobial Resistance Monitoring
USDA	United States Department of Agriculture
VARSS	Veterinary Antimicrobial Resistance and Sales Surveillance
VAV	Spanish Veterinary Antimicrobial Resistance Surveillance Network
WHO	World Health Organization

1 Introduction

Antimicrobials are commonly used for therapeutic, prophylactic, and metaphylactic purposes in livestock and poultry production systems. The administered drugs or its metabolites are secreted into milk, meat, and eggs as residue, mostly due to: (i) failure to monitor the withdrawal periods, (ii) illegal or off-label use of drugs, and (iii) incorrect dosage (Paturkar et al., 2005). The most important concern of antibiotic residues in foods of animal origin is the development of antimicrobial resistance (AMR), which is a global problem. The World Health Organization (WHO) declared AMR as one of the most important public health threats of the twenty-first century. Globally, AMR is estimated to cause about 300 million premature deaths by 2050, with economic loss up to $100 trillion (3.5% reduction in global GDP). By 2050, AMR would cause death of about 2.4 million people in high-income countries alone and 25% increase in health expenditure in low-income countries if current incidence rate of AMR continues (Jonas et al., 2017). Besides, repeated sub-chronic exposure of antibiotic residues causes allergic reaction, toxicity, hypersensitivity, carcinogenicity or mutagenicity, and gastrointestinal

disturbances. For instance, about 5–10% of the population suffers from penicillin-induced allergic reactions even at concentration as low as 1 ppb. Further, 4% of patients with a history of penicillin allergy also experienced an anaphylactic reaction to cephalosporin drugs (Kelkar & Li, 2001). Antibiotic residues in milk also disrupt the milk processing industry by the interference of starter cultures, though it depends on drug levels, species of culture strain, the presence of other natural potential inhibitors, etc. (Packham et al., 2001; Broome et al., 2002). Besides, the excretion of active metabolites of antibiotics through urine and feces causes disturbance on the soil and water microflora.

Irrational antimicrobial use (AMU) is the single most important factor for the development of AMR in human and veterinary medicines (Grave et al., 1999). Though the AMR development is a natural process, inappropriate use of antimicrobials in human and veterinary medicine can speed up the selection and spread of AMR. In the European Union (EU), and in the USA, AMU in food animal production accounted for more than 70% of total antimicrobial consumption (ECDC/EFSA/EMA, 2017). The projected higher demand for animal products and intensive animal farming production systems in low- and middle-income countries is expected to increase the AMU up to 67% by 2030 (Cuong et al., 2018). Understanding AMU is also significant since AMU in humans and animals often overlaps due to the involvement of the same pathogens as a cause of infection. For instance, about 75% of veterinary-approved drugs are essential for human use in the USA, and extensively used drugs in dairy animals like penicillin, cephalosporin, and tetracycline group of antibiotics are also important in treating the same pathogens in humans. Lesser investment by global pharmaceuticals in research and development of antimicrobials when compared to that for noncommunicable diseases like obesity and cardiovascular diseases and the lack of veterinary-specific drugs due to the huge human market are also other causes of concern which put emphasis on the judicious use of available drugs. The health and economic consequences of AMR would be heavier when many infections cannot be treated like in the pre-antibiotic era, if AMR control strategy is not set in place. Therefore, understanding of AMU pattern in dairy animals and consequent prevalence of the veterinary drug residues in milk and other livestock products is a very important prerequisite to control AMR problems. Without detailed information of current AMU in livestock, future strategies to restrict the antimicrobial usage are impossible (Doane & Sarenbo, 2014). The global action plan against AMR also emphasized a multisectoral approach including the understanding of AMU and AMR development in food-producing animals.

2 Indications for AMU and Residue Violation in Animal Production Systems

In dairy animals, among the various reasons for AMU, mastitis, respiratory diseases, infectious foot problems, uterine infections, and parasitism are important driving forces. Among the various groups of antibiotics, β-lactams (penicillins and cephalosporins), aminoglycosides, macrolides and lincosamides, sulfonamides and

trimethoprims, and tetracyclines are mostly used in veterinary medicines (Grave et al., 1999). About 93% of antibiotic residue violations were associated with mastitis treatments, with 30% due to dry cow therapy (DCT) in Michigan dairy farms (Mellenburger, 1998). The majority of antibiotic residue violations was due to intramammary infusions, DCT, and intrauterine administration in UK farms, particularly in herds with more extra label use of antibiotics (McEwen et al., 1991). Herds with separate milking machine for treated animals and practices of following increased withdrawal period during a higher dosage administration were associated with a low residue violation, while accidental transfer of milk collected from treated cows to bulk tanks and prolonged excretion or persistence of drug even after withdrawal period are the most common reasons for residue violation (Tan et al., 2007, 2009). Among the management practices to avoid drug residues, withholding of milk from treated animals for a certain period is an important practice, and it often varies with the type of drugs, dosage, and route of administration. However, it is not strictly followed particularly in low-income countries including India.

The occurrence of antimicrobial residues in food of livestock origin is not only an indicative of AMU but also a predictor of potential threats. In general, residue violations in the USA, the EU, and certain other animal husbandry developed countries are very less due to regular monitoring through national-level programs. For example, the National Milk Drug Residue Data Base (NMDRD) being coordinated by the Food and Drug Administration (FDA) is regularly monitoring and reporting the extent of animal drug residue violation in milk in the USA. When the surveillance was initiated in 1996, it was observed at 6% positive, and it was reduced to 1% by the end of 2002, mostly due to the usage of penicillin and its combinations, followed by tetracyclines and aminoglycosides (Hall et al., 2003). Similarly, the EU reported very less incidence of violation (0.2%) in milk due to antibacterial, anthelmintic, and nonsteroidal anti-inflammatory drugs (NSAIDs), organochlorine compounds, and other chemicals (EFSA, 2015).

It is reported that 19–22% of the raw milk samples were positive with β-lactams and tetracycline residue in Palestine (Al Zuheir, 2012). Mangsi et al. (2014) found that 50% of the marketed milk samples were positive with β-lactam and tetracycline group of antibiotics in Pakistan. More violation of quinolone and penicillin residues was seen in Korea (Kim et al., 2013). The occurrence of tetracycline residues was lesser in milk of Brazilian dairy animals (Prado et al., 2015). The rate of violation due to antibacterial, anthelmintic, and nonsteroidal anti-inflammatory drugs was far less in Lithuania (Serniene et al., 2013). Zheng et al. (2013) found 0.5%, 47%, and 20% positive for βlactams, quinolones, and sulfonamides, respectively, in China. However, only one (0.5%) sample contaminated with βlactams was found to be above the maximum residue limits (MRLs), while sulfonamides and quinolones were found to be below MRLs. Collectively, the available studies indicated the difference in residue prevalence rate between the countries, farming systems, types of antibiotic class, sampling methods, sample size, and methods of screening.

Although, sporadic studies indicated veterinary drug residue violation in milk and milk products in India, the results are based on a lesser sampling size with a small number of targeted group of antibiotics. Sudershan and Bhat (1995) indicated more

usage of oxytetracycline in Hyderabad (India) and found that 73% of the milk samples from private dairies and 9% from market milk vendors had oxytetracycline levels above permissible limits. Patil et al. (2003) found 6% of the pasteurized liquid milk samples had antibiotic residues in the northern part of India. Raghu (2007) using charm detection kits found more of β-lactam residues in raw and processed milk. Bhavadasan & Grover (2002) reported 11% antibiotic residue prevalence in milk samples due to β-lactams and tetracycline group of antibiotics in southern India and also found that milk from the organized farms had more violation than unorganized farms. Chowdhury et al. (2015) also reported that the level of antibiotic residue violation in milk was significantly higher in commercial than local farms. Gaurav et al. (2014) found that 3 out of 133 samples exceeded the MRLs of tetracycline as per the EU and Codex Alimentarius Commission (CAC) in 5 districts of Punjab (India). We also found that only 10% of the samples exceeded the MRL of β-lactams and tetracycline antibiotics as per CAC in southern India (Raosaheb, 2016).

3 Pattern of AMU and Its Influencing Factors in Livestock and Poultry

Monitoring AMU is a basic requirement for AMR surveillance, and several studies correlated the AMU data with AMR development and found a direct relationship between them. Surveillance of AMU along with AMR studies at global and local levels is an important insight to understand AMR pattern (Masterton, 2008). However, the OIE reported that many member countries do not have the perfect and relevant law for the import, manufacturing, distribution, and use of veterinary drugs, including antimicrobial agents. As a result, these products are available extensively with virtually no restriction at all (OIE, 2017). However, in recent times several countries have started programs to monitor AMU. The European Medicines Agency (EMA), a nodal body for the evaluation and supervision of sales of veterinary antimicrobial agents in EU countries through the European Surveillance of Veterinary Antimicrobial Consumption (ESVAC), reported the overall sales of antimicrobials for food animals (including horses) in 26 EU countries in 2013. The studies revealed that the largest proportions of sales (mg/population corrected unit: PCU) were accounted for tetracyclines (37%), penicillin (25%), and sulfonamides and trimethoprim (11%) followed by macrolide and lincosamide groups (11%), polymyxins (6%), aminoglycosides (4%), fluoroquinolones (2%), and other drugs (6%) (ESVAC, 2013). However, the sales of critically important third- and fourth-generation cephalosporins, fluoroquinolones, and macrolides in food-producing animals were proportionately lesser (0.2%, 1.9%, and 7.4%, respectively), in total sales. The data was collected from wholesalers, marketing authorization holders (MAH), pharmacies, and prescriptions which revealed variations in prescribing patterns due to differences in the prescription behavior of the veterinarians, variation in animal species, animal-production systems, the market availability of drugs, prices, and other situations related to infectious diseases between the countries. For instances, Hungary and Bulgaria sold more quantity of tetracyclines, while

Sweden, Norway, and Iceland sold more quantity of penicillins in 2013. The countries with more population of pigs used more quantity of antibiotics (e.g., Spain 317 mg/PCU, Germany 230 mg/PCU, and France 95 mg/PCU). In contrast, Cyprus with a low PCU of food-producing animals sold the highest quantity (426 mg/PCU) among EU countries (EMA, 2015). Cuong et al. (2018) reported more AMU in chickens, followed by swine and dairy cattle. Further, tetracycline and polypeptide classes of antibiotics were highly used in swine, penicillins and cephalosporins in cattle, and tetracyclines and macrolides in poultry. When individual antibiotic was considered, penicillin was the most commonly used antibiotic in pig and cattle, while doxycycline was highly used in poultry.

A survey on the usage of feed additives in poultry farms of Tamil Nadu (India) with special reference to antibiotics revealed that chlortetracycline or oxytetracycline alone or combined supplementation of tylosin with any one of the antibiotics among oxytetracycline, chlortetracycline, bacitracin, and lincomycin was most commonly used as feed additive in broilers. Combined administration of any one of the anti-mycoplasmal drugs (tiamulin, tylosin, tylvalosin, and tilmicosin) and antibacterial agents (oxytetracycline, chlortetracycline, and bacitracin) through feed is a common practice in layer farms. Any one of the fluoroquinolone antibiotics like enrofloxacin, ciprofloxacin, and levofloxacin or other antibiotics such as sulfonamides/trimethoprim combinations, neomycin, and oxytetracycline were frequently administered via drinking water in layer farms (Kavitha, 2021).

The observation of a small-holder dairy production system in Peru revealed that >83% of the affected animals were treated with antibiotics, mostly with oxytetracycline, penicillin, and trimethoprim-sulfamethoxazole antibiotics and antiparasitic drugs (Redding et al., 2014). The study suggested for improvement in farm management practices and prescribed practices for improving animal health and the judicious use of antibiotics. They also suggested incentivizing farmers to withhold antibiotic residue-contaminated milk. Zwald et al. (2004) studied the antibiotic usage based on a farmer's recall of the previous 60 days and found that 85% of farmers treated at least 10% milch cows. Ceftiofur was the most commonly used antibiotic, and 80% of conventional herds used antibiotics to treat mastitis. Similarly, several researchers reported the AMU in dairy animals in the USA and other countries (Sawant et al., 2005; Pol & Ruegg, 2007). Mastitis is the most common reason for antibiotic usage, and about 80% of all antimicrobial drugs were used for the treatment or prevention of mastitis in Wisconsin dairy farms (Pol & Ruegg, 2007). In 2007, about 16% of the approximately nine million cows in the USA were treated for mastitis, which is equal to nearly 1.5 million mastitis cases per annum (USDA, 2008). In general udder health management is carried by preventive (mostly by intramammary DCT) or therapeutic approach (either by local intramammary or systemic therapy). Report indicates that nearly 27% of all intramammary antibiotics were used for clinical mastitis therapy, whereas 73% were used on DCT (Kromker & Leimbach, 2017). A survey on AMU and treatment practices in 809 dairy farms in California, New York, Pennsylvania, Virginia, and Wisconsin revealed that 60% farms had written treatment records and 39% farms conducted on-farm screening tests in these states (Wilson et al., 1998). About 52% of the producers were familiar

with milk quality assurance program and recognized the treated cows. Doane & Sarenbo (2014) reported about 493 kg of AMU in US cattle farm, of which ionophores (monensin and lasalocids) were used predominantly (76%) than penicillin (16%), lincosamides (3%), aminoglycosides (1.6%), and sulfonamides (1.6%). They use a minimum quantity (<1%) of cephalosporin, macrolides, amphenicols, and fluoroquinolones. Most of the antibiotics (excluding ionophores) were administered to milking cows (74 kg), followed by calves (25 kg) and heifers (19 kg). The most common indications were mastitis and bovine respiratory diseases, while general infections, hoof disorders, metritis, ketosis, and calf diarrhea were the other common disorders. Drugs were administered mostly through oral (78%) followed by injectable (11%), intramammary (10%), and topical (1%) routes. Ekakoro et al. (2018) explored the AMU among Tennessee (USA) dairy cattle producers using focus group discussion and survey questionnaires. They observed the presence of disease symptoms, and to ensure the animal welfare, on-farm pathogen surveillance through bacterial culture and sensitivity tests; economic value and lactation stage of animals; veterinarian recommendation; producer's personal experience and their knowledge-based decision-making ability; drug attributes, viz., drug efficacy, cost, and withdrawal period; and the Veterinary Feed Directive were the most common drivers for AMU. They perceived that good animal husbandry practices like udder health management, clean milking practices, vaccinations, usage of immunomodulatory products, and early disease diagnosis using appropriate technology were considered alternatives to AMU. Most of the farmers were moderately concerned about AMR, and they trusted the veterinarian as a source of information for prudent AMU.

In Canada, intramammary administration of antimicrobials accounted for 35% of total AMU in dairy farms (Saini et al., 2012). Further, the study indicated that cephalosporins (especially third-generation ceftiofur), penicillins and its combinations with other antibiotics, tetracyclines, trimethoprim with sulfonamides, and lincosamides were the most commonly used drugs. They observed that herd-level milk production, herd size, and geographic region were significantly associated with variation in AMU. Similarly, about 68% of antibiotics were used for udder health purposes (DCT and treatment of clinical mastitis) in the Netherlands in 2005–2012, where the use of third- and fourth-generation cephalosporins and fluoroquinolones (i.e., third-choice drugs) decreased from 18% of total usage in 2005 to 1% in 2012 (Kuipers et al., 2016). Restricted use of third- and fourth-generation cephalosporins and fluoroquinolones correspondingly increased the use of penicillin and some of the broad-spectrum antibiotics such as trimethoprim and sulfadoxine combinations. These third-choice drugs were banned in Australia and Denmark and restricted in New Zealand (McDougall, 2012; Katholm, 2014). In Denmark, the prescription of antibiotics by veterinarians reduced by about 14% during the period from 2013 to 2018, where pig farming consumes a significant amount of antimicrobials (DANMAP, 2018). Stevens et al. (2016a) reported that fourth-generation cephalosporins were most frequently administered than penicillins and third-generation cephalosporins in Belgian dairy cattle. On the contrary, cephalosporins were the most frequently administered first-choice antibiotic for clinical mastitis treatment in

Germany (Tenhagen et al., 2006). However, for all clinical conditions, tetracyclines were the most frequently used antibiotic followed by trimethoprim/sulfonamide and β-lactam groups in German cattle (Merle et al., 2012). Different dosage regimens of individual drugs are basic reasons for variation in percentages of the consumption of different antibiotic classes in kilogram basis. Based on the number of daily doses per animal year (DDay) metrics, they noted that pigs were more frequently treated than cattle, of which young animals (calves, piglets, and fattening pigs) were treated more frequently than adult animals. As per DDay, β-lactams (38%), aminoglycosides (15%), fluoroquinolones (8%), and cephalosporins (27%) were most commonly used than sulfonamides/trimethoprim (7%). Parenteral and intramammary routes of administration were common in dairy cattle, while oral route was common in piglets, fattening pigs, and calves. Gentamicin and streptomycin were the most frequently used antibiotics as revealed through a questionnaire-based survey in Lebanon; however, their residual levels were below the MRLs of 200 μg/L as set by the FAO/WHO (Zeina & Fawwak, 2013).

Despite the understanding of the relationship between AMU and AMR development, information available is scant regarding the level of antibiotic use in Indian dairy animals. Recently, Van Boeckel et al. (2015) reported that India is the fourth largest consumer of antimicrobials in food animal sector (3%) after China (23%), the USA (13%), and Brazil (9%) in 2010, which is expected to increase to about 4% in India by 2030. This is an indirect estimation based on population density of livestock and has many limitations. Manimaran et al. (2014) estimated the pattern of antibiotic usage for clinical mastitis in organized dairy farms in India and found that enrofloxacin, ampicillin with cloxacillin, gentamicin, and ceftriaxone drugs were most commonly used against clinical mastitis. Studies on the antibiotic use pattern in organized dairy farms and by field veterinarians in southern India by Raosaheb et al. (2020) revealed that mastitis and other udder health-related problems were the most common (34%) followed by gastrointestinal tract (GIT) infections (20%) and postpartum uterine infections (PUI: 20%). Overall, penicillins and its combinations (40%) and tetracycline (33%) group of antibiotics were mostly used for treatment of the above clinical conditions. About 13% of the milk samples were qualitatively positive for antibiotic residues in organized farms. Veterinarian-rated mastitis followed by PUI, respiratory disorders, and GIT problems were the most common reasons for administrating antibiotics in field conditions, based on Garrett's ranking method. Penicillins, cephalosporins, and tetracycline group of antibiotics were the most commonly prescribed for all clinical conditions. In the case of clinical mastitis, veterinarians preferred cephalosporin group followed by penicillins and its combinations. Besides, the available studies on antibiotic use in Indian dairy cattle (Grover & Bhavadasan, 2013; Unnikrishnan et al., 2005) were mostly based on indirect methods such as residue identification in milk rather than direct methods of data collection. Although periodic collection of drug use information from end user would be a more accurate way to understand the specific information (e.g., off-label use), such studies are rarely conducted in India. Similarly, most developing countries have a limited capacity for surveillance of antibiotic use in the animal husbandry system (Rushton et al., 2014).

The lack of data on the pattern of AMU and influencing factors are obstacles to design the measures to tackle the growing AMR problem. Jones et al. (2015) reported that the farmers' intention to reduce AMU was based on veterinarian's guidance, and hence the policymakers need to target veterinarians with information on the benefits and the ways to reduce AMU along with data on potential cost savings from reduced AMU, without affecting milk yield or compromising on the health of dairy animals. It is also noted that farmers had either recently reduced their AMU, or planned to do so, though they perceived that reduced AMU would be good and important to keep treatment records. Hommerich et al. (2019) estimated AMU in German dairy cows, calves, and beef cattle in 2011–2015 and found a decreased treatment frequency in dairy calves and beef cattle, while the treatment range in dairy cows was between 1.9 and 2.3 days. They identified a significant impact of time and farm size on production system, while region had no effect. Holstege et al. (2018) reported that dairy farmers who practiced immediate treatment of their sick calves using antimicrobials had a higher AMU than farmers who treated their sick calves with supportive, non-antimicrobial therapy (e.g., electrolytes, NSAIDs, etc.). Other risk factors associated with a high AMU in young calves were housing of calves on partially slatted floors, a high prevalence rate of respiratory diseases, *Salmonella* infections, and not agreeing with the statement "young calves need specific management" and different mindset of farmers in Dutch dairy farms. Gussmann et al. (2018) studied the determinants of antimicrobial treatment for managing udder health in Danish dairy cattle and found that somatic cell count was the most important health indicator for treatment on some farms, whereas other groups were treated based on production factors (milk yield) or culling status of the cows. However, these determinants varied between farms.

Zuliani et al. (2020) studied the influence of dairy farming systems on AMU in Italy and found that feeding management practices and rearing of local, dual-purpose breeds can reduce the treatment incidence and thus requirement for AMU. They suggested that reduced treatment incidence and AMU in dual-purpose breed could be due to lesser milk yield, smaller herd size, and limited concentrate ration and pasture access provision. Nyman et al. (2007) also reported the influence of breed in that Swedish Red and White breed cows in Sweden had a lower incidence rate of veterinary-treated clinical cases of mastitis than Holstein herds. Several other researchers also investigated the associations between AMU and management practices or farm performance (Stevens et al., 2016b; Hyde et al., 2017) in European countries. Stevens et al. (2016b) quantified the AMU using antimicrobial treatment incidence (ATI; number of defined daily doses animal (DDDA) used per 1,000 cow-days) metric in Flemish dairy herds. They observed a large variation of AMU between herds. Fourth-generation cephalosporins were the most commonly used drug, followed by penicillins and third-generation cephalosporins. The consumption of critically important antimicrobials (i.e., third- and fourth-generation cephalosporins and fluoroquinolones) was lower than other antimicrobials. For udder health management, they used more of systemically administered antimicrobials followed by dry-cow therapy and intramammary treatment of (sub)clinical mastitis. In herds with a low antimicrobial consumption, most of the antimicrobials

were used for dry-cow therapy, while injectable or intramammary mastitis therapies were common in high-antimicrobial-consuming herds. The incidence rate of mastitis treatment was positively correlated with ATI. Herds that practiced blanket DCT had a higher ATI than herds that used selective DCT. The ATI was observed lesser with an increasing number of primiparous cows. Hyde et al. (2017) reported that AMU via oral and foot-bath routes increased the antimicrobial consumption. They also found that the top 25% of farms contribute >50% of AMU by mass, and thus identification and targeted AMU reduction strategies in these farms may facilitate the overall reduction of AMU in British dairy farms. McDougall et al. (2017) reported that dairy veterinarians prescribed antibiotics based on diagnosis and response to previous therapy. Nonclinical factors such as withdrawal period of antibiotics and farmers' preferences also influenced the prescribing pattern where culture and antimicrobial sensitivity testing were not commonly practiced by veterinarians. Alhaji et al. (2019) assessed the knowledge and practices in AMU in lactating cows by pastoralists' and potential AMR transmission pathways from cow milk to humans in Nigeria. They found that improper AMU, non-implementation of regulatory laws, weaker economics, and a low education level and knowledge significantly influenced the antimicrobial misuse in lactating cows. Tetracycline, penicillin, streptomycin, and sulfonamide were the frequently used antimicrobials, and raw milk and milk product consumption, direct contact with contaminated udder, and discarded milk were the recognized risk pathways for AMR transmission from cow milk. Chauhan et al. (2018) explored the drivers of irrational usage of veterinary antibiotics in peri-urban India (Ludhiana, Guwahati, and Bangalore) and identified the following as possible drivers: the low level of knowledge about antibiotics among dairy farmers, active informal service providers like para-vets, animal husbandry assistants, and inseminators; direct marketing of drugs to farmers, including to the abovementioned unauthorized prescribers or users; and easy availability of antibiotics even without proper prescriptions. Mutua et al. (2020) reported that animal disease surveillance and support delivery system are less, and over-the-counter availability of antibiotics to farmers is common in India. Antibiotics were mostly used for mastitis management, but farmers rarely observed withdrawal periods, and thus there is antibiotic residue violation in milk. They also reported less awareness on AMR and a lack of antimicrobial stewardship programs in the Indian livestock sector. Altogether it indicated that the pattern of AMU and their influencing factors vary between countries and production systems, and thus it is very important to understand those factors for better implementation of policies to regulate the AMU in dairy animals and other livestock species.

4 Methods and Metrics Used for AMU Data Collection and Quantification in Veterinary Medicine

Various methods have been explored in veterinary medicines to collect AMU data including usage of mailed questionnaires, surveillance of on-farm treatment records, sales records from pharmaceuticals and pharmacies, residue levels in food of animal

origin, and collection of discarded drug packets in dairy farms (Redding, 2014), and many of these methods are not practiced in developing countries including India. The lack of consensus on the type of data collection and its recording system are the major limitations for AMU-related data collection, and thus harmonization of units and methods of AMU data collection from different sources has been a long goal in AMR research area (Ferreira, 2017). It is suggested that accurate data on AMU, ideally in a digital format, is of paramount importance. The lack of harmonized technical methods or metrics to collect AMU, the insufficient incentives (e.g., tax incentive) to motivate farm producers to report their AMU, and the lack of user-friendly technologies and electronic devices are the major limitations for collecting AMU data. Ferreira (2017) also suggested that the development and adoption of the globally standardized units such as ESVAC-recommended metrics, rewarding the animal producers for less AMU, and the development of suitable app, to which farmers, veterinarians, or pharmacists could orally report the AMU, are the solutions to overcome the current challenges. Implementation of electronic veterinary prescriptions and awareness campaigns through public private partnerships (PPP) mode are also suggested to control AMR problems. The advantages of having digitized data on AMU include:

(i) Species-level differentiation of AMU, which is not currently available for many countries as the same antibiotics in the same commercial name are licensed to be used in multiple species (Postma et al., 2015). Since species-level quantification of AMU based on pharmacy or pharmaceutical sales data is not possible (Bondt et al., 2013), having digital data is critical to understand AMR at species level and for consequent implementation of risk management protocol.
(ii) Based on digitized data, it is also possible to quantify good practices associated with reduced AMU either at the herd level, regional level, or national level, without compromising on the animal productivity (Collineau et al., 2017), and thus the same can be promoted through evidence-based policy interventions (Speksnijder et al., 2015).
(iii) Digital data is also useful for the identification of a temporal association between AMU and AMR development, when use of an antibiotic is terminated, either on a voluntary basis or on legal ban (Aarestrup, 2015). It is also useful for the evaluation of the impact of specific policies related to targeted reduction of AMU.

The animal daily dose (ADD), defined daily dose (DDD), total mg, mg/PCU, mg/kg, treatment frequency, and therapy index are some of the technical units currently used to measure AMU in EU countries (Ferreira & Staerk, 2017). Mills et al. (2018) reported that available metrics for quantification of AMU are somewhat different in interpretations. To facilitate the widespread use of metrics, the method should be explicable and relevant to the veterinarians and farmers who are prescribing and using antimicrobials. Clarity about the number, weight and physiological state of animals, and dose rates and duration of treatment should also be considered during estimation of AMU. The description of various metrics for estimation of

Table 1 Different metrics used for estimation of AMU and their advantages and disadvantages. (Source: Mills et al., 2018)

Metrics used	Data requirements for calculation	Advantages	Disadvantages
Total mgs	Total mg of each active drugs used	Simple method	• Do not consider the variations in animal weight (wt.) and numbers • Do not consider dose rates and duration of different antibiotics
Total mg/kg (or) mg/PCU of 425 kg	Total mg of each active ingredients used in a given population	Simple method and consider animal wt. and numbers	• Animal wt. varies with several factors (breed, age, etc.) • This method also ignores the differences in dose rates and duration of treatment of various antibiotics
Daily dose metrics (e.g., DDDvet)	It is calculated based on total mg of each active ingredients used as per daily dose rate in given (risk) population	• It considers animal wt., numbers, dose rate, and duration of different antibiotics • EU-recommended method • Country-specific dosage regimen and animal wt. may improve accuracy	• Complicated metric • Units such as dose rate and animal wt. vary with countries • Not possible for drugs that lack pharmacokinetics and defined dose rate data • It does not account for duration of treatment across antimicrobials
Course dose metrics (e.g., DCDvet)	It is similar to defined daily dose, but use defined course dose	• In addition to daily dose metrics, it also considers the duration of treatment for different antimicrobials • EU-recommended metric	• More complicated metric • Units vary with countries • Units may not be available for all drugs
Cow calculated courses (CCC)	CCC calculated based on course of each drugs used in 12 months period in the farm considering the young (<24 months) and adult (>24 months) stock, separately	Number of cattle, specific wt., and duration of treatment are considered for all drugs	• It requires information on the number of both young and adult stock • Units may vary with countries

AMU including data required, calculation methods, and advantages and disadvantages for each method used in UK dairy industry are given in Table 1. Cuong et al. (2018) reported that 67% of AMU-related studies in animal production systems reported quantitatively, with "daily doses per animal per administration" being the most common metric.

5 Global Strategies for Regulation of AMU

The OIE (2001) reported a comprehensive strategy to manage AMR arising from the agricultural and veterinary AMU (Fig. 1). They reported animals to be sampled (e.g., potential livestock species that is expected to cause AMR using AMU data), type of sampling (e.g., contaminated sample or sampling at different processing chain), sampling strategies (e.g., active or passive surveillance through simple random, random systematic, stratified random collection, or purposive sampling with optimum sample size), bacteria to be tested (e.g., species-wise animal, zoonotic, and indicator pathogens), and important antimicrobials that may be included in AMR surveillance program.

Several countries have taken measures to reduce AMU in food animal production, and recently approved regulations on veterinary medicines and medicated feed in EU member states are an evidence for such action plan. An outline of new regulations which is expected to come into force in the European Union from January 2022 is given below:

- Ban on the preventive use of antimicrobials in animals as well as in medicated feeds
- Restricted use of antimicrobials for metaphylaxis purpose
- Reinforced ban on the use of antimicrobial growth promoters (AGPs) to increase yield
- Reservation of certain antimicrobials for human use only
- Compulsory collection of data on the sales and AMU in food production system
- Ban on the use of AGPs and restricted use of antimicrobials of human importance on imported animals and products

The ban of all AGPs in Sweden in 1986 was an eye-opening policy to global agencies to reduce AMU in food-producing animals. The withdrawal of specific AGP (e.g., avoparcin) in 1997 followed by the complete ban of all AGPs used in animals by the WHO in 2006 forced a reduction of AMU in food-producing animals (Speksnijder et al., 2015). Upon perceiving potential threats of AMU in food

1. Risk assessment for the potential public health impact of AMR bacteria originated from animals
2. Prudent and responsible AMU in animal production system
3. Monitoring the quantities of antimicrobials used in animal husbandry
4. Standardization and harmonization of laboratory analytical techniques used for the detection and quantification of AMR
5. Harmonization of national AMR monitoring and surveillance programs in animals and food of animal origin

Fig. 1 OIE strategy to reduce AMU in food animals

Table 2 Global AMR surveillance programs. (Source: Walia et al., 2019)

Program and country	Actions	References
DANMAP in Denmark	It is an integrated program to understand the relationship between AMU and development of AMR bacteria in animals and humans	DANMAP, 2018
NARMS in USA	• It is a joint program between local public health departments, universities, FDA, CDC, and USDA • It tracks the changes in the antimicrobial susceptibility of enteric bacteria in humans, animals and its products • Providing information about emerging AMR and the impact of AMR interventions program • NARMS data are extensively used for making regulatory guidelines for AMU by FDA	NARMS, 1996
JVARM in Japan	• Monitor AMU and the development of AMR bacteria in food of animal origin • Identification of the efficacy of antimicrobials in food-producing animals and promote those antimicrobials to reduce public health problems	JVARM, 1999
APVMA in Australia	AMU for veterinary use in Australia in 2005–2010	APVMA, 2014
SWEDRES/SVARM in Sweden	Consumption (including sales) of antibiotics and occurrence of AMR (including zoonotic pathogens) in Sweden	SWEDRES/ SVARM, 2018
NORM-VET in Norway	• Monitoring program for AMR in the veterinary and food animal system. • NORM-VET data provide basis for understanding the relationship between the AMU and AMR. • This program also essential for setting policies, risk assessment, and evaluating interventions.	NORM-VET, 2000
AURES in Austria	Comprehensive data collection and analyses from the human, veterinary, and phytosanitary sectors on AMU and AMR	Strauss et al., 2007
RESAPATH in France	• RESAPATH is a surveillance network for AMR in pathogenic bacteria of food-producing animals. • It is voluntary-based data from network of laboratories.	RESAPATH, 2001
GERM-VET in Germany	AMU and spread of AMR from food of animal origin	GERMVET, 2001
NethMap/MARAN in the Netherlands	Monitoring of Antimicrobial Resistance and Antibiotic Usage in Animals (MARAN) in the Netherlands	NethMap, 2019
FINRES-VET in Finland	• Monitors the antibiotic susceptibility of zoonotic bacteria, animal pathogens, and indicator (i.e., normal gut) bacteria • Monitors AMR, AMU, and feed additive use	FINRES-VET, 2002
ITAVARM in Italy	Report on AMR of zoonotic bacteria and commensal, particularly in poultry	Battisti et al., 2003
CIPARS in Canada	• Monitor the trends in AMU and its relationship with development of AMR in selected bacteria from	CIPARS, 2006

(continued)

Table 2 (continued)

Program and country	Actions	References
	humans and animals • Setting evidence-based antimicrobial stewardship policies and programs in humans and agriculture • Control the spread of AMR bacteria between animals and humans via food chain in Canada	
UK-VARSS in the UK	AMR of veterinary and zoonotic pathogens	Borriello et al., 2014, 2015
ARCH-VET in Switzerland	Monitoring sales of veterinary antimicrobials and resistance rates	ARCH-VET, 2016
VAV in Spain	Spanish Veterinary Antimicrobial Resistance Surveillance Network	Porrero et al., 2006

animals, several countries established monitoring systems for AMU in food animals and occurrence of AMR in bacteria in food-producing animals and its products (Table 2). Most of the programs were implemented by EU member countries through EU funding, industry funding, and direct national support. Though some harmonization was observed on AMR-related information among EU-funded monitoring programs, other national programs applied in livestock had heterogeneous sampling, testing, and reporting methods, and most reports are not publicly available or are written in the local language (Schrijver et al., 2018). New regulations on veterinary drugs and medicated feeds resulted in substantial alteration in antimicrobial prescription or AMU in Europe and in other countries. For example, Speksnijder et al. (2015) reported that the total AMU in farm animals was reduced about 56% in the Netherlands in 2007–2012. A 35% reduction of dispensed antibiotics was reported in the animal health sector in Germany between 2014 and 2015 (Kromker & Leimbach, 2017).

Although sporadic AMR surveillance programs were initiated in India during the past period, most of the schemes focused on the human medicine sector. Even though the recently initiated Indian National Action Plan on AMR emphasized on "one health" approach, there exists no such coordination in the collection of data from the human and animal health sectors on the ground level. Several isolated studies indicated the presence of AMR in animal production systems, but there is a limited national-level integrated AMR surveillance program implemented in animals and food of animal origin. The lack of consideration about AMU is another weakness of the existing AMR surveillance systems in India. Recommendations of various organizations to address the AMU and AMR in the food animal sector in India are presented in Table 3. However, no stringent regulatory framework has been implemented in India to limit the AMU in livestock and food animals. Walia et al. (2019) reported that the lack of a uniform policy; lack of standardized epidemiological studies to collect reliable, quality data in livestock sector; lack of veterinary surveillance of AMR and AMU; and lack of awareness among farmers and veterinary professionals were the major gaps of AMR studies.

Table 3 Recommendations by Indian organizations to address the AMU and AMR in livestock and poultry sector. (Source: Walia et al., 2019)

Organizations	Recommendations	References
Second amendment of the Drugs and Cosmetics Rules (2006)	About 536 drugs classified under Schedule H (i.e., sold only based on the prescription)	REACT Group, 2018
Poultry feed specifications-BIS 2007	Antibiotics with systemic action (e.g., chloramphenicol, doxycycline, tetracycline, nitrofurazone, and furazolidone) should not be used as AGP and phasing out of gut-acting antimicrobials in 5 years	Walia et al., 2019
National Policy for Containment of AMR – MoHFW	• Strengthening of regulatory provision for AMU in human, veterinary, and industry • Promote prudent use of antibiotics via awareness, education, and regulatory policies • Strengthening of diagnostics for AMR monitoring	National Policy for containment of AMR India, 2011
National Programme on Containment of AMR under the 12th 5-year plan (2012–2017)	• AMR surveillance in different geographical regions • Strengthening national IPC guidelines • Training and capacity building in the area of AMR • Promote prudent use of antibiotics • Establishment of national repository of bacterial strains	NPCAR, 2012
Directorate General of Health Services	Introduced a sub-rule for labeling the withdrawal period of antibiotics intended for use in food-producing animals. If this period is not validated, then recommended period of 7 days for egg or milk, 28 days for poultry meat, and 500 days for fish meat were advised	Directorate General of Health Services, 2013
Advisory on use of antibiotics in food-producing animals issued by DAHDF to States	• Requested states to review the use of AGP in food-producing animals • AMU based on veterinarian's prescription or supervision • Promotion of alternatives to antibiotics like probiotics, phytobiotics, etc. • Use of licensed drugs by registered users through registered distributor of veterinary medicine • Establishment of tracking system for antibiotics from manufacturers to users, by state drug controller	Department of Animal Husbandry, Dairying and Fisheries (DAHDF), 2014

(continued)

Table 3 (continued)

Organizations	Recommendations	References
FSSAI, 2015	• Judicious AMU and ban of AGP • Avoid the inclusion of meat meal and blood meal in commercial feed formulations of meat-producing animals and poultry • Separate slaughterhouse for poultry and livestock species • Strict ban on AGP in poultry and regulate the use of only permitted antibiotics	FSSAI, 2015
CDDEP (Center for Disease Dynamics, Economics and Policy) 2016	• Tracking the AMU, AMR, and residue prevalence through a national-level surveillance and monitoring programs in animal production system • Incentives to encourage prudent use of antibiotic in animals • Educate farmers, veterinarians, and consumers about AMR • Discontinue the sub-therapeutic use of antibiotics in animals in phased manner	CDDEP, 2016
2017: National Action Plan on AMR (NAP-AMR) 2017–2022	• Improving awareness about AMR • Strengthening knowledge through evidence-based surveillance program • Reducing the infection rate through efficient IPC measures • Optimizing the AMU in humans and animals • More investments for AMR programs, research, and innovations • Increasing India's commitment on AMR through international collaborations and national-level network projects	NAP-AMR, 2017
2017: ICMR Action plan for AMU in food-producing animals	• Guidelines on prophylactic, therapeutic, and metaphylactic use of antimicrobials by DAHDF • Ban on use of premix and loose antibiotic powder formulation by DAHAD and Drug Controller General of India (DCGI) • Improve awareness and education of farmers and veterinarians, by DAHDF and ICAR • Proper labeling of medicines by pharmaceuticals by DAHDF and DCGI • Regulation of WHO-listed human importance AMU in food animals by DCGI • Fixing MRL for antibiotic residues by DAHDF and ICAR	Walia et al., 2019

6 Conclusions

Understanding the AMU in various livestock sectors and their influencing factors is an important prerequisite to suggest a targeted action plan and regulate the antimicrobial usage. Global studies indicated that mastitis and other udder health management issues are the most important reasons for AMU in dairy production systems, and thus mastitis control programs are inevitable to reduce the AMU. Besides, prudent AMU in animals through improved biosecurity and antibiotic sensitivity testing were shown as important tools to reduce the AMR problems. Harmonized, uniform, and simple methodology to estimate the AMU in food animals is also the need of the hour. Under the changing dairy production conditions from small-holder, less intensive to highly intensive farming system, identification of influencing factors for AMU and suitable metrics for the above systems are important to make evidence-based policy decision. Since the AMR bacteria is expected to cause more damage in economically underdeveloped and developing countries, it is high time to start specific measures to control AMR. Reduction of critically important antibacterial use should be the immediate goal with a long-term aim for the overall reduction of AMU. Replacements of antimicrobials with alternative treatment like prebiotics, probiotics bacteriophages, phytochemicals, etc. are some of the long-term strategies to reduce AMU without compromising the health and welfare of the animals. Rethinking of animal husbandry practices through continuous education and awareness of AMR and by giving more emphasis on the prevention and control of diseases using vaccination or genetic selection rather treating diseased animals is also an important measure to reduce AMU in animal production system.

References

Aarestrup, F. M. (2015). The livestock reservoir for antimicrobial resistance: A personal view on changing patterns of risks, effects of interventions and the way forward. *Philosophical Transactions of the Royal Society B: Biological Sciences, 370*(1670), 20140085.

Al Zuheir, I. M. (2012). Detection of β-lactams and tetracyclines antimicrobial residues in raw dairy milk for human consumption in Palestine. *Walailak Journal of Science and Technology (WJST), 9*(3), 277–279.

Alhaji, N. B., Aliyu, M. B., Ghali-Mohammed, I., & Odetokun, I. A. (2019). Survey on antimicrobial usage in local dairy cows in north-Central Nigeria: Drivers for misuse and public health threats. *PLoS One, 14*(12), 224949.

APVMA (Australian Pesticides and Veterinary Medicines Authority). (2014). Quantity of antimicrobial products sold for veterinary use in Australia. July 2005 to June 2010. ISSN: 2200–3894, ISBN: 978–1–922188-56-4 (electronic) (Viewed 31 March 2021). Available from: https://apvma.gov.au/

Battisti, A., Franco, A., & Busani, L. (2003). Italian Veterinary Antimicrobial Resistance Monitoring (ITAVARM) first report. *IstitutoZooprofilatticoSperimentale Lazio e Toscana, National Reference Center for Antimicrobial Resistance, Italy. Rome, Italy*, 27–31.

Bhavadasan, M. K., & Grover, C. R. (2002). Microbial inhibitory substances (antibiotic residues) in cow and buffalo milk (AP-ICAR Funded project).

Bondt, N., Jensen, V. F., Puister-Jansen, L. F., & van Geijlswijk, I. M. (2013). Comparing antimicrobial exposure based on sales data. *Preventive Veterinary Medicine, 108*(1), 10–20.

Borriello, S.P., Broadfoot, F., Brown, S., Grace, K., Harris, C., Healey, K., & Reeves, H. (2015). UK veterinary antibiotic resistance and sales surveillance report (UK-VARSS). (Viewed 31 March 2021). Available from: https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/582341/1051728-v53-UK-VARSS_2015.pdf.

Borriello, S.P., Grace, K., Harris, C., Reeves, H., Healey, K., & Coyne, L. (2014). UK veterinary antibiotic resistance and sales surveillance report (UK-VARSS). (Viewed 31 March 2021). Available from: https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/477788/Optimisedversion_VARSS_Report_2014_Sales_Resistance_pdf.

Broome, C., Powell, B., & Limsowtin, Y. (2002). Starter cultures: Specific properties. In *Encyclopedia of dairy sciences* (Vol. 1, pp. 269–275). Academic Press.

CDDEP (Centre for Disease Dynamics, Economics and Policy). (2016). Antibiotic use and resistance in food animals' current policy and recommendations. 1–58. (Viewed 31 March 2021).

Chauhan, A. S., George, M. S., Chatterjee, P., Lindahl, J., Grace, D., & Kakkar, M. (2018). The social biography of antibiotic use in smallholder dairy farms in India. *Antimicrobial Resistance and Infection Control, 7*(1), 1–13.

Chowdhury, S., Hassan, M. M., Alam, M., Sattar, S., Bari, M. S., Saifuddin, A. K. M., & Hoque, M. A. (2015). Antibiotic residues in milk and eggs of commercial and local farms at Chittagong, Bangladesh. *Veterinary World, 8*(4), 467–471.

CIPARS (Canadian Integrated Program for Antimicrobial Resistance Surveillance). (2006). (Viewed 31 March 2021). Available from: https://www.canada.ca/en/publichealth/services/surveillance/canadian-integrated-program-antimicrobial resistance-surveillance-cipars.html.

Collineau, L., Rojo-Gimeno, C., Léger, A., Backhans, A., Loesken, S., Nielsen, E. O., Postma, M., Emanuelson, U., Grosse Beilage, E., Sjölund, M., & Wauters, E. (2017). Herd-specific interventions to reduce antimicrobial usage in pig production without jeopardising technical and economic performance. *Preventive Veterinary Medicine, 144*, 167–178.

Cuong, N. V., Padungtod, P., Thwaites, G., & Carrique-Mas, J. J. (2018). Antimicrobial usage in animal production: Areview of the literature with a focus on low-and middle-income countries. *Antibiotics, 7*(3), 75.

DAHDF (Department of Animal Husbandry, Dairying and Fisheries). (2014). (Viewed 31 March 2021). Available from: https://www.farmer.gov.in/dadf/Advisories/Advisory_on_use_of_antibiotics_in_food_producing_animals.pdf

DANMAP. (2018). Use of antimicrobial agents and occurrence of antimicrobial resistance in bacteria from food animals, food and humans in Denmark. ISSN 1600–2032. (Viewed 31 March 2021). Available from www.danmap.org

Directorate General of Health Services. (2013). (Viewed 31 March 2021). Available from: https://www.dfda.goa.gov.in/attachments/article/133/Sub-rule%203A%20of%20Rule%2097%20of%20Drugs%20and%20Cosmetics%20Rules%201945%20regarding%20withdrawal%20period.pdf. (Viewed on 5 April, 2021).

Doane, M., & Sarenbo, S. (2014). Antibiotic usage in 2013 on a dairy CAFO in NY state, USA. *Infection Ecology and Epidemiology, 4*(1), 24259.

ECDC (European Centre for Disease Prevention and Control), EFSA (European Food Safety Authority), & EMA (European Medicines Agency). (2017). ECDC/EFSA/EMA second joint report on the integrated analysis of the consumption of antimicrobial agents and occurrence of antimicrobial resistance in bacteria from humans and food-producing animals – Joint inter agency antimicrobial consumption and resistance analysis (JIACRA) report. *EFSA Journal, 15*(7), 4872, 135.

EFSA (European Food Safety Authority). (2015). Report for 2013 on the results from the monitoring of veterinary medicinal product residues and other substances in live animals and animal products. *EFSA Supporting Publications, 12*(11), 723.

Ekakoro, J. E., Caldwell, M., Strand, E. B., & Okafor, C. C. (2018). Drivers of antimicrobial use practices among Tennessee dairy cattle producers. *Veterinary Medicine International, 2018*, 14.

EMA (European Medicines Agency). (2015). European surveillance of veterinary antimicrobial consumption, 2015. (EMA/387934/2015).

ESVAC (European Surveillance of Veterinary Antimicrobial Consumption). (2013). *Sales of veterinary antimicrobial agents in 26 EU/EEA countries in 2013: Fifth ESVAC report*. European MedicineAgency.

Ferreira, J. P. (2017). Why antibiotic use data in animals needs to be collected and how this can be facilitated. *Frontiers in Veterinary Science, 4*, 213.

Ferreira, J. P., & Staerk, K. (2017). Antimicrobial resistance and antimicrobial use animal monitoring policies in Europe: Where are we? *Journal of Public Health Policy, 38*(2), 185–202.

FINRES-VET (Finnish Veterinary Antimicrobial Resistance Monitoring and Consumption of Antimicrobial Agents). (2002). (Viewed 31 March 2021). Available from: https://www.ruokavirasto.fi/en/farmers/animal-husbandry/animal-medication/monitoring-of-antibiotic-resistance

FSSAI (Food Safety and Standards Authority of India). (2015). (Viewed 31 March 2021). Available from: https://old.fssai.gov.in/Portals/0/Pdf/Draft_Meat_Poultry_Comments.pdf

Gaurav, A., Gill, J. P. S., Aulakh, R. S., & Bedi, J. S. (2014). ELISA based monitoring and analysis of tetracycline residues in cattle milk in various districts of Punjab. *Veterinary World, 7*(1), 26–29.

GERMVET. (2001). The Federal Office for Consumer Protection and Food Safety. (Viewed 31 March 2021). Available from: http://www.bvl.bund.de

Grave, K., Greko, C., Nilsson, L., Odensvik, K., Mork, T., & Ronning, M. (1999). The usage of veterinary antibacterial drugs for mastitis in cattle in Norway and Sweden during 1990–1997. *Preventive Veterinary Medicine, 42*(1), 45–55.

Grover, C. R., & Bhavadasan, M. (2013). Antibiotic residues in milk: Apublic health concern. In *National Conference on food safety and environmental toxins* (p. 47). Centre for Science and Environment.

Gussmann, M., Graesboll, K., Toft, N., Nielsen, S. S., Farre, M., Kirkeby, C., & Halasa, T. (2018). Determinants of antimicrobial treatment for udder health in Danish dairy cattle herds. *Journal of Dairy Science, 101*(1), 505–517.

Hall, H. C., St. John, V. S., Watson, R. S., Padmore, L. J., & Paris, S. M. (2003). Antibiotic residue surveillance at the veterinary services laboratory. The Pine, St. Micheal, Barbados. Ministry of Agriculture & Rural Development, Veterinary Services Laboratory. (Viewed 31 March 2021). Available from: http://www.agriculture.gov.bb

Holstege, M. M. C., de Bont-Smolenaars, A. J. G., Santman-Berends, I. M. G. A., van der Linde-Witteveen, G. M., Van Schaik, G., Velthuis, A. G. J., & Lam, T. J. G. M. (2018). Factors associated with high antimicrobial use in young calves on Dutch dairy farms: A case-control study. *Journal of Dairy Science, 101*(10), 9259–9265.

Hommerich, K., Ruddat, I., Hartmann, M., Werner, N., Käsbohrer, A., & Kreienbrock, L. (2019). Monitoring antibiotic usage in German dairy and beef cattle farms-a longitudinal analysis. *Frontiers in Veterinary Science, 6*, 244.

Hyde, R. M., Remnant, J. G., Bradley, A. J., Breen, J. E., Hudson, C. D., Davies, P. L., Clarke, T., Critchell, Y., Hylands, M., Linton, E., & Wood, E. (2017). Quantitative analysis of antimicrobial use on British dairy farms. *Veterinary Record, 181*(25), 683–691.

Jonas, O. B., Irwin, A., Berthe, F. C. J., Le G., Francois, G., & Marquez, P. V. (2017). *Drug-resistant infections: A threat to our economic future (Vol. 2): Final report (English). HNP/agriculture global antimicrobial resistance initiative*. World Bank Group.

Jones, P. J., Marier, E. A., Tranter, R. B., Wu, G., Watson, E., & Teale, C. J. (2015). Factors affecting dairy farmers' attitudes towards antimicrobial medicine usage in cattle in England and Wales. *Preventive Veterinary Medicine, 121*(1–2), 30–40.

JVARM (Japanese Veterinary Antimicrobial Resistance Monitoring programme). (1999). (Viewed 31 March 2021). Available from: http://www.maff.go.jp/nval/tyosa_kenkyu/taiseiki/monitor/e_index.html.

Katholm, J. (2014). Info about Denmark. Study on reducing antibiotic usage in dairy sector. In T. van Werven, H. Hage, M. Bokma, R. Bergevoet, J. Bakker, & A. Kuipers (Eds.), Agro Management Tools, 2012, Publ. No. 45. More recent info updated by personal communication Feb.-May 2014 (in Dutch). *Agro management tools* (pp 14–15).

Kavitha. (2021). Evaluation of bioactive feed additive for their effect on production performance and health in broilers. Ph.D. Thesis Submitted to the Tamil Nadu Veterinary and Animal Sciences University.

Kelkar, P. S., & Li, J. T. C. (2001). Cephalosporin allergy. *New England Journal of Medicine, 345*(11), 804–809.

Kim, M., Cho, B. H., Lim, C. M., Kim, D. G., Yune, S. Y., Shin, J. Y., Bong, Y. H., Kang, J., Kim, M. A., & Son, S. W. (2013). Chemical residues and contaminants in foods of animal origin in Korea during the past decade. *Journal of Agricultural and Food Chemistry, 61*(10), 2293–2298.

Kromker, V., & Leimbach, S. (2017). Mastitis treatment-reduction in antibiotic usage in dairy cows. *Reproduction in Domestic Animals, 52*, 21–29.

Kuipers, A., Koops, W. J., & Wemmenhove, H. (2016). Antibiotic use in dairy herds in The Netherlands from 2005 to 2012. *Journal of Dairy Science, 99*(2), 1632–1648.

Mangsi, A. S., Khaskheli, M., Soomro, A. H., & Shah, M. G. (2014). Detection of antimicrobial drug residues in milk marketed at different areas of Sindh province. *IOSR Journal of Agriculture and Veterinary Science, 7*, 65–69.

Manimaran, A., Layek, S. S., Kumaresan, A., Prasad, S., Sreela, L., Boopathi, V., Kumar, K., & Venkatasubramanian, V. (2014). Estimation of antimicrobial drug usage for treatment of clinical mastitis cases in organized dairy farm. *Indian Journal of Veterinary and Animal Sciences Research, 43*(2), 140–150.

Masterton, R. (2008). The importance and future of antimicrobial surveillance studies. *Clinical Infectious Diseases, 47*(Supplement_1), 21–31.

McDougall, S. (2012). Info about New Zealand. Study on reducing antibiotic usage in dairy sector. In T. van Werven, H. Hage, M. Bokma, R. Bergevoet, J. Bakker, & A. Kuipers (Eds.), *Agro management tools,* Publ. No. 45. Agro management tools (in Dutch). (pp. 16–17).

McDougall, S., Compton, C. W. R., & Botha, N. (2017). Factors influencing antimicrobial prescribing by veterinarians and usage by dairy farmers in New Zealand. *New Zealand Veterinary Journal, 65*(2), 84–92.

McEwen, S. A., Meek, A. H., & Black, W. D. (1991). A dairy farm survey of antibiotic treatment practices, residue control methods and associations with inhibitors in milk. *Journal of Food Protection, 54*(6), 454–459.

Mellenburger, R. W. (1998). Milk antibiotic violations: 1996 and 1997 (mid-march). *Michigan Dairy Review, 3*(1), 11–14.

Merle, R., Hajek, P., Kasbohrer, A., Hegger-Gravenhorst, C., Mollenhauer, Y., Robanus, M., Ungemach, F. R., & Kreienbrock, L. (2012). Monitoring of antibiotic consumption in livestock: A German feasibility study. *Preventive Veterinary Medicine, 104*(1–2), 34–43.

Mills, H. L., Turner, A., Morgans, L., Massey, J., Schubert, H., Rees, G., Barrett, D., Dowsey, A., & Reyher, K. K. (2018). Evaluation of metrics for benchmarking antimicrobial use in the UK dairy industry. *Veterinary Record, 182*(13), 379.

Mutua, F., Sharma, G., Grace, D., Bandyopadhyay, S., & Shome, B. (2020). A review of animal health and drug use practices in India and their possible link to antimicrobial resistance. *Antimicroial Resistenceand Infection Control, 9*, 103.

NAP-AMR (National Action Plan on Antimicrobial Resistance). (2017). (Viewed 31 March 2021). Available from: http://www.searo.who.int/india/topics/antimicrobial_resistance/nap_amr.pdf.

NARMS (National Antimicrobial Resistance Monitoring System). (1996). (Viewed 31 March 2021). Available from: https://www.fda.gov/animal-veterinary/antimicrobial-resistance/national-antimicrobial-resistance-monitoring-system

National Policy for Containment of Antimicrobial Resistance India. (2011). Available from https://main.mohfw.gov.in/sites/default/files/3203490350abpolicy%20%281%29.pdf (Viewed 31 March 2021).

NethMap/MARAN. (2019). Consumption of antimicrobial agents and antimicrobial resistance among medically important bacteria in the Netherlands, MARAN (Monitoring of antimicrobial resistance and antibiotic usage in the animals in Netherlands in 2018. Available from https://www.rivm.nl/documenten/nethmap-2019. (Viewed 31 March 2021).

NORM-VET. (2000). Monitoring programme for antimicrobial resistance in the veterinary and food production sectors. (Viewed 31 March 2021). Available from: https://www.vetinst.no/en/surveillance-programmes/norm-norm-vet-report

NPCAR (National Programme on Containment of Antimicrobial Resistance). (2012). (Viewed 31 March 2021). Available from: https://www.nhp.gov.in/national-programme-on-containment-of-anti-microbial-resistance-(amr)_pg

Nyman, A. K., Ekman, T., Emanuelson, U., Gustafsson, A. H., Holtenius, K., Waller, K. P., & Sandgren, C. H. (2007). Risk factors associated with the incidence of veterinary-treated clinical mastitis in Swedish dairy herds with a high milk yield and a low prevalence of subclinical mastitis. *Preventive Veterinary Medicine, 78*(2), 142–160.

OIE (Office International des Epizooties). (2001). OIE guidelines. 2nd OIE International Conference on Antimicrobial Resistance. Use of antimicrobials and protection of public health. (Viewed 31 March 2021). Available from: http://www.anmv.afssa.fr/oiecc/conference/guidelines.htm

OIE (Office International des Epizooties). (2017). OIE annual report on antimicrobial agents intended for use in animals. (Viewed 31 March 2021). Available from: https://www.oie.int/fileadmin/Home/eng/Our_scientific_expertise/docs/pdf/AMR/Annual_Report_AMR_2.pdf

Packham, W., Broome, M. C., Limsowtin, G. K. Y., & Roginski, H. (2001). Limitations of standard antibiotic screening assays when applied to milk for cheesemaking. *Australian Journal of Dairy Technology, 56*(1), 15.

Patil, G. R., Kumar, N., & Ram, J. (2003). WHO assisted project on quality survey of milk and milk products in NDRI.

Paturkar, A. M., Waskar, V. S., Mokal, K. V., & Zende, R. Z. (2005). Antimicrobial drug residues in meat and their public health significance – A review. *The Indian Journal of Animal Sciences, 75*(9), 1103–1111.

Pol, M., & Ruegg, P. L. (2007). Treatment practices and quantification of antimicrobial drug usage in conventional and organic dairy farms in Wisconsin. *Journal of Dairy Science, 90*(1), 249–261.

Porrero, M. C., Garcia, M., Teshager, T., Cubillo, I., Rivero, E., Rigaut, D., Moreno, M. A., & Dominguez, L. (2006). *Veterinary monitoring of antimicrobial resistance in Spain, VAV 2005, 12threport, Ed. 1*. C.E.R.S.A.

Postma, M., Sjolund, M., Collineau, L., Losken, S., Stark, K. D., & Dewulf, J. (2015). Assigning defined daily doses animal: A European multi-country experience for antimicrobial products authorized for usage in pigs. *Journal of Antimicrobial Chemotherapy, 70*(1), 294–302.

Prado, C. K., Ferreira, F. D., Bando, E., & Machinski, M., Jr. (2015). Oxytetracycline, tetracycline, chlortetracycline and doxycycline in pasteurised cow's milk commercialised in Brazil. *Food Additives and Contaminants: Part B, 8*(2), 81–84.

Raghu, H. V. (2007). Performance of lab kit for semi-quantitative detection of antibiotic residues in milk and dried products. Ph.D. Thesis Submitted to the ICAR-NDRI.

Raosaheb, C. V. (2016). Surveillance of antibiotic usage in dairy animals and stability of residues in pasteurized milk. Master's Thesis Submitted to the ICAR-NDRI.

Raosaheb, C. V., Manimaran, A., Sivaram, M., & Jeyakumar, S. (2020). Antimicrobials use pattern under organized and unorganized dairy production conditions in southern India. *Indian Journal of Animal Sciences, 90*(3), 362–366.

REACT Group. (2018). Antibiotic use in food animals: India overview. (Viewed 31 March 2021). Available from: https://www.reactgroup.org/wpcontent/uploads/2018/11/Antibiotic_Use_in_Food_Animals_India_LIGHT_2018_web.pdf

Redding, L. E. (2014). Understanding the use of antibiotics on small dairy farms in rural Peru. Publicly Accessible Penn Dissertations. 1414.

Redding, L. E., Cubas-Delgado, F., Sammel, M. D., Smith, G., Galligan, D. T., Levy, M. Z., & Hennessy, S. (2014). The use of antibiotics on small dairy farms in rural Peru. *Preventive Veterinary Medicine, 113*(1), 88–95.

RESAPATH. (2001). The RESAPATH network monitors the antimicrobial resistance of pathogenic bacteria isolated from animals in France. (Viewed 31 March 2021). Available from: https://resapath.anses.fr/

Rushton, J., Ferreira, J. P., & Stärk, K. D. (2014). Antimicrobial resistance: The use of antimicrobials in the livestock sector. In *OECD food, agriculture and fisheries papers, n*o. 68. OECD Publishing.

Saini, V., McClure, J. T., Léger, D., Dufour, S., Sheldon, A. G., Scholl, D. T., & Barkema, H. W. (2012). Antimicrobial use on Canadian dairy farms. *Journal of Dairy Science, 95*(3), 1209–1221.

Sawant, A. A., Sordillo, L. M., & Jayarao, B. M. (2005). A survey on antibiotic usage in dairy herds in Pennsylvania. *Journal of Dairy Science, 88*(8), 2991–2999.

Schrijver, R., Stijntjes, M., Rodríguez-Bano, J., Tacconelli, E., Rajendran, N. B., & Voss, A. (2018). Review of antimicrobial resistance surveillance programmes in livestock and meat in EU with focus on humans. *Clinical Microbiology and Infection, 24*(6), 577–590.

Serniene, L., Stimbirys, A., & Daunoras, G. (2013). Trends in monitoring residues of pharmacologically active group B substances in products of animal origin in Lithuania from 1999 to 2008. *Food Additives and Contaminants: Part B, 6*(3), 187–195.

Speksnijder, D. C., Mevius, D. J., Bruschke, C. J. M., & Wagenaar, J. A. (2015). Reduction of veterinary antimicrobial use in The Netherlands. The Dutch success model. *Zoonoses and PublicHealth, 62*, 79–87.

Stevens, M., Piepers, S., & De Vliegher, S. (2016a). Mastitis prevention and control practices and mastitis treatment strategies associated with the consumption of (critically important) antimicrobials on dairy herds in Flanders, Belgium. *Journal of Dairy Science, 99*(4), 2896–2903.

Stevens, M., Piepers, S., Supré, K., Dewulf, J., & De Vliegher, S. (2016b). Quantification of antimicrobial consumption in adult cattle on dairy herds in Flanders, Belgium, and associations with udder health, milk quality, and production performance. *Journal of Dairy Science, 99*(3), 2118–2130.

Strauss, R., Muchl, R., Metz-Gercek, S., Sagl, M., Allerberger, F., Hrabcik, H., & Mittermayer, H. (2007). AURES-the first Austrian report on antimicrobial resistance–perspective of the human sector. *Weekly Releases (1997–2007), 12*(50), 3329.

Sudershan, R. V., & Bhat, R. V. (1995). A survey on veterinary drug use and residues in milk in Hyderabad. *Food Additives and Contaminants, 12*(5), 645–650.

Swedres-Svarm. (2018). Consumption of antibiotics and occurrence of resistance in Sweden. Solna/Uppsala ISSN1650–6332 (Viewed 31 March 2021). Available from: at www.folkhalsomyndigheten.se/publicerat-material/ or at www.sva.se/swedres-svarm/

Swiss Antibiotic Resistance Report. (2016). Federal Office of PublicHealth and FederalFoodSafety and VeterinaryOffice. Usage of antibiotics and occurrence of antibiotic resistance in bacteria from humans and animals in Switzerland. Anresischd ARCH-VET, November 2016.

Tan, X., Huang, Y. J., Jiang, Y. W., & Hu, S. H. (2007). Persistence of oxytetracycline residues in milk after the intrauterine treatment of lactating cows for endometritis. *Veterinary Record, 161*(17), 585–587.

Tan, X., Jiang, Y. W., Huang, Y. J., & Hu, S. H. (2009). Persistence of gentamicin residues in milk after the intramammary treatment of lactating cows for mastitis. *Journal of Zhejiang University Science B, 10*(4), 280–284.

Tenhagen, B. A., Köster, G., Wallmann, J., & Heuwieser, W. (2006). Prevalence of mastitis pathogens and their resistance against antimicrobial agents in dairy cows in Brandenburg, Germany. *Journal of Dairy Science, 89*(7), 2542–2551.

Unnikrishnan, V., Bhavadassan, M. K., Nath, B. S., & Ram, C. (2005). Chemical residues and contaminants in milk: A review. *The Indian Journal of Animal Sciences, 75*(5), 592–598.

USDA (United States Department of Agriculture). (2008). APHIS (Animal and Plant HealthInspectionService) VeterinaryServices (Centre for Epidemiology and Animal Health) Informationsheet. October 2008.

Van Boeckel, T. P., Brower, C., Gilbert, M., Grenfell, B. T., Levin, S. A., Robinson, T. P., Teillant, A., & Laxminarayan, R. (2015). Global trends in antimicrobial use in food animals. *Proceedings of the National Academy of Sciences, 112*(18), 5649–5654.

Walia, K., Sharma, M., Vijay, S., & Shome, B. R. (2019). Understanding policy dilemmas around antibiotic use in food animals and offering potential solutions. *The Indian Journal of Medical Research, 149*(2), 107–118.

Wilson, D. J., Sears, P. M., & Hutchinson, L. J. (1998). Dairy producer attitudes and farm practices used to reduce the likelihood of antibiotic residues in milk and dairy beef: Afive-state survey. *Large Animal Practice, 19*(5), 24.

Zeina, K., & Fawwak, S. (2013). Quantification of antibiotic residues and determination of antimicrobial resistance profiles of microorganisms isolated from bovine milk in Lebanon. *Food and Nutrition Sciences, 4*(7), 1–9.

Zheng, N., Wang, J., Han, R., Xu, X., Zhen, Y., Qu, X., Sun, P., Li, S., & Yu, Z. (2013). Occurrence of several main antibiotic residues in raw milk in 10 provinces of China. *Food Additives and Contaminants: Part B, 6*(2), 84–89.

Zuliani, A., Lora, I., Brscic, M., Rossi, A., Piasentier, E., Gottardo, F., Contiero, B., & Bovolenta, S. (2020). Do dairy farming systems differ in antimicrobial use? *Animals, 10*(1), 47.

Zwald, A. G., Ruegg, P. L., Kaneene, J. B., Warnick, L. D., Wells, S. J., Fossler, C., & Halbert, L. W. (2004). Management practices and reported antimicrobial usage on conventional and organic dairy farms. *Journal of Dairy Science, 87*(1), 191–201.

Diseases and Antimicrobial Use in Aquaculture

Jerusha Stephen, Susmita Mukherjee, Manjusha Lekshmi, and Sanath H. Kumar

Contents

Abstract

Farming of finfish and shellfish is a growing industry in the developing world, owing to the increasing global demand for food fish. The artificial rearing conditions are stressful to fish, making them susceptible to infections by opportunistic microorganisms that inhabit the farm environment. Among the limited options available to prevent and control disease outbreaks in fish farms is the

J. Stephen · M. Lekshmi · S. H. Kumar (✉)
Post-Harvest Technology, ICAR-Central Institute of Fisheries Education, Seven Bungalows, Andheri (W), Mumbai, India
e-mail: sanathkumar@cife.edu.in

S. Mukherjee
Fish Processing Technology, Faculty of Fisheries Sciences, West Bengal University of Animal and Fishery Sciences, Kolkata, India

M. P. Mothadaka et al. (eds.), *Handbook on Antimicrobial Resistance*,
https://doi.org/10.1007/978-981-19-9279-7_15

application of antibiotics. Unregulated and excessive use of antibiotics can be counterproductive, leading to the development of antibiotic resistance in bacteria in fish farms and the surrounding environments, transfer of resistance mechanisms to zoonotic bacterial pathogens, and the presence of antibiotic residues in fish. The application of antibiotics that are important in the treatment of human infections can diminish the clinical efficacy of these antibiotics when bacteria that develop resistance in the farm environment eventually spread in the community through food fish, farm workers, and the environmental contamination of farm effluents. Several countries have adopted stringent measures to reduce or end the use of antibiotics without affecting the economic prospects of fish farming, while also being able to gradually alleviate the bacterial resistance to clinically important antibiotics. With the scientific management of fish farms, use of vaccines, immune-stimulants, and biocontrol measures involving bacteriophages for pathogen control, the growing industry of aquaculture can remain viable and sustain a harmonious association with the environment.

Keywords

Antibiotics · Resistance · Aquaculture · Genes · Environment · Pathogen · Fish

1 Introduction

Fish is an integral part of human diet, with a vast portion of it contributed from the natural sources. About 88% of 177.8 million metric ton fish produced globally is consumed directly. The demand for food fish is constantly on the rise, whereas the availability of fish from the natural sources is declining. The global demand for food fish has dramatically increased, and an estimated 232 million metric tons of fish is required to meet the demand for food fish by 2030. Due to overexploitation, the wild stock of fish has drastically decreased and cannot meet the demands of rising global population. On the other hand, the production of fish by aquaculture is increasing at a rapid pace and is expected to contribute to most of dietary fish in the near future. While capture fisheries have remained nearly constant in the last two decades, the production of farmed fish has increased by over 50 times. In 2018, aquaculture produced 82 million metric tons of food fish, and the sector continues to grow rapidly, especially in developing countries of Asia, Africa, and the Latin America. The world's aquaculture production of fish is expected to reach 109 million tons in 2030 from the current 82 million tons (FAO, 2020). The farmed fish will be a major component of human diet in the near future. Farming of fish and shellfish has evolved from the traditional extensive faming system in which little or no human interference is involved to the modern semi-intensive and intensive methods in which several inputs including fish seed, artificial feed, probiotics, vaccines, etc. are involved in addition to a high stocking density of animals per unit area. The intensive system aims at maximizing production and profit. However, high-intensive rearing creates unnatural living conditions for fish and tends to disturb the fragile

balance between the host and the environment. Microorganisms, being an integral component of this biological system, can often present formidable problems as disease-causing agents when fish reared in crowded conditions are subjected to physiological stress that makes them vulnerable to disease outbreaks. Microbial diseases are a major problem affecting the aquaculture systems responsible for production and economic losses. Microorganisms cohabiting with fish in intensive aquaculture systems are opportunistic pathogens that can cause infections of varying intensities, from mild to acute, often leading to mass mortalities. Unlike in terrestrial animals, the spread of disease is rapid in aquatic systems, with little or no scope for diagnosis and implementation of treatment regimes. The emergence of new infectious agents causes considerable crop losses before they are identified and control measures are implemented. Diverse and multiple infectious agents combined with the appearance of new pathogens complicate the development of vaccines. The rapid growth of aquaculture of commercial scale has inadvertently prompted unscientific management approaches involving the application of antimicrobial chemicals including antibiotics to control pathogens. Here, we discuss the current global trends of antimicrobial use in aquaculture, its impact on the environment and human health in terms of antimicrobial resistance development, and the strategies to make aquaculture an eco-friendly and sustainable activity.

2 Aquaculture: The Global Scenario

With the increasing demand for fish, aquaculture meets almost 50% of the food fish need (Miao & Wang, 2020). Globally, the average per capita fish consumption is 20.5 kg as of 2017. The type of aquaculture practices carried out, being an intensive or extensive type of system, type of species cultured, and the technology used for aquaculture depend on various factors like agroclimatic conditions, economic status, social and spatial factors, etc. The aquaculture is dominated by culture of food fish like carps which caters to the domestic requirements. Though the quantity of carps produced is enormous, it has a feeble role in international trade. On the other hand, the shrimp culture system is designed to meet the international market demand, with *Litopenaeus vannamei* being a classic example (Miao & Wang, 2020).

3 Current Global Status

According to the United Nation's Food and Agriculture Organization (FAO), from 2004 to 2017, China's grass carp (white amur) ranked first among the farmed species, in value. In 2018, the red swamp crawfish (*Procambarus clarkii*) overtook the grass carp and became the number one commodity in value at 14.23 billion US dollars. In China, the average annual growth rate of red swamp crawfish is very high at 30.33% for over 15 years (2003–2018). In the ranking based on the aquaculture produce live weight, China and Indonesia are among the top countries in seaweed production. China's Japanese kelp and Indonesia's Eucheuma seaweed are

competing for the top rank in terms of live weight of the aquaculture produce. Norway leads in the production of Atlantic salmon. Since the seaweed tops the chart in terms of quantity, the marine environment contributes the most to aquaculture production. As expected, the freshwater environment contributed most to aquaculture in terms of value realized since seven out of the top ten species belong to this environment.

4 Types of Aquaculture

The aquaculture systems can be classified based on the culture environment into freshwater, brackishwater, and marine aquaculture. Based on the organism cultured, it could be monoculture or polyculture. The structure used for the cultured organism confinement could be a pond, pen, cage, or raceway system. The traditional aquaculture systems are developed based on socio-environmental conditions. For example, in rural areas, where agriculture and animal husbandry practices are the predominant livelihood sources, aquaculture is integrated with them as in rice-cum-fish culture, integrated aquaculture with poultry, duckery, piggery, and cattle. Waste-fed fish culture in suburban setup and trash fish-fed aquaculture in coastal regions reflect the socio-environmental conditions behind the development of such traditional aquaculture systems. Modern aquaculture practices like recirculatory aquaculture and biofloc technology aim at making intensive aquaculture eco-friendlier (Edwards, 2015).

The system of aquaculture classification into intensive and extensive aquaculture is based on the stocking density and the input intensity. In extensive systems, the stocking density is low, and inputs like feed and fertilizers are not usually provided, leading to a low yield. Usually, low-value fish and carps for domestic consumption are cultured extensively. Filter-feeder silver carp is the most cultured fish in non-fed aquaculture system. The contribution of non-fed aquaculture to the total aquaculture production was 30.5% in 2018 and is facing a declining trend compared with fed aquaculture productions (FAO, 2020). Like in any other production system, aquaculture also requires intervention at different stages to increase the system's yield. Inputs, viz., fertilizer, feed, aeration, and prophylactics, are essential to sustain a high stocking density of intensive aquaculture systems. In extensive fish culture, however, there is a lesser requirement for the addition of chemicals.

5 Chemicals Used in Aquaculture

Aquaculture practices make use of an array of chemicals for various purposes. These chemicals can be broadly categorized into water and soil quality management chemicals, disinfectants, pesticides, fertilizers, antibiotics, feed additives, and probiotics. There has been a boom in the aqua chemical industry to cater to the needs of the farmers. More than 100 companies provide about 400 types of aquaculture

chemicals in Bangladesh alone, including medicines (Alam & Rashid, 2014; Ali et al., 2016). In Mexico, nearly 106–134 different chemicals are used in shrimp aquaculture (Lyle-Fritch et al., 2006). In Bangladesh, aquaculture farms of various capacities were surveyed, and almost all the farmers used some type of chemicals in their farming practices (Ali et al., 2016). In Bangladesh, the extensive type of rice-cum-fish farming system uses chemicals like lime for water quality management, and the use of other chemicals is negligible (Ali et al., 2016). On the other hand, Mexico uses more of feed additives with negligible use of zeolite, pesticides, and malachite green, when compared to Asian countries (Lyle-Fritch et al., 2006). Although the use of chemicals in an intensive aquaculture system is inevitable, the farmers need to take into consideration their detrimental effects on the environment and, hence, adopt environmentally friendly substances (Lieke et al., 2020).

It is essential to understand the fate of the chemicals used and their bioaccumulative potentials. Use of self-degrading chemicals in food fish culture considerably reduces the threat to consumer health from bioaccumulation of such chemicals in food chain (Boyd & Massaut, 1999). However, certain chemicals like malachite green, methylene blue, and formalin are toxic to the cultured organism and the consumers as well (Lieke et al., 2020). Malachite green and its reduced form leucomalachite green are known carcinogens and mutagens (Henderson et al., 1997; Srivastava et al., 2004). Thus, the use of malachite green in aquaculture was banned in the EU from 2002 (Jennings et al., 2016). The use of manures could pose a food safety risk in terms of heavy metals and pathogens (Boyd & Massaut, 1999). Formalin is a frequently reported disinfectant in the aquaculture industry (Mishra et al., 2017; Akter et al., 2020). Formalin is toxic not only to microorganisms but also to fish and humans. Damage to fish gills, skin, liver, kidney, and spleen has been reported at higher concentrations (Ayuba et al., 2013; Andem et al., 2015; Tavares-Dias, 2021).

6 Microbial Problems in Aquaculture

6.1 Bacterial Diseases

Bacterial pathogens are among the most significant disease-causing organisms in farmed finfish and shellfish. A majority of the diseases reported from warm- and cold-water fisheries are influenced by poor water quality parameters, environmental stress, malnutrition, excess waste deposition, etc., and outbreaks can occur in nursery, nearing, or stocking ponds (Park, 2009). Some of the very common and important diseases caused by gram-negative bacteria in finfish are vibriosis (in both freshwater and seawater fish, shrimp, prawn, etc.), aeromoniasis (furunculosis), edwardsiellosis, pseudomonasis, flavobacteriosis (columnaris disease), eye disease, tail rot, fin rot, etc. (Austin, 2012). Gram-positive bacterial diseases include mycobacteriosis, streptococcosis, renibacteriosis (bacterial kidney disease), and

infections with anaerobic bacteria like *Clostridium botulinum*, enterobacterium, *Catenibacterium*, etc. Some of the very prominent bacterial diseases reported from crustaceans are vibriosis, penaeid bacterial septicemia, luminescent vibriosis, gaffkemia, bacterial shell disease, etc. (Ibrahim et al., 2020).

6.2 Viral Diseases

Virus carried by wild fish readily transmits to a large number of hosts in intensive aquaculture system, where the presence of chronic stress triggers outbreak of the viral diseases. The outbreaks are also influenced by environmental factors, host animal factors, and viral factors (Kibenge, 2019). The important DNA viruses infecting teleost fish (carp, catfish, eel, grouper, salmon, trout, etc.) belong to the families of *Adenoviridae*, *Iridoviridae*, and *Herpesviridae*, while *Reoviridae*, *Picornaviridae*, *Aquareoviridae*, *Togaviridae*, *Nodaviridae*, *Paramyxoviridae*, *Rhabdoviridae*, and *Orthomyxoviridae* are the families of RNA viruses that infect grass carp, channel catfish, cyprinids, salmon, flounder, halibut, striped bass, milkfish, ayu, grouper, and sea bass (Austin, 2012). Retroviruses belonging to the reverse transcriptase group infect Atlantic salmon, chinook salmon, and damsel fish (Lepa & Siwicki, 2011). Monodon baculovirus (MBV), baculoviral midgut gland necrosis virus (BMNV), hemocycte-infecting baculovirus (HB), type C baculovirus (TCBV), infectious hypodermal and hematopoietic necrosis virus (IHHNV), hepatopancreatic parvo-like virus (HPV), Taura syndrome virus (TSV), lymphoid parvo-like virus (LOPV), yellow head virus (YHV), lymphoid organ vacuolization virus (LOVV), and white spot syndrome virus (WSSV) are few of the important viruses that cause diseases in shrimp aquaculture (Murray, 2013).

6.3 Fungal Diseases

Fungal diseases occur in fish and shellfish as secondary manifestations to infections caused by bacteria, virus, parasites, poor water quality, environmental stress, etc. Fungi can infect eggs, larvae, or adult fish. A majority of the infective fungi are multicellular and possess hyphae for infection. Some common fungal diseases include saprolegniasis, epizootic ulcerative syndrome (EUS), branchiomycosis, ichthyophoniasis, and infections caused by *Fusarium* sp. (Yanong, 2003). *Achlya*, *Calyptralegnia*, *Leptolegnia*, *Dictyuchus*, *Pythiopsis*, and *Thraustotheca* sp. are important fungi belonging to the *Saprolegniales* that infect eggs as well as adult fish. *Ichthyophonus gasterophilus* and *Ichthyophonus hoferi* have been reported to be the major causative agents of infections in salmonids (Austin, 2012). Marine oomycetes cause disease in marine abalones and shellfish. These include *Lagenidium*, *Halocrusticida*, *Haliphthoros*, *Halioticida*, *Pythium*, and *Atkinsiella*. *Fusarium*, *Ochroconis*, *Plectosporium*, *Exophiala*, etc. are mitosporic fungal species that infect marine fish and shellfish (Hatai, 2012).

6.4 Parasitic Diseases

Parasitic diseases are one of the major constraints in aquaculture systems of tropical and subtropical countries. Both ectoparasites (in direct contact with external environment) and endoparasites (no direct contact with the external environment) are reported to cause infections in cultured fish (Bellay et al., 2015). Parasitic groups such as Protista, Myxozoa, Monogenea, Digenea, Cestoda, Nematoda, Acanthocephala, Arthropoda (copepods, isopods, branchiurans) etc. constitute the major parasitic organisms infecting aquaculture systems across the world (Paladini et al., 2017). Protistans can translocate without an intermediate host, and *Amyloodinium ocellatum* (marine dinoflagellate), *Ichthyobodo necator*, and *Chilodonella cyprini* (freshwater flagellates) are some important species of this group that attach to host epithelial cells and feed on it causing focal erosion of the cell (Hoffman, 1999). Other members of this group affecting finfish are *Ichthyophthirius multifiliis*, *Trypanosoma* spp. (blood-borne), *Trichodina* spp., etc. *Perkinsus marinus*, *Bonamia ostreae*, etc. are some protistans affecting shellfish (bivalves) (Hine & Thorne, 2000). Myxozoans alternate between vertebrates and invertebrates in their lifecycle. Examples are *Tetracapsuloides bryosalmonae* (etiological agent of proliferative kidney disease (PKD)), *Myxobolus cerebralis* (etiological agent of whirling disease (WD)), *Ceratonova* spp., *Chloromyxum* spp., and *Thelohanellus* spp. which target cyprinids (carps, goldfish, etc.) along with other species like *Anabas*, etc. (Yokoyama et al., 2012).

Dactylogyrus spp. (gill fluke), *Gyrodactylus* spp. (skin fluke), and *Lamellodiscus* spp. are important monogeneans with hyper-parasitic activity (Reed et al., 2012). Prime examples of Digenea affecting finfish include *Clonorchis sinensis*, *Opisthorchis viverrini*, and *Opisthorchis felineus* (dos Santos & Howgate, 2011). *Paragonimus* and *Metagonimus* target shellfish (mollusks and crustaceans). Helminths of other families are found in tropical countries in among 2400 families of fish, and some of them act as biological markers (Pakdee et al., 2018). Some significant examples of helminth group are *Anisakis* spp., *Diplostomum* spp., *Bothriocephalus* spp., *Haplorchis* spp., *Anguillicola* spp., *Gnathostoma* spp., and *Acanthocephalus* spp. Crustacean parasites include *Ergasilus*, *Argulus* (carp lice), and *Lernaea* which cause severe morbidity and mortality in the culture stock (Sahoo et al., 2013). These parasites cause hyperplasia in fish and trigger inflammation and erosions on the skin and gills. Table 1 summarizes the most common diseases encountered in farmed fish and shellfish.

7 Antibiotic Use in Livestock

The global use of antibiotics in agriculture exceeds that of human use (Van Boeckel et al., 2015). According to an estimate, 105,600 tons of antibiotics will be used annually in livestock by 2030, which is a 67% increase from 2010 (Van Boeckel et al., 2015). The drastic increase in the consumption of antibiotics is due to the

Table 1 Diseases of farmed fish and shellfish and their causative agents

Category	Disease	Etiological agent	Susceptible species
Bacterial diseases	Furunculosis	*Aeromonas salmonicida*	Freshwater salmon, rohu
	Columnaris	*Flavobacterium columnare*	Ayu, tilapia, carp, gold fish, channel catfish
	Eye disease	*Staphylococcus aureus*, *Aeromonas liquefaciens*	Rainbow trout, carps
	Edwardsiellosis	*Edwardsiella tarda*	Tilapia, channel catfish, mullet, carps
	Mycobacteriosis (piscine tuberculosis)	*Mycobacterium marinum* *Mycobacterium piscium*	Siamese fighting fish, bettas, piranhas, barbs
	Brown/black spot (shell disease)	*Vibrio, Aeromonas*, and *Pseudomonas* groups	*Penaeus monodon*, *P. merguiensis*, *P. indicus*
	Luminous bacterial disease	*Vibrio harveyi,* *Vibrio splendidus*	*P. monodon,* *P. merguiensis,* *P. indicus*
Viral diseases	Infectious pancreatic necrosis virus (IPNV)	Birnavirus (*Birnaviridae*)	Salmonids (rainbow trout, brook trout)
	Infectious spleen and kidney necrosis virus (ISKNV)	*Megalocytivirus* (*Iridoviridae*)	Freshwater fish
	Infectious hematopoietic necrosis (IHN)	*Novirhabdovirus* (*Rhabdoviridae*)	Salmonids (Atlantic chum, chinook, sockeye, rainbow trout)
	Viral hemorrhagic septicemia (VHS)	*Novirhabdovirus* (*Rhabdoviridae*)	Lake, brook, and rainbow trout, brown and golden trout, Atlantic salmon, pike turbot, sea bass
	White spot syndrome virus (WSSV)	*Whispovirus* (*Nimaviridae*)	Penaeid shrimps (specially *Penaeus monodon*)
	Monodon baculovirus (MBV)	*Baculoviridae*	Shrimps and freshwater prawns
	Taura syndrome virus (TSV)	Picornaviridae	Penaeid shrimps (specifically white leg shrimp)
	Yellowhead virus (YHV)	*Okavirus* (*Roniviridae*)	Penaeid shrimps and prawns, Antarctic krill, mysis shrimps, etc.
Fungal diseases	Saprolegniasis	*Saprolegnia* spp., *Achlya* spp., *Aphanomyces* spp.	Freshwater fish (carps and goldfish)
	Branchiomycosis (gill rot)	*Branchiomyces* spp.	Carps, gold fish, eels

(continued)

Table 1 (continued)

Category	Disease	Etiological agent	Susceptible species
	Epizootic ulcerative syndrome (EUS)	*Aphanomyces invadans* (along with rhabdovirus and *Aeromonas hydrophila*)	Catfish, snakeheads, tilapia, goby, gourami, etc.
	Ichthyophoniasis/ ichthyosporidiasis	*Ichthyophonus* spp.	Trouts, groupers, flounders, herrings, and cods
	Aspergillomycosis (red disease)	*Aspergillus flavus*, *Aspergillus* spp.	*Penaeus monodon* and other *Penaeus* spp., tilapia, etc.
	Black gill disease (fusarium disease)	*Fusarium solani*	All *Penaeus* species
Parasitic diseases	Ichthyophthiriasis (Ich/white spot)	*Ichthyophthirius multifiliis*	All fish species
	Trichodiniasis	*Trichodina* spp.	Mainly freshwater fish (tilapia, etc.)
	Dactylogyrosis	*Dactylogyrus* spp.	Freshwater and salt water fish
	Gyrodactylosis	*Gyrodactylus* spp.	Most freshwater species
	Black spot disease	*Diplostomum* spp.	Salmonids, freshwater and marine fish
	Argulosis	*Argulus* spp.	Mainly freshwater species of tropical waters
	Lernaesis	*Lernaea* spp.	Freshwater fish (carp, catfish, rare in tilapia)

increase in the animal production as a consequence of increasing population and the demand for meat. Antibiotics in low doses in feed enhance the general health and feed efficiency of animals resulting in improved growth and meat production (Angulo et al., 2005; Lekshmi et al., 2017). Currently, antibiotics in livestock and poultry are intended for growth promotion, disease prophylaxis, and disease control as well (Landers et al., 2012). Antibiotics such as tetracycline, sulfasuxidine, streptothricin, and streptomycin as feed additives showed growth-promoting effects in chicken and pig prompting their quick adoption as antibiotic growth promoters (Moore et al., 1946; Jukes & Williams, 1953). Following this, several antibiotics were employed in poultry and animal husbandry in the western countries. The inappropriate use of antibiotics exposes bacteria to sublethal concentration promoting the development of antibiotic resistance. The use of antibiotics for growth promotion has been banned in the European Union since January 2006 (Castanon, 2007).

The continuous use of antibiotics in agricultural settings leads to the development of resistance. The problem is more confounding when antibiotics are used in sublethal concentrations that allow a clonal population of resistant bacteria to proliferate, which eventually overgrow the susceptible population under selection pressure. Populations of resistant bacteria can persist in the environments where antibiotics are used. The genetic factors responsible for resistance usually reside on

transmissible elements such as the plasmids and transposons. Exchange of genetic materials among bacteria living in the same ecosystem usually involves mechanisms such as transformation and conjugation. Phage-mediated transfer of antibiotic resistance via transduction can also occur among closely related bacteria (Kirchhelle, 2018).

8 Antibiotic Use in Aquaculture

The developing economies of Asia and South America have witnessed a phenomenal growth of aquaculture industry in the last two decades. Farming of shrimp and fish is considered a viable and remunerative economic activity due to the intense demand for food fish, both in domestic and international markets. Although the increasing global population and decreasing wild capture from the oceans are considered as important drivers of this demand, increase in per capita consumption and preference for food fish over animal meat owing to its health benefits are also important reasons. The modern aquaculture has transformed into a highly intensive, profit-oriented activity with high inputs of seed, feed, chemical, and biological agents for health and water quality management. Disease outbreaks in such systems can have detrimental effects on the economic prospects of the producer. This eventually compels the industry to use chemotherapeutic agents such as antibiotics to prevent or treat disease outbreaks in fish farms. Antibiotic use in aquaculture is of similar proportion to that of livestock with most antibiotics classified as critically important for human health and being employed for uncertain purposes. The extensive use of antibiotics in aquaculture and agriculture involves 51 antibiotics, 39 (76%) of which are important in human medicine (Done et al., 2015). Studies have found associations between antibiotic resistance and antimicrobial use in aquaculture (Sapkota et al., 2008; Ryu et al., 2012; Shah et al., 2014).

The information on the use of antibiotics in aquaculture is critically lacking. The main reason is that most of the world aquaculture is concentrated in developing economies of Asia and the South America where strict monitoring of antibiotic usage is still lacking. According to a study, antibiotic use ranges from 1 g per metric ton of fish produced in Norway to 700 g per metric ton in Vietnam (Defoirdt et al., 2011; Watts et al., 2017). At least 12 different classes of antibiotics are applied in aquaculture globally, a majority of which belong to the group of “highly” or “critically important” antibiotics to human medicine as classified by the World Health Organization (WHO) (Heuer et al., 2009; Defoirdt et al., 2011). Antibiotics are commonly used in intensive farming systems owing to disease risks, poor farm management practices, and a lack of access to vaccines and other preventive therapeutics (Lulijwa et al., 2020). A study in 2012 reported the application of diverse antibiotics in shrimp (*Penaeus monodon*), Nile tilapia (*Oreochromis niloticus*), and catfish (*Pangasianodon hypophthalmus*) farms in Asia, with Vietnam

and China in forefront of antibiotic use, predominantly tetracycline, quinolone, and sulfonamides (Rico et al., 2012).

A recent survey of published literature on antibiotics usage in aquaculture between 2008 and 2018 revealed that 67 antibiotics were used in 11 countries out of 15 surveyed, with oxytetracycline, sulfadiazine, and florfenicol being the most commonly used antibiotics (Lulijwa et al., 2020). Vietnam, China, and Bangladesh were the leading users of antibiotics in aquaculture. Antibiotics such as oxytetracycline, sulfadiazine, and florfenicol were employed by more than two-thirds of the surveyed countries; more than half of them used amoxicillin, erythromycin, sulfadimethoxine, and enrofloxacin. Antibiotics are used in all *Pangasius* farms in Vietnam. While 17 antibiotics are used commonly in farms, 24 antibiotics are applied in hatcheries for bacterial control (Phu et al., 2016). Limited studies are available on the use of antibiotics in African aquaculture farms (Limbu, 2020). Oxytetracycline, chloramphenicol, and gentamicin are some of the most common antibiotics used in African catfish (*Clarias gariepinus*) farms in Nigeria, with nearly 85% of the fish tested carrying detectable levels of antibiotic residues (Okocha et al., 2021). Studies have found residues of antibiotics such as erythromycin in farmed shrimp in India (Swapna et al., 2012), chloramphenicol in Bangladesh (Hassan et al., 2013), and tetracyclines, sulfonamides, fluoroquinolones, and florfenicol in Iran (Mahmoudi et al., 2014; Barani & Fallah, 2015).

Some of the antibiotics such as fluoroquinolones and tetracyclines can remain in water for a considerable period of time, and if adequate withdrawal period is not allowed after the application of antibiotics in fish farms, antibiotic residues could be found in farmed fish after harvest (Pham et al., 2015). These antibiotic residues can have serious ill effects on human health such as allergy, carcinogenicity, mutagenicity, aplastic anemia, and changes in the gut microbiota apart from promoting antibiotic resistance development in human-associated bacteria (Hu & Cheng, 2014).

The direct effect of antibiotic use is the development of antibiotic resistance in the farm environment, while the presence of sublethal levels of antibiotic residues in farmed fish and shellfish can promote resistance development in human microbiome. Long-term exposure to antibiotic residues and their metabolites in food can induce several chronic disorders (Fig. 1).

The bactericidal compounds used in aquaculture definitely pose a food safety risk in terms of antimicrobial-resistant bacteria and adverse drug reactions (Liu et al., 2017) and need to be discouraged (Boyd & Massaut, 1999). In a study conducted in Vietnam among different livestock producers, only 7% of the aquaculture farmers were aware of antibiotics in specific commercial feeds compared to 43% of poultry producers and 32% of piggery producers (Pham-Duc et al., 2019). However, most aquaculture farmers (57.5%) knew about the prohibition of certain antibiotics compared to 27.5% piggery producers and 16.5% poultry producers. It was observed in the study that poultry farmers and aquaculture farmers use antibiotics as a means to prevent infection rather than to treat the infection as in the case of piggery farmers (Pham-Duc et al., 2019).

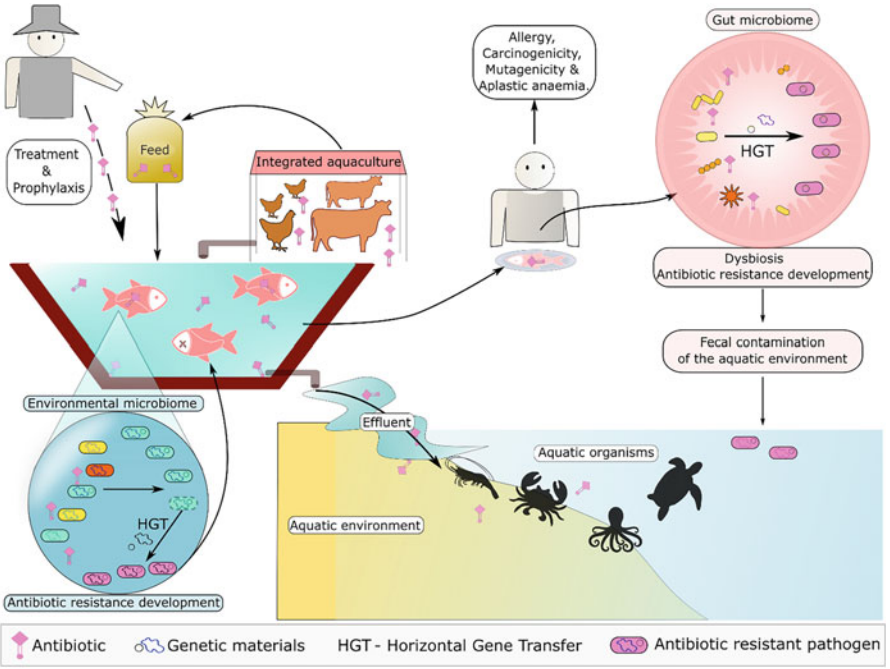


Fig. 1 Antibiotic use in aquaculture, resistance development, and dissemination of resistance genes through horizontal transfer

9 Antimicrobial Use and Resistance Development

The problem of indiscriminate application of antibiotics in fish and shrimp farms is confounded by poor quality of drugs employed in aquaculture, which actually contain lower concentrations of active ingredients. This results in the application of sublethal concentration of antibiotics leading to the development of resistance in bacteria (Lulijwa et al., 2020). Bacteria exposed to concentrations lower than the cidal concentrations gradually evolve to resist the antibiotic. The general mechanisms by which bacteria resist antimicrobial compounds include the production of enzymes that hydrolyze or modify the antibiotic making them ineffective, alterations in antibiotic targets thorough mutations in genes encoding them, and active efflux of antibiotic molecules (Kumar & Varela, 2013; Kumar et al., 2017). Farmers use antibiotics arbitrarily without any guidance from veterinarians, and in most cases, antibiotics are incorporated in feed at suboptimal conditions often leading to treatment failures and exposure of general population of bacteria in water and sediment to sub-lethal levels of antibiotics (Phu et al., 2016). The antibiotic residues remain in the sediment or are released to the environment through farm effluents. The farmers are also exposed to the ill effects of antibiotic residues, and there are high chances of farm workers being the carriers of antibiotic-resistant bacteria. The release of

antibiotic-resistant bacteria and the residues into the aquatic environment can eventually result in the wider spread of resistant bacterial populations. Resistant bacteria in farm workers can contribute to the dissemination of such bacteria in the community. Thus, the use of antibiotics in aquaculture settings can have far-reaching consequences on the resistance development and spread of resistant bacteria via food, humans, and the environment. The use of antibiotics in fish farms can result in antibiotic residues and antibiotic-resistant bacteria being found in products derived from nonedible portions of fish as well. A study revealed the presence of antimicrobial resistance genes (ARGs) in mariculture sediments, and these were derived from the fish meal used in mariculture (Han et al., 2017). Fish pond water and sediment could act as reservoirs of ARGs, which can be transmitted to related and unrelated bacteria in the environment leading to the development of clonal populations of resistant bacteria that eventually replace the antibiotic-sensitive populations due to the continued application of antibiotics. This horizontal spread of antibiotic resistance genes is of particular concern, as the antimicrobial resistance developed in farm environments could soon be found in human pathogenic bacteria, since many bacteria found in aquaculture environments are opportunistic human pathogens of zoonotic potentials (Ma et al., 2021). Since the antibiotics used in aquaculture in many cases are similar to those employed for treatment of infectious diseases, the development of resistance in aquaculture environments may soon translate into a clinical problem. Bacteria such as the livestock-associated methicillin-resistant *Staphylococcus aureus* (LA-MRSA), extended-spectrum β-lactamase-producing *E. coli* and *Klebsiella* spp. and carbapenem-resistant *Enterobacteriaceae* (CRE) represent some of the highly resistant groups of bacteria that are difficult to treat when associated with human infections.

Several studies have suggested that the antimicrobial resistances found in bacteria associated with aquaculture can be transferred to human pathogenic bacteria (Kruse & Sørum, 1994; Rhodes et al., 2000). Zoonotic pathogens resistant to multiple antibiotics and carrying resistance genes have been isolated from aquaculture farms including water, sediment, shrimp or fish, farm workers, probiotics, feed, etc. (Miranda & Zemelman, 2001; Cabello, 2006; Grema et al., 2015; Chuah et al., 2016; Santos & Ramos, 2018). Similarities in ARGs between fish and human pathogenic bacteria have speculated the human health implications of antibiotic use and resistance development in aquaculture settings (Kim et al., 2004; Obayashi et al., 2020). A study reported high levels of resistance to tetracycline, amoxicillin, and augmentin among *Aeromonas* spp. isolated from tilapia, trout, and koi aquaculture systems, and these bacteria harbored transmissible Class 1 integron-associated antibiotic resistance genes (Jacobs & Chenia, 2007). In Nigerian fish farms where antibiotics such as chloramphenicol, tetracycline, and gentamicin are used, aquaculture-associated bacteria were highly resistant to these antibiotics (Olatoye & Afisu, 2013; Fakorede et al., 2020). The association of ARGs with highly transmissible genetic elements such as the plasmids or integrons has been reported in several zoonotic bacterial pathogens such as *Aeromonas hydrophila*, *Vibrio cholerae*, and *Salmonella enterica* (Cabello, 2006; Ishida et al., 2010; Defoirdt et al., 2011; Zhang et al., 2015). The use of antibiotics in Chilean salmon

aquaculture resulted in antibiotic residues and the presence of resistant bacteria harboring ARGs in 8-km-distant marine sediments. A metagenomic study of Chinese mitten crab *Eriocheir sinensis from* farm environment revealed plasmids as the predominant mobile genetic elements carrying the ARGs (Fang et al., 2019). The resistance of *Aeromonas* isolates from the aquaculture environment in Israel correlated with the use of sulfadiazine and trimethoprim and tetracycline antibiotics (Patil et al., 2016). Multidrug resistance and the presence of ARGs were more frequently encountered in *Aeromonas* spp. isolated during the culture period compared to those isolated prior to stocking. The occurrence of antibiotic-resistant bacteria in fish gut correlated with the high use of antibiotics florfenicol and oxytetracycline in Chilean salmon farms (Higuera-Llantén et al., 2018). Similarly, mass mortality of *Penaeus monodon* larvae in a hatchery in India was attributed to antibiotic-resistant *V. harveyi* (Karunasagar et al., 1994). Multiple antibiotic-resistant *Vibrio* spp. resistant to antibiotics such as ampicillin, cefoxitin, streptomycin, aztreonam, and sulfamethoxazole (21%) were detected in aquaculture facilities, a few of which harbored transferable SXT element (García-Aljaro et al., 2014). *Vibrio* spp. such as *Vibrio aestuarianus* and *Vibrio harveyi* isolated from gilthead sea bream reared in Italian mariculture were resistant to ampicillin, amoxicillin, erythromycin, and sulfadiazine (Scarano et al., 2014).

The spread of colistin resistance mediated by plasmid-borne *mcr-1* gene from pigs in China indicates how antimicrobial resistance development in livestock can eventually spread in the community (Liu et al., 2016). This was the first instance of plasmid-mediated resistance mechanism to polymyxins that could be horizontally transmitted. The presence of colistin resistance gene on a transmissible plasmid resulted in its faster dissemination in livestock and, finally, to humans. Subsequently, the use of colistin as a growth promoter was banned in China in 2016. However, the colistin resistance has already been reported from more than 30 countries (Walsh & Wu, 2016). Multidrug-resistant, *mcr-1*-positive *E. coli* was isolated from farmed rainbow trout in Lebanon (Hassan et al., 2020). The *mcr-1* gene was present on IncX4 plasmids, and in addition to colistin, the isolates were resistant to as many as 14 antibiotics (Hassan et al., 2020). The emergence of resistance to last-resort antibiotics such as polymyxins could mean a dead end to antimicrobial efficacy against pathogenic bacteria. The World Health Organization (WHO) has marked colistin as a "reserved" antibiotic and classified it as a highest priority critically important antibiotic (HPCIA), meaning this antibiotic should be used only when all other chemotherapeutic options have failed. On the contrary, colistin is widely used in livestock and poultry for growth promotion and treatment of infections. Although colistin is propagated as a therapeutic agent in poultry, its use is not restricted to treatment. The antibiotic is used for growth promotion and prophylaxis as well (Apostolakos & Piccirillo, 2018).

The colistin resistance gene *mcr-1* has spread from livestock to humans, and this theory has been strengthened by the predominance of *mcr-1* harboring bacteria in livestock and poultry compared to the hospitals (Walsh & Wu, 2016). A study from India reported the occurrence of colistin-resistant bacteria in meat, fish, fruits, and vegetables (Ghafur et al., 2019), suggesting that these diverse foods can disseminate

mcr-1 E. coli in the community. A study from China found a higher prevalence of *mcr-1 E. coli* in provinces with high aquaculture activities (Shen et al., 2018). Colistin-resistant *Edwardsiella ictaluri* has been reported from Vietnamese *Pangasius* farms (Tu et al., 2008). Aquaculture environments are thus increasingly being viewed as hotspots of antibiotic resistance development and reservoirs of ARGs, although more studies are necessary to establish a direct link between aquaculture and antibiotic resistance in human pathogens.

Although the ill effects of antibiotic use in food animals are evident, a majority in the meat industry presume a steep decline in the production if antibiotics are withdrawn (Manyi-Loh et al., 2018) The USA, the European Union, and Japan strictly regulate the use of antibiotics in fish farms, and a very few antibiotics are allowed for treatment purposes. Countries such as Norway have completely banned the use of antibiotics in agriculture without significantly affecting the livestock production. In Norway, the antibiotic use in aquaculture has decreased by 99% since 1980 with the help of a strong legal framework and farmer education. Further, the long-term beneficial effects of antibiotic withdrawal from the animal production would make the industry more productive, environment-friendly, and sustainable. These examples have shown that good farming practices combined with prophylactic measures involving the use of vaccines can effectively replace antibiotic use without compromising on the economic gains (Watts et al., 2017). The developed countries have either completely banned the use of antibiotics or reduced their application to minimum essential quantities; however, the situation is contrasting in developing countries where most of the aquaculture occurs. A majority of these countries experiencing rapid growth of aquaculture industry, however, lack legislation and antibiotic stewardship to control antibiotic use in fish and shrimp farming. Nevertheless, developing countries such as China, India, Thailand, and Vietnam are taking rapid measures to regulate and minimize the use of antibiotics in aquaculture.

Antibiotic growth promoters have least or no effect on the health as well as the growth of animals, and the same could be achieved without their use. A study investigated the potential impact of withdrawal of antibiotic growth promoters (AGPs) from livestock (Laxminarayan et al., 2015) and found that AGPs have a very low or no effect on growth in well-managed farming systems in developed countries, while the impact was more significant in developing countries with least efficient farming systems. Optimized farming practices are oblivious to the effect of withdrawal of AGPs and can attain the same production output without them. The developed countries are gradually eliminating the antibiotics as growth promoters and restricting them only to treatment purposes. The focus is on the developing economies where the demand for animal food products is expected to double by the next decade. While the animal production in developed countries has remained stagnant or decreased, it continues to grow rapidly in developing economies. The global meat production is estimated to increase by 76%, from 258 million tons in 2005–2007 to 455 million tons in 2050, most of which will occur in developing countries. However, this growth is characterized by factors such as the high-intensity production systems that are profit-oriented, disease-prone environments, public health risks, etc., all of which are expected to promote the use of antibiotics as a

routine management practice. This situation calls for a strong legal framework in developing countries that restricts the use of antibiotics in animal production systems. The dependency on antibiotics will be phased out gradually when farmers realize better productivity with scientific management practices without the use of antibiotics.

10 Legislation

Vietnam, China, and Bangladesh are among the top countries using antibiotics in food animals (Lulijwa et al., 2020). The data available on the type and quantity of antibiotics used in aquaculture farms across the world is scarce. Nevertheless, surveillance reports and statistical studies indicate direct relationship between antibiotic use in animals like cattle, pigs, and aquaculture sector and antibiotic resistance in bacteria isolated from food-producing animals (Van et al., 2008). With an increase in the global perception towards the devastating effects of antibiotic overuse in aquaculture, more and more regulations are being framed to restrict their use. The local regulations for the use of antibiotics in aquaculture and other animal rearing practices vary widely depending on the countries. Many countries continue to use antibiotics inadvertently as growth promoters in food animals. The first country to ban the use of antibiotics for nontherapeutic purposes is Sweden. This was followed by Denmark, the Netherlands, the UK, and other EU countries which banned the use of antibiotics for prophylactic purposes as well (Agyare et al., 2018). Some countries like Japan, the USA, and European countries have strict regulations, and only few antibiotics are licensed for use in aquaculture. Many antibiotics with profound effect on the development of bacterial antibiotic resistance such as chloramphenicol, which is classified as a suspected carcinogen (Lees et al., 2020), have been banned; however, they are allowed to be used for prophylactic purposes.

Antibiotics are used with twin goals of growth enhancement and prophylaxis. The European Union has banned the application of antibiotics for these two purposes effective from 2006. In certain countries, prescriptions to use antibiotics are mandatory, and the antibiotic use should be reported to the government agencies. From January 2017, the use of antibiotics for growth promotion has been completely banned in the USA. Further, the rule says that all clinically relevant antibiotics must be prescribed by a veterinarian. Following the administration of antibiotics, a withdrawal period must be observed strictly before the animal is prepared for consumption.

Aquaculture produce intended for export from countries with liberal regulations on antibiotic use will have to comply with the stringent regulations of importing countries. This has resulted in many countries coming forward with strict regulations for antibiotic use in aquaculture sector (Defoirdt et al., 2011). In addition, many countries have imposed maximum residue levels (MRLs) for aquaculture produce, which will further control the routine use of antibiotics for growth and prophylactic purposes (Codex Alimentarius, 2018). The European Commission (EC), the Food and Drug Administration (FDA), the Norwegian Food Safety Authority (NFSA),

Codex, and government ministries are the important regulatory authorities involved in framing and implementation of regulations with regard to the use of antibiotics in aquaculture farms (Lulijwa et al., 2020). In Europe, EU Council Regulations No 37/2010 of 22 December 2009 and No 470/2009 of 06 May 2009 regulate the use of veterinary drugs by establishing MRLs for veterinary medicinal products in foods of animal origin. The use of chloramphenicol, nitrofurans, and furazolidone in aquaculture is banned, and MRLs are set via Regulation No 37/2010. Norway controls the use of antibiotics in aquaculture by making it mandatory for pharmacies to sell these antibiotics only on prescription by veterinarians or fish pharmacologists. The antibiotic drugs such as chloramphenicol, enrofloxacin, and rifampin are banned for use in aquaculture in the USA. The list of drugs authorized by FDA includes oxytetracycline, sulfadimethoxine-ormetoprim, florfenicol, sarafloxacin, erythromycin, and sulfonamides potentiated with trimethoprim (Chuah et al., 2016). In other countries, namely, India, Thailand, the Philippines, Brazil, Vietnam, and Bangladesh, respective government authorities regulate and control the use of antibiotics and have listed the banned and licensed drugs for use in aquaculture (Lulijwa et al., 2020). These countries, however, make use of the FDA and EC regulations and MRLs as the baseline for setting up regulations taking into consideration the regional requirements and preferences. The use of antibiotics categorized as critically important in human treatment should be treated as noncompliance and such farms/industries be denied certification. The use of vaccines and immunestimulants should be propagated as health management tools in place of antibiotics.

11 Conclusions and Way Forward

The rising global population has put food production sector under tremendous pressure while also contributing to its rapid growth. Aquaculture is one such sector which has grown phenomenally in the last few decades, from traditionally extensive, sustenance-oriented farming to highly intensive, commercial farming. The use of banned antibiotics for growth promotion, prophylaxis, and treatment of diseases in aqua farms has raised the fear of antimicrobial resistance development and dissemination among human pathogens. The problem is more severe in developing countries which lack strong legal and infrastructural framework to regulate antimicrobial use. In the context of one health concept, it is imperative that the aquaculture sector finds alternate, environment-friendly methods of disease management and avoids the use of antibiotics identified as critical in human medicine.

References

Agyare, C., Boamah, V. E., Zumbi, C. N., & Osei, F. B. (2018). Antibiotic use in poultry production and its effects on bacterial resistance. In Y. Kumar (Ed.), *Antimicrobial resistance: A global threat* (pp. 33–42). IntechOpen.

Akter, M. N., Sarker, G., Ali, M. M., & Zafar, M. A. (2020). Present status of using aqua medicines and chemicals on fish health management in Bogura district, Bangladesh. *Research in Agriculture Livestock and Fisheries, 7*(1), 129–138.

Alam, M. A., & Rashid, M. M. (2014). Use of aqua-medicines and chemicals in aquaculture in Shatkhira district, Bangladesh. *IOSR Journal of Pharmacy and Biological Sciences, 9*, 05–09.

Ali, H., Rico, A., Murshed-e-Jahan, K., & Belton, B. (2016). An assessment of chemical and biological product use in aquaculture in Bangladesh. *Aquaculture, 454*, 199–209.

Andem, A. B., Esenowo, I. K., Ibor, O. R., & Abosi, A. (2015). Toxicological effects of formaldehyde concentrations on African Catfish, *Clarias gariepinus* fingerlings. *International Journal of Fisheries and Aquatic Studies, 2*(5), 75–79.

Angulo, F. J., Collignon, P., Wegener, H. C., Braam, P., & Butler, C. D. (2005). The routine use of antibiotics to promote animal growth does little to benefit protein undernutrition in the developing world. *Clinical Infectious Diseases, 41*(7), 1007–1013.

Apostolakos, I., & Piccirillo, A. (2018). A review on the current situation and challenges of colistin resistance in poultry production. *Avian Pathology, 47*(6), 546–558.

Austin, B. (Ed.). (2012). *Infectious disease in aquaculture: Prevention and control*. Woodhead Publishing.

Ayuba, V. O., Iyakwari, S. P., & Oyeniyi, M. E. (2013). Acute toxicity of formalin on *Clarias gariepinus* juveniles. *Publication of Nasarawa State University, 9*(1), 21–28.

Barani, A., & Fallah, A. A. (2015). Occurrence of tetracyclines, sulfonamides, fluoroquinolones and florfenicol in farmed rainbow trout in Iran. *Food and Agricultural Immunology, 26*(3), 420–429.

Bellay, S., De Oliveira, E. F., Almeida-Neto, M., Mello, M. A. R., Takemoto, R. M., & Luque, J. L. (2015). Ectoparasites and endoparasites of fish form networks with different structures. *Parasitology, 142*(7), 901–909.

Boyd, C. E., & Massaut, L. (1999). Risks associated with the use of chemicals in pond aquaculture. *Aquacultural Engineering, 20*(2), 113–132.

Cabello, F. C. (2006). Heavy use of prophylactic antibiotics in aquaculture: A growing problem for human and animal health and for the environment. *Environmental Microbiology, 8*(7), 1137–1144.

Castanon, J. I. R. (2007). History of the use of antibiotic as growth promoters in European poultry feeds. *Poultry Science, 86*(11), 2466–2471.

Chuah, L. O., Effarizah, M. E., Goni, A. M., & Rusul, G. (2016). Antibiotic application and emergence of multiple antibiotic resistance (MAR) in global catfish aquaculture. *Current Environmental Health Reports, 3*(2), 118–127.

Codex Alimentarius. (2018). *Maximum residue limits (MRLS) and risk management recommendations (RMRs) for residues of veterinary drugs in foods*. Alimentarius.

Defoirdt, T., Sorgeloos, P., & Bossier, P. (2011). Alternatives to antibiotics for the control of bacterial disease in aquaculture. *Current Opinion in Microbiology, 14*(3), 251–258.

Done, H. Y., Venkatesan, A. K., & Halden, R. U. (2015). Does the recent growth of aquaculture create antibiotic resistance threats different from those associated with land animal production in agriculture? *The AAPS Journal, 17*(3), 513–524.

dos Santos, C. A. M. L., & Howgate, P. (2011). Fish-borne zoonotic parasites and aquaculture: A review. *Aquaculture, 318*(3), 253–261.

Edwards, P. (2015). Aquaculture environment interactions: Past, present and likely future trends. *Aquaculture, 447*, 2–14.

Fakorede, C. N., Fatokun, E. N., Philip-Kantiok, B., Iwu, C. J., & Jaja, I. F. (2020). Bacteriological quality and antibiotics susceptibility profile of small-medium scale commercial fish farms in Nigeria. *The Open Agriculture Journal, 14*(1). [Online] https://doi.org/10.2174/1874331502014010198. Accessed February 15, 2021.

Fang, H., Huang, K., Yu, J., Ding, C., Wang, Z., Zhao, C., Yuan, H., Wang, Z., Wang, S., Hu, J., & Cui, Y. (2019). Metagenomic analysis of bacterial communities and antibiotic resistance genes in the *Eriocheir sinensis* freshwater aquaculture environment. *Chemosphere, 224*, 202–211.

FAO. (2020). *The state of world fisheries and aquaculture 2020. Sustainability in action*. Food and Agriculture Organization of the United Nations (FAO). Available online: http://www.fao.org/documents/card/en/c/ca9229en

García-Aljaro, C., Riera-Heredia, J., & Blanch, A. R. (2014). Antimicrobial resistance and presence of the SXT mobile element in *Vibrio* spp. isolated from aquaculture facilities. *The New Microbiologica, 37*(3), 339–346.

Ghafur, A., Shankar, C., Gnana Soundari, P., Venkatesan, M., Mani, D., Thirunarayanan, M. A., & Veeraraghavan, B. (2019). Detection of chromosomal and plasmid-mediated mechanisms of colistin resistance in *Escherichia coli* and *Klebsiella pneumoniae* from Indian food samples. *Journal of Global Antimicrobial Resistance, 16*, 48–52. [Online] https://doi.org/10.1016/j.jgar.2018.09.005. Accessed February 15, 2021.

Grema, H. A., Geidam, Y. A., Suleiman, A., Suleiman, A., & Birma, R. B. (2015). Multi-drug resistant bacteria isolated from fish and fish handlers in Maiduguri, Nigeria. *International Journal of Animal and Veterinary Advances, 7*(3), 49–54.

Han, Y., Wang, J., Zhao, Z., Chen, J., Lu, H., & Liu, G. (2017). Fishmeal application induces antibiotic resistance gene propagation in mariculture sediment. *Environmental Science and Technology, 51*(18), 10850–10860.

Hassan, J., Eddine, R. Z., Mann, D., Li, S., Deng, X., Saoud, I. P., & Kassem, I. I. (2020). The mobile colistin resistance gene, *mcr-1.1*, is carried on IncX4 plasmids in multidrug resistant *E. coli* isolated from Rainbow trout Aquaculture. *Microorganisms, 8*(11), 1636. [Online] https://doi.org/10.3390/microorganisms8111636. Accessed February 15, 2021.

Hassan, M. N., Rahman, M., Hossain, M. B., Hossain, M. M., Mendes, R., & Nowsad, A. A. K. M. (2013). Monitoring the presence of chloramphenicol and nitrofuran metabolites in cultured prawn, shrimp and feed in the Southwest coastal region of Bangladesh. *The Egyptian Journal of Aquatic Research, 39*(1), 51–58.

Hatai, K. (2012). Diseases of fish and shellfish caused by marine fungi. In C. Raghukumar (Ed.), *Progress in molecular and subcellular biology* (Vol. 53, pp. 15–52). Springer.

Henderson, A. L., Schmitt, T. C., Heinze, T. M., & Cerniglia, C. E. (1997). Reduction of malachite green to leucomalachite green by intestinal bacteria. *Applied and Environmental Microbiology, 63*(10), 4099–4101.

Heuer, O. E., Kruse, H., Grave, K., Collignon, P., Karunasagar, I., & Angulo, F. J. (2009). Human health consequences of use of antimicrobial agents in aquaculture. *Clinical Infectious Diseases, 49*(8), 1248–1253.

Higuera-Llantén, S., Vásquez-Ponce, F., Barrientos-Espinoza, B., Mardones, F. O., Marshall, S. H., & Olivares-Pacheco, J. (2018). Extended antibiotic treatment in salmon farms select multiresistant gut bacteria with a high prevalence of antibiotic resistance genes. *PloS One, 13*(9), e0203641. [Online] https://doi.org/10.1371/journal.pone.0203641. Accessed February 15, 2021.

Hine, P. M., & Thorne, T. (2000). Survey of some parasites and diseases of several species of bivalve mollusc in northern Western Australia. *Diseases of Aquatic Organisms, 40*, 67–78.

Hoffman, G. L. (1999). *Parasites of North American freshwater fishes*. Cornell University Press.

Hu, Y., & Cheng, H. (2014). Research opportunities for antimicrobial resistance control in China's factory farming. *Environmental Science & Technology, 48*(10), 5364–5365.

Ibrahim, M., Ahmad, F., Yaqub, B., Ramzan, A., Imran, A., Afzaal, M., Mirza, S. A., Mazhar, I., Younus, M., Akram, Q., Taseer, M. S. A., Ahmad, A., & Ahmed, S. (2020). Current trends of antimicrobials used in food animals and aquaculture. In M. Z. Hashmi (Ed.), *Antibiotics and antimicrobial resistance genes in the environment* (pp. 39–69). Elsevier.

Ishida, Y., Ahmed, A. M., Mahfouz, N. B., Kimura, T., El-Khodery, S. A., Moawad, A. A., & Shimamoto, T. (2010). Molecular analysis of antimicrobial resistance in gram-negative bacteria isolated from fish farms in Egypt. *The Journal of Veterinary Medical Science, 72*(6), 727–734.

Jacobs, L., & Chenia, H. Y. (2007). Characterization of integrons and tetracycline resistance determinants in *Aeromonas* spp. isolated from South African aquaculture systems. *International Journal of Food Microbiology, 114*(3), 295–306.

Jennings, S., Stentiford, G. D., Leocadio, A. M., Jeffery, K. R., Metcalfe, J. D., Katsiadaki, I., Auchterlonie, N. A., Mangi, S. C., Pinnegar, J. K., Ellis, T., Peeler, E. J., Luisetti, T., Baker-Austin, C., Brown, M., Catchpole, T. L., Clyne, F. J., Dye, S. R., Edmonds, N. J., Hyder, K., & Jeffreys, D. W. (2016). Aquatic food security: Insights into challenges and solutions from an analysis of interactions between fisheries, aquaculture, food safety, human health, fish and human welfare, economy and environment. *Fish and Fisheries, 17*(4), 893–938.

Jukes, T. H., & Williams, W. L. (1953). Nutritional effects of antibiotics. *Pharmacological Reviews, 5*(4), 381–420.

Karunasagar, I., Pai, R., Malathi, G. R., & Karunasagar, I. (1994). Mass mortality of *Penaeus monodon* larvae due to antibiotic-resistant *Vibrio harveyi* infection. *Aquaculture, 128*(3), 203–209.

Kibenge, F. S. (2019). Emerging viruses in aquaculture. *Current Opinion in Virology, 34*, 97–103.

Kim, S. R., Nonaka, L., & Suzuki, S. (2004). Occurrence of tetracycline resistance genes *tet*(M) and *tet*(S) in bacteria from marine aquaculture sites. *FEMS Microbiology Letters, 237*(1), 147–156.

Kirchhelle, C. (2018). Pharming animals: A global history of antibiotics in food production (1935–2017). *Palgrave Communications, 4*(1), 1–13. [Online] https://doi.org/10.1057/s41599-018-0152-2. Accessed May 22, 2021.

Kruse, H., & Sørum, H. (1994). Transfer of multiple drug resistance plasmids between bacteria of diverse origins in natural microenvironments. *Applied and Environmental Microbiology, 60*(11), 4015–4021.

Kumar, S., & Varela, M. F. (2013). Molecular mechanisms of bacterial resistance to antimicrobial agents. In A. Méndez-Vilas (Ed.), *Microbial pathogens and strategies for combating them: Science, technology and education* (pp. 522–534). Badajoz, Spain.

Kumar, S., Lekshmi, M., Parvathi, A., Nayak, B. B., & Varela, M. F. (2017). Antibiotic resistance in seafood-borne pathogens. In O. V. Singh (Ed.), *Foodborne pathogens and antibiotic resistance* (pp. 397–415). Wiley.

Landers, T. F., Cohen, B., Wittum, T. E., & Larson, E. L. (2012). A review of antibiotic use in food animals: Perspective, policy, and potential. *Public Health Reports, 127*(1), 4–22.

Laxminarayan, R., Boeckel, T. V., & Teillant, A. (2015). *The economic costs of withdrawing antimicrobial growth promoters from the livestock sector*. Food, Agriculture and Fisheries Papers, No. 78. OECD Publishing.

Lees, P., Pelligand, L., Giraud, E., & Toutain, P. L. (2020). A history of antimicrobial drugs in animals: Evolution and revolution. *Journal of Veterinary Pharmacology and Therapeutics, 44*(2), 137–147.

Lekshmi, M., Ammini, P., Kumar, S., & Varela, M. F. (2017). The food production environment and the development of antimicrobial resistance in human pathogens of animal origin. *Microorganisms, 5*(1), 11. [Online] https://doi.org/10.3390/microorganisms5010011. Accessed February 15, 2021.

Lepa, A., & Siwicki, A. (2011). Retroviruses of wild and cultured fish. *Polish Journal of Veterinary Sciences, 14*(4), 703–709.

Lieke, T., Meinelt, T., Hoseinifar, S. H., Pan, B., Straus, D. L., & Steinberg, C. E. W. (2020). Sustainable aquaculture requires environmental-friendly treatment strategies for fish diseases. *Reviews in Aquaculture, 12*(2), 943–965.

Limbu, S. M. (2020). Antibiotics use in African aquaculture: Their potential risks on fish and human health. In A. L. K. Abia & G. R. Lanza (Eds.), *Current microbiological research in Africa: Selected applications for sustainable environmental management* (pp. 203–221). Springer.

Liu, X., Steele, J. C., & Meng, X. Z. (2017). Usage, residue, and human health risk of antibiotics in Chinese aquaculture: A review. *Environmental Pollution, 223*, 161–169.

Liu, Y. Y., Wang, Y., Walsh, T. R., Yi, L. X., Zhang, R., Spencer, J., Doi, Y., Tian, G., Dong, B., Huang, X., Yu, L. F., Gu, D., Ren, H., Chen, X., Lv, L., He, D., Zhou, H., Liang, Z., Liu, J. H., & Shen, J. (2016). Emergence of plasmid-mediated colistin resistance mechanism *mcr*-1 in animals and human beings in China: A microbiological and molecular biological study. *The Lancet Infectious Diseases, 16*(2), 161–168.

Lulijwa, R., Rupia, E. J., & Alfaro, A. C. (2020). Antibiotic use in aquaculture, policies and regulation, health and environmental risks: A review of the top 15 major producers. *Reviews in Aquaculture, 12*(2), 640–663.

Lyle-Fritch, L. P., Romero-Beltrán, E., & Páez-Osuna, F. (2006). A survey on use of the chemical and biological products for shrimp farming in Sinaloa (NW Mexico). *Aquacultural Engineering, 35*(2), 135–146.

Ma, F., Xu, S., Tang, Z., Li, Z., & Zhang, L. (2021). Use of antimicrobials in food animals and impact of transmission of antimicrobial resistance on humans. *Biosafety and Health, 3*(1), 32–38.

Mahmoudi, R., Gajarbeygi, P., Norian, R., & Farhoodi, K. (2014). Chloramphenicol, sulfonamide and tetracycline residues in cultured rainbow trout meat (*Oncorhynchus mykiss*). *Bulgarian Journal of Veterinary Medicine, 17*, 147–152.

Manyi-Loh, C., Mamphweli, S., Meyer, E., & Okoh, A. (2018), Antibiotic use in agriculture and its consequential resistance in environmental sources: Potential public health implications. *Molecules, 23*(4), 795. [Online] https://doi.org/10.3390/molecules23040795. Accessed May 22, 2021.

Miao, W., & Wang, W. (2020). Trends of aquaculture production and trade: Carp, tilapia, and shrimp. *Asian Fisheries Science, 33*(S1), 1–10.

Miranda, C. D., & Zemelman, R. (2001). Antibiotic resistant bacteria in fish from the Concepción Bay, Chile. *Marine Pollution Bulletin, 42*(11), 1096–1102.

Mishra, S. S., Das, R., Das, B. K., Choudhary, P., Rathod, R., Giri, B. S., Debbarma, J., Sahoo, S. N., Barua, A., Sahu, A., & Patil, P. K. (2017). Status of aqua-medicines, drugs and chemicals use in India: A survey report. *Journal of Aquaculture & Fisheries, 1*(004). [Online] https://doi.org/10.24966/AAF-5523/100004. Accessed February 11, 2021.

Moore, P. R., Evenson, A., Luckey, T. D., McCoy, E., Elvehjem, C. A., & Hart, E. B. (1946). Use of sulfasuxidine, streptothricin, and streptomycin in nutritional studies with the chick. *Journal of Biological Chemistry, 165*(2), 437–441.

Murray, A. G. (2013). Epidemiology of the spread of viral diseases under aquaculture. *Current Opinion in Virology, 3*(1), 74–78.

Obayashi, Y., Kadoya, A., Kataoka, N., Kanda, K., Bak, S. M., Iwata, H., & Suzuki, S. (2020). Tetracycline resistance gene profiles in red seabream (*Pagrus major*) intestine and rearing water after oxytetracycline administration. *Frontiers in Microbiology, 11*, 1764.

Okocha, R. C., Olatoye, I. O., Alabi, P. I., Ogunnoiki, M. G., & Adedeji, O. B. (2021). Aquaculture management practices associated with antimicrobial residues in Southwestern Nigeria. *Aquaculture, 533*, 736195.

Olatoye, O., & Afisu, B. (2013). Antibiotic usage and oxytetracycline residue in African catfish (*Clarias gariepinus*) in Ibadan, Nigeria. *World Journal of Fish and Marine Sciences, 5*, 302–309.

Pakdee, W., Yeemin, T., Sa-ngunkiet, S., Chullasorn, S., & Sutthacheep, M. (2018). Comparison of helminth fauna from three different habitats in the Andaman Sea coastal ecosystem, southern Thailand. *Agriculture and Natural Resources, 52*(2), 176–181.

Paladini, G., Longshaw, M., Gustinelli, A., & Shinn, A. P. (2017). Parasitic diseases in aquaculture: Their biology, diagnosis and control. In B. Austin & A. Newaj-Fyzul (Eds.), *Diagnosis and control of diseases of fish and shellfish* (pp. 37–107). Wiley.

Park, S. I. (2009). Disease control in Korean aquaculture. *Fish Pathology, 44*(1), 19–23.

Patil, H. J., Benet-Perelberg, A., Naor, A., Smirnov, M., Ofek, T., Nasser, A., Minz, D., & Cytryn, E. (2016). Evidence of increased antibiotic resistance in phylogenetically-diverse *Aeromonas* isolates from semi-intensive fish ponds treated with antibiotics. *Frontiers in Microbiology, 7*, 1875. [Online] https://doi.org/10.3389/fmicb.2016.01875. Accessed February 10, 2021.

Pham, D. K., Chu, J., Do, N. T., Brose, F., Degand, G., Delahaut, P., De Pauw, E., Douny, C., Van Nguyen, K., Vu, T. D., Scippo, M. L., & Wertheim, H. F. L. (2015). Monitoring antibiotic use and residue in freshwater aquaculture for domestic use in Vietnam. *EcoHealth, 12*(3), 480–489.

Pham-Duc, P., Cook, M. A., Cong-Hong, H., Nguyen-Thuy, H., Padungtod, P., Nguyen-Thi, H., & Dang-Xuan, S. (2019). Knowledge, attitudes and practices of livestock and aquaculture producers regarding antimicrobial use and resistance in Vietnam. *PLoS One, 14*(9), e0223115. [Online] https://doi.org/10.1371/journal.pone.0223115. Accessed February 27, 2021.

Phu, T. M., Phuong, N. T., Dung, T. T., Hai, D. M., Son, V. N., Rico, A., Clausen, J. H., Madsen, H., Murray, F., & Dalsgaard, A. (2016). An evaluation of fish health-management practices and occupational health hazards associated with Pangasius catfish (*Pangasianodon hypophthalmus*) aquaculture in the Mekong Delta, Vietnam. *Aquaculture Research, 47*(9), 2778–2794.

Reed, P., Francis-Floyd, R., Klinger, R., & Petty, D. (2012). Monogenean parasites of fish. *EDIS, 2012*(8).

Rhodes, G., Huys, G., Swings, J., McGann, P., Hiney, M., Smith, P., & Pickup, R. W. (2000). Distribution of oxytetracycline resistance plasmids between aeromonads in hospital and aquaculture environments: Implication of Tn1721 in dissemination of the tetracycline resistance determinant *tetA*. *Applied and Environmental Microbiology, 66*(9), 3883–3890.

Rico, A., Satapornvanit, K., Haque, M. M., Min, J., Nguyen, P. T., Telfer, T. C., & van den Brink, P. J. (2012). Use of chemicals and biological products in Asian aquaculture and their potential environmental risks: A critical review. *Reviews in Aquaculture, 4*(2), 75–93.

Ryu, S. H., Lee, J. H., Park, S. H., Song, M. O., Park, S. H., Jung, H. W., Park, G. Y., Choi, S. M., Kim, M. S., Chae, Y. Z., Park, S. G., & Lee, Y. K. (2012). Antimicrobial resistance profiles among *Escherichia coli* strains isolated from commercial and cooked foods. *International Journal of Food Microbiology, 159*(3), 263–266.

Sahoo, P. K., Mohanty, J., Garnayak, S. K., Mohanty, B. R., Kar, B., Prasanth, H., & Jena, J. K. (2013). Estimation of loss due to argulosis in carp culture ponds in India. *Indian Journal of Fisheries, 60*, 99–102.

Santos, L., & Ramos, F. (2018). Antimicrobial resistance in aquaculture: Current knowledge and alternatives to tackle the problem. *International Journal of Antimicrobial Agents, 52*(2), 135–143.

Sapkota, A., Sapkota, A. R., Kucharski, M., Burke, J., McKenzie, S., Walker, P., & Lawrence, R. (2008). Aquaculture practices and potential human health risks: Current knowledge and future priorities. *Environment International, 34*(8), 1215–1226.

Scarano, C., Spanu, C., Ziino, G., Pedonese, F., Dalmasso, A., Spanu, V., Virdis, S., & De Santis, E. P. L. (2014). Antibiotic resistance of *Vibrio* species isolated from *Sparus aurata* reared in Italian mariculture. *The New Microbiologica, 37*(3), 329–337.

Shah, S. Q. A., Cabello, F. C., L'abée-Lund, T. M., Tomova, A., Godfrey, H. P., Buschmann, A. H., & Sørum, H. (2014). Antimicrobial resistance and antimicrobial resistance genes in marine bacteria from salmon aquaculture and non-aquaculture sites. *Environmental Microbiology, 16*(5), 1310–1320.

Shen, Y., Zhou, H., Xu, J., Wang, Y., Zhang, Q., Walsh, T. R., Shao, B., Wu, C., Hu, Y., Yang, L., Shen, Z., Wu, Z., Sun, Q., Ou, Y., Wang, Y., Wang, S., Wu, Y., Cai, C., Li, J., et al. (2018). Anthropogenic and environmental factors associated with high incidence of *mcr*-1 carriage in humans across China. *Nature Microbiology, 3*(9), 1054–1062.

Srivastava, S., Sinha, R., & Roy, D. (2004). Toxicological effects of malachite green. *Aquatic Toxicology, 66*(3), 319–329.

Swapna, K. M., Rajesh, R., & Lakshmanan, P. T. (2012). Incidence of antibiotic residues in farmed shrimps from the southern states of India. *Indian Journal of Geo-Marine Sciences, 41*(4), 344–347.

Tavares-Dias, M. (2021). Toxicity, physiological, histopathological and antiparasitic effects of the formalin, a chemotherapeutic of fish aquaculture. *Aquaculture Research, 52*(5), 1803–1823.

Tu, T. D., Haesebrouck, F., Nguyen, A. T., Sorgeloos, P., Baele, M., & Decostere, A. (2008). Antimicrobial susceptibility pattern of *Edwardsiella ictaluri* isolates from natural outbreaks of bacillary necrosis of *Pangasianodon hypophthalmus* in Vietnam. *Microbial Drug Resistance, 14*(4), 311–316.

Van Boeckel, T. P., Brower, C., Gilbert, M., Grenfell, B. T., Levin, S. A., Robinson, T. P., Teillant, A., & Laxminarayan, R. (2015). Global trends in antimicrobial use in food animals. *Proceedings of the National Academy of Sciences of the United States of America, 112*(18), 5649–5654.

Van, T. T. H., Chin, J., Chapman, T., Tran, L. T., & Coloe, P. J. (2008). Safety of raw meat and shellfish in Vietnam: An analysis of *Escherichia coli* isolations for antibiotic resistance and virulence genes. *International Journal of Food Microbiology, 124*(3), 217–223.

Walsh, T. R., & Wu, Y. (2016). China bans colistin as a feed additive for animals. *The Lancet Infectious Diseases, 16*(10), 1102–1103.

Watts, J. E. M., Schreier, H. J., Lanska, L., & Hale, M. S. (2017). The rising tide of antimicrobial resistance in aquaculture: Sources, sinks and solutions. *Marine Drugs, 15*(6), 158. [Online] https://doi.org/10.3390/md15060158. Accessed February 27, 2021.

Yanong, R. P. E. (2003). Fungal diseases of fish. *Veterinary Clinics: Exotic Animal Practice, 6*(2), 377–400.

Yokoyama, H., Grabner, D., & Shirakashi, S. (2012). Transmission biology of the Myxozoa. In E. Carvalho (Ed.), *Health and environment in aquaculture* (pp. 1–42). InTech Europe.

Zhang, J., Yang, X., Kuang, D., Shi, X., Xiao, W., Zhang, J., Gu, Z., Xu, X., & Meng, J. (2015). Prevalence of antimicrobial resistance of non-typhoidal *Salmonella* serovars in retail aquaculture products. *International Journal of Food Microbiology, 210*, 47–52.

Part III

Insights on Antimicrobial Resistance in Pathogens

Evolution and Milestones in the Development of AMR in Bacteria

V. A. Minimol, Abhay Kumar, and Mukteswar Prasad Mothadaka

Contents

Abstract

Antimicrobial resistance is a global challenge to human and veterinary medical world to address the treatments of infection, namely, multidrug-resistant bacteria. The fight against antibiotic resistance by antibiotics continues to fail, when employed in surplus. Further, the emergence of antibiotic-resistant bacteria reduces the efficacy of the antibiotics available in the market. It is estimated that approximately 0.7 million people will decease due to AMR-related bacterial infections. In this chapter, an attempt was taken to describe the evolution of AMR and major milestones to prevent the AMR burden prevailing in the ecosystems including human, animals, and environment.

V. A. Minimol (✉) · A. Kumar · M. P. Mothadaka
Microbiology, Fermentation and Biotechnology Division, ICAR-Central Institute of Fisheries Technology (ICAR-CIFT), Cochin, Kerala, India
e-mail: minimol.a@icar.gov.in; minimattath@gmail.com; abhay.kumar2@icar.gov.in; mm.prasad@icar.gov.in; prasadmm@hotmail.com

M. P. Mothadaka et al. (eds.), *Handbook on Antimicrobial Resistance*,
https://doi.org/10.1007/978-981-19-9279-7_2

Keywords

Antibiotics · Evolution · Bacteria · Fitness cost · Surveillance

1 Introduction

The existence of bacteria and their evolution on earth have been recorded from a period of nearly 3.5 billion years (Beveridge, 1995). However, the development of microbial resistance started with concomitant discovery of antimicrobials. The development of resistance occurred mainly through evolutionary pressure. In general, the evolution of AMR can be either from the microbial producers of antibiotics or through genetic engineering of the microbes to novel resistance strains due to human activities (Ventola, 2015). The AMR is also resultant of biofilm ability of the bacteria where the survival dynamics of mixed or single population in group influence the growth of various clones, enhance their genetic diversity through molecular mechanism of plasmid-mediated horizontal gene transfer, and their persistence of resistance genes in the environment (Ahmed et al., 2018). The AMR via *de novo* mutation is associated with drug resistance in *Mycobacterium tuberculosis* (Ragheb et al., 2019).

2 History of Antimicrobial Resistance in Bacteria

The antibiotic era started long before the discovery of penicillin by Fleming in 1929, and the first antibiotic traces detected in the histological human studies was tetracycline (Aminov, 2010). The first antibiotic bacterium identified was penicillin-resistant *Staphylococcus* in 1940 (Alanis, 2005). Since then, an increased penicillin resistance was observed soon in clinical isolates of *S. aureus*, and eventually, pandemic penicillin-resistant staphylococcal infections occurred worldwide. Ever since, there has been the emergence of several AMR bacteria alongside the discovery of new synthetic antibiotics. The resistance is attributed to the presence of class A β-lactamase enzyme in their plasmids. By the late 1950s, a semisynthetic penicillin called methicillin was introduced for preventing asepsis and cross infection caused by the pandemic staphylococcal infections (Wise, 1973). In the 1960s, the first-line treatments to this resistance have been overcome by the introduction of broad-spectrum penicillins such as Ampicillin, Oxacillin, and Nafcillin. However, the drug-resistant *Enterobacteriaceae* (TEM-1 and SHV-1) and *Pseudomonas* species (PSE-1) are eventually identified from clinical isolates (Neu, 1984). The increased resistance of Gram-negative bacteria towards β-lactam antibiotics through transfer of resistance factor from one bacterium to another bacterium by direct cell-to-cell contact (conjugation) further urged the development of more powerful antibiotics. The first-generation cephalosporins such as Cephalothin, Carbenicillin, Cefazolin, and Ticarcillin introduced during the 1970s are more effective than broad-spectrum penicillins and are shown to be more beta lactamase-resistant and rapid permeable to the target bacteria (Livermore, 1995). Shortly after this, several bacteria of clinical

importance have shown resistance towards cephalosporins, and they include *S. marcescens* and *Acinetobacter* species, TEM-1-producing *Salmonella,* and TEM-1 plus multiple antibiotic resistance genes producing *K. pneumonia (*Medeiros, 1997). The first derivatives of β-lactam antibiotics cephamycins called cefoxitin were discovered from *Streptomyces clavuligerus* in 1978 and were resistant to plasmid-mediated β-lactamases (Essack, 2001). During the 1970s and the 1980s, several synthetic additions of β-lactam antibiotics such as Cefuroxime, Ceftriaxone, Ceftazidime were tried to inhibit the resistance in several clinically important bacteria such as *Enterobacter species, K. oxytoca, Morganella morganii*, and *S. marcescens* (Neu, 1986). The golden era of antibiotics started with the discovery of β-lactam compounds in bacteria such as *Streptomyces* and *Nocardia*, *Agrobacterium*, *Flavobacterium, Gluconobacter*, *Chromobacterium* species, and *Pseudomonas acidophila* (Sykes & Bonner, 1985).

The first effective antibiotic, namely, sulfonamides were hindered by the development of resistance mechanisms from the introduction of the antibiotics in late 1937 onwards until recently (Davies & Davies, 2010). The development of resistance to sulfonamides was reported soon after the discovery of sulfonamides in the late 1930s (Sköld, 2000). In 1944, the discovery of streptomycin for the treatment of tuberculosis was again caused by the development of resistant strains of *Mycobacterium tuberculosis* (Gillespie, 2002.). However, in 1950, the discovery of the genetically transferable antibiotic-resistance mechanism has become a turning point in the research of antibiotic resistance in several bacteria ((Davies & Davies, 2010). Subsequently, the horizontal gene transfers of virulence and other functional genes including antibiotic resistance genes have become major focus for the scientific community. The major breakthrough events in antibiotic history are given in Table 1.

3 Evolution of Antimicrobial Resistance in Public Health Significant Bacteria

The discovery of antibiotics causes the antibiotic resistances in several public health significant bacteria in which many of them reform to multidrug-resistant bacteria. The bacteria which show high level of resistance to several antibiotic classes are termed as super-resistant or superbug bacteria. The superbug bacteria include *M. tuberculosis*, *Pseudomonas aeruginosa, Acinetobacter baumannii, Clostridium difficile, methicillin-resistant S. aureus* (MRSA) (Robicsek et al., 2008). Recently, the treatment options for tuberculosis disease has become obsolete in case of XDR (extremely drug-resistant) strains as well as TDR (totally drug-resistant) strains. It is reported that the antibiotic resistance in these superbugs are due to spontaneous mutation and horizontal gene transfer has no role on it (Velayati et al., 2009). However, in many Gram-negative bacteria such as *Escherichia coli, Salmonella enterica,* and *Klebsiella pneumonia*, the evolution and transmission of antibiotic resistance happens mainly through horizontal gene transfer. The development of resistance towards beta-lactam classes of antibiotics and inactivation of beta-lactamases is found to be major in *Enterobacteriaceae*. Similarly, beta-lactams and aminoglycosides-resistant *Pseudomonas aeruginosa* are

Table 1 List of major breakthrough events in antibiotics history

Year	Major event
1929	Discovery of Penicillin
1940	Penicillin used as therapeutic agent for infection control
1941	Tetracycline was purified from *Streptomyces aureofaciens* and used in antibiotic therapy
1944	Introduced Streptomycin against tuberculosis (*Mycobacterium tuberculosis)* disease
1948	Penicillin resistance (PR) in *Staphylococcus aureus* was reported
1950's	Genetic exchange of transferable mechanism of resistance via bacterial conjugation is first noticed
1954	Introduction of glycopeptides
1959	Introduction of methicillin to fight against PR-resistant *Staphylococcus aureus*
1962	Reported methicillin resistance in *Staphylococcus aureus*
1976	Meropenem (Carbapenem class) was first obtained from Actinomycete, *Streptomyces cattleya*
1980	Extended-spectrum beta-lactamase-producing *Escherichia coli* isolate was first reported
1987	Integron as a mobile genetic element in antibiotic resistance was first identified
1988	Vancomycin resistance in *Enterococcus* is first reported
2001	Vancomycin-Resistant *Staphylococcus aureus* (VRSA) was first reported
2011	First report of Colistin resistance in *Enterobacteriaceae*
2013	Carbapenem-resistant *Enterobacteriaceae* (CRE) and multidrug-resistant *Acinetobacter* were designated as prioritized pathogens by U.S. Centers for Disease Control
2015	WHO's Global Antimicrobial Surveillance System (GLASS) came into effect
2015	Introduced world antibiotic awareness week concept by World health organization to facilitate the better antibiotic use in health, food, and environment sector

considered as major concern in antibiotic treatments in chronic cystic fibrosis (Saiman et al., 1996). The emergence of imipenem- and carbapenem-resistant strains of *A. baumannii* during the late 1990s decreased the effectiveness of antimicrobial treatments in infection control. The mechanism of resistance evolved from the vigorous survival through the inactivation of antimicrobial enzymes, inhibiting the target of operation by reducing membrane permeability, as well as the inherent mutation abilities of *A. baumannii* (Fernández-Cuenca et al., 2003). The emergence of Methicillin-resistant strains of *Staphylococcus aureus* in the early 1960s has led to the discovery of multidrug-resistant strain of *Staphylococcus* with higher virulence characteristics. Subsequently, this strain gradually transformed from nosocomial infection to community-acquired infection (Tarai et al., 2013). Antibiotic resistance plays an important role in the emergence of highly virulent types of *Clostiridum difficile* (Spigaglia, 2016). The mechanism of resistance is mainly attributed to acquisition of genetic elements and alteration of antibiotic target sites. The main causes of emerging antibiotic-resistant bacteria are the overuse of antibiotics, poor hygiene and sanitation,

and excessive use of new generation antibiotics for the treatment of mild infections, etc. However, the molecular mechanisms of resistance have been well documented.

4 Evolution of Antimicrobial Resistance in Gram-Negative Bacteria

The major AMR-resistant Gram-negative bacteria includes *Enterobacteriaceae* (*E. coli*, *Klebsiella pneumonia*, and *Enterobacter* Species), *Pseudomonas aeruginosa, Acinetobacter baumannii, Helicobacter pylori, Campylobacter, Salmonella* species, etc. The acquisition of antibiotic resistance genes to the bacterial genome resulted in the formation of novel genes via random mutation which causes extended spectrum of resistance in the bacteria. Antimicrobial effect of penicillin in Gram-negative bacterial cell is achieved by its β-lactam ring which kills or inhibits the bacterial enzyme for the bacterial cell wall synthesis. However, the mutation in bacterial gene causes resistance to penicillin. Penicillinase enzymes in mutated strain are capable of breaking down the β-lactam ring. Moreover, it is evident that the rapid spread of resistance genes from hospitals to community-level outbreak is due to the selective pressure of antibiotic use as well as extra chromosomal transfer mechanisms. The penicillinase gene in the mutated strain is not the part of bacterial chromosomal DNA but on extra chromosomal DNA termed as plasmid. Plasmids are considered as the vectors for the transfer of antibiotic resistance genes in Gram-negative bacteria. The ESBL-producing bacteria can also be able to hydrolyze early generation cephalosporins, monobactam, and penicillin antibiotics and the major gene encoding β-lactamases includes TEM 1, TEM2, SHV 1, etc. Resistances to these antibiotics are also emerging out via mutation in these genes to become resistance to third-generation cephalosporins. The new β-lactamase genes including the new extended-spectrum β-lactamase (CTX-M) to hydrolyze the expanded-spectrum cephalosporins have been evolved (Allen et al., 2009; Livermore et al., 2007). Another plasmid-mediated species-specific chromosomally encoded β-lactamase, namely, AmpC cephalosporinases are common in *Enterobacteriaceae* and *Pseudomonadaceae* (Jacoby, 2009).

The metallo β-lactamases activity in carbapenem-resistant Gram-negative bacteria (*P. aeruginosa* and *K. pneumoniae*) has reduced the clinical use of these antibiotics to a large extent. The greater production of ESBL or *AmpC* together with pore formation and efflux resulted in the evolution of carbapenem resistance (Walsh, 2008). The metallo-β-lactamase enzymes have the potential to degrade all the β-lactam substrates, except in Aztreonam antibiotics. The dominant genotypes of MBL in Gram-negative bacteria include VIM-1, SPM-1, GIM-1, and NDM-1. Recently, the increased use of carbapenem exerted selective pressure for the spread of carbapenemase-producing strains (Young et al., 2008). Carbapenem-resistant *Enterobacteriaceae* was first reported in 1990 where AmpC β-lactamase production was noticed. The major carbapenemases found in different Gram-negative bacteria are class A serine-based β-lactamases, class B New Delhi Metallo-β-lactamases

(NDM), Verona integrin-encoded Metallo-β-lactamase (VIM), class D oxacillinases, or OXA-48-like carbapenemases and imipenemase against IMP.

5 Evolution of Antimicrobial Resistance in Gram-Positive Bacteria

Staphyloccous aureus is one of the important nosocomial pathogens of Gram-positive category. The methicillin resistance in this bacterium was first reported in the 1960s which causes major threat to medical microbiology field for the diagnostics and treatment options of Staphylococcal infections (Ventola, 2015). The other clinically important multidrug-resistant Gram-positive bacteria include Vancomycin-Resistant *Enterococcus faecium* (VRE) and drug-resistant *Streptococcus pneumonia* (Guan et al., 2019). The resistance patterns of these Gram-positive bacteria are evolved from multiple factors such as the frequent changes in ecological conditions, overuse of antibiotics, anthropogenic activities, and exchange of genetic factors through molecular mechanisms. For example, the global distribution of the multidrug resistance clone of *Streptococcus pneumonia* is due to the increased use of antibiotics and the greater movement of people. The multidrug resistance in *Streptococcus pneumoniae* is mediated by a single base mutation in the chromosomal gene (Mathers et al., 2015). The major form of resistance in Gram-positive bacteria includes β-lactam, Glycopeptide, Oxazolidinone, and Daptomycin resistance (Falagas et al., 2008). The emergence of resistances of these compounds in the frontline antibiotics highlights the huge genetic flexibility among the Gram-positive bacteria. The first β-lactam derivatives called penicillin was first used in the 1940s for the treatment of Gram-positive bacteria particularly in staphylococcal infections. The resistance mechanism of penicillin was evolved by the production of penicillinase enzymes by Gram-positive bacteria decreasing the usefulness of this compound for the treatment. The new compounds of β-lactam classes have been rapidly evolved, such as methicillin, cephalosporins, and carbapenems, etc. (Fuda et al., 2004).

The enterococci infections during 1994 were primarily treated with combined dosage of penicillin and Streptomycin. However, MDR clade in *Enterococcus faecium* emerged from animal sources soon after the discovery of Streptomycin, and large-scale use of antibiotics in twentieth century led the development of vancomycin resistance in *Enterococci*. Vancomycin was mostly used for the treatment of MRSA and *Enterococci* infections. The gradual increase in vancomycin-resistant *Enterococci* (VRE) since its first discovery in 1988 in clinical sector poses a serious threat to public health. The glycopeptides category of vancomycin and teicoplanin forms the last resort against multidrug resistance in Gram-positive bacteria (Walsh & Amyes, 2004). Penicillin resistance was quite common in *Streptococcus pneumoniae.* However, the macrolide resistance in penicillin-resistant *Streptococcus pneumonia* harboring both *mefA* and *ermB* genes since the 1990s have been reported. Similarly, fluoroquinolone resistance in *Clostridium difficile* has emerged from 2005 onwards, which will further suppress all the antibiotic treatment options in epidemiological outbreak (Fair & Tor, 2014).

6 Genomic Evolution of Antimicrobial Resistance in Bacteria

The spread of antibiotic resistance among bacteria happens either through natural selection or through lateral gene transfer. The genetic mechanism for natural selection and lateral gene transfer were unclear before twentieth century. The consequences of random selection of genes were unable to predict the phenotypical characteristics of the bacterial population. With the invention of modern biotechnological tools, the genomic history and clonal lineages were easily predicted and reduced the genetic bottleneck to a greater extent. Microevolutionary changes via mutation of DNA sequences are common in bacteria when it is exposed to a sudden change in the growth condition. Hence, the mutation can develop antibiotic resistance traits in bacteria (Martinez, 2009). The genes responsible for resistance traits were recovered from ancient cave microbiomes, and metagenomic DNA analysis helps in the detection of ancient gene lineages for tetracycline, beta lactams resistance, etc. (Nesme & Simonet, 2015). The recombinant DNA or the acquisition of foreign DNA via transient hypermutation increases the mutation rate up to 30% (Galhardo et al., 2007). The mobile genetic elements for antibiotic resistance include conjugative plasmids, integrative conjugating elements, mobile integrons, etc. Integrons are recognized as hotspots for recombination in which gene encoding resistance traits are inserted into a new sequence of DNA. Integrons have unique attachment site, promoter site, and integrase enzyme. The integrons play great role in the genomic evolution of antibiotic resistance due to its integrase enzyme *Intl*, which shows extensive diversity in the gene cassettes. This might be the reason for the emergence of integron variant for the spread of antibiotic resistance genes among bacteria (Bennett, 2008). The class I integron accounts 130 resistance genes was the first among the integron discovered and widely distributed in Gram-negative environmental pathogens (Koczura et al., 2016). The environmental class 1 integron possesses outer membrane efflux protein of quaternary ammonium compounds (*qac)* family in which it is able to interact with commensal bacteria via transposon of the Tn402 family when it is exposed to antimicrobial agents (Hegstad et al., 2010). Further, several variants of class 1 integrons were formed as a result of the capture of chromosomal integrons by transposons (Chen et al., 2019).

The integrative and conjugative elements transfer the antibiotic resistance either through vertical or horizontal ways by integration, excision, and conjugation process, which is mediated by several sets of genes (Salyers et al., 1995). The emergence of ICE families depends upon the specificity of integration sites, conjugation and regulation modules. The SXT/R391 ICE family is an example of variant of ICE in which it conferred resistance to sulfamethoxazole and trimethoprim and was first reported in *Vibrio cholerae* in Bay of Bengal during 1978 to 1984 (Das et al., 2016). The SXT/R391 ICE family possesses tyrosine recombinase enzyme (*Int*) which inserts the ICE into peptide chain release factor (prfC) (Wozniak et al., 2009). The source of SXT/R391 ICEs in *V. cholerae* is believed to be originated from Gammaproteobacteria. Several variants of SXT/R391 ICEs have been evolved between 1980 and 2000. The resistance genes for tetracycline, furazolidone, and trimethoprim-sulfamethoxazole were found in SXT elements.

In the present context of evolution of AMR, the identification of resistance types or mutants became easy with the advent of high-throughput technologies such as whole genome sequencing, next-generation sequencing, and genotyping arrays etc., together with series of bioinformatic tools for the prediction and analysis of voluminous sequence reads. Computational tools available for predicting mutations and evolutionary relationships are protein variation effect analyzer (PROVEAN), multivariate analysis of protein polymorphism (MAPP), protein analysis through evolutionary relationships position-specific evolutionary preservation (PANTHER-SEP), structure-based prediction of protein stability changes upon single point mutation (STRUM), etc. (Tunstall et al., 2020).

7 Fitness Cost and Evolution of AMR in Bacteria

Fitness cost or the way in which resistant bacteria established in a system is usually defined in terms of bacterial growth rate where the development of resistance in particular bacteria occurs with a gradual reduction of growth rate before it is being established in the system. However, the rate of developing resistance can be slowed down if antimicrobial use were reduced. In this way, the reduction of antibiotic use will favor the growth of susceptible bacteria over resistant bacteria. Various factors to reduce the fitness costs of antibiotic resistance, and the possibility of exploiting them were well documented (Andersson & Hughes, 2010). A decade later, Andersson et al. (2020) consolidated the latest information on the progression and ecology of AMR into a roadmap for future investigations and also for mitigating AMR both at clinical and environmental settings. At community level, emergence, transmission and dissemination of antibiotic resistance and at individual level adaptation involving bacterial physiology and host resilience were well assessed (Andersson et al., 2020). Further, new methods and know-hows for refining diagnosis and treatment and curtailing the spread of AMR were well documented (Andersson et al., 2020).

One of the unsolved problems is understanding the bacteria during the process of evolution in terms of fitness, especially in persistent and inconsistent environs. In this context, AMR of bacteria, namely, multidrug resistance (MDR) can provide a vital clue when we ask why only certain group of bacteria possess MDR and others not. This also helps us develop strategies of AMR alleviation. The MDR of AMR is scourge of first order, albeit from the perspective of evolution it is fascinating to note the bacteria is evolved in skill development of handling multiple antibiotics simultaneously. The possible reason for this cost of fitness is well explained (Chavhan et al., 2021). In general, pattern of fitness of the bacteria is confined to particular environment. This observation holds good only to smaller populations of bacteria; however, for the larger populations in fluctuating environments this opinion differs as these groups overcome hindrances. Studies have been undertaken both in perpetual and unpredictable environs, subjecting the evolved populations to WGS and WPS (Whole Population Sequence) analyses revealed very interesting results. In case of smaller

populations the mutations in a particular set helped to survive but in the differed environments the cost of fitness was severe. The additional compensatory mutations helped larger population to survive in unstable environments. The population size played an important role in determining the availability of mutations leading to evolving the fitness costs. The same study subjected *E. coli* of nearly 480 generations to constant environments of four carbon sources namely, galactose, thymidine, maltose, and sorbitol. In addition to this, in one unpredictable environment, the carbon source too was made inconsistent among the four. Since bacteria tend to utilize only one carbon source at one time, the attempt was made to assess which available carbon source influences and facilitates the bacterium to survive and grow. In this also, population size played a predominant role in the evolution of bacteria. The WGS and WPS assessment of the bacteria revealed that the larger group harbored more mutations than smaller populations, and these groups paid less fitness cost in their evolution. On the contrary, the small population bacteria had mutations pertaining one carbon source, thus lessening chances of survival and the resultant evolution.

The bacterial reaction upon coming in contact with antibiotics depends the way they co-survive in intricate multispecies and in intra-communities' communication. This has significant bearing on clinical, ecological, and environmental settings, shifting the tolerance intensity, the range of resistance to antimicrobials, and the course of evolution of resistance. In general, the inference of antimicrobial susceptibility is drawn based on pure cultures and that will be useful for treating the infections at clinical level. On the contrary, this type of information is of less significance when the infection originates from multi-microbials entrenched in bacterial communities of commensals. From this view point, Bottery et al. (2021) recommended a strategy for successful antimicrobial stewardship to combat microbes that occur in communities. The study suggested to treat AMR as evolving property as outcome of collective effect of challenge to antibiotics and intra-community communication among bacteria.

8 Cause Factors for the Evolution of Antimicrobial Resistance

The cause factors for the evolution of antibiotic resistance are listed here. The antibiotic use in the selected geographical location and seasons plays a major role in the evolution of resistance in case of environmental pathogens. The migration of antibiotic residues from already existing geographical location to the adjacent location accelerates the evolution of resistance by natural selection via point mutation. The overdose and self-medication promote antimicrobial resistance in clinically relevant pathogens. Poor sanitation and failure in the management of infection control in many cases causes a serious risk factor for the development of antimicrobial resistance. The use of multiple antibiotics in a host population pose the variation in the consumption pattern of antibiotics in the host populations, and the structure of the host population in terms of sensitive and resistant bacteria etc. determine the

evolution of antibiotic resistance. The ability of certain genetic elements to transfer the resistance traits to other bacteria by several molecular mechanisms is yet another cause factor of antibiotic resistance. The main cause for the spread of methicillin-resistant *Staphylococcus aureus* (MRSA) and the vancomycin-resistant enterococci (VRE) is the use of broad-spectrum antibiotics for hospitalization and treatment (Florescu et al., 2008).

9 Emergence of Antimicrobial-Resistant Bacteria in Seafood and Aquatic Environment

Aquatic environment is bestowed with vast bioresources and biodiversity, and the main factors influencing productivity are availability of light and nutrients. In addition to these, the functional metabolites from various agricultural and municipal runoff to aquatic ecosystem have profound influence on the productivity of aquatic ecosystem. The coastal waters, waste water habitats, the water bodies which are direct contact with human/animal husbandries form major source of AMR bacteria. Aquaculture system is now considered as hot spot for AMR genes in which more than 90% of the bacterial populations are resistant to one or more antibiotics. The multidrug resistance has been reported in various seafood pathogens such as *Salmonella, Campylobacter, Listeria, Staphylococcus,* and *Escherichia coli*. The development of resistant clones in various seafood pathogens is rapidly evolving at alarming rate (Methicillin-resistant *Staphylococcus aureus* (MRSA), *E. coli* ST131, *Klebsiella* ST 258). Transmission of resistance from fish to human occurs via direct contact or with the ingestion of seafood. The major health concern in terms of drug resistance is increased frequency of treatment failure and higher severity of AMR infection which further complicate other treatment interventions such as surgery, chemotherapy, transplantations, etc.

10 Milestones in Antimicrobial Resistance

The collective responsibility of all human beings is the rational and wise use and disposal of antibiotics to prevent the emergence of AMR globally. Many regulatory and government agencies have been constantly working on issues related to AMR both regionally at state level as well as globally. The World Health Organization (WHO) sets global action plan on AMR with five strategic objectives to tackle AMR: (a) to improve awareness and understanding of AMR; (b) to strengthen knowledge through surveillance and research; (c) to reduce the incidence of infection; (d) to optimize the use of antimicrobial agents; and (e) to ensure sustainable investment in countering AMR. Recently, WHO proposed the One Health approach for combating AMR in ecological systems. The key factors such as vulnerability to bacterial disease, AM access, disease diagnostic capacity, AMR, target markets and food safety regulations, and certification should be taken in consideration while using the antibiotic use in aquaculture, and the rigid monitoring of the quantity and

quality of antibiotics used by farmers and its residues in the farmed species and in the ecosystems should be enforced. The setting up of a national program for containment of AMR for the efficient surveillance and containment of AMR in public health sectors in the country by the Government of India, National Antimicrobial Resistance Research and Surveillance Network was established by Indian Council of Medical Research (ICMR) in this regard to stop the development of AMR.

The Food and Drug Administration (FDA) or their equivalent in other countries has approved antimicrobial agents in food animal production only after reviewing the manufacturers' data about efficacy, safety, and quality of the product. The FDA-approved antibiotics are beta-lactams (including cephalosporins), tetracyclines, macrolides, aminoglycosides, lincosamides, sulfonamides, streptogramins, and fluoroquinolones for use in both humans and animals. Antibiotics such as avilamycin, bacitracin, carbadox, flavomycin, and pleuromutilins are not supposed to be used in humans, but can be employed in food animals.

11 Conclusion

Antibiotic resistance is a complex issue in terms of mortality and morbidity. Effective intervention needs to be in place for the proper diagnosis and infection control. Development of antimicrobial agents further adds antimicrobial resistance in bacteria. The surveillance program for the antibiotic resistance need to be strengthened by improving the testing efficiency of the laboratories, awareness among the officials as well as public about the appropriate strategies on real-time basis. Developing the multispectral approaches to address the issues of antimicrobial resistance is the need of the hour for better well-being of the ecosystem. In this context, the predictive models incorporating genomic tools for the evolution of resistance may help to improve diagnostic and treatment strategies for preventing resistance evolution in bacteria. The assessment of AMR in bacterial community is daunting task due to diverse nature and intricate interaction networks of microbes. However, this is possible through meticulous consolidation of harmonizing methods including (Bottery et al., 2021):

1. The need for implementation of AST (antibiotic susceptibility testing) on communities of microbes apart from single-cell cultures, wherever necessitated, for the reason resistance is more to do with the interactions occurring in intraspecific community.
2. This needs to be collective evaluation reconnoitering the significances of antibiotic treatments on community structure and functioning, that could additionally alter community proneness to antibiotics during long term or frequent cures which are common in lingering, polymicrobial infections.
3. The microbial community plays a pivotal role in influencing the selection dynamics or as source of AMR genes. For these reasons, the evolutionary reactions to antibiotic treatments besides concentrating on central pathogen also need to center on the community of its environs.

References

Ahmed, M. N., Porse, A., Sommer, M. O. A., Høiby, N., & Ciofu, O. (2018). Evolution of antibiotic resistance in biofilm and planktonic P. aeruginosa populations exposed to sub-inhibitory levels of ciprofloxacin. *Antimicrobial Agents and Chemotherapy, 62*(8), e00320-18. https://doi.org/10.1128/AAC.00320-18

Alanis, A. J. (2005). Resistance to antibiotics: Are we in the post-antibiotic era? *Archives of Medical Research, 36*(6), 697–705. https://doi.org/10.1016/j.arcmed.2005.06.009. PMID: 16216651.

Allen, H. K., Moe, L. A., Rodumrer, J., Gaarder, A., & Handelsman, J. (2009). Functional metagenomics reveals diverse beta-lactamases in a remote Alaskan soil. *ISME Journal, 3*, 243–251. https://doi.org/10.1038/ismej.2008.86

Aminov, R. I. (2010). A brief history of the antibiotic era: Lessons learned and challenges for the future. *Frontiers in Microbiology, 1*, 134. https://doi.org/10.3389/fmicb.2010.00134. PMID: PMC3109405.

Andersson, D. I., & Hughes, D. (2010). Antibiotic resistance and its cost: Is it possible to reverse resistance. *Nature Reviews Microbiology, 8*(4), 260–271. https://doi.org/10.1038/nrmicro2319. PMID: 20208551.

Andersson, D. I., Balaban, N. Q., Baquero, F., Courvalin, P., Glaser, P., Gophna, U., Kishony, R., Molin, S., & Tonjum, T. (2020). Antibiotic resistance: Turning evolutionary principles into clinical reality. *FEMS Microbiological Review, 44*(2), 171–188. https://doi.org/10.1093/femsre/fuaa001. PMID: 31981358.

Bennett, P. M. (2008). Plasmid encoded antibiotic resistance: Acquisition and transfer of antibiotic resistance genes in bacteria. *British Journal of Pharmacology, 153*(S1), S347–S357. https://doi.org/10.1038/sj.bjp.0707607. PMID: 18193080.

Beveridge, T. J. (1995). The periplasmic space and the periplasm in Gram-positive and Gram-negative bacteria. *ASM News, 61*, 125–130. https://doi.org/10.1038/sj.bjp.0707607. PMID: 18193080.

Bottery, M. J., Pitchford, J. W., & Ville-Petri Friman, V. P. (2021). Ecology and evolution of antimicrobial resistance in bacterial communities. *The ISME Journal, 15*, 939–948. https://doi.org/10.1038/s41396-020-00832-7

Chavhan, Y., Malusare, S., & Dey, S. (2021). Interplay of population size and environmental fluctuations: A new explanation for fitness cost rarity in asexuals. *Ecology Letters*. https://doi.org/10.1111/ele.1383

Chen, J., Su, Z., Dai, T., Huang, B., Mu, Q., Zhang, Y., & Wen, D. (2019). Occurrence and distribution of antibiotic resistance genes in the sediments of the East China Sea bays. *Journal of Environmental Sciences, 81*, 156–167. https://doi.org/10.1007/s11356-018-2664-0. PMID: 29974445.

Das, B., Pazhani, G. P., Sarkar, A., Mukhopadhyay, A. K., Nair, G. B., & Ramamurthy, T. (2016). Molecular evolution and functional divergence of *vibrio cholerae*. *Current Opinion in Infectious Diseases, 29*(5), 520–527. https://doi.org/10.1097/QCO.0000000000000306

Davies, J., & Davies, D. (2010). Origins and evolution of antibiotic resistance. *Microbiology and Molecular Biology Reviews, 74*(3), 417–433. https://doi.org/10.1128/MMBR.00016-10

Essack, S. Y. (2001). The development of β-lactam antibiotics in response to the evolution of β-lactamases. *Pharmaceutical Research, 18*(10), 1391–1399. https://doi.org/10.1023/a:1012272403776. PMID: 11697463.

Fair, R. J., & Tor, Y. (2014). Antibiotics and bacterial resistance in the 21st century. *Perspect Medicinal Chemistry, 6*, PMC-S14459. https://doi.org/10.4137/PMC.S14459

Falagas, M. E., Siempos, I. I., & Vardakas, K. Z. (2008). Linezolid versus glycopeptide or β-lactam for treatment of Gram-positive bacterial infections: Meta-analysis of randomised controlled trials. *The Lancet Infectious Diseases, 8*(1), 53–66. https://doi.org/10.1016/S1473-3099(07)70312-2

Fernández-Cuenca, F., Martínez-Martínez, L., Conejo, M. C., Ayala, J. A., Perea, E. J., & Pascual, A. (2003). Relationship between β-lactamase production, outer membrane protein and penicillin-binding protein profiles on the activity of carbapenems against clinical isolates of

Acinetobacter baumannii. *Journal of Antimicrobial Chemotherapy, 51*(3), 565–574. https://doi.org/10.1093/jac/dkg097

Florescu, I., Beuran, M., Dimov, R., Razbadauskas, A., Bochan, M., Fichev, G., Dukart, G., Babinchak, T., Cooper, C. A., Ellis-Grosse, E. J., & Dartois, N. (2008). Efficacy and safety of tigecycline compared with vancomycin or linezolid for treatment of serious infections with methicillin-resistant *Staphylococcus aureus* or vancomycin-resistant *Enterococci*: A phase 3, multicentre, double-blind, randomized study. *Journal of Antimicrobial Chemotherapy, 62*(suppl 1), i17–i28. https://doi.org/10.1093/jac/dkn250

Fuda, C., Suvorov, M., Vakulenko, S. B., & Mobashery, S. (2004). The basis for resistance to β-lactam antibiotics by penicillin-binding protein 2a of methicillin-resistant *Staphylococcus aureus*. *Journal of Biological Chemistry, 279*(39), 40802–40806. https://doi.org/10.1074/jbc.M403589200

Galhardo, R. S., Hastings, P. J., & Rosenberg, S. M. (2007). Mutation as a stress response and the regulation of evolvability. *Critical Reviews in Biochemistry and Molecular Biology, 42*(5), 399–435. PMID: 17917874

Gillespie, S. H. (2002). Evolution of drug resistance in Mycobacterium tuberculosis: Clinical and molecular perspective. *Antimicrobial Agents and Chemotherapy, 46*(2), 267–274. https://doi.org/10.1128/AAC.46.2.267-274.2002. PMID: 11796329.

Guan, D., Chen, F., Qiu, Y., Jiang, B., Gong, L., Lan, L., & Huang, W. (2019). Sulfonium, an underestimated moiety for structural modification, alters the antibacterial profile of vancomycin against multidrug resistant bacteria. *Angewandte Chemie International Edition, 58*(20), 6678–6682. https://doi.org/10.1002/anie.201902210. PMID: 30908776.

Hegstad, K., Langsrud, S., Lunestad, B. T., Scheie, A. A., Sunde, M., & Yazdankhah, S. P. (2010). Does the wide use of quaternary ammonium compounds enhance the selection and spread of antimicrobial resistance and thus threaten our health? *Microbial Drug Resistance, 16*(2), 91–104. https://doi.org/10.1089/mdr.2009.0120. PMID: 20370507.

Jacoby, G. A. (2009). AmpC β-lactamases. *Clinical Microbiological Reviews, 22*, 161–182. https://doi.org/10.1128/CMR.00036-08

Koczura, R., Mokracka, J., Taraszewska, A., & Łopacinska, N. (2016). Abundance of class 1 integron-integrase and sulfonamide resistance genes in river water and sediment is affected by anthropogenic pressure and environmental factors. *Microbial Ecology, 72*(4), 909–916. https://doi.org/10.1007/s00248-016-0843-4. PMID: 27599709

Livermore, D. M. (1995). Beta-lactamases in laboratory and clinical resistance. *Clinical Microbiology Reviews, 8*(4), 557–584. https://doi.org/10.1128/CMR.8.4.557. PMID: 8665470.

Livermore, D. M., Canton, R., Gniadkowski, M., Nordmann, P., Rossolini, G. M., Arlet, G., Ayala, J., Cosque, T. M., Kern-Zdanowicz, I., Luzzaro, F., Poirel, L., & Woodford, N. (2007). CTX-M: Changing the face of ESBLs in Europe. *Journal of Antimicrobial Chemotherapy, 59*, 165–174. https://doi.org/10.1093/jac/dkl483

Martinez, J. L. (2009). The role of natural environments in the evolution of resistance traits in pathogenic bacteria. *Proceedings of the Royal Society B: Biological Sciences, 276*(1667), 2521–2530. https://doi.org/10.1098/rspb.2009.0320. PMID: 19364732

Mathers, A. J., Peirano, G., & Pitout, J. D. (2015). The role of epidemic resistance plasmids and international high-risk clones in the spread of multidrug-resistant *Enterobacteriaceae*. *Clinical Microbiology Reviews, 28*(3), 565–591. https://doi.org/10.1128/CMR.00116-14. PMID: 25926236.

Medeiros, A. A. (1997). Evolution and dissemination of β-lactamases accelerated by generations of β-lactam antibiotics. *Clinical Infectious Diseases, 24*(Supplement 1), S19–S45. https://doi.org/10.1093/clinids/24.supplement_1.s19. PMID: 8994778.

Nesme, J., & Simonet, P. (2015). The soil resistome: A critical review on antibiotic resistance origins, ecology and dissemination potential in telluric bacteria. *Environmental Microbiology, 17*(4), 913–930. https://doi.org/10.1111/1462-2920.12631. PMID: 25286745.

Neu, H. C. (1984). Current mechanisms of resistance to antimicrobial agents in microorganisms causing infection in the patient at risk for infection. *The American Journal of Medicine, 76*(5), 11–27. https://doi.org/10.1016/0002-9343(84)90240-7

Neu, H. C. (1986). β-Lactam antibiotics: Structural relationships affecting in vitro activity and pharmacologic properties. *Reviews of Infectious Diseases, 8*(Supplement 3), S237–S259. https://doi.org/10.1093/clinids/8.supplement_3.s237. PMID: 3529320.

Ragheb, M. N., Thomason, M. K., Hsu, C., Nugent, P., Gage, J., Samadpour, A. N., Kariisa, A., Merrikh, C. N., Miller, S. I., Sherman, D. R., & Merrikh, H. (2019). Inhibiting the evolution of antibiotic resistance. *Molecular Cell, 73*(1), 157–165. https://doi.org/10.1016/j.molcel.2018.10.015. PMID: 30449724.

Robicsek, A., Beaumont, J. L., Paule, S. M., Hacek, D. M., Thomson, R. B., Jr., Kaul, K. L., King, P., & Peterson, L. R. (2008). Universal surveillance for methicillin-resistant Staphylococcus aureus in 3 affiliated hospitals. *Annals of Internal Medicine, 148*(6), 409–418. https://doi.org/10.7326/0003-4819-148-6-200803180-00003. PMID: 18347349.

Saiman, L., Mehar, F., Niu, W. W., Neu, H. C., Shaw, K. J., Miller, G., & Prince, A. (1996). Antibiotic susceptibility of multiply resistant *Pseudomonas aeruginosa* isolated from patients with cystic fibrosis, including candidates for transplantation. *Clinical Infectious Diseases, 23*(3), 532–537. https://doi.org/10.1093/clinids/23.3.532. PMID: 8879776.

Salyers, A. A., Shoemaker, N. B., Stevens, A. M., & Li, L. Y. (1995). Conjugative transposons: An unusual and diverse set of integrated gene transfer elements. *Microbiological Reviews, 59*(4), 579–590. PMID: 8531886

Sköld, O. (2000). Sulfonamide resistance: Mechanisms and trends. *Drug Resistance Updates, 3*(3), 155–160. https://doi.org/10.1054/drup.2000.0146. PMID: 11498380.

Spigaglia, P. (2016). Recent advances in the understanding of antibiotic resistance in Clostridium difficile infection. *Therapeutic Advances in Infectious Disease, 3*(1), 23–42. https://doi.org/10.1177/2049936115622891. PMID: 26862400.

Sykes, R. B., & Bonner, D. P. (1985). Discovery and development of the monobactams. *Reviews of Infectious Diseases, 7*(Supplement 4), S579–S593. https://doi.org/10.1093/clinids/7.supplement_4.s579. PMID: 3909315.

Tarai, B., Das, P., & Kumar, D. (2013). Recurrent challenges for clinicians: Emergence of methicillin-resistant Staphylococcus aureus, vancomycin resistance, and current treatment options. *Journal of Laboratory Physicians, 5*(2), 71. https://doi.org/10.4103/0974-2727.119843. PMID: 24701097.

Tunstall, T., Portelli, S., Phelan, J., Clark, T. G., Ascher, D. B., & Furnham, N. (2020). Combining structure and genomics to understand antimicrobial resistance. *Computational and Structural Biotechnology Journal, 18*, 3377–3394. https://doi.org/10.1016/J.CSBJ.2020.10.017

Velayati, A. A., Masjedi, M. R., Farnia, P., Tabarsi, P., Ghanavi, J., ZiaZarifi, A. H., & Hoffner, S. E. (2009). Emergence of new forms of totally drug-resistant tuberculosis bacilli: Super extensively drug-resistant tuberculosis or totally drug-resistant strains in Iran. *Chest, 136*(2), 420–425. https://doi.org/10.1378/chest.08-2427. PMID: 19349380.

Ventola, C. L. (2015). The antibiotic resistance crisis: Part 1: Causes and threats. *Pharmacy and Therapeutics, 40*(4), 277.

Walsh, T. R. (2008). Clinically significant carbapenemases: An update. *Current Opinion in Infectious Diseases, 21*, 367–371. https://doi.org/10.1097/QCO.0b013e328303670b

Walsh, F. M., & Amyes, S. G. (2004). Microbiology and drug resistance mechanisms of fully resistant pathogens. *Current Opinion in Microbiology, 7*(5), 439–444. https://doi.org/10.1016/j.mib.2004.08.007. PMID: 15451497.

Wise, R. I. (1973). Modern management of severe staphylococcal disease. *Medicine (Baltimore), 52*, 295–304. https://doi.org/10.1097/00005792-197307000-00008

Wozniak, R. A., Fouts, D. E., Spagnoletti, M., Colombo, M. M., Ceccarelli, D., Garriss, G., Déry, C., Burrus, V., & Waldor, M. K. (2009). Comparative ICE genomics: Insights into the evolution of the SXT/R391 family of ICEs. *PLoS Genetics, 5*(12), e1000786. https://doi.org/10.1371/journal.pgen.1000786

Toward One Health Approach: Linking Enteric Pathogens from Diverse Sources with Widespread Dissemination of Antimicrobial Resistance

Shanta Dutta, Goutam Chowdhury, Asish Kumar Mukhopadhyay, and Thandavarayan Ramamurthy

Contents

Abstract

One Health is a multidisciplinary concept to provide optimal health for humans and animals and to protect the environment. Antimicrobial resistance (AMR) is one of the components of One Health that cover its use and misuse in humans,

S. Dutta · G. Chowdhury · A. K. Mukhopadhyay · T. Ramamurthy (✉)
Division of Bacteriology, ICMR-National Institute of Cholera and Enteric Diseases, Kolkata, West Bengal, India
e-mail: ramamurthy.t@icmr.gov.in

M. P. Mothadaka et al. (eds.), *Handbook on Antimicrobial Resistance*,
https://doi.org/10.1007/978-981-19-9279-7_48

animals, and environmental sectors, monitoring and generation of knowledge about the spread of resistant bacteria and resistance determinants within and between these sectors at the national and global levels. These activities might give many vital information to take appropriate measures that can reduce the risk factors in public health. The indiscriminate use of antibiotics in animal husbandry and their abundance in the environment generate substantial pressure on bacteria for the development of resistance. These antibiotics enter the food chain and affect various ecological niches due to bioaccumulation. Once selected, the AMR can persist for longer periods in many bacterial species. In several pathogens, it was shown that the persistence of resistance is directly related to the clinical use of antimicrobials. From the public health perspective, the patient/asymptomatic carriers act as a reservoir of antimicrobial resistant bacteria (ARB). There is a rapid increase in the burden of ARB in India impacting several sectors. In this context, it is important to focus on the trends in the AMR status of important enteric pathogens, the possible resistance determinants, and expansion of resistant clones among human, animal, and environment. Considering the Indian scenario, this chapter focuses on the AMR status on important enteric pathogens, antimicrobial resistance genes (ARGs) with reference to different hosts, and possible transmission by identifying the molecular fingerprints.

Keywords

Antibiotic resistance · Antimicrobial resistance genes · Enteric pathogens · Multidrug resistance · One health

1 Introduction

Diarrheal diseases continue as a significant cause of morbidity and mortality worldwide. Globally, about 1.9 billion people are getting infected each year and 715,000 die. About one-third of these infections are spread through food/water. The epidemiology of foodborne pathogens shows an escalating trend due to mass production and consumption of various foods, increase in industrialization and globalization of the food supply, raising the need for ready-to-eat foods for all age groups, etc. In addition, animals and the environments are acting as reservoirs of pathogens. Worldwide, there has been a constant increase in the consumption of antibiotics. Between 2000 and 2010 alone, antibiotic use has increased by 36%. This increase was recorded to be up to 76% in India and other developing countries (Van Boeckel et al., 2014). A study in India showed that about 50% of families are spending on unnecessary medications or clinical investigations (Porter & Grills, 2016). This seems to be contributing to the increasing rates of antibiotic resistance (AMR).

AMR increases the cost and duration of treatments, rendering the patient's infectious status for longer time that sometimes leads to adverse consequences. A disparity between the empirical therapeutic drug and subsequent susceptibility results for a suspected pathogen is one of the main factors that delay the effective

therapy. The emergence of resistance during antimicrobial therapy due to improper dosage has also been known to cause negative outcomes.

The length of hospitalization and mortality determine the short-term direct effect of AMR. However, indirect and long-term consequences of AMR-associated infections may have several important implications, including the use of other antibiotics, future health, emotional impact, and the loss of work and family time due to increased hospitalization period. Use of antimicrobials such as cefuroxime and ceftriaxone can have substantial long-term effects on antimicrobial resistance gene (ARG)-$bla_{\text{CTX-M}}$ carriage duration of gut microbiome. Similarly, implementation of mass drug administration with azithromycin may lead to selection of multiresistant bacteria residing in the gut and respiratory track. Once selected, the AMR can persist for longer periods of time in bacterial species. From the public health perspective, the patient/asymptomatic carriers act as a reservoir of infection caused by the ARB, thus placing members of the community and health care workers at risk.

Although the name "One Health" is recently being used both nationally and globally, the concept was first adapted by Rudolf Virchow in the nineteenth century, linking human and animal health with the term "zoonosis." The first One Health approach was recommended for avian and pandemic influenza preparedness in New Delhi during 2007, involving human and animal health systems. Subsequently, the One Health framework included AMR to address a complex multidisciplinary problem. In 2015, a global action plan for AMR was adopted by the World Health Assembly to improve awareness and understanding of AMR through effective communication, strengthen the surveillance and research, reduce the incidence of infection, and optimize the use of antimicrobials in human and animal health.

RB exist in humans, animals, and the ecosystems. Based on the frequency and type of interactions, these bacteria get transferred between hosts and ecosystems. Several factors are closely interwoven to contribute to the emergence and spread of AMR, which include exposure to antibiotics and disinfectants at individual as well as community levels, water sources, and food animals. The indiscriminate use of antibiotics in animal husbandry generates significant pressure on bacteria for the development of resistance. The half-life of antibiotics varied from <1 day to >9 years in an environment. Most of the antibiotics enters the food chain and affect various ecological niches due to bioaccumulation. ARB transfer resistance genes to other bacterial populations, through several genetic mechanisms. Using PCR/whole-genome sequencing (WGS), several ARGs have been identified globally and typed/subtyped. Table 1 shows prevailing ARGs among enteric pathogens, covering different classes of antimicrobials. Nearly, 60% of infectious diseases in humans are caused by zoonotic pathogens that have the potential to carry ARGs, which could be transmitted to humans.

Several independent studies have been conducted on antibiotics resistant bacteria in humans, animals, and their associated environment. Considering the One Health aspect, integrated studies are seldom made in India. *Escherichia coli* is a widely studied bacterium due to its quick spread in humans, animals, and the environment, long survival, easy detection, and for mobility of ARGs. Generated information suggests that there is a rapid increase in the burden of AMR in India impacting several sectors/ecosystems.

Table 1 Reported antimicrobial resistance encoding genes among enteric pathogens isolated from different sources

	Class of antimicrobial/resistance gene								
Pathogen	Aminoglycosides	Beta-Lactams (Carbapenem/ Cephalosporin/ Penicillin)	Chloramphenicol	Macrolides	Polypeptides	Quinolones/ Fluoroquinolones	Sulfonamides	Tetracyclines	Rifampicin/ Florfenicol/ Oxazolidinones/ Lincosamides/ Linezolid/ Vancomycin/ Fosfomycin Deptomycin *Pleuromutilin*
Aeromonas spp.	*aadA1, aadA2, aacA4, aadA5, aadA13, aadA17, aac (3′)- IIa, aac (6′)Ia, aac(6′)-Ib, aac(6′)-Ib-cr, ant (3′′)Ia, aphAI-IAB, aph(3′)IIIa, strA, strB*	*ampC*, bla_{ACC}, bla_{cphA}, $bla_{CTX-M-3}$, $bla_{CTX-M-15}$, $bla_{CTX-M-27}$, $bla_{CTX-M-98}$, $bla_{FOX-2\ like}$, bla_{FOX-3}, $bla_{FOX-4\ like}$, bla_{FOX-9}, $bla_{FOX-10-like}$, $bla_{FOX-13-like}$, bla_{MOX3}, bla_{NDM-1}, bla_{OXA-1}, bla_{OXA-10}, $bla_{OXA-101}$, $bla_{OXA-181}$, bla_{PER-4}, bla_{SHV}, bla_{SHV-11}, bla_{TEM}, bla_{TEM-1}, bla_{VEB}	*catB, catB3, catB8, floR*	*ermB, mphA, mphR*	*mcr-1, mcr-3, mcr-5, mcr-7*	*qnrA, qnrB, qnrD, qnrS2*	*dfrA1, dfrA5, dfrA12, dfrA14 dfrA17, dfrA28, dfrB1, dfrB3, dfrB4, sul1, sul2*	*tetA, tetB, tetC, tetD tetE, tetH, tetM*	*arr-3*

Bacillus cereus		bla_1, bla_2, $bla_{CTX\text{-}M\text{-}1}$, $bla_{CTX\text{-}M\text{-}15}$, $bla_{VIM\text{-}like}$	*catA15*	*ermA, ermB, mph*				*tetA, tetB, tetK, tetL, tetM, tetO, otrA*	*rph*
Campylobacter spp.	*aacA, aadA1, aadE, aad9, aac(6′)-Ie, aac(6′)-Im, ant(6)-Ia, aphD, aph(2″)-Ia, aph(2″)-Ib, aph(2″)-Ic, aph(2″)-if, aph(2″)-Ig, aph(2″)-Ih, aph(2″)-ii, aph(3′)-III, aph(3′)-Ic, aph(3″)-VIIa, strA*	$bla_{CTX\text{-}M3}$, bla_{OXA}, $bla_{OXA\text{-}1}$, $bla_{OXA\text{-}61}$, $bla_{OXA\text{-}184}$, $bla_{OXA\text{-}193}$, $bla_{OXA\text{-}449}$, $bla_{OXA\text{-}461}$, $bla_{OXA\text{-}580}$, bla_{SHV}, bla_{TEM}	*catA9*	*ermB*			*dfrA1, sul2*	*tetB, tetM tetO*	*cfrC, fexA, optrA lnuC*
Clostridium difficile		bla_{XCDD}	*catP, catQ*	*ermB, ermQ*				*tetL, tetM, tetW*	*cfrB, cfrC vanZ, vanG-link*
Clostridium perfringens	*ant(6′)-Ia, aph(3′)-III, ksg*A, *sat4*	bla_2, bla_{OXA}, bla_{SHI}, bla_{TEM}	*catP, catQ, floR*	*ermB, ermQ*	*bcrA, bcrB, bcrD, bcrR*	*qnrB*, *qnrD, qnrS*	*dfrK, sul1*	*tetA, tetB, tetK, tetL, tetM*	*lunA, lunB, lnuP vanR*
ETEC	*aad, aadA1, aadA5, aac(3″)-IIa, aac(3)-IV, ant(2″)-Ia, aph(3′)-Ia, aph(3′)-IIa, aph(3″)-Ib, aph(6′)-id, aph(4′)-Ia, strA*	$bla_{CMY\text{-}2}$, $bla_{CTX\text{-}M\text{-}3}$, $bla_{CTX\text{-}M\text{-}14}$, $bla_{CTX\text{-}M\text{-}15}$, $bla_{CTX\text{-}M\text{-}27}$, $bla_{CTX\text{-}M\text{-}55}$, $bla_{OXA\text{-}1}$, $bla_{OXA\text{-}7}$, bla_{PSE}, bla_{SHV}, $bla_{SHV\text{-}12}$, $_{-1}$, bla_{TEM}, $bla_{TEM\text{-}176}$	*catA, floR*	*mphA*	*mcr-1, mcr-4, mcr-5*	*qnrS1*	*dfrA, dfr1, dfrA8, dfrA17, sul1, sul2*	*tetA, tetD, tetE, tetY*	

(continued)

Table 1 (continued)

Pathogen	Class of antimicrobial/resistance gene								
	Aminoglycosides	Beta-Lactams (Carbapenem/ Cephalosporin/ Penicillin)	Chloramphenicol	Macrolides	Polypeptides	Quinolones/ Fluoroquinolones	Sulfonamides	Tetracyclines	Rifampicin/ Florfenicol/ Oxazolidinones/ Lincosamides/ Linezolid/ Vancomycin/ Fosfomycin Deptomycin *Pleuromutilin*
EPEC	*aadA1, aacC1, aac(6′)-Ib, ant (3″)-1a, aph (6)-Ib, aph(6)-Ic, aph(3″)-I, strA, strB*	*bla*$_{ACT}$, *bla*$_{DHA}$, *bla*$_{CMY}$, *bla*$_{CMY-2}$, *bla*$_{CTX-M}$, *bla*$_{CTX-M-1}$, *bla*$_{CTX-M-3}$, *bla*$_{CTX-M-9}$, *bla*$_{CTX-M-14}$, *bla*$_{CTX-M-15}$, *bla*$_{CTX-M-55}$, *bla*$_{CTX-M-137}$, *bla*$_{NDM-1}$, *bla*$_{PER-2}$, *bla*$_{SHV}$, *bla*$_{TEM}$, *bla*$_{TEM-116}$, *bla*$_{TEM-214}$, *bla*$_{VIM}$	*catA1*	*ermB*		*qnrB, qnrS*	*dfrA1, dfrA7, sul1, sul2, sul3*	*tetA, tetB, tetC, tetD*	
STEC	*aadA1, aadA2, aadA5, aadA9, aadA12, aadB, ant(2′)-Ia, aph (3′)IIA/XV, aac (3)-I, aac(3)-II, aac(3)-IId, aac (3)-IV, aac*	*ampC, bla*$_{AMPC}$, *bla*$_{CMY-2}$, *bla*$_{CTX-M}$, *bla*$_{CTX-M-1}$, *bla*$_{CTX-M-3}$, *bla*$_{CTX-M-15}$, *bla*$_{DHA-1}$, *bla*$_{OXA1}$, *bla*$_{OXA-4}$, *bla*$_{SHV}$, *bla*$_{SHV-12}$., *bla*$_{TEM-1}$,	*catA, catB, cmlA, floR*	*ermB, mphA, mphB, mphE, mphG*	*mcr-1, mcr-2, mcr-3, mcr-4, mcr-5*	*qnrA,qnrB, qnrS*	*dfrA1, dfrA5, dfrA7, dfrA8, dfrA12, dfrA17, dfrA36, sul1, sul2, sul3*	*tetA, tetB, tetC*	*fosA*

	(3)-Iva, aac(6′) Ib-cr, aph(3″)-Ib, aph6-id, strA, strB	*bla*$_{TEM-1B}$, *bla*$_{TEM-1C}$, *bla*$_{TEM-116}$, *bla*$_{TEM-117}$, *bla*$_{TEM-191}$							
EAEC	*aadA1, aac(3)-IV, aac(6′)-I, aac (6′)Ib-cr*	*bla*$_{AAC}$, *bla*$_{CIT}$, *bla*$_{CMY}$, *bla*$_{CMY-2}$, *bla*$_{CTX-M-1}$, *bla*$_{CTX-M-2}$, *bla*$_{CTX-M-8}$, *bla*$_{CTX-M-14}$, *bla*$_{CTX-M-15}$, *Bla*$_{CTX-M-27}$, *bla*$_{CTX-M-55}$, *bla*$_{CTX-M-101}$, *bla*$_{DHA}$, *bla*$_{OXA}$, *bla*$_{OXA-5}$, *bla*$_{SHV}$, *bla*$_{TEM}$, *bla*$_{TEM-1}$, *bla*$_{TEM-214}$	*cat1, cat2, floR*	*ereA*		*qnrA, qnrB, qnrS*	*dfrA7, dfrA13, sul1, sul2, sul3*	*tetA*	
Listeria monocytogenes	*aadA, aadA6, adA10, aadB, aadE ant9, aac (3″)-IIa, aac(6′)-Ib, aphA, strB*	*ampC, bla*$_{AmpC}$, *bla*$_{CIT}$, *bla*$_{DHA-1}$, *bla*$_{CTX-M}$, *bla*$_{CTX-M9}$, *bla*$_{OXA-1}$, *bla*$_{TEM}$, *bla*$_{TEM-101}$, *bla*$_{TEM-105}$, *bla*$_{SHV}$, *bla*$_{VEB}$, *cfxA, ftsW, mecA, penA, rodA*	*catII, catA8, cmlA, floR*	*ereA, ereB, ermA, ermB, ermC*		*fepA, lde, norB, oqxB, qnrA, qnrB, qnrS*	*dfrA, dfrD, dfrGsul1, sul2, sul4*	*tetA, tetB, tetC, tetD, tetE, tetG, tetM, tetS*	*fosA, fosB, fosX* lin, *mprF, abc-f vanA, vanB, vanZ lnuA, lnuB isaE*

(continued)

Table 1 (continued)

Pathogen	Class of antimicrobial/resistance gene								
	Aminoglycosides	Beta-Lactams (Carbapenem/ Cephalosporin/ Penicillin)	Chloramphenicol	Macrolides	Polypeptides	Quinolones/ Fluoroquinolones	Sulfonamides	Tetracyclines	Rifampicin/ Florfenicol/ Oxazolidinones/ Lincosamides/ Linezolid/ Vancomycin/ Fosfomycin Deptomycin *Pleuromutilin*
Salmonella spp.	*aadA1, aadA1b, aadA2, aadA5, aadA7, aadA12, aadA22, aadA23, aadA24, aadB, aac(3)-I, aac(3)-II, aac(3)-id, aac (3)-Ie, aac (3) Iva, aac(3)-IV, aac(3)-VI, aac(6′)-Ia, aac (6′)-Ib-cr, aac (6′)-I30, ant(3′)-Ia, aph(3′)-Ia, aph(3′)-IIa, aph (3′)-Ib, aph(4)-Ia, aph(6′)-Ib, aph(6)-id, aphA7, strA, strB*	$bla_{\text{ACC-1}}$, bla_{AmpC}, $bla_{\text{CARB-2}}$, $bla_{\text{CARB-8}}$, $bla_{\text{CMY-2}}$, $bla_{\text{CMY-30}}$, $bla_{\text{CMY-32}}$, $bla_{\text{CTX-M-1}}$, $bla_{\text{CTX-M-3}}$, $bla_{\text{CTX-M-4}}$, $bla_{\text{CTX-M-5}}$, $bla_{\text{CTX-M-6}}$, $bla_{\text{CTX-M-7}}$, $bla_{\text{CTX-M-14}}$, $bla_{\text{CTX-M-15}}$, $bla_{\text{CTX-M-37}}$, $bla_{\text{CTX-M-55}}$, $bla_{\text{CTX-M-65}}$, $bla_{\text{-DHA-1}}$, $bla_{\text{FOX-6}}$, bla_{IMP}, $bla_{\text{KPC-1}}$, $bla_{\text{LAP-1}}$, $bla_{\text{NDM-1}}$, $bla_{\text{NDM-5}}$, $bla_{\text{NDM-9}}$, bla_{OXA}, $bla_{\text{OXA-2}}$, $bla_{\text{OXA-10}}$, $bla_{\text{OXA-30}}$,	*catA1, catA2, catB2, catB3, catB8, cmlA, cmlA4, cmlB, floR*	*ermB, ermK, mphA*	*mcr-3, mcr-9*	*qnrA, qnrB19, qnrD1, qnrS, qnrS1, oqxA/B*	*dfrA1, dfrA5, dfrA7, dfrA10, dfrA12, dfrA14, dfrA17, dfrA19, dfrA21, dfrA23, dfrA34, sul1, sul2, sul3*	*tetA, tetB, tetC, tetD, tetG*	*fosA*

		bla$_{\text{OXA-53}}$, *bla*$_{\text{PSE}}$, *bla*$_{\text{SHV-2}}$, *bla*$_{\text{SHV-5}}$, *bla*$_{\text{SHV-12}}$, *bla*$_{\text{TEM}}$, *bla*$_{\text{OXA}}$, *bla*$_{\text{TEM-1b}}$, *bla*$_{\text{TEM-1c}}$, *bla*$_{\text{TEM-131}}$, *bla*$_{\text{TEM-135}}$, *bla*$_{\text{TEM-137}}$„ *bla*$_{\text{TEM-150}}$, *bla*$_{\text{TEM-191}}$							
Shigella spp.	*aadA1, aadA2, aadA5, aph(3″)-Ib, aac(3″)-IId, aac(6′)-Ib-cr, aph(6)-id, sat1, sat2, strA, strB*	*bla*$_{\text{AmpC}}$, *bla*$_{\text{CIT}}$, *bla*$_{\textit{CARB-like}}$, *bla*$_{\text{EC}}$, *bla*$_{\text{EC-8}}$, *bla*$_{\text{CMY}}$, *bla*$_{\text{CMY-2}}$, *bla*$_{\text{CTX-M-1}}$, *bla*$_{\text{CTX-M-2}}$, *bla*$_{\text{CTX-M-3}}$, *bla*$_{\text{CTX-M-9}}$, *bla*$_{\text{CTX-M-14}}$, *bla*$_{\text{CTX-M-15}}$, *bla*$_{\text{CTX-M-24}}$, *bla*$_{\text{CTX-M-28}}$, *bla*$_{\text{CTX-M-39}}$, *bla*$_{\text{CTX-M-55}}$, *bla*$_{\text{CTX-M-57}}$, *bla*$_{\text{CTX-M-64}}$, *bla*$_{\text{CTX-M-79}}$, *bla*$_{\text{CTX-M-123}}$, *bla*$_{\text{DHA1}}$, *bla*$_{\text{NDM-5}}$, *bla*$_{\text{OXA}}$, *bla*$_{\text{OXA-1}}$, *bla*$_{\text{OXA-30}}$, *bla*$_{\text{SHV}}$,	*catA1, catB1, cmlA1*	*ermB, mphA*	*mcr-1*	*oqxA, oqxB, qepA, qnrB, qnrC, qnrB4, qnrB6, qnrB10, qnrB19, qnrC, qnrS1*	*dfrA1, dfrA3, dfrA5, dfrA7, dfrA12, dfrA14, dfrA17, sul1, sul2, sul3*	*tetB, tetC, tetG, tetO, tetR, tetX*	*fosA3*

(continued)

Table 1 (continued)

	Class of antimicrobial/resistance gene								
Pathogen	Aminoglycosides	Beta-Lactams (Carbapenem/ Cephalosporin/ Penicillin)	Chloramphenicol	Macrolides	Polypeptides	Quinolones/ Fluoroquinolones	Sulfonamides	Tetracyclines	Rifampicin/ Florfenicol/ Oxazolidinones/ Lincosamides/ Linezolid/ Vancomycin/ Fosfomycin Deptomycin *Pleuromutilin*
		bla$_{TEM}$, *bla*$_{TEM-1}$, *bla*$_{TEM-1B}$							
Vibrio cholerae	*aacA4, aadA1, aadA2, aadA5, aac(3″)-IId, aph (3″)-Ib, aph(6)-id strA, strB*	*bla*$_{CARB-7}$, *bla*$_{CARB-9}$, *bla*$_{CMY}$, *bla*$_{CTX-M-14}$, *bla*$_{CTX-M-15}$, *bla*$_{CTX-M-99}$, *bla*$_{DHA-1}$, *bla*$_{NDM}$, *bla*$_{NDM-1}$, *bla*$_{P1}$, *bla*$_{OXA-48}$, *bla*$_{TEM}$, *bla*$_{TEM-1}$, *bla*$_{TEM-20}$, *bla*$_{TEM-63}$, *varG*	*catB3, catB9, floR*	*ereA2, ermB, ermA, ermB, mphA, mphB, mphR, msrA, msrB, msrD*	*almE, almF, almG*	*qnrVC, qnrVC5, qnrVC7*	*dfrA1, dfrA6, dfrA12, dfrA15, dfrA17, dfrA18, sul1, sul2*	*tetA, tetC, tetD, tetT, tetM, tetQ*	*aar-3, vanE, vanHD, vanHO, vanRI, vanT*
Vibrio Parahaemolyticus	*aadA1, aacA3, aadB, aphA-3 aph(3″)-Ib, aph (6)-id, strA, strB*	*bla*$_{AmpC}$, *bla*$_{CARB-17}$, *bla*$_{CARB-19}$, *bla*$_{CMY}$, *bla*$_{CMY-2}$, *bla*$_{CTX-M}$,	*catA2, catB2, catB3, catB9, catC, cmlA, floR*	*emrD*		*oqxB, qnrA, qnrD, qnrS1, qnrVC*	*dfrA1, dfrA6, dfrA14, dfrA15,*	*tetA, tetB, tetC, tetM, tetR*	*arr2*

		bla$_{CTX}$, *bla*$_{NDM-1}$, *bla*$_{OXA}$, *bla*$_{OXA-10}$, *bla*$_{PER-1a}$, *bla*$_{SHV}$, *bla*$_{TEM}$, *bla*$_{TEM-6}$, *bla*$_{TEM-116}$, *bla*$_{VEB-1}$					*dfrA18, sul1, sul2, sul3*		
Vibrio fluvialis	*aadA, aadA1, aadA13, aadB, aac(3)-id, aac (6′)-Ib, aac(6′)-Ib-cr, strA, strB*	*ampC*, *bla*$_{CTX-M-3}$, *bla*$_{IMP}$, *bla*$_{NDM-1}$, *bla*$_{OXA-1}$, *bla*$_{OXA-7}$, *bla*$_{SHV}$, *bla*$_{TEM}$, *bla*$_{TEM-9}$, *bla*$_{OXA-48}$, *bla*$_{VIM}$	*catII*, *catB3, cm1A5, floR*	*ere*A, *mph*A		*qnrA, qnrVC, qnrVC5*	*dfrA1*, *sul, sul1, sul2, sul3*	*tetB, tetC*	*arr2*
Yersinia enterocolitica	*aadA, aadA1, aadA1a*	*bla*$_{A}$, *bla*$_{B}$, *bla*$_{DHA}$, *bla*$_{TEM-1}$	*catA1*	*emrD*			*dfr1, sul1*	*tetB*	

ARGs in color represent the respective antimicrobial class

This chapter reviews the status and the prevalence of enteric pathogens in diverse sources and their role in the dissemination of AMR in India.

2 Antibiotic Residues in the Environment and Animals

The existence of antibiotic residues in the riverine environment and food animals is due to anthropogenic activities that help the exposed bacterial population to acquire resistance through several mechanisms. A study conducted on antimicrobial concentrations in the Kshipra river in Ujjain, Madhya Pradesh, indicated the maximal levels of norfloxacin (0.98 μg/L), ofloxacin (1.46 μg/L), metronidazole (0.27 μg/L), sulfamethoxazole (4.66 μg/L), and β-lactams (>5 ppb) in the water samples and ofloxacin (9.74 μg/kg), sulfamethoxazole (8.23 μg/kg), and β-lactam (>5 ppb) in the sediments (Hanna et al., 2020). In groundwater and wastewater samples from West Bengal, the measured highest concentration of ciprofloxacin and ofloxacin was 5.75 μg/L and 17.84 μg/L, respectively (Barbhuiya & Adak, 2021). In cultured shrimps, the concentration of fluoroquinolones varied from 108 to 134 ng/g, which is above the maximum residual limit as given by Council Directive 2377/90 European Commission 2006 (Palaniyappan et al., 2013). Concentration of doxycycline residue in some of the chicken meat samples ranged from 125 to 186 ppm (EU MRL 100 ppm) (https://arccarticles.s3.amazonaws.com/webArticle/Final-attachment-published-B-3899.pdf Accessed on November 18, 2022). The above information gives an idea of how the environment and animal foods are acting as a source and triggering the emergence of AMR bacteria.

3 Importance of Commensal Bacteria in the Gut

Commensal bacteria, mostly represented by MDR *E. coli*, are the reservoir of several antibiotic resistance genes. They acquire mobile genetic elements either from the pathogens or through the other commensal bacteria. *E. coli* is also capable of disseminating these genetic elements containing ARGs through horizontal transfer. Commensal *E. coli* isolated from stools from children and adults were resistant to ampicillin, cephalosporin, quinolone, ceftazidime, cefoxitin, streptomycin, co-trimoxazole, and tetracycline-harbored bla_{TEM}, $bla_{\text{CTX-M9}}$ (in ESBL producers); *qnrA, qnrB, qnrS*, and *aac(6ɸ)-Ib-cr* genes (in quinolone resistant isolates) (Chandran et al., 2017, Saksena et al., 2018).

4 *Aeromonas* spp.

Aeromonas spp. is a Gram-negative bacteria belonging to the Aeromonadaceae. Of the 36 species, about 20 are considered as pathogens to humans, causing a wide spectrum of illnesses. The pathogenic potential of *Aeromonas* is multifactorial due to the expression of adhere, proteases, lipases, enterotoxins, hemolysins, and Shiga-like

toxins. *Aeromonas* spp. are generally found in many habitats, including fish, environments, and food products. *A. hydrophila*, *A. sobria*, *A. caviae*, and *A. veronii* are the commonly identified species in humans and other sources in India. Aeromonads exist as the normal microbial flora as well as cause several diseases in cold blooded and warm blooded animals including fishes, birds, and domestic animals. Fish and chicken play an important role in the transmission of this pathogen to humans.

Many of the *Aeromonas* spp. isolated from diarrheal patients exhibited resistance to commonly used antibiotics such as ampicillin, furazolidone, nalidixic acid, ciprofloxacin, norfloxacin, chloramphenicol, co-trimoxazole, and tetracycline (Sinha et al., 2004). In addition to the above antibiotics, *Aeromonas* spp. from North-Indian pediatric patients were also resistant to amikacin, cefuroxime, cefepime, ceftazidime, ceftriaxone, cefoperazone/sulbactam, piperacillin/tazobactam, imipenem, cefotaxime, cefixime, and azithromycin (Verma et al., 2019).

Transferable plasmids encoding resistance to ampicillin, cephalexin, cefoxitin, erythromycin, and furazolidone, either alone or in combination, were detected in *A. hydrophila* and *A. caviae* from clinical and environmental samples. *A. caviae* isolated from hospitalized acute diarrhea cases was resistant to ciprofloxacin and norfloxacin due to the double mutations in the quinolone resistance-determining regions (QRDRs). Extended-spectrum β-lactamase (ESBL)-producing *Aeromonas* spp. from stool samples harbored $bla_{\text{CTX-M}}$ gene and were also expressed ceftriaxone resistance. Carbapenem resistance in clinical *A. caviae* was due to the presence of bla_{NDM}, and bla_{VEB}, $bla_{\text{OXA-181}}$. The $bla_{\text{OXA-181}}$-positive isolate harbored an incompatibility plasmid, IncQ2 with a plasmid-mediated quinolone resistance (PMQR) gene *qnrS2*.

A. veronii and *A. dhakensis* from urban sewage in Delhi found to harbor a mobilized colistin resistance gene (*mcr-1*). These isolates also had ESBL-resistant determinants CTX-M and TEM. *A. caviae* from effluent waters from Delhi-NCR harboring $bla_{\text{CTX-M-15}}$ was found to be multidrug resistant (MDR) to colistin, chloramphenicol, trimethoprim, ampicillin, aztreonam, ceftazidime, cefotaxime, and ceftriaxone (Ali et al., 2021).

A. hydrophila isolated from fish and prawns was resistant to methicillin and rifampicin followed by bacitracin and novobiocin. From the shrimp culture hatcheries, *Aeromonas* spp. were found to be resistant to ampicillin and chlortetracycline and erythromycin. *A. hydrophila* isolated from cultured freshwater Nile Tilapia in Kerala was resistant to several antibiotics (amoxycillin, amoxiclav, ampicillin, bacitracin, cefazolin, cefixime, cefoperazone, cefoxitin, cephalothin, co-trimoxazole, doripenem, imipenem, meropenem, nitrofurantoin), which is comparatively higher than *A. sobria* (resistant to ampicillin, bacitracin, cefazolin, cephalothin, nalidixic acid, tetracycline, vancomycin).

5 *Bacillus cereus*

Bacillus cereus is a Gram-positive, spore-forming bacterium, which is widely distributed and also an important human pathogen. This species is associated with food poisoning and increasingly reported to cause serious enteric and non-

gastrointestinal-tract infections. Several toxins have been implicated in disease, including pore-forming hemolysins, phospholipases, an emesis-inducing toxin, and proteases. Studies conducted in food samples indicated that the AMR pattern of *B. cereus* depends on the type of food and geographical location. The prevalence of clonally distinct toxigenic-type *B. cereus* among diarrheal cases in Kolkata was 3.5% and was highly resistant to amoxiclav and cefixime (Banerjee et al., 2011). Several enterotoxin-encoding gene-positive *B. cereus* were detected in seafood and fermented foods (Carter et al., 2018; Keisam et al., 2019).

B. cereus isolates from the milk and milk products in and around the Jammu region showed resistance to penicillin-G and cephalexin. In ready-to-eat food items from Himachal Pradesh, *B. cereus* often detected in cheese, khoa, cream, milk, and paneer-based foods with high levels of antimicrobial resistance to penicillin, amoxicillin, ampicillin, cefixime, and ceftazidime (Rana et al., 2020). Tetracycline resistance encoding genes *(tetA* and *tetB*) were mostly found on plasmids of *B. cereus* isolated from various food samples.

6 *Campylobacter* spp.

The Gram-negative bacteria *Campylobacter* is the most common cause of acute bacterial enteritis in humans, in both developing and developed countries. The genus *Campylobacter* consists of 32 species and 9 subspecies. About 20 species are associated with infections in humans and animals. The thermotolerant species *C. jejuni* and *C. coli* are phylogenetically related and often cause gastroenteritis in humans. Various virulence-associated bacterial determinants include the flagellum, secretion systems, flagellar secreted factors, adhesins, cytolethal distending toxin, lipooligosaccharide, and serine protease HtrA.

The global disease study based on the burden of diarrhea, etiologies, and risk factors in India from 1990 to 2019 has shown that among all the death cases, the most prevalent disease-causing pathogen was *Campylobacter* (Behera & Mishra, 2022). In developing countries, *Campylobacter* infections are common in children under the age of two. A longitudinal birth cohort study on enteric infections and malnutrition and the consequences for child health and development showed the association of *Campylobacter* infection with poor growth and increased intestinal inflammation (Haque et al., 2019). *C. jejuni* is known to be an antecedent of Guillain-Barré syndrome (GBS). Higher risk of GBS with a history of *Campylobacter*-related diarrhea in children has been documented.

With the exception of streptomycin, campylobacters isolated from diarrheal children in Kolkata were susceptible to all the antibiotics during 1985–88 (Bhadra et al., 1992). Nalidixic acid resistance was reported during late 1990 in *Campylobacter* from diarrheal children in Chennai (Ananthan et al., 1998). A sharp increase in fluoroquinolone resistance has been reported, from fully susceptible in 1994 to 97% resistant during 2008–2010 (Mukherjee et al., 2013). In Kolkata, *C. jejuni* isolated from the hospitalized patients with diarrhea were highly resistant to

co-trimoxazole during 2008–2010 and to azithromycin in the following years (Mukherjee et al., 2013, 2014).

Poultry, in particular the broiler chickens, is the main source of campylobacteriosis in humans. Consumption of contaminated chicken meat is one of the common modes of transmission. Studies conducted in North India indicated that resistance to ampicillin was higher in *C. jejuni* from humans compared to chickens, but the frequency of resistance to tetracycline was higher in chicken strains than from humans. *C. jejuni* from poultry meat and poultry-related samples was resistant to co-trimoxazole, cephalothin, and tetracycline and few isolates showed resistant to nalidixic acid, ciprofloxacin, erythromycin, gentamicin, and azithromycin (Khan et al., 2018). Some of the other studies indicated the importance of asymptomatic carriers and animal handlers in the spread of campylobacteriosis.

The prevalence rate of *Campylobacte*r was comparatively less in raw milk samples from Gujarat, but the AMR is almost similar to other sources, that is, resistant against nalidixic acid, ciprofloxacin, and tetracycline (Modi et al., 2015). Higher prevalence of thermophilic *Campylobacter* has been reported in water samples, humans, and poultry chickens with MDR phenotype (resistant to ciprofloxacin, erythromycin, nalidixic acid, norfloxacin, tetracycline).

7 *Clostridioides difficile*

Clostridioides difficile (formerly *Clostridium difficile,* reclassified in 2016) is a Gram-positive, spore-forming, anaerobic bacillus, which is commonly found in the intestinal tract of humans and animals. Since this pathogen is widely present, its transmission occurs by the fecal-oral route. The spread of *C. difficile*-mediated infection is reportedly increasing mostly as a hospital-acquired infection as a result of prolonged antibiotic therapy. Antibiotic-associated diarrhea is one of the common symptoms due to the loss of indigenous gut microorganisms (microbial dysbiosis) and intense non-invasive colonization of *C. difficile*. Life-threatening colitis caused by this pathogen usually results in death. Enzymes, such as collagenase, chondroitin-sulfatase, and hyaluronidase as well as toxins (enterotoxin A, cytotoxin B, and *C. difficile* transferase or binary toxin), act as major virulence factors. The toxins damage the epithelial cell cytoskeleton, which leads to disruption of tight junctions, fluid secretion, neutrophil adhesion, and local inflammation.

C. difficile-associated diarrhea in hospitalized patients ranged from 3 to 18%. It was also reported that about 15–30% of the patients experience recurrent infections after discontinuation of antibiotics. Clonally distinct toxigenic *C. difficile* isolated from the hospitalized antibiotic-associated diarrhea cases were identified as MDR to imipenem and moxifloxacin, clindamycin, and tetracycline (Rituparna et al., 2016). *C. difficile* isolated from diarrheal patients was found to resist clindamycin and levofloxacin irrespective of toxigenic status (Abuderman et al., 2018).

Metronidazole and vancomycin are the effective drugs for the treatment of *C. difficile*-associated diarrhea. In certain strains, the transferable plasmids pCD-METRO with high copy number replicons and pX18–498 are responsible for

resistance to metronidazole and decreased sensitivity to vancomycin. Presence of these plasmids supports increased fitness and expression of virulence in a humanized mouse model. WGS and phylogenetic analysis has identified global spread of two distinct epidemic lineages, fluoroquinolone resistance FQR1 and FQR2. Reports on the detection of toxigenic *C. difficile* from other sources are rare in India. One report showed detection of toxigenic *C. difficile* in cattle, pig, and poultry from Northeast India (Hussain et al., 2016).

8 *Clostridium perfringens*

Clostridium perfringens is a Gram-positive, spore-forming, anaerobic bacterium. This pathogen produces various histotoxic infections and gastroenteritis in humans, necrotic enteritis in animals. Based on their ability to produce a combination of α, β, ε, and ι-toxins, *C. perfringens* strains are classified into toxin types A to E. Many of these toxins are located on the plasmids and frequently associated with specific hosts and diseases. Recently, two new toxinotypes have been established, including type F (isolates that produce *C. perfringens* enterotoxin, but not β-toxin, ε-toxin, or ι-toxin), which are responsible for *C. perfringens*-mediated human food poisoning and antibiotic-associated diarrhea and type G (isolates that produce necrotic enteritis B-like *toxin* in chickens).

Toxinotype A seems predominant in humans, animals, and several other sources in India. Prevalence of *C. perfringens* with toxinotypes A and D was reported from Kashmir valley sheep and goats (Nazki et al., 2017). Pulsed-field gel electrophoresis (PFGE) indicated that *C. perfringens* type A Kolkata strains from human diarrheal cases exhibited close lineage to goat, pig diarrhea, as well as meats of pork and chevon (Yadav et al., 2017). Multilocus sequence typing (MLST)-based phylogeny clearly indicated the link between *C. perfringens* toxinotypes A and F strains from calves, dairy workers, and the environment (Verma et al., 2020).

C. perfringens from livestock and poultry were largely resistant to gentamicin, erythromycin, bacitracin, and tetracycline. Poultry feed ingredients such as fish meal, bone meal, meat and bone meal, and dry fish were found to be highly contaminated with *C. perfringens*. These isolates were resistant to penicillin-G with low degree of susceptibility to neomycin, co-trimoxazole, and bacitracin (Udhayavel et al., 2017). MDR (co-trimoxazole, ceftriaxone ampicillin, ceftazidime, and tetracycline) strains of *C. perfringens* type A were identified from diarrheal patients, animals (co-trimoxazole, ceftriaxone, ampicillin tetracycline, ceftazidime), and fishes (ceftriaxone, ampicillin, tetracycline, and co-trimoxazole) (Yadav et al., 2017).

9 Diarrheagenic *Escherichia coli*

Escherichia coli is one of the most widely studied etiologic agents of diarrhea. This organism belongs to the family Enterobacteriaceae and normally resides in the intestine as a commensal microbe. *E. coli* acquire horizontally transferred virulence

genes that convert them as a pathogen to instigate diarrhea in humans and animals. The diarrheagenic *E. coli* (DEC) contributes about 21% among diarrheal cases. DEC has different combinations of virulence traits and based on which they are categorized into five main pathotypes, viz., enterotoxigenic *E. coli* (ETEC), enteropathogenic *E. coli* (EPEC), enteroaggregative *E. coli* (EAEC), *Shiga* toxin-producing *E. coli* (STEC), and enteroinvasive *E. coli* (EIEC). Table 2 shows different virulence factors that specifically prevail in each of these pathotypes. Serological characterization is based on the somatic (O) and flagellar (H) antigens.

In many investigations, prevalence and MDR have been reported among DEC pathotypes. Amoxicillin, aztreonam, cefotaxime, cefixime, cefalotin, colistin, gentamicin, ceftazidime, cefalexin, imipenem, doripenem, ertapenem, tetracycline, neomycin,

Table 2 Phenotypic and virulence characteristics of diarrheagenic *Escherichia coli*

Pathotype	Phenotypic character	Virulence factor	Virulence encoding gene/ subtype
Enterotoxigenic *E. coli* (ETEC)	Heat-labile toxin (LT) Heat-stable toxin (ST)	Plasmid-LT subunits Plasmid-ST	*eltA* and *eltB* *est* (*sta*)
Enteropathogenic *E. coli* (EPEC)	Locus of enterocyte effacement (LEE) pathogenicity island has attaching and effacing gene to cause lesions on enterocytes Strains may also have the plasmid-encoded *bfp* bundle-forming pilus gene	Chromosomal-attaching and effacing (LEE-encoded intimin protein) Plasmid-major structural subunit of bundle-forming pilus	*eae* *bfpA* tEPEC (*eae* + *bfpA*) aEPEC (*eae* only)
Enteroaggregative *E. coli* (EAEC)	Colonization is facilitated by fimbriae with typical stacked-brick pattern, biofilm formation, and toxin release	Plasmid-transcriptional activator Pathogenicity island-type VI secretion system Plasmid-ABC transporter EAEC heat-labile toxin	*aggR* *aaiC* *aat* tEAEC (with *aggR*) aEAEC (without *aggR*) *east1*
Shiga toxin-producing *E. coli* (STEC)		Chromosomal-Shiga-like toxin variants Chromosomal-attaching and effacing (LEE-encoded intimin protein) Plasmid-enterohemolysin Autoagglutinating protein	*stx1, stx2* *eae* *ehxA* *saa*
Enteroinvasive *E. coli* (EIEC)		Type-III effector protein	*ipaH*

streptomycin, amikacin, vancomycin, novobiocin, and kanamycin resistance exists in DEC from humans, vegetables, drinking water, several rivers, and estuarine waters.

9.1 Enterotoxigenic *E. coli*

ETEC is one of the most widely recognized and an important cause of diarrhea in children in low- and middle-income countries. Before 2000s, ETEC from diarrheal patients and the environment remained resistant to streptomycin, tetracycline, ampicillin, kanamycin, chloramphenicol, and carbenicillin but susceptible for nalidixic acid, ciprofloxacin, and norfloxacin (Ghosh et al., 1996). Clonally distinct ETEC was highly resistant to ciprofloxacin, norfloxacin, and nalidixic acid and was detected from a diarrheal outbreak in Ahmedabad during 2000 (Chakraborty et al., 2001). ETEC from diarrheic calves and lambs in Kashmir was resistant to co-trimoxazole, ampicillin, cefalexin, and co-amoxiclav (Wani et al., 2013).

Transfer of virulence and MDR features of ETEC from animal sources has been demonstrated *in vitro* to indicate the zoonotic importance. Along with mutations in the QRDR, several antimicrobial resistance encoding genes were identified in ETEC that include *aac(6′)-Ib-cr* encoding a fluoroquinolone-modifying enzyme (aminoglycoside N-acetyltransferase), *dfrA17, aadA1, aadA5* in class 1, and *dfrA1, sat1, aadA1* in class 2 integrons. In addition, the other resistance genes such as *tet* gene alleles, *catAI, strA,* $bla_{\text{TEM-1}}$, and *aphA1-Ia* were detected in most of the isolates (Pazhani et al., 2011). ETEC sequence type ST38 with $bla_{\text{CTX-M-15}}$ has been identified among travelers.

9.2 Enteropathogenic *E. coli*

Typical enteropathogenic *E. coli* (tEPEC) produces a specific histopathology known as attaching and effacing on intestinal cells due to the presence of a virulence plasmid known as the EPEC adherence factor, while atypical EPEC (aEPEC) does not possess this plasmid. Among diarrheal cases, aEPEC has been more frequently reported than the tEPEC. EPEC from diarrheal children was resistant to ampicillin, ceftriaxone, cefoperazone-tazobactam, cefixime, doxycycline, co-trimoxazole, norfloxacin, and nalidixic acid. aEPEC is the most common cause of diarrhea in children and exhibited resistance to norfloxacin, nalidixic acid, cefotaxime, amikacin, and gentamicin. A comparative study on tEPEC and aEPEC from diarrheal patients indicated that resistant to chloramphenicol, nalidixic acid, norfloxacin, furoxone, ceftriaxone, and azithromycin was more in aEPEC (Malvi et al., 2015). EPEC isolated from poultry meat samples showed different MDR, that is, to novobiocin, cefixime, sulfafurazole, and vancomycin (Jana & Mondal, 2013). aEPEC resistant to amoxicillin and cephalexin was also identified in piglets kept in the unorganized farms (Kylla et al., 2020). EPEC with bla_{VIM}, $bla_{\text{CTX-M-15}}$, and $bla_{\text{NDM-1}}$ was also detected in this finding.

EPEC detected in diarrheal cases was MDR (β-lactams, quinolone, aminoglycosides, tetracycline with ARGs such as *bla*$_{CTX}$, *bla*$_{TEM}$, *bla*$_{SHV}$, *qnrB, qnrS, aac(6′)-1b, aac(3)-IV,* and *tet) (*Natarajan et al., 2018). Almost similar ARG profile was reported in EPEC isolated from humans and domestic animals in Imphal (Lalhruaipuii et al., 2021).

9.3 Shiga Toxin-Producing *E. coli*

Shiga toxin-producing *E. coli* (STEC) is mostly associated with foodborne outbreaks, which contain genes coding for the Shiga toxin (*stx*). STEC also known as verotoxin-producing *E. coli* (VTEC) is a subset of the group enterohemorrhagic *E. coli* (EHEC), which causes severe bloody diarrhea and the hemolytic uremic syndrome in humans. Of the several serotypes, O157:H7 is widely distributed and caused several foodborne outbreaks in developed countries. A retrospective analysis of STEC collected over a period of 10 years has identified the serogroup O157 from humans, meat, milk and milk products, seafood, and surface waters (Sehgal et al. 2008). In most of the investigations, Shiga toxin-1 (*stx1*)-producing STEC was predominantly detected with or without *stx2* and other virulence factors such as intimin (*eaeA*), enterohemolysin (*ehxA*), and autoagglutinating adhesin (*saa*).

Nalidixic acid-resistant non-O157 serogroups commonly prevailed among hospitalized children with diarrhea or dysentery in Mumbai. They also suffered from hemolytic uremic syndrome and acute renal failure (Lanjewar et al., 2010). The non-O157 serogroups identified from the diarrheal patients as well as animal sources in Kolkata were resistant to tetracycline, ampicillin, cephalothin, and co-trimoxazole (Khan et al., 2002).

STEC belonging to O157 and non-O157 serogroups has been reported from the potable water distribution systems, dairy farm, slaughtered cattle, retail fish/shrimp, eggs, raw milk, vegetables, and fruits. Most of these STEC were resistant to cephalothin, neomycin, tetracycline, streptomycin, and ampicillin. Majority of STEC from non-human sources were resistant to cloxacillin and some isolates showed resistance to ampicillin, co-trimoxazole, amoxycillin, cephalothin, nalidixic, ciprofloxacin, neomycin, cefadroxil, cefoxitin, gentamicin, and tetracycline.

Diarrheic and neonatal calves act as a reservoir of STEC that can spread MDR strains (kanamycin, cephalexin, cephaloridine, cefotaxime, imipenem, amikacin, ampicillin, tetracycline, ceftiofur, nalidixic acid, ciprofloxacin, colistin, streptomycin, and co-trimoxazole resistance) to other farm animals as well as humans. Presence of STEC in healthy buffaloes and goats was reported in Kolkata, which are resistant to erythromycin, cephalothin, amikacin, kanamycin, and gentamicin, but not to β-lactam antibiotics (Mahanti et al., 2013). However, ESBL-producing STEC from raw milk samples contained *bla*$_{TEM}$ and resistant to penicillin, cefalexin, rifampicin, methicillin, and novobiocin (Joseph & Kalyanikutty, 2022).

In porcine, non-O157 either with *stx1* in combination or with *stx2* is more common than O157 serogroup. This STEC was highly resistant to ampicillin, tetracycline, streptomycin, lincomycin, nalidixic acid, sulfadiazine, penicillin,

gentamicin, kanamycin, and ceftriaxone. Farm piglets were positive for STEC with carbapenem-resistant (with $bla_{\text{OXA-48}}$) and ESBL producers with bla_{TEM}, $bla_{\text{CTX-M-1}}$, and $bla_{\text{CTX-M-15}}$, with the other ARGs (*qnrA, qnrB, qnrS, teB*, and *sul1*). STEC from piglets and animal handlers remain resistant to cephalexin, aztreonam, and amoxicillin, third-generation cephalosporins, except ceftriaxone (Puii et al., 2019). These ESBL producers harbored bla_{TEM}, bla_{SHV}, $bla_{\text{CMY-2}}$, and $bla_{\text{CTX-M}}$.

Multilocus variable number tandem repeat analysis (MLVA) showed the possible transmission of STEC to humans from contaminated meat and bovine sources. Analysis of global STEC categorized 61 STs, of which ST21, ST33, and ST416 remained either host-specific (ST33 and ST416) or can be shared among human and bovine hosts (ST21). Some of the STEC identified as ST58 of STEC from neonatal calves and dairy workers indicates their zoonotic potential (Angappan et al., 2021).

9.4 Enteroaggregative *E. coli*

EAEC is known to cause persistent and inflammatory diarrhea. Adherence to epithelial cells in a stacked brick-like pattern is the specific attribute of this pathogen when cultured with HEp-2 cells. The main virulence factors that could promote the pathogenicity includes adhesions and a heat-stable toxin. Epidemic diarrhea due to EAEC has been reported in several places of India. EAEC is classified as typical (tEAEC) and atypical (aEAEC) strains based on the presence or absence of the transcriptional regulator (AggR regulon).

Among the DEC, EAEC is the predominant pathogroup mainly found in developing countries. However, in many studies, prevalence of EAEC remained almost the same in diarrheal cases and control children. Detailed characterization of the isolates indicated that aEAEC strains were more common in the control group without diarrhea. From the diarrheal group, tEAEC isolates showed higher antibiotic resistance than aEAEC.

Generally, EAEC is resistant to several antibiotics compared to other pathotypes of DEC. From diarrheal children below 5 years of age, EAEC was resistant to co-trimoxazole, ampicillin, furazolidone, chloramphenicol, amoxicillin/clavulanic acid, nalidixic acid, norfloxacin, and ciprofloxacin (Raju and Ballal, 2009). In EAEC, $bla_{\text{CTX-M-27}}$ was most commonly reported ARG. Reports on EAEC from the environment and foods are limited. In a report it was shown contamination of leafy greens by MDR EAEC (Priyanka, et al., 2021).

10 *Listeria monocytogenes*

The Gram-positive *Listeria monocytogenes* belong to the phylum *Firmicute*s and family Listeriaceae. The genus *Listeria* consists of 20 species. Based on somatic and flagellar antigens, *L. monocytogenes* has been divided into 13 serotypes, which are grouped into 4 genetic lineages. Serotypes ½b, 3b, 4b, 4d, 4e, and 7 form lineage I, which commonly exist in food and human samples. Lineage II comprises serotypes

½a, ½c, 3a, and 3c. Serotypes 4b, ½a, 4a, and 4c belong to lineage III and 4a, 4c, and atypical 4b serotypes are considered as lineage IV. *L. monocytogenes* has several virulence factors including internalin (*inlAB*), positive regulatory factor A (*pfrA*), phospholipase C-PC-PLC (*plcB*), invasion, listeriolysin O (*hly*), metalloprotease (*mpl*), actin assembly-inducing protein (*actA*)-associated protein-p60 (*iap*). The *inlAB* locus and the *Listeria* pathogenicity islands LIPI-1, LIPI-3, and LIPI-4 harbor many virulence factors. LIPI-1 has been detected between the genes *prs* and *orfX* and comprises *prfA*, *plcA*, *hly*, *mpl*, *actA*, and *plcB.*

L. monocytogenes is one of the most invasive foodborne pathogens and is associated with a number of clinical syndromes such as mild to acute diarrhea, sepsis, meningitis, and abortion in human and animals. Infection often occurs after ingestion of contaminated foods that include dairy products, meat products, seafood, and raw vegetables. Reports on isolation of *L. monocytogenes* from diarrheal cases are rare in India. Serotypes 1/2a and 4b were predominantly reported from milk and milk products of Tamil Nadu. *L. monocytogenes* (serogroups 4b, 4d, 4e, 1/2a, 3a and 1/2b, and 3b) isolated from human, animal, and foods were mostly susceptible to most of the tested antibiotics except co-trimoxazole (Negi et al., 2015).

L. monocytogenes isolated from Ganges water (serogroups 1/2c and 3c) was uniformly resistant to ampicillin along with variable resistance to gentamicin, co-trimoxazole, ofloxacin, rifampicin, and tetracycline (Soni et al., 2013). Some of the *L. monocytogenes* isolated from domestic animals is mostly susceptible to gentamicin, co-trimoxazole, and erythromycin, but resistant to amoxicillin, doxycycline, erythromycin, and ampicillin (Barman et al., 2020). Serotypes 1/2a, 1/2b, 1/2c,, 3a, 3b, and 4b from bovine raw milk and beef samples were resistant to ampicillin, penicillin-G, amoxicillin–clavulanic acid, nalidixic acid, oxacillin, ceftriaxone, rifampicin, ciprofloxacin, clindamycin, and tetracycline (Soni et al., 2013, Swetha et al., 2021). Pathogenic *L. monocytogenes* 1/2a, 3a, 4b serogroups isolated from marine fish and fish products from Kerala showed resistance to ampicillin, cefixime, penicillin, erythromycin, tetracycline, and clindamycin (Menon et al., 2021).

Clonal analysis indicated that *L. monocytogenes* serotypes 1/2b, 2, 4b, 4d detected from clinical samples were not genetically related. In the PFGE, serotype 4b isolated from different animals, humans, foods, and the environment showed a single, indistinguishable pattern (Ind-4b-dom-pulsotype). Based on these results, it is evident that *L. monocytogens* serotype 4b is highly clonal distributed in several sources. PFGE results of *L. monocytogenes* from different sources in India indicate that foods of animal origin may act as a significant source of listeriosis among human.

11 *Salmonella* spp.

The genus *Salmonella* is one of the members of the family Enterobacteriaceae. This genus contains two species, *S. enterica* and *S. bongori*. On the basis of the somatic O (lipopolysaccharide) and flagellar H antigens, *S. enterica* has been further divided into more than 2500 serovars/serotypes (Kauffman–White serotyping scheme). The

full name of a serotype is given as, for example, *Salmonella enterica* subsp. *enterica* serotype Poona, but can be abbreviated to *Salmonella* Poona or *S*. Poona. Enteric fever caused by the host-adapted serovars *S*. Typhi and *S*. Paratyphi A, B and C, whereas the non-typhoidal salmonellae (NTS) have a broad host range. Epidemiologically, the proportion of non-typhoidal cases (52%) are more than that of typhoid cases (37%). Many serovars of *S. enterica* are distributed in the environment and are associated with infection in both humans and animals.

Enteric fever is caused by *S.* Typhi and *S.* Paratyphi (A, B, and C) has several features at various stages of the infection that include fever, bradycardia, abdominal pain, hepatosplenomegaly, intestinal bleeding, and perforation and translocation. The major virulence mechanisms of *Salmonella* spp. include internalization of host cells, endotoxins, exotoxins, virulence plasmids, type I (T1SS) and III secretion systems (T3SS), fimbriae, flagella, and ion transporters. Compared to strains from other countries, the Indian *S*. Typhi strains additionally had colicin V and bacteriocin production; multidrug resistance efflux pumps; ABC transporters; T3SS and T6SS, siderophore aerobactin, pathogenicity islands and Vi polysaccharide biosynthesis and transport, making them more virulent.

Chloramphenicol was widely used to treat enteric fever during the 1970s. Due to the emergence of resistant strains, this drug was substituted by ampicillin and co-trimoxazole. *S*. Typhi from typhoid cases during this period were susceptible to chloramphenicol, streptomycin, tetracycline, ampicillin, and co-trimoxazole. Resistance to these antibiotics started emerging during late 1970s. Due to the emergence of MDR in typhoid endemic Asian regions, most of these drugs became less effective.

Chloramphenicol resistance in *S.* Typhi is medicated by a conjugative plasmid of the IncHI1, which is very similar in different geographic isolates. In the early 1980s, there was a rapid emergence of strains in India with IncHI1 plasmid-mediated resistance to all these three drugs and this has advocated the use of fluoroquinolones, which were highly efficacious till 1990s. Using the plasmid transfer system, it was demonstrated that ampicillin, chloramphenicol, co-trimoxazole, and tetracycline resistance has been transferred to antibiotic-sensitive *S*. Typhi strains. Resistance of *S.* Typhi to chloramphenicol, ampicillin, and co-trimoxazole continued till mid-2000s in several parts of the country. Higher prevalence of *S.* Typhi resistance to chloramphenicol, ampicillin, tetracycline, and co-trimoxazole was seen in children with enteric fever in Kolkata during 1990–92 (Rasaily et al., 1994).

An increase in resistance was also noticed in *S.* Typhi to cephalosporins and β-lactams. No fluoroquinolone resistance was observed in 1990, but there was a steady increase of resistance during the late 1990s to ciprofloxacin, sparfloxacin, and ofloxacin (Kumar et al., 2008). Decreased susceptibility to ciprofloxacin has been reported in India during the late 1990s, and as a consequence, several treatment failures have been reported. Mutations in the QRDR regions were found to be associated with decreased susceptibility to ciprofloxacin. Patients with nalidixic acid-resistant *S*. Typhi (NRST) had a significantly longer duration of infection compared to nalidixic acid-susceptible *S*. Typhi group. In addition, higher levels of aspartate aminotransferase, higher frequency of hepatomegaly, and clinical failure of

fluoroquinolone therapy were found to be associated with NRST. Furazolidone was recommended as an alternative drug to chloramphenicol resistance enteric fever caused by *S*. Typhi. Third-generation cephalosporins was also used in the treatment of typhoid. Complementing azithromycin with cephalosporins is advocated in non-responsive patients.

From the early 2000s, there was a sharp decrease in *S*. Typhi resistance toward chloramphenicol, ampicillin, and co-trimoxazole. Ceftriaxone and azithromycin have become the drugs of choice for treating salmonellosis due to the emergence of fluoroquinolone resistance during this time. These drugs have been increasingly used either as a mono or dual antibiotic therapy, as the susceptibility of the pathogen to these drugs was high. An increase in resistance to ceftriaxone has been noticed in 2016 (Kokare et al., 2021). Several reports suggest the emergence of ceftriaxone- and azithromycin-resistant isolates due to selective pressure. Several *S*. Typhi isolates from South India were found to be resistant to amikacin, gentamicin, imipenem, meropenem, colistin, and cefepime. A multicentric study conducted during 2017–20 has shown an overall declining trend of MDR in typhoidal *Salmonella* isolates, including ampicillin, chloramphenicol, β-lactams, and co-trimoxazole (Veeraraghavan et al., 2021). However, resistance to ciprofloxacin still remains high in several typhoid endemic areas.

Several aquatic sources act as a reservoir of *S*. Typhi. Outbreaks of typhoid have been reported due to MDR *S*. typhi and *S*. Paratyphi A. In Chandigarh, a typhoid outbreak was linked with fecally contaminated drinking water (Gupta et al., 1986). Asymptomatic carriers of the pathogen play a crucial role in the outbreaks of typhoid. As an evidence, suspected food handlers were found to carry MDR *S.* Typhi (Senthilkumar & Prabakaran, 2005).

Since typhoid is caused by a diverse population of *S*. Typhi, knowledge on ARGs and genetic differences are important to better understand its epidemiology. MLST of clinical strains collected during 2010–2013 indicated the existence of sequence types ST1, ST2, and ST3 in *S*. Typhi and ST85 and ST129 in S. Paratyphi A, suggesting the common spread of the sequence types across the globe. Of these, ST1 and ST2 are prevailing since 1990 in India. An antimicrobial resistance surveillance network report also highlighted the Indian clinical *S.* Typhi isolates belonged to sequence types ST1 and ST2 and the S. Paratyphi A was clustered in ST85 and ST129 (Dahiya et al., 2017). Haplotyping is a genetic marker that uses a combination of alleles with different chromosomal single nucleotide polymorphisms (SNPs). This scheme has divided the *S*. Typhi population into more than 80 haplotypes (H). *S.* Typhi H58 was found mainly associated with MDR that has undergone a global clonal expansion. H58 has emerged 30 years before and spread to many Asian and African countries. In India, this haplotype prevailed since the early 1990s. Fluoroquinolone-resistant *S*. Typhi H58 was predominantly detected in India (Samajpati et al., 2021). Genomic analysis of ceftriaxone-resistant *S*. Typhi H58 has shown persistence of resistance plasmids such as IncX3 with $bla_{\text{SHV-12}}$ or IncN with $bla_{\text{TEM-1B}}$ and $bla_{\text{DHA-1}}$ (Samajpati et al., 2021).

In addition to the mutations in the topoisomerase genes *gyrA*, *gyrB*, and *parC,* fluoroquinolone resistance in *S*. Typhi is also due to the presence of plasmid-

mediated quinolone resistance (PMQR). Ciprofloxacin-resistant *S*. Typhi blood isolates carried the PMQR gene *qnrB*. *S.* Typhi harboring $bla_{\text{SHV-12}}$ has been detected in travelers who had a history of visiting India. Cefixime-resistant *S*. Typhi strain was negative for cephalosporin resistance encoding gene $bla_{\text{CTX-M}}$ but had $bla_{\text{TEM-1B}}$ and $bla_{\text{DHA-1}}$ along with *qnrB4* and *sul1*. Overexpression of bla_{TEM} by these isolates might have caused cephalosporin resistance (Devanga Ragupathi et al., 2016).

In NTS, the presence of virulence encoding genes such as *invA* (invasive), *stn* (*Salmonella* enterotoxin), *spvR/spvC* (*Salmonella* plasmid virulence), and *fimA* (fimbriae) is associated with their pathogenicity. NTS serovars such as *S*. Choleraesuis, *S*. Typhimurium, and *S*. Worthington were reported to cause typical enteric fever with the clinical features of meningitis and septicemia. *S.* Typhimurium has been detected almost equally in diarrheal and non-diarrheal children, indicating its wide occurrence in the community (Saha et al., 1992). *S*. Kentucky, *S*. Infantis, *S.* Typhimurium *S*. Seftenberg, and *S*. Virchow were the serovars associated with children with diarrhea and displayed resistant to chloramphenicol, tetracycline, furazolidone, co-trimoxazole, nalidixic acid, ciprofloxacin, ampicillin, norfloxacin, and gentamicin. Many reports indicated an association of *S*. Weltevreden in several outbreaks (Wattal et al., 1994, Chowdhury et al., 2013).

Salmonella serovars (Enteritidis, Newport, Paratyphi B, Teko, Virchow, Saintpaul, Typhimurium) from several street vended foods were found to be resistant to nalidixic acid, cephalothin, cefoperazone, sulfamethizole, furazolidone, kanamycin, oxacillin, cefoxitin, ampicillin, doxycycline, and cefotaxime. NTS isolated from humans, animals, raw chevon and chicken meats, and slaughtered food animals were resistant against erythromycin and metronidazole, clindamycin, ampicillin, co-trimoxazole, oxytetracycline, carbapenem, and colistin. The presence of salmonellae from the waters was correlated with higher levels of biochemical oxygen demand, total and fecal coliforms. *Salmonella* spp. from such environments showed resistance to cefoperazone, cefotaxime, cefixime, moxifloxacin, piperacillin/tazobactam, co-trimoxazole, levofloxacin, trimethoprim, and ceftazidime. *S.* Senftenberg, *S*. Typhimurium, *S*. Agon, *S*. Paratyphi, and *S*. Newport isolates from lake waters and backwater environments and water/sediment samples from Bays were resistant to oxytetracycline, tetracycline, streptomycin, ampicillin, amikacin, kanamycin, nalidixic acid, amoxicillin/clavulanic acid, co-trimoxazole, chloramphenicol, cephalothin, and cephalexin.

S. Gallinarum caused several major outbreaks in different poultry farms. Most of the isolates were resistant to penicillin, erythromycin, streptomycin, co-trimoxazole, cloxacillin, ampicillin, azithromycin, cefepime, cefixime, ceftazidime, chloramphenicol, and tetracycline (Sannat et al., 2017). *S.* Weltevreden and *S*. Typhimurium from poultry meat and poultry farm environments showed resistance to doxycycline, oxytetracycline, neomycin, nalidixic acid, co-trimoxazole, nitrofurantoin, erythromycin, tetracycline, and ceftizoxime (Olukemi Adesiji et al., 2017). Apart from the QRDR mutations, PMQR was detected in *S.* Weltevreden and *S*. Typhimurium from poultry with the presence of *qnrA*, *qnrB,* and *qnrS* (Olukemi Adesiji et al., 2017).

S. Typhimurium resistant to cefalexin was identified in piglets with diarrhea. Pathogenic *S*. Weltevreden and *S*. Enteritidis isolated from swines were mainly

resistant to tetracycline and ampicillin, and a few were also to streptomycin, amoxicillin–clavulanate, cefotaxime, and ceftriaxone. These NTS harbored ARGs for ESBL (bla_{TEM} and bla_{OXA}), aminoglycoside (*strA, strB*, and *aadA1*), sulfonamide (*sul1, sul2*, and *dfrA1*), tetracycline (*tetA* and *tetB*), and a plasmid with AmpC β-lactamase (Karabasanavar et al., 2022).

NTS (Weltevreden, Rissen, Bareilly, Irumu, Newport, Ohio, and Oslo) from seafood including exported products were resistant to chloramphenicol, amoxicillin–clavulanic acid, cefalotin, streptomycin, co-trimoxazole, carbenicillin, and oxytetracycline. *S.* Anatum, *S.* Weltevreden, *S.* Rostock, *S.* Typhimurium, and *S.* Gallinarum from buffalo meat and milk products were resistant to streptomycin, kanamycin, gentamicin, ampicillin, and oxytetracycline (Singh et al., 2010). Several serotypes of *Salmonella* isolated from leafy greens and other vegetables were resistant to amoxicillin/clavulanic acid, ampicillin, gentamycin, colistin, sulfamethoxazole, nalidixic acid, kanamycin, imipenem, and amikacin (Singh et al., 2007, Priyanka et al., 2021).

Genetically dissimilar NTS was found to have several AMR mechanisms. ESBL-positive NTS from various clinical specimens harbored genes encoding for TEM-1, SHV-12, DHA-1, OXA-1-like, and CTX-M-15. Mostly, the $bla_{CTX\text{-}M\text{-}15}$ has been commonly reported in various NTS. In the clinical isolates of *S.* Senftenberg, β-lactamase activity was due to the presence of $bla_{CTX\text{-}M\text{-}15}$ and $bla_{CMY\text{-}2}$ and in other NTS *S.* Thompson, *S.* Infantis**,** and *S.* Newport, only the $bla_{CTXM\text{-}15}$ was detected (Taneja *et al.,* 2014). ESBL-producing *Salmonella* spp. from diarrheal patients in Mizoram mostly harbored non-transferable plasmid having a $bla_{CTX\text{-}M\text{-}1}$ gene (Warjri *et al.,* 2015). A study conducted in Kolkata showed dominance of S. Worthington, S. Enteritidis, and S. Typhimurium among diarrheal children during 2000–16 (Jain *et al.,* 2020). Generally, these serovars were resistant to nalidixic acid, ampicillin, third-generation cephalosporins, and azithromycin but remained susceptible to fluoroquinolones. $bla_{CTX\text{-}M\text{-}15}$ and $bla_{SHV\text{-}12}$ and *mphA* were the most common ARGs in S. Worthington. The most frequent STs identified in this study include ST36 and ST19 in *S.* Typhimurium and ST11 and ST1975 in *S.* Enteritidis. ST36 and ST19 S. Typhimurium were also detected in both clinical and livestock/food samples, of which ST19 isolates were MDR. Widely circulating fluoroquinolone-resistant *S.* Kentucky ST198 reported in Europe and North America was identified in India from several sources during 2014–2017 (Mahindroo et al., 2019).

An IncA/C plasmid harboring clinical *S.* Senftenberg ST14 isolate was resistant to β-lactams due to the presence of $bla_{TEM\text{-}1}$, $bla_{OXA\text{-}9}$, $bla_{CMY\text{-}2}$, and $bla_{NDM\text{-}1}$ genes. In addition, resistance to aminoglycoside [*aac(6′)-Ia, aac(6′)-Ib, aph(3′)-Ib, aph(6′)-Ib*, and *ant(3′)-Ia*], sulfonamide (*sul-1* and *sul-2*), and chloramphenicol (*florR*) was also detected along with mutations in the QRDR for fluoroquinolone resistance (Veeraraghavan et al., 2019). The proportion of ceftriaxone resistance in clinical NTS (serogroups B, E, and C1/C2) was about 5% due to the presence of $bla_{CTX\text{-}M\text{-}1}$, bla_{TEM}, bla_{CMY}, and bla_{SHV} in the IncH12 plasmid.

The emergence of azithromycin resistance, conferred by a point mutation in a multidrug efflux pump encoding *acrB* (AcrB-R717Q/L), was first reported in Bangladesh in 2019, followed by many reports from the other countries.

S. Typhimurium ST313 causes invasive disease in sub-Saharan African and other gastrointestinal infections in the UK and Brazil. This lineage in India had an aminoglycoside-modifying enzyme encoding *aac(6′)-Ia* in the genome, which was genotypically distinct from the African, UK, and Brazilian lineages that holds the *bstA* virulence gene as a part of a novel repertory of prophage Blantyre Prophage BTP-1.

12 *Shigella* spp.

Shigella spp. is a Gram-negative, non-motile bacterium and is a member of the family Enterobacteriaceae. The genus *Shigella* is divided into four species and subdivided into serogroups/serotypes on the basis of O-specific polysaccharide of the LPS that include *S. dysenteriae* (serogroup A, 15 serotypes), *S. flexneri* (serogroup B, 19 serotypes), *S. boydii* (serogroup C, 20 serotypes), and *S. sonnei* (serogroup D, single serotype). The symptoms of shigellosis include diarrhea and/or dysentery with frequent mucoid bloody stools, abdominal cramps, and tenesmus. Dysentery is caused due to invasion of bacterial cells in the colonic mucosa, multiplication of the pathogen within the epithelial cells, inflammation, bleeding, and cell death. Pathogenesis of *Shigella* is associated with the virulence plasmid, which contain most of the important virulence factors such as T3SS encoding *mxi-spa* locus and effector/translocator protein encoding *ipa–ipg* operon. In addition, distinct chromosomal regions have also been known to contribute to infection, as it contains several pathogenicity islands encoding the other virulence factors. Virulence genes such as *ipaD, ipaH, virF, senB, iha, capU, lpfA, sigA, pic, sepA, celb*, and *gad* have been identified either in the plasmid or on the chromosome. Shiga-like toxin produced by *S. dysenteriae* serotype 1 causes the most serious hemolytic uremic syndrome. Transmission of *Shigella* occurs through contaminated food and water or through person-to-person contact. A low infectious dose (10–100 organisms) helps *Shigella* to spread effectively. Due to less dehydration during the course of infection, antimicrobials are extensively used in the treatment of shigellosis.

Shigella is the second leading cause of bacterial diarrhea worldwide and has been categorized as a priority pathogen among enteric bacteria by the Global Antimicrobial Resistance Surveillance System of the World Health Organization. Most of the outbreaks reported till early 2000s are mainly associated with *S. dysenteriae* serotype 1. Nalidixic acid-resistant strain was predominant among dysentery cases during early 1990s. The newer clone of *S. dysenteriae* serotype 1 that emerged after 14 years in India had increased MDR (ampicillin, co-trimoxazole, tetracycline, chloramphenicol, nalidixic acid, norfloxacin, lomefloxacin, pefloxacin, and ofloxacin resistance) and showed reduced susceptibility to ciprofloxacin. However, this serotype has perpetually disappeared from the mid-2000s all over the world. *S. flexneri* type 2a and *S. sonnei* were also reported to cause outbreaks. The trend of shigellosis till 2010 was dominated by *S. flexneri* > *S. dysenteriae* > *S. sonnei* > *S. boydii*. In the following years, *S. flexneri* was replaced by *S. sonnei.*

The initial drugs used to treat *Shigella* infections include ampicillin and sulfonamides, which were replaced by tetracycline and then by chloramphenicol. Conversely, treatment recommendations were again changed to nalidixic acid because

Shigella developed resistance to the former drugs. In clinical trials conducted in Kolkata, nalidixic acid or norfloxacin were found to be efficacious for the treatment of shigellosis. Later, resistance to nalidixic acid was developed and, for this, fluoroquinolones were introduced. After 2005, resistance to newer generation fluoroquinolones and third-generation cephalosporins have been increasingly reported in several States of India. The ESBL-producing strains were also resistant to ampicillin, ampicillin/sulbactam, co-trimoxazole, ciprofloxacin, ofloxacin, and gentamicin. *S. flexneri* from diarrheal patients has become resistant to ceftriaxone.

Decreased susceptibility to azithromycin appeared from 2001. In the following years, MDR *Shigella* spp. (nalidixic acid, ciprofloxacin, co-trimoxazole, azithromycin, ceftriaxone, and cefotaxime) had the azithromycin resistance gene (*mphA*) that encodes macrolide 2′-phosphotransferase. Some of the azithromycin-resistant *S. sonnei* carried *sul1,* bla_{DHA1}*, qnrB4, mphA, tetR* and in an IncFII plasmid. Ceftriaxone resistance encoding genes bla_{TEM} $bla_{\text{CTX-M-15}}$, $bla_{\text{CMY-2}}$, and bla_{OXA} were also frequently detected. The presence of the quinolone and cephalosporin resistance genes is common in *Shigella* spp. Among β-lactamases, $bla_{\text{OXA-1}}$ was predominantly detected, followed by the $bla_{\text{TEM-1B}}$, bla_{EC}, and $bla_{\text{CTX-M-15}}$ along with plasmid-mediated AmpC β-lactamases genes (Taneja et al., 2012). Several mutations in the QRDR, the efflux pumps, and PMQR genes (*qnrB* and *qnrC*) increased fluoroquinolone resistance in *S. sonnei*. *aac(6′)-Ib-cr* and *qnrS1* were predominantly detected in *S. flexneri* isolated since 2002 (Taneja et al., 2014). Fluoroquinolone resistance, integrons, and PMQR genes such as *aac(6′)-Ib-cr* and *qnrS1* were detected more in diarrheal children than the control group.

The disposal of sewage into natural water bodies is an immense public health problem. Contaminated water was responsible for epidemic dysentery in Vellore and the responsible pathogen identified was *S. dysenteriae* serotype 1 and other species of *Shigella. S. dysenteriae, S. flexneri*, and *S. sonnei* isolated from river Narmada during 2005–2006 were resistant to ciprofloxacin, chloramphenicol, trimethoprim, cefotaxime, and amikacin. bla_{TEM}-positive *S. sonnei* from river water, β-lactam-resistant *S. dysentery* from sewage effluents, and $bla_{\text{NDM-5}}$-positive *S. boydii* from hospital sewage water were identified. *Shigella* spp. has also been identified from domestic animals, supplementary milk feeds. Tetracycline, cefalotin, streptomycin, amoxicillin–clavulanic acid, ampicillin, ceftriaxone-resistant *Shigella* spp. was reported in exported seafood (Obaidat & Bani Salman, 2017).

Phylogenetic and evolutionary analysis revealed that the majority of the isolates belonged to phylogenetic group 3 within the predominance of *S. flexneri* serotype 2 and the ciprofloxacin-resistant *S. sonnei* included along with the lineage III MDR clones of Central Asia. *S. sonnei* strains were more clonally related when compared to the other *Shigella* spp.

13 Pathogenic vibrios

Members of the genus *Vibrio* mostly reside in the aquatic environment and are distinguished as Gram-negative rods, motile, and have a single polar flagellum. It has 4 different families and more than 70 species, of which 11 are considered clinically

important. *Vibrio cholerae*, *V. parahaemolyticus*, and *V. fluvialis* are the important pathogens as they cause epidemics and severe gastroenteritis in humans.

13.1 *Vibrio cholerae*

Cholera is caused by toxigenic strains of *Vibrio cholerae* due to the expression of cholera toxin (CT, encoded in the gene *ctxAB*) in the intestinal milieu. The sequels of this infection include the excretion of substantial volumes of watery stool, loss of electrolyte, dehydration that results in hypovolemic shock, and metabolic acidosis. The global burden of cholera is projected between 1.4 and 4.3 million cases with about 21,000–143,000 deaths per year. Of the 200 O-serogroups of *V. cholerae*, epidemic cholera is caused by the O1 and O139 serogroups. The rest are known as non-O1, non-O139 serogroups, which cause cholera-like diarrhea. The serogroup O1 is classified into two biotypes, classical and El Tor and each biotype into Ogawa and Inaba serotypes. The O1 serogroup has caused seven cholera pandemics, the first six pandemics being caused by the classical biotype. The current seventh cholera pandemic instigated by the El Tor biotype in 1961. In 1992, a new serogroup O139 emerged in the Indian subcontinent and briefly replaced the El Tor vibrios in Asia until mid-2000s. The major virulence factors involved in cholera/acute diarrheal infection include CT, hemolysin, heat-stable enterotoxin, T3SS, and T6SS. Genetic changes in the conserved genes like *ctxB, tcpA* have been used as markers of clonal expansion of *V. cholerae*.

In India, more than 500 outbreaks of cholera have been recorded between 2009 and 2020, affecting about 30 states and union territories of India (Muzembo et al., 2022). Of these, 62 were identified with MDR *V. cholerae*. Cholera outbreaks associated with natural disasters and *V. cholerae* has been found in water samples, including *ctx*-positive non-O1/non-O139 strains. During 1992–93, serogroup O139 dominated cholera scenario in India, but waned during 1995–1996 and appeared again in 1997 with the sporadic isolation until 2005. The re-emerged *V. cholerae* O139 displayed a dramatic shift in patterns of AMR to co-trimoxazole, neomycin, and streptomycin (Basu et al. 2000). Serogroup involving both the O1 and O139 in a cholera outbreak has also been reported (Chakraborty et al., 2001). The cholera burden in the slums is high due to multiple risk factors such as infected household members, young age, and low educational level. A large proportion of outbreak isolates of *V. cholerae* O1 from South India during 2006–2009 were resistant to ampicillin, polymyxin B, nalidixic acid, co-trimoxazole, norfloxacin, ciprofloxacin, and doxycycline.

The non-O1 and non-O139 serogroups are clinically important, as their contribution is significant in the epidemiology of acute diarrhea. These serogroups are present in various aquatic environments with different putative virulence factors. *V. cholerae* O1 as well as the non-toxigenic non-O1 and non-O139 serogroups have been isolated from the fresh waters (Mishra et al., 2012). Fresh and raw seafood from Cochin contained toxigenic *V. cholerae* O1 as well as the non-toxigenic non-O1 and

non-O139 serogroups. The non-toxigenic strains were resistant to cefpodoxime and colistin (Kumar and Lalitha, 2013).

AMR pattern of *V. cholerae* has been constantly changing. The changing AMR trend in *V. cholerae* has been depicted in Fig. 1. Sulfamethoxazole trimethoprim-constin (SXT) is an integrating conjugative element (ICE) that was identified in a *V. cholerae* O139 from India. The SXT is a self-transferring large genomic segment, which carry many functions related to bacterial adaptation as well as AMR. These ICEs comprise several ARG arrays and its configuration might change over the years at the regional level. *V. cholerae* O1 isolated between 2008 and 2015 in Kolkata carried two types of ICEs, one with tetracycline (*tetA*) and the other with chloramphenicol (*floR*) resistance (Sarkar et al., 2019). In *V. cholerae* class 1 integron, resistance gene cassettes containing *aadA* (streptomycin and spectinomycin), *blaP1* (β-lactams), *aar-3* (rifampicin), *aacA4* (kanamycin and gentamicin), *dfrA* (trimethoprim), and *ereA2* (erythromycin) have also been reported (Thungapathra et al., 2002).

Tetracycline is the important drug used in the treatment of cholera. Resistance to this drug was dominant during 1989 in Kolkata, 2007 in Delhi, Kolkata, 2008 in Chandigarh, 2010 in Karnataka, Odisha, Port Blair, 2003–2010 in Assam. Resistant to quinolones and fluroquinolones in *V. cholerae* O1 are due to mutations in the QRDRs as well as the presence of PMQR *qnrVC*. Tetracycline, cefotaxime, ciprofloxacin- and azithromycin-resistant strains of *V. cholerae* from North India also harbored bla_{TEM}, bla_{CMY}, $bla_{\text{CTX-M-15}}$, $bla_{\text{OXA-48}}$, and *ermB* and rarely the bla_{NDM} (Gupta et al., 2022). *V. cholerae* O1 sensitive to polymyxin B was reported in isolates collected between 2005 (*ctxB1*) and 2019 (*ctxB7*) (Samanta et al., 2020). Mutation in the two-component system encoded by *carRS* and downregulation of the *almEFG*

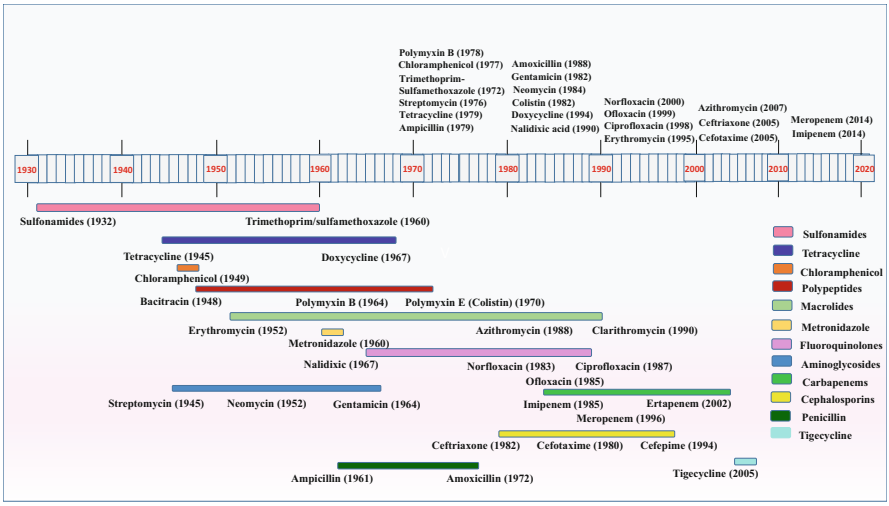


Fig. 1 Changing antimicrobial resistance in clinical isolates of *V. cholerae*. Years in parentheses above and below the timeline bar indicate emergence of resistance in *V. cholerae* and commencing year for clinical use, respectively

operon has been identified for the shift of polymyxin B resistance to sensitive phenotype (Samanta et al., 2020). This change might affect the conventional classification of classical and El Tor biovars.

Intermittent appearance of fluoroquinolone resistance in *V. cholerae* has been reported in various states of India. *V. cholerae* non-O1/non-O139 from potable/coastal water samples were highly resistant to cefotaxime, nalidixic acid, streptomycin, and tetracycline, and few are also resistant to ciprofloxacin, norfloxacin, spectinomycin, gentamicin, chloramphenicol, ampicillin, and erythromycin (Jagadeeshan et al., 2009). Chromosomally mediated $bla_{\text{NDM-1}}$ was reported in *V. cholerae* isolated from water samples (Walsh et al., 2011).

Constant shift in the genome of *V. cholerae* leads to the emergence of genetically and phenotypically varied pandemic clones of *V. cholerae* in Asia and Africa. Toxigenic *V. cholerae* O1 El Tor Ogawa strains with *ctxB1, ctxB3,* and *ctxB7* genotypes and ampicillin, nalidixic acid, furazolidone, and co-trimoxazole resistance prevails in India. *V. cholerae* from the Asian region always harbors ICE*Vch*Ind5. Analysis of WGS of MDR *V. cholerae* has identified three waves of seventh pandemic cholera with ST69 emerged from South Asia and caused outbreaks in East Africa, Western Asia, and Latin America (Mutreja et al., 2011). Since 1970, a single expanded lineage was introduced more than 10 times into West Africa and East/Southern Africa, instigating epidemics that lasted 28 years. Recent MDR sublineages from Asia spread to Africa and replaced antibiotic-susceptible native sublineages after 2000 (Weill et al., 2017).

13.2 *V. parahaemolyticus*

Vibrio parahaemolyticus is a leading cause of seafood-associated illness. The disorders caused by this pathogen include gastroenteritis with or without blood in the stools, wound infections, and septicemia. Serotyping of *V. parahaemolyticus* is based on somatic (O) and capsular (K) antigens using a combination of 11 different O and 71 K antisera. *V. parahaemolyticus* express a variety of virulence factors, including the thermostable direct hemolysin (TDH) responsible for the Kanagawa hemolysin, TDH-related hemolysin, and T3TSS located on both the chromosomes.

The pandemic serovar O3:K6 was detected for the first time in 1996 from diarrheal patients in Kolkata and spread to several South-Asian countries, Europe, and Latin America (Nair et al., 2007). Pandemic *V. parahaemolyticus* has a unique *toxRS* sequence, a filamentous phage f237, and belongs to ST3. Other serovars, like O4:K68, O1:K25, and O1:KUT (untypable), with pandemic strain genetic traits subsequently reported in the following years. Several findings indicated the prevalence of *V. parahaemolyticus* among hospitalized diarrheal patients as well as in the community. AMR is not very common in clinical isolates of *V. parahaemolyticus*. Fee reports indicated that clinical isolates of *V. parahaemolyticus* were resistant to ampicillin, streptomycin, nalidixic acid, and chloramphenicol.

Abundance of *V. parahaemolyticus* finfish and shellfish is due to the excess time difference between catch and sale and also lack of cold temperature in the markets. MDR in *V. parahaemolyticus* has emerged because of mismanagement of antibiotics to control infections in aquaculture production. The most frequently observed AMR includes ampicillin, penicillin, and tetracycline. Cephalothin and nitrofurantoin resistance also reported in isolates from the water and sediment samples of South India. *V. parahaemolyticus* was highly prevalent in finfish samples obtained from retail outlets and showed resistant to ampicillin, streptomycin, carbenicillin, cefpodoxime, cephalothin, colistin, and *amoxycillin*. *V. parahaemolyticus* isolates from finfish samples imported to Jordan were resistant to colistin, neomycin, kanamycin, tetracycline, and ampicillin. *V. parahaemolyticus* from cultured shrimps in brackish water aquaculture farms were not only pathogenic by harboring potential virulence genes, but also exhibited resistance to β-lactams, erythromycin, and nalidixic acid. ESBL and carbapenemase producers were identified from retail seafood. Pandemic serovars of *V. parahaemolyticus* were identified from the oysters and majority of the isolates exhibited resistant to cefpodoxime, ampicillin, cefotaxime, ceftizoxime, tetracycline, and ceftriaxone.

13.3 *Vibrio fluvialis*

V. fluvialis is known to cause cholera-like diarrhea with different virulence factors including hemolysin and T3SS. Several outbreaks and sporadic cases of acute diarrhea caused by this emerging pathogen have been reported. A hospital-based study indicated the prevalence of *V. fluvialis* among all age groups of acute diarrheal cases and also caused an outbreak (Chowdhury et al., 2013). Long-term studies conducted in Kolkata showed that AMR in this pathogen is very dynamic. *V. fluvialis* with class 1 integron contained a novel aminoglycoside acetyltransferase gene (*aac (3)-Id*) and an aminoglycoside adenyltransferase gene (*aadA7*). Azithromycin resistance encoding gene *mphA* harboring *V. fluvialis* isolated from diarrheal patients was predominant during 2014–15 in Kolkata (Chowdhury et al., 2019). These MDR isolates belonging to different genetic lineages were also resistant to β-lactam antibiotics ($bla_{\text{OXA-1}}$, $bla_{\text{OXA-7}}$, and $bla_{\text{TEM-9}}$) and aminoglycoside acetyltransferase, conferring resistance to ciprofloxacin-modifying enzyme (*aac[6']Ib-cr*). The NDM-1-positive isolates were resistant to all the tested antimicrobial drugs except doxycycline. The class 1 integron located on a low copy number plasmid harbored variable region of *arr3-cmlA-*bla_{OXA10}*-aadA1* gene cassettes.

MDR *V. fluvialis* had mutations in the QRDRs of GyrA and carried a transferable plasmid harboring the quinolone resistance gene *qnrA1* in a complex *sul1*-type integron, *aac(6')-Ib-cr*, and genes encoding for extended-spectrum β-lactamases such as bla_{SHV} and $bla_{\text{CTX-M-3}}$ (Chowdhury et al., 2011). Quinolone resistance *V. fluvialis* harbored *qnrVC5*. In addition, the efflux pumps make the isolates resistant to chloramphenicol, kanamycin, streptomycin, and tetracycline.

14 *Yersinia enterocolitica*

Yersiniosis is caused by Gram-negative bacterium *Yersinia enterocolitica*, which belongs to the Enterobacteriaceae family. *Y. enterocolitica* causes gastrointestinal infection with fever, abdominal pain, and diarrhea. The other clinical manifestations include mesenteric lymphadenitis and endocarditis. This pathogen is psychrotrophic and hence can well replicate at low temperatures and persists in frozen foods/liquids for a long period. *Y. enterocolitica* has been isolated from a variety of domestic animals, and pigs are considered as the main reservoir. *Y. enterocolitica* has about 60 serotypes and six biovars (1A, 1B, 2, 3, 4, and 5), which differ in geographical dissemination, ecological niche, and pathogenicity. Biovar 1A has been described as a non-pathogenic found in healthy people. Serotypes O3, O8, O9, and O5. 27 are mostly reported in human yersiniosis, of which serotype O8 causes acute infection with severe ulceration of the gastrointestinal tract.

Both plasmid and chromosome of *Y. enterocolitica* involved in the expression of several virulence factors, including Ysc (a T3SS), several immunomodulatory *Yersinia* outer proteins (Yops) and invasion (adhesion and invasion), YstA,B (heat-stable enterotoxins), and yersiniabactin (catecholtyle). Of these, *ystB* gene was more prevalent in *Y. enterocolitica* biotype 1A. In some of the clinical strains of *Y. enterocolitica* biovar 1A, iron acquisition and storage and flagellar proteins are also considered as virulence factors.

Y. enterocolitica has been detected in clinical, environmental samples, including raw milk, traditional fermented foods, fast foods, meat, and meat products. An outbreak associated with consumption of buttermilk contaminated with *Y. enterocolitica* has also been reported (Abraham et al., 1997). Multilocus enzyme electrophoresis and multilocus restriction typing indicated the genetic relationships between some of the clinical and food isolates of *Y. enterocolitica* biovar 1A.

Ofloxacin resistance was common in most of the *Y. enterocolitica* isolated from gastroenteritis cases (Lal et al., 2003). Genetically diverse *Y. enterocolitica* from fish and chicken sources were resistant to ciprofloxacin and amoxicillin. *Y. enterocolitica* biovar 1A belonging to the serovars O:6, 30–6, 31 had an association between virulence factors and amoxicillin–clavulanate resistance (Singhal et al., 2016). *Y. enterocolitica* biotype 1A isolated from clinical and non-clinical strains were resistant to the first-generation cephalosporin–cefazolin and third-generation cephalosporins–cefixime and macrolides–erythromycin. The weak biofilm-producing isolates were mostly resistant to amoxicillin and cefazolin.

15 Conclusion and Future Perspectives

Long-term multi-sector use of antimicrobials has changed the susceptibility patterns of several enteric pathogens. For some AMR pathogens, there is a constant healthcare challenge due to limited treatment options. The genetic milieu of these microbes is constantly changing and the ARGs are accumulating in their genome. As a consequence, their reversion to natural susceptibility might take years, even in the

absence of any antimicrobial pressure. Considering the AMR burden, it is important to implement the One Health program. This effort needs steps to eliminate mass medication of animals with antimicrobials; reduction in the over-prescription of the antimicrobials, improvement in the sanitation and hygiene, implementation of appropriate vaccines in humans and animals, adequate treatment of industrial, residential, and farm waste are needed to benefit humans and animals. Mandatory actions for the improvement in antimicrobial use regulation and policy, surveillance, stewardship, and infection control programs should be in place, acquiring necessary help from public authorities, political leaders, and economic actors. Research should focus on complex bacterial resistance structures in humans, animals, and the environment using cost-effective and rapid methods.

Acknowledgments This study was supported in part by the Indian National Science Academy (INSA/SP/SS/2019) and the Indian Council of Medical Research (AMR/TF/55/13ECDII).

References

Abraham, M., Pai, M., Kang, G., Asokan, G. V., Magesh, S. R., Bhattacharji, S., et al. (1997). An outbreak of food poisoning in Tamil Nadu associated with *Yersinia enterocolitica*. *The Indian Journal of Medical Research, 106*, 465–468.

Abuderman, A. A., Mateen, A., Syed, R., & Sawsan Aloahd, M. (2018). Molecular characterization of *Clostridium difficile* isolated from carriage and association of its pathogenicity to prevalent toxic genes. *Microbial Pathogenesis, 120*, 1–7. https://doi.org/10.1016/j.micpath.2018.04.013

Ali, A., Sultan, I., Mondal, A. H., Siddiqui, M. T., Gogry, F. A., & Haq, Q. M. R. (2021). Lentic and effluent water of Delhi-NCR: A reservoir of multidrug-resistant bacteria harbouring $bla_{\text{CTX-M}}$, bla_{TEM} and bla_{SHV} type ESBL genes. *Journal of Water and Health, 19*(4), 592–603. https://doi.org/10.2166/wh.2021.085

Ananthan, S., Swarna, S. R., & Alavandi, S. V. (1998). Isolation of nalidixic acid resistant campylobacters from cases of paediatric diarrhoea in Chennai. *The Journal of Communicable Diseases, 30*(3), 159–162.

Angappan, M., Ghatak, S., Milton, A. A. P., Verma, A. K., Inbaraj, S., Abhishek Chaudhuri, P., et al. (2021). Detection of novel sequence types and zoonotic transmission potentiality among strains of Shiga toxigenic *Escherichia coli* (STEC) from dairy calves, animal handlers and associated environments. *Brazilian Journal of Microbiology, 52*(4), 2541–2546. https://doi.org/10.1007/s42770-021-00561-9

Banerjee, M., Nair, G. B., & Ramamurthy, T. (2011). Phenotypic & genetic characterization of *Bacillus cereus* isolated from the acute diarrhoeal patients. *The Indian Journal of Medical Research, 133*(1), 88–95.

Barbhuiya, N. H., & Adak, A. (2021). Determination of antimicrobial concentration and associated risk in water sources in West Bengal state of India. *Environmental Monitoring and Assessment, 193*(2), 77. https://doi.org/10.1007/s10661-020-08801-5

Barman, N. N., Nath, A. J., Doley, S., Begum, S. A., Kakati, P., Das, S. K., et al. (2020). Listeriosis in a peri-urban area: Cultural and molecular characterization of *listeria monocytogenes* isolated from encephalitic goats. *Vet World, 13*(9), 1743–1749. https://doi.org/10.14202/vetworld.2020.1743-1749

Basu, A., Garg, P., Datta, S., Chakraborty, S., Bhattacharya, T., Khan, A., et al. (2000). *Vibrio cholerae* O139 in Calcutta, 1992–1998: Incidence, antibiograms, and genotypes. *Emerging Infectious Diseases, 6*(2), 139–147. https://doi.org/10.3201/eid0602.000206

Behera, D. K., & Mishra, S. (2022). The burden of diarrhea, etiologies, and risk factors in India from 1990 to 2019: Evidence from the global burden of disease study. *BMC Public Health, 22*(1), 92. https://doi.org/10.1186/s12889-022-12515-3

Bhadra, R. K., Dutta, P., Bhattacharya, S. K., Dutta, S. K., Pal, S. C., & Nair, G. B. (1992). *Campylobacter* species as a cause of diarrhoea in children in Calcutta. *The Journal of Infection, 24*(1), 55–62. https://doi.org/10.1016/0163-4453(92)90982-c

Carter, L., Chase, H. R., Gieseker, C. M., Hasbrouck, N. R., Stine, C. B., Khan, A., et al. (2018). Analysis of enterotoxigenic *Bacillus cereus* strains from dried foods using whole genome sequencing, multi-locus sequence analysis and toxin gene prevalence and distribution using endpoint PCR analysis. *International Journal of Food Microbiology, 284*, 31–39. https://doi.org/10.1016/j.ijfoodmicro.2018.06.016

Chakraborty, S., Deokule, J. S., Garg, P., Bhattacharya, S. K., Nandy, R. K., Nair, G. B., et al. (2001). Concomitant infection of enterotoxigenic *Escherichia coli* in an outbreak of cholera caused by *Vibrio cholerae* O1 and O139 in Ahmedabad, India. *Journal of Clinical Microbiology, 39*(9), 3241–3246. https://doi.org/10.1128/JCM.39.9.3241-3246.2001

Chandran, S. P., Sarkar, S., Diwan, V., Pathak, A., Shah, H., Tamhankar, A. J., et al. (2017). Detection of virulence genes in ESBL producing, quinolone resistant commensal *Escherichia coli* from rural Indian children. *Journal of Infection in Developing Countries, 11*(5), 387–392. https://doi.org/10.3855/jidc.8574

Chowdhury, G., Pazhani, G. P., Nair, G. B., Ghosh, A., & Ramamurthy, T. (2011). Transferable plasmid-mediated quinolone resistance in association with extended-spectrum β-lactamases and fluoroquinolone-acetylating aminoglycoside-6'-N-acetyltransferase in clinical isolates of *Vibrio fluvialis*. *International Journal of Antimicrobial Agents, 38*(2), 169–173. https://doi.org/10.1016/j.ijantimicag.2011.04.013

Chowdhury, G., Sarkar, A., Pazhani, G. P., Mukhopadhyay, A. K., Bhattacharya, M. K., & Ramamurthy, T. (2013). An outbreak of foodborne gastroenteritis caused by dual pathogens, *Salmonella enterica* serovar Weltevreden and *Vibrio fluvialis* in Kolkata, India. *Foodborne Pathogens and Disease, 10*(10), 904–906. https://doi.org/10.1089/fpd.2013.1491

Chowdhury, G., Ramamurthy, T., Ghosh, A., Dutta, S., Takahashi, E., & Mukhopadhyay, A. K. (2019). Emergence of azithromycin resistance mediated by phosphotransferase-encoding *mph*(-A) in diarrheagenic *Vibrio fluvialis*. *mSphere 4*(3):e00215–19. https://doi.org/10.1128/mSphere.00215-19.

Dahiya, S., Sharma, P., Kumari, B., Pandey, S., Malik, R., Manral, N., et al. (2017). Characterisation of antimicrobial resistance in salmonellae during 2014–2015 from four centres across India: An ICMR antimicrobial resistance surveillance network report. *Indian Journal of Medical Microbiology, 35*(1), 61–68. https://doi.org/10.4103/ijmm.IJMM_16_382

Devanga Ragupathi, N. K., Muthuirulandi Sethuvel, D. P., Shankar, B. A., Munusamy, E., Anandan, S., & Veeraraghavan, B. (2016). Draft genome sequence of bla_{TEM-1}-mediated cephalosporin-resistant *Salmonella enterica* serovar Typhi from bloodstream infection. *J Glob Antimicrob Resist, 7*, 11–12. https://doi.org/10.1016/j.jgar.2016.06.003

Ghosh, A. R., Koley, H., De, D., Paul, M., Nair, G. B., & Sen, D. (1996). Enterotoxigenic *Escherichia coli* associated diarrhoea among infants aged less than six months in Calcutta, India. *European Journal of Epidemiology, 12*(1), 81–84. https://doi.org/10.1007/BF00144433

Gupta, V., Kaur, U., Singh, G., Prakash, C., Sharma, M., & Aggarwal, K. C. (1986). An outbreak of typhoid fever in Chandigarh, North India. *Tropical and Geographical Medicine, 38*(1), 51–54.

Gupta, P., Modgil, V., Kant, V., Kaur, H., Narayan, C., Mahindroo, J., et al. (2022). Phenotypic and genotypic characterization of antimicrobial resistance in clinical isolates of *Vibrio cholerae* over a decade (2002–2016). *Indian Journal of Medical Microbiology, 40*(1), 24–29. https://doi.org/10.1016/j.ijmmb.2021.11.008

Haque, M. A., Platts-Mills, J. A., Mduma, E., Bodhidatta, L., Bessong, P., Shakoor, S., et al. (2019). Determinants of *Campylobacter* infection and association with growth and enteric inflammation in children under 2 years of age in low-resource settings. *Scientific Reports, 9*(1), 17124. https://doi.org/10.1038/s41598-019-53533-3

Hanna, N., Purohit, M., Diwan, V., Chandran, S. P., Riggi, E., Parashar, V., et al. (2020). (2020) monitoring of water quality, antibiotic residues, and antibiotic-resistant *Escherichia coli* in the Kshipra river in India over a 3-year period. *International Journal of Environmental Research and Public Health, 17*(21), 7706. https://doi.org/10.3390/ijerph17217706

Hussain, I., Borah, P., Sharma, R. K., Rajkhowa, S., Rupnik, M., Saikia, D. P., et al. (2016). Molecular characteristics of *Clostridium difficile* isolates from human and animals in the north eastern region of India. *Molecular and Cellular Probes, 30*(5), 306–311. https://doi.org/10.1016/j.mcp.2016.08.010

Jagadeeshan, S., Kumar, P., Abraham, W. P., & Thomas, S. (2009). Multiresistant *Vibrio cholerae* non-O1/non-O139 from waters in South India: Resistance patterns and virulence-associated gene profiles. *Journal of Basic Microbiology, 49*(6), 538–544. https://doi.org/10.1002/jobm.200900085

Jain, P., Chowdhury, G., Samajpati, S., Basak, S., Ganai, A., Samanta, S., et al. (2020). Characterization of non-typhoidal *Salmonella* isolates from children with acute gastroenteritis, Kolkata, India, during 2000–2016. *Brazilian Journal of Microbiology, 51*(2), 613–627. https://doi.org/10.1007/s42770-019-00213-z

Jana, A., & Mondal, A. (2013). Serotyping, pathogenicity and antibiogram of *Escherichia coli* isolated from raw poultry meat in West Bengal, India. *Veterinaria Italiana, 49*(4), 361–365. https://doi.org/10.12834/VetIt.1215.10

Joseph, J., & Kalyanikutty, S. (2022). Occurrence of multiple drug-resistant Shiga toxigenic *Escherichia coli* in raw milk samples collected from retail outlets in South India. *Journal of Food Science and Technology, 59*(6), 2150–2159. https://doi.org/10.1007/s13197-021-05226-x

Karabasanavar, N., Sivaraman, G. K., S P S, Nair, A. S., Vijayan, A., & Rajan, V. (2022). Non-diarrhoeic pigs as source of highly virulent and multidrug-resistant non-typhoidal *Salmonella. Brazilian Journal of Microbiology*: [publication of the Brazilian Society for Microbiology], *53*(2), 1039–1049. https://doi.org/10.1007/s42770-022-00700-w.

Keisam, S., Tuikhar, N., Ahmed, G., & Jeyaram, K. (2019). Toxigenic and pathogenic potential of enteric bacterial pathogens prevalent in the traditional fermented foods marketed in the northeast region of India. *International Journal of Food Microbiology, 296*, 21–30. https://doi.org/10.1016/j.ijfoodmicro.2019.02.012

Khan, A., Das, S. C., Ramamurthy, T., Sikdar, A., Khanam, J., Yamasaki, S., et al. (2002). Antibiotic resistance, virulence gene, and molecular profiles of Shiga toxin-producing *Escherichia coli* isolates from diverse sources in Calcutta, India. *Journal of Clinical Microbiology, 40*(6), 2009–2015. https://doi.org/10.1128/JCM.40.6.2009-2015.2002

Khan, J. A., Rathore, R. S., Abulreesh, H. H., Qais, F. A., & Ahmad, I. (2018). Prevalence and antibiotic resistance profiles of *Campylobacter jejuni* isolated from poultry meat and related samples at retail shops in northern India. *Foodborne Pathogens and Disease, 15*(4), 218–225. https://doi.org/10.1089/fpd.2017.2344

Kokare, R. S., Bari, A. K., Pereira, J. V., Patel, K., & Poojary, A. (2021). Minimum inhibitory concentration (MIC) of ceftriaxone and azithromycin for blood culture isolates of *Salmonella enterica* spp. *Journal of Infection in Developing Countries, 15*(4), 538–543. https://doi.org/10.3855/jidc.13454

Kumar, R., & Lalitha, K. V. (2013). Prevalence and molecular characterization of *Vibrio cholerae* O1, non-O1 and non-O139 in tropical seafood in Cochin, India. *Foodborne Pathogens and Disease, 10*(3), 278–283. https://doi.org/10.1089/fpd.2012.1310

Kumar, S., Rizvi, M., & Berry, N. (2008). Rising prevalence of enteric fever due to multidrug-resistant *Salmonella*: An epidemiological study. *Journal of Medical Microbiology, 57*(Pt 10), 1247–1250. https://doi.org/10.1099/jmm.0.2008/001719-0

Kylla, H., Dutta, T. K., Roychoudhury, P., Subudhi, P. K., Lalhruaipuii, L. J., et al. (2020). Characterisation of porcine enteropathogenic *Escherichia coli* isolated in northeastern India. *Journal of Veterinary Research, 64*(3), 391–397. https://doi.org/10.2478/jvetres-2020-0046

Lal, M., Kaur, H., & Gupta, L. K. (2003). *Y. Enterocolitica* gastroenteritis-a prospective study. *Indian Journal of Medical Microbiology, 21*(3), 186–188.

Lalhruaipuii, K., Dutta, T. K., Roychoudhury, P., Chakraborty, S., Subudhi, P. K., & Samanta, I. (2021). Multidrug-resistant extended-spectrum β-lactamase-producing *Escherichia coli* pathotypes in north eastern region of India: Backyard small ruminants-human-water interface. *Microbial Drug Resistance, 27*(12), 1664–1671. https://doi.org/10.1089/mdr.2020.0365

Lanjewar, M., De, A. S., & Mathur, M. (2010). Diarrheagenic *E. coli* in hospitalized patients: Special reference to Shiga-like toxin producing *Escherichia coli*. *Indian Journal of Pathology & Microbiology, 53*(1), 75–78. https://doi.org/10.4103/0377-4929.59188

Mahanti, A., Samanta, I., Bandopaddhay, S., Joardar, S. N., Dutta, T. K., Batabyal, S., et al. (2013). Isolation, molecular characterization and antibiotic resistance of Shiga toxin-producing *Escherichia coli* (STEC) from buffalo in India. *Letters in Applied Microbiology, 56*(4), 291–298. https://doi.org/10.1111/lam.12048

Mahindroo, J., Thanh, D. P., Nguyen, T. N. T., Mohan, B., Thakur, S., Baker, S., et al. (2019). Endemic fluoroquinolone-resistant *Salmonella enterica* serovar Kentucky ST198 in northern India. *Microbial Genomics, 5*(7), e000275. https://doi.org/10.1099/mgen.0.000275

Malvi, S., Appannanavar, S., Mohan, B., Kaur, H., Gautam, N., Bharti, B., et al. (2015). Comparative analysis of virulence determinants, antibiotic susceptibility patterns and serogrouping of atypical enteropathogenic *Escherichia coli* versus typical enteropathogenic E. coli in India. *Journal of Medical Microbiology, 64*(10), 1208–1215. https://doi.org/10.1099/jmm.0.000131

Menon, K. V., Sunil, B., & Latha, C. (2021). Prevalence and antibiotic resistance profile of *Listeria* spp. associated with seafoods from fish catchment areas in Kerala, India. *Vet World, 14*(3), 777–783. https://doi.org/10.14202/vetworld.2021

Mishra, A., Taneja, N., & Sharma, M. (2012). Environmental and epidemiological surveillance of *Vibrio cholerae* in a cholera-endemic region in India with freshwater environs. *Journal of Applied Microbiology, 112*(1), 225–237. https://doi.org/10.1111/j.1365-2672.2011.05191.x

Modi, S., Brahmbhatt, M. N., Chatur, Y. A., & Nayak, J. B. (2015). Prevalence of *Campylobacter* species in milk and milk products, their virulence gene profile and anti-bio gram. *Vet World, 8*(1), 1–8. https://doi.org/10.14202/vetworld.2015.1-8

Mukherjee, P., Ramamurthy, T., Bhattacharya, M. K., Rajendran, K., & Mukhopadhyay, A. K. (2013). *Campylobacter jejuni* in hospitalized patients with diarrhea, Kolkata, India. *Emerging Infectious Diseases, 19*(7), 1155–1156. https://doi.org/10.3201/eid1907.121278

Mukherjee, P., Ramamurthy, T., Mitra, U., & Mukhopadhyay, A. K. (2014). Emergence of high-level azithromycin resistance in *Campylobacter jejuni* isolates from pediatric diarrhea patients in Kolkata, India. *Antimicrobial Agents and Chemotherapy, 58*(7), 4248. https://doi.org/10.1128/AAC.02931-14

Mutreja, A., Kim, D. W., Thomson, N. R., Connor, T. R., Lee, J. H., Kariuki, S., et al. (2011). Evidence for several waves of global transmission in the seventh cholera pandemic. *Nature, 477*(7365), 462–465. https://doi.org/10.1038/nature10392

Muzembo, B. A., Kitahara, K., Debnath, A., Ohno, A., Okamoto, K., & Miyoshi, S. I. (2022). Cholera outbreaks in India, 2011-2020: A systematic review. *International Journal of Environmental Research and Public Health, 19*(9), 5738. https://doi.org/10.3390/ijerph19095738

Nair, G. B., Ramamurthy, T., Bhattacharya, S. K., Dutta, B., Takeda, Y., & Sack, D. A. (2007). Global dissemination of *Vibrio parahaemolyticus* serotype O3:K6 and its serovariants. *Clinical Microbiology Reviews, 20*(1), 39–48. https://doi.org/10.1128/CMR.00025-06

Natarajan, M., Kumar, D., Mandal, J., Biswal, N., & Stephen, S. (2018). A study of virulence and antimicrobial resistance pattern in diarrhoeagenic *Escherichia coli* isolated from diarrhoeal stool specimens from children and adults in a tertiary hospital, Puducherry, India. *Journal of Health, Population, and Nutrition, 37*(1), 17. https://doi.org/10.1186/s41043-018-0147-z

Nazki, S., Wani, S. A., Parveen, R., Ahangar, S. A., Kashoo, Z. A., Hamid, S., et al. (2017). Isolation, molecular characterization and prevalence of *Clostridium perfringens* in sheep and goats of Kashmir Himalayas, India. *Vet World, 10*(12), 1501–1507. https://doi.org/10.14202/vetworld.2017.1501-1507

Negi, M., Vergis, J., Vijay, D., Dhaka, P., Malik, S. V., Kumar, A., et al. (2015). Genetic diversity, virulence potential and antimicrobial susceptibility of *Listeria monocytogenes* recovered from different sources in India. *Pathog Dis, 73*(9), ftv093. https://doi.org/10.1093/femspd/ftv093

Obaidat, M. M., & Bani Salman, A. E. (2017). Antimicrobial resistance percentages of *Salmonella* and *Shigella* in seafood imported to Jordan: Higher percentages and more diverse profiles in *Shigella*. *Journal of Food Protection, 80*(3), 414–419. https://doi.org/10.4315/0362-028X.JFP-16-322

Olukemi Adesiji, Y., Kogaluru Shivakumaraswamy, S., Kumar Deekshit, V., Shivani Kallappa, G., & Karunasagar, I. (2017). Molecular characterization of antimicrobial multi-drug resistance in non-typhoidal salmonellae from chicken and clam in Mangalore, India. *Journal of Biomedical Research, 32*(3), 237–244. https://doi.org/10.7555/JBR.31.20160094

Palaniyappan, V., Nagalingam, A. K., Ranganathan, H. P., Kandhikuppam, K. B., Kothandam, H. P., & Vasu, S. (2013). Antibiotics in south Indian coastal sea and farmed prawns (*Penaeus monodon*). *Food Additives & Contaminants. Part B, Surveillance, 6*(3), 196–199. https://doi.org/10.1080/19393210.2013.787555

Pazhani, G. P., Chakraborty, S., Fujihara, K., Yamasaki, S., Ghosh, A., & Nair, G. B. (2011). QRDR mutations, efflux system & antimicrobial resistance genes in enterotoxigenic *Escherichia coli* isolated from an outbreak of diarrhoea in Ahmedabad, India. *The Indian Journal of Medical Research, 134*(2), 214–223.

Porter, G., & Grills, N. (2016). Medication misuse in India: A major public health issue in India. *Journal of Public Health (Oxford, England), 38*(2), e150–e157.

Priyanka, M. P. R., Meghwanshi, K. K., Rana, A., & Singh, A. P. (2021). Leafy greens as a potential source of multidrug-resistant diarrhoeagenic *Escherichia coli* and *Salmonella*. *Microbiology (Reading), 167*(6). https://doi.org/10.1099/mic.0.001059

Puii, L. H., Dutta, T. K., Roychoudhury, P., Kylla, H., Chakraborty, S., & Mandakini, R. (2019). Extended spectrum beta-lactamase producing Shiga-toxin producing-*Escherichia coli* in piglets, humans and water sources in north east region of India. *Letters in Applied Microbiology, 69*(5), 373–378. https://doi.org/10.1111/lam.13216

Raju, B., & Ballal, M. (2009). Multidrug resistant enteroaggregative *Escherichia coli* diarrhoea in rural southern Indian population. *Scandinavian Journal of Infectious Diseases, 41*(2), 105–108. https://doi.org/10.1080/00365540802641856

Rana, N., Panda, A. K., Pathak, N., Gupta, T., & Thakur, S. D. (2020). *Bacillus cereus*: Public health burden associated with ready-to-eat foods in Himachal Pradesh, India. *Journal of Food Science and Technology, 57*(6), 2293–2302. https://doi.org/10.1007/s13197-020-04267-y

Rasaily, R., Dutta, P., Saha, M. R., Mitra, U., Lahiri, M., & Pal, S. C. (1994). Multi-drug resistant typhoid fever in hospitalised children. Clinical, bacteriological and epidemiological profiles. *European Journal of Epidemiology, 10*(1), 41–46. https://doi.org/10.1007/BF01717450

Rituparna, C., Mamatha, B., Mukhyaprana, P. M., Manjunatha, H. H., Gururaja, P. P., & Thandavarayan, R. (2016). Characterization of *Clostridium difficile* isolated from diarrheal patients in a tertiary-care hospital, Karnataka, South India. *The Southeast Asian Journal of Tropical Medicine and Public Health, 47*(6), 1221–1230.

Saha, M. R., Sircar, B. K., Dutta, P., & Pal, S. C. (1992). Occurrence of multi-resistant *Salmonella typhimurium* infection in a pediatric hospital at Calcutta. *Indian Pediatrics, 29*(3), 307–311.

Saksena, R., Gaind, R., Sinha, A., Kothari, C., Chellani, H., & Deb, M. (2018). High prevalence of fluoroquinolone resistance amongst commensal flora of antibiotic naïve neonates: A study from India. *Journal of Medical Microbiology, 67*(4), 481–488. https://doi.org/10.1099/jmm.0.000686

Samajpati, S., Pragasam, A. K., Mandal, S., Balaji, V., & Dutta, S. (2021). Emergence of ceftriaxone resistant *Salmonella enterica* serovar Typhi in eastern India. *Infection, Genetics and Evolution, 96*, 105093. https://doi.org/10.1016/j.meegid.2021.105093

Samanta, P., Mandal, R. S., Saha, R. N., Shaw, S., Ghosh, P., Dutta, S., et al. (2020). A point mutation in *carR* is involved in the emergence of polymyxin B-sensitive *Vibrio cholerae* O1 El tor biotype by influencing gene transcription. *Infection and Immunity, 88*(5), e00080–e00020. https://doi.org/10.1128/IAI.00080-20

Sannat, C., Patyal, A., Rawat, N., Ghosh, R. C., Jolhe, D. K., & Shende, R. K. (2017). Characterization of *Salmonella* Gallinarum from an outbreak in Raigarh, Chhattisgarh. *Vet World, 10*(2), 144–148. https://doi.org/10.14202/vetworld.2017.144-148

Sarkar, A., Morita, D., Ghosh, A., Chowdhury, G., Mukhopadhyay, A. K., & Okamoto, K. (2019). Altered integrative and conjugative elements (ICEs) in recent *Vibrio cholerae* O1 isolated from cholera cases, Kolkata, India. *Frontiers in Microbiology, 6*(10), 2072. https://doi.org/10.3389/fmicb.2019.02072

Sehgal, R., Kumar, Y., & Kumar, S. (2008). Prevalence and geographical distribution of *Escherichia coli* O157 in India: A 10-year survey. *Transactions of the Royal Society of Tropical Medicine and Hygiene, 102*(4), 380–383. https://doi.org/10.1016/j.trstmh.2008.01.015

Senthilkumar, B., & Prabakaran, G. (2005). Multidrug resistant *Salmonella typhi* in asymptomatic typhoid carriers among food handlers in Namakkal district, Tamil Nadu. *Indian Journal of Medical Microbiology, 23*(2), 92–94. https://doi.org/10.4103/0255-0857.16046

Singh, B. R., Singh, P., Agrawal, S., Teotia, U., Verma, A., Sharma, S., et al. (2007). Prevalence of multidrug resistant *Salmonella* in coriander, mint, carrot, and radish in Bareilly and Kanpur, northern India. *Foodborne Pathogens and Disease, 4*(2), 233–240. https://doi.org/10.1089/fpd.2006.0082

Singh, B. R., Agarwal, M., Chandra, M., Verma, M., Sharma, G., Verma, J. C., et al. (2010). Plasmid profile and drug resistance pattern of zoonotic *Salmonella* isolates from Indian buffaloes. *Journal of Infection in Developing Countries, 4*(8), 477–483. https://doi.org/10.3855/jidc.713

Singhal, N., Kumar, M., & Virdi, J. S. (2016). Resistance to amoxicillin-clavulanate and its relation to virulence-related factors in *Yersinia enterocolitica* biovar 1A. *Indian Journal of Medical Microbiology, 34*(1), 85–87. https://doi.org/10.4103/0255-0857.174125

Sinha, S., Shimada, T., Ramamurthy, T., Bhattacharya, S. K., Yamasaki, S., Takeda, Y., et al. (2004). Prevalence, serotype distribution, antibiotic susceptibility and genetic profiles of mesophilic *Aeromonas* species isolated from hospitalized diarrhoeal cases in Kolkata, India. *Journal of Medical Microbiology, 53*(Pt 6), 527–534. https://doi.org/10.1099/jmm.0.05269-0

Soni, D. K., Singh, R. K., Singh, D. V., & Dubey, S. K. (2013). Characterization of *Listeria monocytogenes* isolated from Ganges water, human clinical and milk samples at Varanasi, India. *Infection, Genetics and Evolution, 14*, 83–91. https://doi.org/10.1016/j.meegid.2012.09.019

Swetha, C. S., Porteen, K., Elango, A., Ronald, B. S. M., Senthil Kumar, T. M. A., Milton, A. P., et al. (2021). Genetic diversity, virulence and distribution of antimicrobial resistance among *Listeria monocytogenes* isolated from milk, beef, and bovine farm environment. *Iranian Journal of Veterinary Research, 22*(1), 1–8. https://doi.org/10.22099/ijvr.2020.37618.5472

Taneja, N., Appannanavar, S. B., Kumar, A., Varma, G., Kumar, Y., Mohan, B., et al. (2014). Serotype profile and molecular characterization of antimicrobial resistance in non-typhoidal *Salmonella* isolated from gastroenteritis cases over nine years. *Journal of Medical Microbiology, 63*(Pt 1), 66–73. https://doi.org/10.1099/jmm.0.061416-0

Taneja, N., Kumar, A., Appannanavar, S., Verma, G., & Sharma, M. (2014). Plasmid-mediated quinolone resistance in *Shigella* isolates over a decade in India. *Journal of Global Antimicrobial Resistance, 2*(1), 59–60. https://doi.org/10.1016/j.jgar.2013.10.006

Taneja, N., Mewara, A., Kumar, A., Verma, G., & Sharma, M. (2012). Cephalosporin-resistant *Shigella flexneri* over 9 years (2001-09) in India. *The Journal of Antimicrobial Chemotherapy, 67*(6), 1347–1353. https://doi.org/10.1093/jac/dks061

Thungapathra, M., Amita, S. K. K., Chaudhuri, S. R., Garg, P., Ramamurthy, T., et al. (2002). Occurrence of antibiotic resistance gene cassettes *aac(6′)-Ib, dfrA5, dfrA12*, and *ereA2* in class I integrons in non-O1, non-O139 *Vibrio cholerae* strains in India. *Antimicrobial Agents and Chemotherapy, 46*(9), 2948–2955. https://doi.org/10.1128/AAC.46.9.2948-2955.2002

Udhayavel, S., Thippichettypalayam Ramasamy, G., Gowthaman, V., Malmarugan, S., & Senthilvel, K. (2017). Occurrence of *Clostridium perfringens* contamination in poultry feed ingredients: Isolation, identification and its antibiotic sensitivity pattern. *Animal Nutrition, 3*(3), 309–312. https://doi.org/10.1016/j.aninu.2017.05.006

Van Boeckel, T. P., Gandra, S., Ashok, A., Caudron, Q., Grenfell, B. T., Levin, S. A., et al. (2014). Global antibiotic consumption 2000 to 2010: An analysis of national pharmaceutical sales data. *The Lancet Infectious Diseases, 14*(8), 742–750.

Veeraraghavan, B., Jacob, J. J., Prakash, J. A. J., Pragasam, A. K., Neeravi, A., Narasimman, V., et al. (2019). Extensive drug resistant *Salmonella enterica* serovar Senftenberg carrying bla_{NDM}

encoding plasmid p5558 (IncA/C) from India. *Pathogens and Global Health, 113*(1), 20–26. https://doi.org/10.1080/20477724.2019.1574112

Veeraraghavan, B., Pragasam, A. K., Ray, P., Kapil, A., Nagaraj, S., Perumal, S. P. B., et al. (2021). Evaluation of antimicrobial susceptibility profile in *Salmonella* Typhi and *Salmonella* Paratyphi a: Presenting the current scenario in India and strategy for future management. *The Journal of Infectious Diseases, 224(Supple 5):S502–S516*, S502. https://doi.org/10.1093/infdis/jiab144

Verma, A. K., Abdel-Glil, M. Y., Madesh, A., Gupta, S., Karunakaran, A. C., Inbaraj, S., et al. (2020). Multilocus sequence typing of *Clostridium perfringens* strains from neonatal calves, dairy workers and associated environment in India. *Anaerobe, 63*, 102212. https://doi.org/10.1016/j.anaerobe.2020.102212

Verma, S., Venkatesh, V., Kumar, R., Kashyap, S., Kumar, M., Maurya, A. K., et al. (2019). Etiological agents of diarrhea in hospitalized pediatric patients with special emphasis on diarrheagenic *Escherichia coli* in North India. *Journal of Laboratory Physicians, 11*(1), 68–74. https://doi.org/10.4103/JLP.JLP_123_18

Walsh, T. R., Weeks, J., Livermore, D. M., & Toleman, M. A. (2011). Dissemination of NDM-1 positive bacteria in the New Delhi environment and its implications for human health: An environmental point prevalence study. *The Lancet Infectious Diseases, 11*(5), 355–362. https://doi.org/10.1016/S1473-3099(11)70059-7

Wani, S. A., Hussain, I., Beg, S. A., Rather, M. A., Kabli, Z. A., Mir, M. A., et al. (2013). Diarrhoeagenic *Escherichia coli* and salmonellae in calves and lambs in Kashmir absence, prevalence and antibiogram. *Revue Scientifique et Technique, 32*(3), 833–840. https://doi.org/10.20506/rst.32.2.2213

Warjri, I., Dutta, T. K., Lalzampuia, H., & Chandra, R. (2015). Detection and characterization of extended-spectrum β-lactamases ($bla_{\text{CTX-M-1}}$ and $bla_{\text{SHV)}}$ producing *Escherichia coli*, *Salmonella* spp. and *Klebsiella pneumoniae* isolated from humans in Mizoram. *Vet World, 8*(5), 599–604. https://doi.org/10.14202/vetworld.2015

Wattal, C., Kaul, V., Chugh, T. D., Kler, N., & Bhandari, S. K. (1994). An outbreak of multidrug resistant *Salmonella typhimurium* in Delhi (India). *The Indian Journal of Medical Research, 100*, 266–267.

Weill, F. X., Domman, D., Njamkepo, E., Tarr, C., Rauzier, J., Fawal, N., et al. (2017). Genomic history of the seventh pandemic of cholera in Africa. *Science, 358*(6364), 785–789. https://doi.org/10.1126/science.aad5901

Yadav, J. P., Das, S. C., Dhaka, P., Mukhopadhyay, A. K., Chowdhury, G., & Naskar, S. (2017). Pulsed-field gel electrophoresis of enterotoxic *Clostridium perfringens* type A isolates recovered from humans and animals in Kolkata, India. *International Journal of Veterinary Science and Medicine, 6*(1), 123–126. https://doi.org/10.1016/j.ijvsm.2017.11.002

Yadav, J. P., Das, S. C., Dhaka, P., Vijay, D., Kumar, M., Mukhopadhyay, A. K., et al. (2017). Molecular characterization and antimicrobial resistance profile of *Clostridium perfringens* type A isolates from humans, animals, fish and their environment. *Anaerobe, 47*, 120–124. https://doi.org/10.1016/j.anaerobe.2017.05.009

Antimicrobial Resistance Associated with Infectious Diseases

Sanjeev Saini, Nivedita Thass, Jyoti Kayesth, and Welile Sikhondze

Contents

S. Saini (✉) · N. Thass · J. Kayesth · W. Sikhondze
Infectious Diseases Detection and Surveillance, ICF, Delhi, India
e-mail: sanjeev.saini@icf.com; jyoti.kayesth@icf.com

M. P. Mothadaka et al. (eds.), *Handbook on Antimicrobial Resistance*,
https://doi.org/10.1007/978-981-19-9279-7_49

Abstract

The silent pandemic of antimicrobial resistance (AMR) has been a lethal enemy of mankind for years. Unfortunately, humans have themselves been responsible for the troublesome and worsening trends of AMR. The lack of sanitation and hygiene, lack of awareness among the public, inadequate infection prevention, and control policies in hospitals, indiscriminate antimicrobial use in humans, animals, as well as the environment, and irresponsible disposal of these antibiotics into the environment have made matters worse. Our armamentarium against these pathogens is diminishing gradually with hardly any antibiotics left to treat the patients. Thus, the World Health Organization recently developed the significance pathogen list to rank the development of drugs for the most common but difficult-to-treat pathogens across the world. Carbapenem-unresponsive; *Klebsiella* spp., *Escherichia coli*, *Pseudomonas aeruginosa,* and *Acinetobacter baumannii*, MRSA, and VRE are some of the organisms on the list. Although research is ongoing to discover new molecules to fight these superbugs and cure the infections caused by them, the current pressing main concern is to rectify our practices by following judicious use and proper disposal of antibiotics, working toward the strategic priorities of creating awareness, strictly complying with infection prevention and control protocols along with integration and collaboration among all the sectors (human, animal, environment, research) as identified under the country's accomplishment strategy on fight against drug-unresponsive superbugs (NAP-AMR) in India.

Keywords

Resistance · NAP AMR · WHO Priority pathogens · Antibiotics · Infection

1 Introduction

The antibiotic unresponsiveness is a health problem across the globe and a major challenge to public well-being. Worldwide hazard of superbugs in humans and animals has resulted in contagious infections becoming the vital ground for diseases (Dhingra et al., 2020). As we enter the post-antibiotic era, the rapidly developing resistance among human pathogens and limited newer antimicrobials is interfering with the inhibition and cure of transmissible maladies (CDC, 2019). AMR has not only been responsible for causing lethal infections, overuse of antimicrobials, treatment failures, as well as increased morbidity and mortality in patients but also it has been associated with the requirement of extended hospital care, thus, leading to an unnecessary economic burden (Dadgostar, 2019). AMR is a rapidly spreading

silent global pandemic prevalent across high- and middle-income-income countries (Hay et al., 2018).

2 Global Scenario

A study to estimate the liability of drug-unaffected diseases throughout the world was conducted in 2019, together with an assessment of pathogen–drug (88) groups, which observed a near five million mortalities, of which 1.27 million deaths per annum were attributed to antimicrobial resistance (Laxminarayan et al., 2020). A research study commissioned by the UK government predicted that at the advent of 2050 AMR will burden the global economy with a hundred trillion USD and could be responsible for ten million human mortalities per annum (O'Neill, 2016).

The average length of hospital stays for a patient infected with a multidrug-resistant pathogen is around 13 days and contributes to an additional eight million hospital days, which is approximately US$29,000 per patient every year (Majumder et al., 2020).

At the same time, infectious diseases due to multidrug-resistant organisms (MDROs) are now the major contributors to mortalities among the pediatric age group. Most reported pathogens associated with high mortality rates in this age group are drug-resistant infections, such as extended-spectrum beta-lactamase (ESBL)-generating microbes and ailments associated with methicillin-resistant Staphylococcus aureus (MRSA) (Kayange et al., 2010).

In concordance with a global opinion that antimicrobial resistance is a risk to public well-being, a plan of action at global level (GAP) was formulated by the Assembly of World Health (WHA) in May 2014 (WHO, 2014). Furthermore, the WHA appeals its member states to draft their National Action Plans in such a way that they are in alignment with the GAP-AMR by May 2017. To strengthen the fight against AMR, a strong commitment by global leaders was endorsed at a meeting on AMR at the UNGA on September 21, 2016. In alignment with the 2016 announcement of the UN, the system of Conscience of Antimicrobial Resistance Accountability (CARA), was an initiative propelled to supervise the steps taken by countries to conserve the potency of antibiotics (Gelband, 2016).

3 Infectious Diseases and AMR

3.1 Indian Scenario

The disease burden owing to highly prevalent infectious diseases in India today is indicated by the simple mortality rate of 417 per 100,000 persons. Pneumonia alone accounts for nearly 25% of pediatric deaths (approximately 410,000 deaths) in India annually. As per the country's Accomplishment Strategy on Antimicrobial Obduracy 2017, the frequency of occurrence of pathogens immune to drugs in India is accelerating at a frightening pace. Infections of MRSA rose from 29% to 45%

during the period from 2008 to 2014 in a span of 6 years, among which 65% and 42% of pseudomonads were obdurate to ceftazidime and imipenem, respectively. While 51% of *Klebsiella* spp. were unresponsive to carbapenems (National Centre for Disease Control & World Health Organization, 2017; Taneja & Sharma, 2019).

Indiscriminate application of drugs in other sectors, namely, veterinary and agriculture, contributed massively to the problem of AMR as highlighted by the report of MoHFW. The worldwide intake of drugs in faunal feed in 2010 is assessed at 63.15×10^3 tons, and India at 3% is ranked the fourth highest antimicrobial-employing nation in the world. The continuous trend of unfettered consumption of antimicrobials in the food and animal sectors in India could lead to a twofold escalation by 2030 (National Centre for Disease Control & World Health Organization, 2017).

One of the major challenges in our fight against AMR remains low in report and deficiency of adequate data from economically underprivileged nations (Antimicrobial Resistance Collaborators, 2022). Research to understand the drug resistance mechanisms, better diagnostic methods, and vigilant AMR surveillance in hospitals will play a key role in curbing the morbidity and mortality rates due to infectious diseases.

3.2 Diagnosing Antimicrobial Resistance

Rapid and accurate laboratory methods to detect antimicrobial resistance among pathogens are indispensable in regulating and monitoring the development of resistance and ensuring effective treatments.

In most settings, approximately 50% of infectious disease cases are started on empirical antibiotics as the causative organism is identified late due to a lack of rapid and sensitive antimicrobial susceptibility testing (Vasala et al., 2020).

Despite the availability of effective diagnostic methods, clinicians still opt for empirical treatment, especially in outpatient departments, resulting in the overuse of antimicrobials (Li et al., 2016).

However, in practical experience across Indian hospitals, most of the rapid diagnostic methods are unaffordable for the public and not available for use in clinics and hospitals. The standalone labs take at least 1–2 days to release the required reports. Hence, empirical antibiotics are unavoidable in Outpatient Department (OPD) patients mostly. The cost of these diagnostics is, thus, a hindrance and a challenge to be resolved.

Conventional susceptibility testing requires the growth of organisms on culture media, followed by the identification of the organism and susceptibility testing by disc diffusion or automated systems like VITEK (Biomerieux, France) and Phoenix (Becton Dickinson, USA). The former is time-consuming, and by the time the report is available, empirical therapy is already started. Another system popularly known as Matrix Assisted Laser Desorption/Ionization Time of Flight MALDI-ToF is presently employed in some labs for the identification of organisms. Susceptibility testing using this technique is still being researched and not widely done yet.

Rapid molecular methods can guide effective treatment strategies even at the initial stage of the disease. There is no dearth of upcoming molecular methods available today such as nucleic acid amplification technology (NAAT), micro- and nanoparticles, microarrays, electrochemical methods, and mass spectrometry. However, only a few systems so far have been approved by the USFDA. These methods also help distinguish viral infections from bacterial infections, thereby reducing the chances of unnecessary antibiotic use in patients. These diagnostics can also identify colonizers, where the organism has been isolated by the laboratory but may not be pathogenic. Such cases are of critical importance in the hospital setting since these may not require treatment, thus reducing antibiotic overuse (Burnham et al., 2017).

3.3 Gaps in Diagnosing AMR

The currently available rapid tests for detecting AMR are mostly genotypic methods, that is, they identify certain resistance genes for a particular drug–pathogen combination. Although less time-consuming, their major drawback is the unavailability of screening outputs of proneness to drugs, which is indispensable for curing regimes (Burnham et al., 2017). Contrastingly, conventional tests are based on phenotypic methods that provide both susceptibility and resistance patterns as well as reproducible results. The conventional technology is time-consuming and has inadequate clinical predictive value as it does not consider host response, biofilm formation, or bioavailability at the tissue level, etc. (Doern & Brecher, 2011).

Despite the utility of rapid tests, culture correlation is indispensable. Polymerase Chain Reaction PCR detects a variety of genetic material in the specimen. When multiple organisms are detected along with multiple resistance genes, the automated molecular systems are unable to distinguish the source of the gene and, hence, a correlation with culture is recommended even by systems like Biofire Film array (Biomerieux, France).

Antimicrobial resistance is a natural phenomenon. The reckless and inconsiderate employment of drugs resulted in the evolution and transmission of superbugs that are immune to most classes of drugs (bacteria, viruses, parasites, and fungi). Other factors that have facilitated the proliferation of drug-resistant strains globally include nonadherence to infection control practices, inadequate sanitary conditions, misuse of antimicrobials in the veterinary sector, and inappropriate food handling (Hay et al., 2018).

4 Factors Contributing to AMR

The rising issue of AMR has highlighted that it is a multifaceted problem and has made us realize the significance of intersectoral collaboration: human health, animal health, food and hygiene, and environmental health in our fight against AMR. The rates of resistance have been rising disproportionately across these sectors and necessitate research in the field of AMR. The lack of standardized surveillance

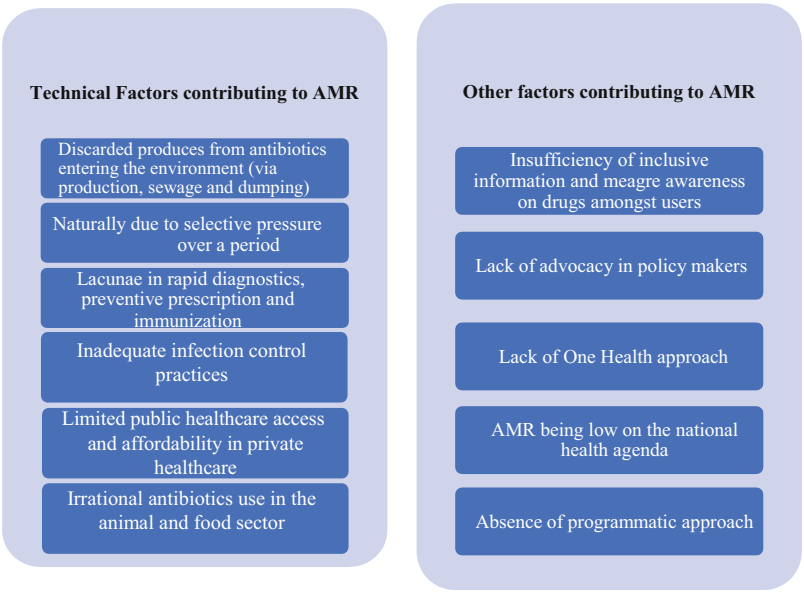


Fig. 1 Factors contributing to antimicrobial resistance

data makes gauging the extent and scope of AMR difficult (Taneja & Sharma, 2019). Some of the factors contributing to AMR are given below and are depicted in Fig. 1.

4.1 Antimicrobial Resistance Is a Natural Phenomenon

It takes place due to the selective pressure over a period when a particular antibiotic is in use. The strains that carry the resistance gene survive and replicate, thus resulting in the emergence of multidrug-resistant microbes. Numerous additional factors have contributed to the rapid acquisition of resistance by pathogens globally, and some are listed below.

4.2 Antibiotic Misuse/Overuse

Owing to inadequate regulatory systems to monitor antibiotic use, self-medication and ease in accessibility in buying drugs have exacerbated the spread of obduracy to drugs. The lack of antibiotic stewardship programs in India has enabled resistance to develop among the microbes (National Centre for Disease Control & World Health Organization, 2017; Prestinaci et al., 2015). Moreover, the pandemic of COVID-19 also fueled the immeasurable application of drugs for the last 2 years, increasing the rates of AMR globally. Despite the guidelines given by health authorities the world

over, including WHO, which discouraged the use of antibiotics for mild cases of COVID-19, the irrational and unsupervised use of antibiotics continued during the pandemic. This has only worsened the silent pandemic of AMR in the last 2 years by increasing the rates of hospitalizations and the emergence of drug-resistant pathogens (Majumder et al., 2020).

4.3 Inadequate Infection Control Practices

Lack of awareness and well-trained staff, infrastructure, and extraordinary workload, all have contributed to poor infection control practices. This directly leads to the nosocomial transmission of drug-resistant pathogens.

4.4 Environmental Pollution

The wastes generated from antimicrobials enter into the environment through manufacture, sewage, and disposal. Out of all Gram-negative bacteria isolated from the two important rivers of India, Ganges and Yamuna, 17.4% were ESBL producers, and all the *E. coli* totaling to a number of 283 isolated from the Cauvery River of Karnataka a south Indian state, were resistant to the third-generation cephalosporin (Taneja & Sharma, 2019). However, when discussing the AMR among humans, the effect of resistance genes present in environmental bacteria is rarely considered. The horizontal transfer of environmental resistance genes into pathogens causes infectious diseases and leads to treatment failures that will be given emphasis on the environmental aspect of AMR. The need of the hour is to fill the evidence gap that will enable policymakers and environmental regulators to deliver environmental protection from AMR (Taneja & Sharma, 2019).

4.5 Poor Diagnostics

As discussed earlier, due to the unavailability of good diagnostics there is a lack of early identification and diagnosis of pathogens and their susceptibility profile. This interferes with the initiation of appropriate antimicrobial therapy and forces physicians to initiate empirical treatment using broad-spectrum antimicrobials.

4.6 Irrational Antibiotics Use in the Animal and Food Sector

The absence of stringent rules in the application of drugs in farmed animals and cattle results in the indiscriminate use as growth promoters and for disease treatment, making these animals another reservoir of resistance genes. Since 2006, Europe has excluded the application of drugs as growth supporters, but the United States and several other countries continue to have this unrestricted practice (Prestinaci et al.,

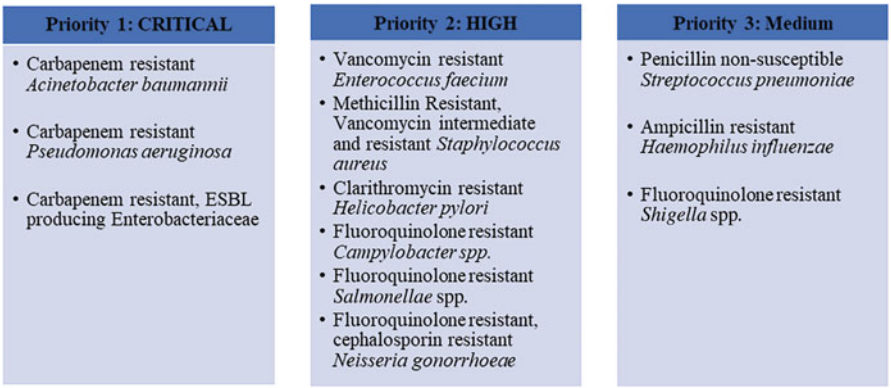


Fig. 2 WHO list of priority pathogens

2015). Establishing better infection control measures and surveillance networks to monitor resistance in both sectors, that is, animal and agriculture sectors, is essential.

Since infectious diseases are caused by superbugs are the primary reason for death all over the world, the WHO documented drug obdurate "priority pathogens" in 2017 (WHO, 2017) (Fig. 2), which includes 12 genera of microbes that are most commonly being reported across the globe and are recognized as public health threats. The CDC of the United States in 2019 authenticated AMR microbes categorized into three groups. There are a total of eight microbes on this list; most of these are also on the WHO list (CDC, 2019).

As per the WHO published worldwide information on AMR surveillance, the increasing rates of antimicrobial resistance among malarial parasites, human immunodeficiency virus, and MDR/XDR *Mycobacterium tuberculosis* are being reported worldwide, especially from China, India, and the Russian Federation cannot be ignored and public health strategies to fight antimicrobial resistance should also include these (WHO, 2014).

5 WHO Priority Pathogens

5.1 Carbapenem-Resistant *Acinetobacter baumannii* (CRAB)

The Problem Statement Among drug-resistant nosocomial microbes, CRAB is the main cause of maximum rate of demises. Various studies across the world have reported carbapenem unresponsiveness amounts to very high levels to an extent of 90%, and the death frequency allied to CRAB infections is approximately 60% (Isler et al., 2019). The biofilm-forming capacity of this organism in biomedical relevant devices leading to its persistence in hospital settings and its formidable drug resistance profile are the reasons behind frequent nosocomial outbreaks caused by CRAB (Rosales-Reyes et al., 2017). CRAB mostly affects most vulnerable patients

in ICU settings and is concomitant to life-threatening infections like ventilator-associated pneumonia and bacteremia (Isler et al., 2019).

Mechanism of Resistance Studies have shown various mechanisms for this resistance pattern of CRAB, (a) loss of outer membrane porins expression, (b) horizontal transfer of some resistance factors like OXA-23; NDM carbapenemases and aminoglycoside-altering enzymes; (c) they intrinsically express some β-lactamases; AmpC cephalosporinases, carbapenemases, and β-lactamases of OXA kind; (d) they form "resistance island" made of multiple mobile resistance gene elements; and (e) isoform of efflux pumps, viz., AbeABC, AbeFGH, etc. (Wong et al., 2017).

Treatment Selections: Widely drug-obdurate CRAB infections are commonly treated using tigecycline, polymyxin, and sulbactam. Tigecycline, although used widely against CRAB infections, is not effective against bloodstream infections due to its pharmacokinetic property of achieving low plasma levels. However, increasing cases of resistance to this drug are being reported globally, thus discouraging the use of tigecycline (Taccone et al., 2006). Minocycline too has shown good clinical efficacy in infections due to CRAB (Wong et al., 2017). Sulbactam also has intrinsic activity against CRAB, so sulbactam-containing regimens can be a treatment option. Its use is again limited due to high rates of resistance being reported worldwide (Viehman et al., 2014). Amikacin also carries anti-CRAB activity, but being nephrotoxic its use for systemic infections is not recommended. In vitro susceptibility tests showed that polymyxins have potent activity against *A. baumannii* strains, but clinical efficacy is unreliable due to the absence of susceptibility breakpoints, no therapeutic window, and their nephrotoxic and neurotoxic nature (Isler et al., 2019). Hence, there is an urgent need for alternative therapeutic options against CRAB.

Research is ongoing to find newer antimicrobials to treat such multidrug-resistant isolates of *Acinetobacter baumannii*. Until then, the need is to judiciously use the available antimicrobials and keep resistance rates under check. The need of the hour is a multidisciplinary approach, involving strict infection control practices, antimicrobial stewardship, and increased awareness among healthcare providers and patients (Wong et al., 2017).

5.2 Carbapenem-Resistant Enterobacteriaceae (CRE)

The Problem Statement In recent years, carbapenem-resistant Enterobacteriaceae (CRE) has been identified as one of the main reasons for epidemics and management failures of both nosocomial and community-acquired infections (Elshamy & Aboshanab, 2020).

With CRE demonstrating unresponsiveness to key classes of antibiotics, for example, fluoroquinolones β-lactams and aminoglycosides, the last resort for

treatment are polymyxins. In some cases, aminoglycosides and tigecycline have shown some efficacy (Elshamy & Aboshanab, 2020).

A US study reported a prevalence rate of colonized CRE ranging from 3% to 30.4%; meanwhile, in Asia, it ranged from 13% to 22.7% (Jean et al., 2022). The European Antimicrobial Resistance Surveillance Network (EARS-Net) described considerable variability across EEA/EU countries. The carbapenem resistance in *K. pneumoniae* aggressive isolates was observed to be ranging from 0% to 65% in 2017 (ECDC, 2018).

A national report from the surveillance network in China reported the prevalence of *K. pneumoniae* isolates unresponsiveness to meropenem and imipenem enhanced from 2.9% to 24.0% and 3.0%, to 20.9%, respectively, between the years 2005 and 2017 (Ding et al., 2019).

Indian studies have shown that the CRE prevalence rate varies from 13% in south India to 31% in western India. There may be varying factors influencing this prevalence. The density of the population in India, the ability of the organisms to spread through the intestinal flora of healthy carriers, the lack of adequate public health infrastructure, and the lack of antimicrobial stewardship (AMS) policies are a few of them. The lack of an Antimicrobial Stewardship Programme (AMSP) is related to nonjudicious application of drugs in healthcare settings, thus leading to selection pressure for resistant strains. These strains are eventually transmitted in the hospital, as well as the community, through various routes due to poor infection control practices (Modi et al., 2021). The surveillance data of the Indian Council of Medical Research (ICMR) Antimicrobials has shown a steady fall in Imipenem susceptibility among *E. coli* isolates (86% in 2016 to 63% in 2019), which in 2020 slightly improved to 72%. *Klebsiella pneumonia*-susceptible isolates plunged from 65% to 45% during the period 2016–2020 (ICMR, 2020).

Furthermore, with the emergence of ESBL-producing Enterobacteriaceae around the year 2000, carbapenems were increasingly being used as a treatment option. Overuse of this class of antibiotics quickly resulted in the spread of carbapenemase-producing isolates of Enterobacteriaceae globally at an alarming rate (Elshamy & Aboshanab, 2020).

Mechanism of Resistance The resistance to carbapenems among Enterobacteriaceae is based on three main mechanisms: (a) enzymatic hydrolysis of carbapenems by carbapenemases. Carbapenemase enzymes are categorized into three key categories established on their molecular configuration: Ambler Classes A, B, and D. Class A carbapenemases are utmost shared and include *Klebsiella pneumoniae* carbapenemase (KPC) and imipenem-hydrolyzing beta-lactamase (IML). Class B metallo-beta-lactamases (MBL), namely, New Delhi Metallo-beta-lactamase (NDM), imipenem-unresponsive *Pseudomonas* (IMP), and Verona integron-encrypted metallo-lactamase (VIM), whereas oxacillin-hydrolyzing carbapenemase (OXA)) enzymes comprise class D carbapenemases. (b) Isoform of efflux pumps that drive away carbapenems from the cells of bacteria, and (c) lessening the outer membrane penetrability by creation of beta-lactamases

(AmpC) in amalgamating with modifications in cell membrane of bacteria through mutations of porin (Sheu et al., 2019).

Among the carbapenems, isolates continue to show susceptibility to meropenem and imipenem; however, the susceptibility of the organism toward ertapenem is dreadful. This is most likely due to the presence of AmpC/ESBL and altered porins (Codjoe & Donkor, 2017).

Detection of carbapenemases can be done by phenotypic as well as genotypic methods. There are various methods for phenotypic detection, namely, automatic methods or disc diffusion, modified Hodge test, and discerning agar and tests of combined effect such as double disc tests. These approaches can help in detecting the carbapenem unresponsiveness but may not necessarily indicate the mechanism in effect. Tests for molecular identification of genes related to carbapenemase include PCR, LAMP, MLST, MLE electrophoresis, and DNA fingerprinting methods, namely, AFLP and PFGE (Codjoe & Donkor, 2017; Elshamy & Aboshanab, 2020).

Treatment Options The recent surge in cases of CRE infections across the globe is a cause of concern. Until a few years back, only polymyxins and aminoglycosides formed a major part of our armamentarium against these pathogens (Doi, 2019). Few other agents have been recently introduced. For instance, ceftazidime/avibactam which has been reported to be active against KPC and OXA-48 producers plazomicin, and eravacycline, the next-generation antibiotics from the aminoglycosides and tetracycline group, respectively, contained CRE in laboratory conditions (Sheu et al., 2019). Other possible emerging therapeutic options are ceftolozane, meropenem, imipenem in combination with tazobactam, vaborbactam, cilastatin-relebactam, and cefiderocol, respectively, being employed (Doi, 2019). However, studies are being carried out to assess the efficacy of these antimicrobials against lethal infections.

Timely identification and differentiation between clinical pathogens and carriers are of critical importance in tackling CRE cases. Rational use of antibiotics and active screening of carriers accompanied by better infection prevention practices and improved surveillance network will be vital in curbing CRE infection rates.

5.3 Carbapenem-Resistant *Pseudomonas aeruginosa* (CRPA)

The Problem Statement *Pseudomonas aeruginosa,* a major opportunistic microbe, is concomitant to hospital outbreaks and most nosocomial ailments. This pathogen is commonly allied with pneumonia, bacteremia, and infections of skin, soft tissue, and urinary tract, particularly among immunocompromised groups. It can form biofilms and continue to survive on various exteriors such as medical equipment; it is resistant to most disinfectants and easily transmitted from patient to patient. It is intrinsically resistant to multiple antibiotics and acquires genes encoding resistance determinants (Losito et al., 2022).

As per 2020 EARS-Net data, out of all *P. aeruginosa* isolates, 30.1% were unresponsive to a minimum of one set of drugs, viz., fluoroquinolones, ceftazidime, piperacillin-tazobactam, etc., and further unresponsiveness to carbapenem was reported in 17.8% of isolates (ECDPC, 2020) As per ICMR for 2020, the prevalence of CRPA in India is around 30–40% (ICMR, 2020).

Mechanism of Resistance Carbapenem resistance in *P. aeruginosa* develops due to multiple factors, including the attainment of movable genes encrypting carbapenemases, such as the metallo-β-lactamases (MBLs), KPC, increased expression of the chromosomal cephalosporinase AmpC, porin loss due to mutations of OprD gene, overexpression of MexA-MexB-OprM efflux pump, and/or penicillin-binding protein alterations (Xu et al., 2020).

Due to changes in the porin expression, carbapenem resistance was first reported in *Pseudomonas aeruginosa* in the mid-1980s. In comparison, meropenem is less prone to developing porin-mediated resistance mechanism as it passes more swiftly through the OprD porin; however, upregulation of efflux pumps can lead to meropenem resistance. On the other hand, ertapenem has little or no activity against *Pseudomonas aeruginosa* (Doi, 2019).

Treatment Options Ceftolozane–tazobactam and ceftazidime–avibactam have good safety profiles and are efficient in treating carbapenem-resistant *Pseudomonas aeruginosa*. However, in contemporary period, intermittent circumstances of unresponsiveness were recorded for these antibiotics (Nichols et al., 2016; Teo et al., 2021). Ceftolozane–tazobactam-non-susceptible isolates can be treated using imipenem–cilastatin–relebactam, another novel drug combination. Cefiderocol is another effective option with excellent in vitro activity and stability, especially in cases with more complex mechanisms of resistance (Losito et al., 2022).

In view of inadequate opportunities for managing CRPA ailments, monitoring and controlling the spread of genes that cause resistance to these drugs through strict stewardship of drugs and stringent resistor procedures for nosocomial infections is the need of the hour.

5.4 Vancomycin-Resistant *Enterococci* (VRE)

The Problem Statement During the late 1970s, *Enterococci* were first identified as a common nosocomial pathogen due to overuse of third-generation cephalosporins to which enterococci are intrinsically obdurate (Cetinkaya et al., 2000). As per the National Healthcare Safety Network data from 2011 to 2014, *Enterococci* was the second most common organism causing healthcare-associated infections. *E. faecalis* isolation rate was 7.4%; however, vancomycin resistance reportedly is more common among *E. faecium* strains. From 2011 to 2014, approximately 83.8% of isolates causing CLABSI and 86.2% of isolates causing CAUTI were found to be vancomycin-resistant *E. faecium* strains (Levitus et al., 2022). The studies in India revealed that that the rate of VRE in *E. faecalis* is far less (2.8%), whereas it was

higher in *E. faecium* (22.7%) (ICMR, 2020). Studies from Europe have reported a substantial proliferation in the incidence of vancomycin-resistant *Enterococcus faecium* isolated from bloodstream infections (2015: 10.5% vs. 2019: 18.3%) (Correa-Martínez et al., 2022). Often vancomycin-sensitive strains isolated from patients do not respond to the treatment because of inducible resistance genes, *VanA* and *VanB*. Such isolates should be re-cultured in a few days to review the susceptibility (Levitus et al., 2022).

Research has shown that VRE isolates are capable of surviving on surfaces like countertops for up to 7 days and can be recovered from bedrails, telephone handpieces, or stethoscope diaphragms for up to 24 hours or more. It can stay in the hands of healthcare workers for around 60 minutes after inoculation. Further surveys have found that as many as 26–41% of healthcare workers were VRE carriers (Levitus et al., 2022; Cetinkaya et al., 2000).

The hazards associated with VRE colonization are observed to be high among the patients who are hospitalized, especially the ones who are being treated in intensive care units, have co-morbid conditions, and have undergone invasive procedures (Davis et al., 2020).

Mechanism of Resistance The foremost mode of glycopeptide unresponsiveness, for instance, vancomycin in Enterococci is due to replacement of D-alanine-D-alanine, to D-alanine-D-lactate or D-alanine-D-serine that ultimately alters the peptidoglycan synthesis pathway. This is coded by genotypes identified alphabetically as *VanA* to *VanG*. Of these, *VanA* and *VanB* genotypes are plasmid coded and by far the most common (Ahmed & Baptiste, 2018; Levitus et al., 2022).

Treatment Options Intrinsic resistance to multiple antibiotics and the inducible resistance gene have made treatment options for VRE very scarce. Over the past decade, linezolid, daptomycin, quinupristin–dalfopristin, and tigecycline application substantially enhanced as an ultimate solution in the management of VRE isolates (Ahmed & Baptiste, 2018).

The increased prevalence of VRE in hospitals worldwide points to the lack of appropriate infection control programs surveillance systems and inefficient antibiotic stewardship. Active improvement in these aspects is a critical step toward curbing the further rise of VRE.

5.5 Drug-Resistant *Neisseria*

The Problem Statement Among the sexually transmitted diseases, *Neisseria gonorrhoeae* ranks second with considerably high morbidity (St. Cyr et al., 2020).

In the United States, annual drug-resistant *N. gonorrhoeae* infections are approximately 550,000 and 1.14 million new cases every year as documented in the CDC report of 2019 (CDC, 2021). Studies across Europe have reported high cefixime-resistant *N. gonorrhea*; Slovakia, Austria, Poland, Germany, Belgium, Luxembourg, and Croatia at 3.6%, 4.2%, 5.2%, 6.4%, 8.1%, 10%, and 11.1%, respectively

(Młynarczyk-Bonikowska et al., 2019). The data seems to be scarce from economically underprivileged nations. However, the WHO worldwide AMR assessment for *Neisseria gonorrhea* during the years 2017–18 showed that less than 5% isolates had been reported as having decreased susceptibility or resistance to ceftriaxone (Unemo et al., 2021).

The majority of cases of *N. gonorrhoeae* are asymptomatic and can be missed; therefore, routine screening for prompt diagnosis and effective treatment is of significance. Cases that are missed and left untreated often lead to complications such as sterility in females, ectopic pregnancies, and pelvic inflammatory infections (Kueakulpattana et al., 2021).

Mechanism of Action The increasing trend of *Neisseria gonorrhoeae* being reported unresponsive to ceftriaxone and cefixime that come under the category of extended spectrum of cephalosporins (ESC), is a cause of grave concern. The characteristic feature of *Neisseria* genus is to receive DNA of chromosomes through the process of transformation from the other *Neisseria* triggering number of mutations in its chromosomal genes. The resistance mechanisms seen in *N. gonorrhoeae* isolates are alterations in the chromosomal area of penA gene (encrypting the PBP2 protein's transpeptidase sphere), which has contributed the furthermost to the expansion of chromosomal unresponsiveness or condensed vulnerability to ESC group of bacteria. Other mechanisms involve overexpression of efflux pumps like MtrCDE membrane pump proteins (Młynarczyk-Bonikowska et al., 2019).

In the past decade, NAAT (molecular methods) has become the test of choice for the diagnosis of gonorrhea. Conventional culture methods are not used that often, and this presents a major challenge when dealing with emerging drug resistance because the existing amplifications processing of screening with nucleic acids will not facilitate susceptibility to drugs. Furthermore, the lack of standard or established breakpoints and different sampling strategies across countries has resulted in skewed epidemiological and resistance rates; therefore, comparison of data like epidemiological patterns cannot be done effectively (Costa-Lourenço et al., 2017). Thus, the need of the hour is enhanced molecular diagnostics that can guide antibiotic therapy by providing antimicrobial-susceptibility patterns. Novel know-hows such as WGS methods that can detect the drug-resistant isolates can help in resolving this issue (Cristillo et al., 2019).

5.6 Methicillin-Resistant *Staphylococcus aureus* (MRSA)

The Problem Statement *Staphylococcus aureus* is one of the commonly encountered organisms in hospital settings. In the past few decades, a more notorious form of MRSA has developed. The first case reports of MRSA came in 1961 from the United Kingdom (Jevons, 1961). It is a superbug with a multitude of virulence characters and the capability to obtain obduracy to most drugs, namely, β-lactams viz., penicillins, chloramphenicol, cephalosporins, tetracyclines, quinophthalones, aminoglycosides, sulfonamides, etc. Thus, it is frequently associated with treatment failures and fatal infections in patients (Guo et al., 2020; Lakhundi & Zhang, 2018).

Over the years, two types of MRSA have emerged, namely, community-acquired MRSA and hospital-acquired MRSA. Although they evolve from a single bacterium, they have a distinct genetic reservoir and so differ widely in terms of resistance patterns, the population affected, toxins, virulence factors, and resistance genes. The wide spectrum of infections caused by MRSA ranges from mild diseases related to skin and soft tissue to lethal illnesses, namely, infective endocarditis, osteomyelitis, bacteremia, etc. Studies have shown that mortality due to systemic MRSA infections can be as high as 60% (Guo et al., 2020).

In India, the pervasiveness of MRSA in nosocomial and community settings is high and varies between 40% and 70% (ICMR, 2020; NCDC, 2021). The CDC report of 2019–2020 showed an enhancement of 15% bacteremia associated with MRSA picked up from hospital sources (CDC, 2021).

Mechanism of Resistance The evolution of MRSA is due to the existence of the mecA exogenous gene, which is integral to staphylococcal cassette chromosome SCCmec that produces a transpeptidase PB2a, which in turn alters the affinity of the organism toward beta-lactam class of antibiotics. This penicillin-binding protein is one of the unique and medically relevant chromosome-mediated drug resistances that occurs via phage transduction. Based on antibiotic susceptibility testing guidelines, a *Staphylococcus aureus* isolate found resistant to oxacillin is called MRSA (Lakhundi & Zhang, 2018).

Treatment Options Vancomycin is the ideal drug for the medication for MRSA (Brown and Brown, 2021; ICMR, 2019). But many cases of vancomycin treatment failure and the emergence of strains, namely, VRSA, VISA, and Hetero-VRSA, are being reported worldwide (Guo et al., 2020).

In cases where nephrotoxicity is a concern, teicoplanin can be an alternative to vancomycin. Other treatment options for MRSA include linezolid, daptomycin, ceftaroline, and combination therapies (Brown and Brown, 2021; ICMR, 2019). MRSA spread in hospital settings usually happens due to lack of infection prevention practices. Therefore, the implementation of infection-control steps such as hand hygiene compliance and adherence to contact precautions are imperative in the deterrence and governing of MRSA infections. Other critical steps to curb healthcare-associated MRSA infection rates are prompt isolation or cohort of patients in wards, regular screening of MRSA carriers, and identifying colonized healthcare workers through surveillance, decolonization of carriers using mupirocin and chlorhexidine body washes, and environmental decontamination, as almost 20% of populace are carriers of *S. aureus* on a long-term basis (Guo et al., 2020).

5.7 Clarithromycin-Resistant *Helicobacter pylori* (CRHP)

The Problem Statement *Helicobacter pylori* is responsible for communal protracted bacterial infection among humans, leading to 4.4 billion cases per year around the globe. A study done to find the prevalence of *H. pylori* reported a

prevalence rate ranging between 28% to 84% across populations (Saleem & Howden, 2020). The annual relapse hazard was 3.4% and 8.7% for high- and low-income category countries, respectively (Miftahussurur et al., 2019).

Since *H. pylori* has been the etiological agent associated with gastric adenocarcinomas, peptic ulcers chronic atrophic gastritis, and B-cell mucosa-associated lymphoid tissue (MALT) lymphomas, it is a lingering major problematic organism across the world, and hence, the need of the hour is complete suppression (Hu et al., 2017; Kocsmár et al., 2021; Saleem & Howden, 2020). However, transmission of drug obdurate strains has led to the failure of triple-drug treatment over the years. Although monoresistance to clarithromycin, amoxicillin, and metronidazole is reported, the most common and rapidly increasing resistance is reported to clarithromycin, and thus, clarithromycin-immuned *H. pylori* incorporated in the top preeminence pathogen group by the WHO (2017).

Mechanism of Resistance Clarithromycin is a bacteriostatic macrolide that acts by adhering to the 50S ribosomal subunit of *H. pylori* and inhibits production of proteins. The unresponsiveness to clarithromycin (Cla-res) in *H. pylori* takes place owing to topical transmutations of specific codons in the peptidyl transferase area of the 23S rRNA, lowering the affinity of the drug toward the bacterial ribosome. In these 23s mutant strains additionally, efflux pumps synergistically offer resistance by pushing the drug out of the cells (Kocsmár et al., 2021).

Treatment Options Recommended first-line treatment options include quadruple regimens either bismuth-based (two antibiotics, plus bismuth, and proton pump inhibitors) or concomitant/non-bismuth-based. However, the potential toxicity of bismuth as well as the scarcity of bismuth salts in a few countries has been a cause of concern (Goderska et al., 2017; Chey et al., 2017). Newer drug combinations are also being introduced like a highly effective rifabutin-centered blend, permitted lately by the USFDA. A potassium-competitive acid blocker vonoprazan has shown promising results as part of dual-/triple-combination regimens and is still under evaluation (Hu et al., 2017; Saleem & Howden, 2020). Of late, the treatment of *H. pylori*-related infections with probiotics, along with routine antimicrobial therapy, has garnered significant attention. It helps by facilitating eradication and improving tolerability for treatment-related side effects (Goderska et al., 2017).

The most frequently used screening methods in the identification of *H. pylori* are the tests of urea breath and the fecal antigen kind as they are noninvasive and have great accuracy and specificity. These tests can also be employed to make initial diagnosis as well as know the eradication status post-treatment; however, they do not provide the resistance profile of the organism. Invasive methods include endoscopy to obtain biopsy samples to test for urease activity, histopathology, and culture. The culture method can guide susceptibility-based therapy, avoids the use of unnecessary antibiotics, and is a good alternative in the present scenario of increasing resistance (Hu et al., 2017; Saleem & Howden, 2020).

5.8 Fluoroquinolone-Resistant *Salmonella* (FRS)

The Problem Statement *Salmonella* infection lingers to be a predominant apprehension of public health across the world and places an increased economic burden, especially in developing countries of South and Southeast Asia. *Salmonella* genus has over 2600 *Salmonella* serotypes, mostly belonging to *Salmonella enterica* subsp. *enterica*, which are responsible for the maximum number of infirmities in humans. Human Salmonellosis can present clinically as bacteremia, enteric fever, and gastroenteritis, and sometimes lead to extraintestinal problems and a lingering carrier state. Across the world, nearly 93.8×10^6 foodborne infections and 1.55×10^5 mortalities per annum are linked to nontyphoidal *Salmonella* (NTS) as one of the shared pathogens that is the root cause of bacterial enteritis (Gong et al., 2022). Typhoidal *Salmonella* is the leading cause of typhoid fever, accounting for approximately 21.7 million cases and 217,000 deaths every year (Cuypers et al., 2018). The incidence of culture-confirmed typhoid cases in India is around 377 per 100,000 population and case fatality rate of 1% (Veeraraghavan et al., 2021).

In order to term an isolate as Multidrug-resistant (MDR) *Salmonella*, there should be co-resistance to the first-line antibiotics ampicillin, chloramphenicol, and trimethoprim/sulfamethoxazole. The emergence of this strain led to the rampant use of fluoroquinolones. However, by 2010, this indiscriminate use of fluoroquinolones gave rise to complete fluoroquinolone-resistant isolates, including resistance to even the third-generation fluoroquinolone gatifloxacin, and subsequently causing treatment failures and various problems such as gastrointestinal bleeding, intestinal perforation, and less frequently encephalopathy and shock (Crump et al., 2015; Eng et al., 2015). Thus in 2017, the WHO included FQ-resistant *Salmonella* in the list of high-priority pathogens (WHO, 2017).

Mechanism of Resistance The fluoroquinolones unresponsiveness is due to transmutation at the quinolone unresponsive defining locations known as *"Quinolone Resistance-Determining Regions"* (*QRDRs*) of the gene gyrA, which decreases quinolone-binding affinity of topoisomerase enzymes, and by means of upregulation or downregulation and of chromosome-encoded porins or multidrug efflux pumps (Crump et al., 2015; Eng et al., 2015). The quinolone unresponsiveness occurring through plasmids is called "*Plasmid-Mediated Quinolone Resistance (PMQR)*" has also been observed via three genes: (i) *qnr* genes that encrypt topoisomerase-binding proteins responsible for causing a physical barrier for the drug; (ii) genes encoding a modifying enzyme that decreases FQ activity (the *aac (6′)-lb-cr* gene); and (iii) genes that encode quinolone efflux pumps (*oqxAB* and *qepA*) (Cuypers et al., 2018; Li et al., 2018).

Treatment Options Azithromycin and ceftriaxone have become the treatment of choice due to rapidly developing fluoroquinolone-resistant strains. Although the susceptibility for these two drugs is still good due to selective pressure, sporadic occurrence of ceftriaxone and azithromycin-unresponsive strains was recorded in the last few years (Veeraraghavan et al., 2021).

Limiting the use of fluoroquinolones, together with the judicious use of azithromycin and ceftriaxone, should be implemented strictly as we have very few treatment options for Salmonellosis (Li et al., 2018). Despite the dose recommendation for azithromycin being changed years ago, many clinicians are still prescribing suboptimal doses (NCDC, 2016; ICMR, 2019).

Lack of diagnostic tests complicates the controlling of typhoid infection, and, furthermore, makes it problematic to differentiate these contagions the fibrile-associated infections. The mainstay of laboratory diagnosis for typhoid fever is microbiological confirmation by blood or bone marrow culture (Crump et al., 2015). However, this method lacks good sensitivity (ranging between 40% and 80%) (Eng et al., 2015). Antibiotics being used as growth promoters in animal feed, and their unregulated use in the veterinary field to treat various infections, have both been indirectly responsible for the development of drug unresponsiveness in *Salmonella* (Vercelli et al., 2022).

5.9 Fluoroquinolone-Unresponsive *Campylobacter jejuni*

The Problem Statement *Campylobacter jejuni* is a grim civic well-being hazard worldwide as a source of gastroenteritis. The rapid spread of fluoroquinolone-resistant strains has only added to the disease burden. *Campylobacter* is a commensal found in the gut flora of chicken and is transmitted to humans upon ingestion of uncooked/raw poultry (Sproston et al., 2018; Whelan et al., 2019). Despite the fact that it is highly invasive in human intestine, most diseases due to *Campylobacter* are self-limiting. However, due to the unsupervised use of fluoroquinolones to treat every undiagnosed cases of gastroenteritis in humans and rampant misuse of fluoroquinolones in poultry, the resistant isolates have been associated with abdominal and general ailments. The persistent sequelae in communities include serious diseases, namely, Guillain–Barré syndrome hemolytic uremic syndrome, Miller–Fisher syndrome, Reiter's syndrome, reactive arthritis, and septicemia (Sierra-Arguello et al., 2018; Whelan et al., 2019).

Campylobacteriosis affects an estimated 400 and 500 million individuals across the globe annually. Various studies have reported a high prevalence of fluoroquinolone-resistant *Campylobacter* isolates among humans and animals (Kaakoush et al., 2015). Studies indicate that in the United States and Canada, Europe and Africa, and Asia, the prevalence rates are 19–47%, 17–99%, and >80%, respectively (Khademi & Sahebkar, 2020).

Mechanism of Resistance Modifications in gyrA gene encrypting fragment of the GyrA subunit of DNA gyrase, which is one of the target bacterial enzymes of quinolones, lead to fluoroquinolone resistance among the *Campylobacter* strains. The other mechanism of resistance is reduced outer membrane permeability and efflux pump existence, CmeABC. CmeABC is a multidrug efflux pump responsible for antimicrobial resistance against fluoroquinolones and macrolides and works synergistically with the mutation in Gyr A mutations (Lin et al., 2002; Wieczorek & Osek, 2013).

Treatment Options Although not all cases of campylobacteriosis require antimicrobial therapy, only the immunocompromised with complications would need to be treated with antibiotics. Gentamicin and macrolides have been still found to be effective against *Campylobacter*-related ailments (Sproston et al., 2018). However, the rapid emergence of resistance to fluoroquinolones among *Campylobacter* strains led to its inclusion in the WHO priority list of pathogens.

Multiple studies have established the relationship between the misapplication of drugs in all animal sectors, especially fluoroquinolones being used as growth promoters among poultry, and an increase in the number of resistant isolates of *Campylobacter* in humans (Sierra-Arguello et al., 2018; Wieczorek & Osek, 2013).

As per a report by the CDC, the number of fluoroquinolone-resistant *C. jejuni* strains increased in the United States by 8.55% between the years 1997 and 2015 (CDC, 2018).

Some emerging pathogens like *Clostridioides difficile* and *Candida auris* have also become a foremost health hazard in the past few years; however, they are not yet incorporated into the WHO priority pathogen list. However, the CDC of the United States has categorized these as high-threat pathogens (CDC, 2019).

5.10 *Clostridiodes difficile*

Problem Statement *Clostridioides difficile* is the most frequently reported hospital-acquired intestinal infection globally (Peng et al., 2017). Nearly all pseudomembranous colitis-related ailments and approximately 15–25% of diarrheal drug-related infections are caused by this organism. This organism is responsible for the rates of demises to an extent of 17% and an even higher rate of 25% in immunocompromised elderly citizens (Dilnessa et al., 2022).

Inappropriate and prolonged use of broad-spectrum antibiotics like ampicillin, amoxicillin, cephalosporins, fluoroquinolones, and clindamycin leads to the disruption of human intestinal flora and the consequent proliferation of *C. difficile* (Leffler & Lamont, 2015). Hypervirulent strains of *C. difficile* are notorious and becoming a major nosocomial pathogen (Dilnessa et al., 2022). The molecular studies from the last decade have shown that hypervirulent drug-resistant strains ribotype (RT) 027 and 078 were responsible for major outbreaks across, Europe, North America, and South Africa (Harnvoravongchai et al., 2017). Numerous epidemics were recorded in Europe, North America, Oceania, and South Africa during the last decade (Borren et al., 2017), whereas outbreaks in Asia were linked to multidrug-resistant *C. difficile* PCR ribotypes 017 and 018. Other less common but reported to have multidrug-resistant activity are ribotypes 053 and 078 (Harnvoravongchai et al., 2017).

Although it has been established that *C. difficile* infection occurs due to antibiotic misuse, its spore-forming nature helps protect against the antibiotic activity and germinate, thereby leading to cases of relapse of *C. difficile* infection (CDI) post-treatment completion (Peng et al., 2017).

Mechanism of Resistance *C. difficile* develops drug resistance mainly by three mechanisms: suppression of the drugs, alteration of the target drug, and active efflux pump. Modification of the target drug occurs through methylation, protection, or some genetic mutation that leads to decreased binding affinity and limited target access. *C. difficile* could make antibiotics nonfunctional by degrading or modifying them via enzymatic degradation and modification. Furthermore, *C. difficile* has also been seen to modulate metabolic pathways to respond to antibiotics. Genome flexibility in *C. difficile* is due to the mobile genetic elements that comprise more than 10% of its genome. Mobile genetic elements contribute to its pathogenicity, virulence, and resistance mechanisms (Harnvoravongchai et al., 2017).

Treatment Options Presently, metronidazole, vancomycin, and fidaxomicin are effective drugs and are projected for managing primary and recurrent CDI. Because only a small number of antibiotics are available as treatment options for CDI, surveillance of circulating strains and their resistance profiles is critical to tackling this pathogen. Some alternatives are also available as treatment options, namely, tigecycline and rifampicin, out of which tigecycline had a lower resistance rate (Sholeh et al., 2020).

From the infection control perspective in the hospital, patients need to be isolated and put under contact precautions to avoid spread in the hospital. Moreover, the bacteria are resistant to commonly used hand sanitizers, and hence, handwashing with soap and water is recommended for all personnel involved in the care of these patients (Turner & Anderson, 2020).

Knowledge of circulating strains, their resistance mechanisms, strict monitoring of the broad-spectrum antibiotics use among hospitalized patients, and adherence to hospital infection control practices are indispensable practices toward curbing these infections.

5.11 *Candida auris*

The Problem Statement *C. auris* was isolated for the first time in Japan in 2009, with a specimen collected from a patient's ear suffering from external otitis media. Before that most cases of invasive candidiasis were caused by *Candida albicans,* but over the last decade, it has shifted to non-albicans *Candida*. The injudicious use of fluconazole to empirically treat cases of invasive candidiasis is responsible for the occurrence of drug-obdurate strains of *Candida auris*. The organism has been found to be associated *with various nosocomial* outbreaks and deep-seated infections in intensive care units of several hospitals. It exhibits resistance to multiple classes of antifungals (Garcia-Bustos et al., 2021; Du et al., 2020).

Therefore, this infection is often associated with treatment failures in the ICUs, especially among the immunocompromised group (Garcia-Bustos et al., 2021). Unlike other *Candida* spp. that colonize the gut, *C. auris* is postulated to primarily inhabit the skin and rarely the gut. Nearly most of its unique

characteristics, viz., high transmissibility, prolonged persistence in the healthcare settings despite the use of common disinfectants, ability to colonize patients indefinitely, and development of multidrug unresponsiveness to most classes of antifungals, made this organism a serious global health hazard. It has been seen to form dry biofilms that resist disinfectants and decontamination procedures done routinely in hospitals. For this reason, *C. auris* is a major concern from an infection prevention and control perspective (Du et al., 2020). In nosocomial conditions, *C. auris* most commonly causes diseases related to bloodstream. Deep-seated 30–60% infections due to *C. auris* are accountable for global mortalities (Du et al., 2020).

Mechanism of Resistance The primary ways of obduracy against triazoles are (i) overregulation that hinders expression of ERG11, (ii) alterations in the ERG11 gene that is responsible for antifungal character, and (iii) overregulation of efflux pumps (Frías-De-León et al., 2020). Data regarding the molecular mechanism responsible for resistance against AMB are still indistinguishable. Nonetheless, considering the mode of action of polyenes, alteration in the pathway of ergosterol through gene mutation in *ERG2*, *ERG3*, and *ERG6* has been assumed to be the most important possibility (Frías-De-León et al., 2020). Information on echinocandin unresponsiveness has also been reported due to mutations observed in *FKS1* and *FKS2* genes (Frías-De-León et al., 2020). More research studies are being carried out to discover and comprehend various means of unresponsiveness in this notorious organism.

Treatment Options *C. auris* isolates have shown a higher frequency of unresponsiveness to the most important and repeatedly employed antifungals in medical practice, namely, azoles and amphotericin B (ICMR, 2020), although resistance patterns differ between clades. Echinocandins are still the drugs of choice for this organism, despite drug-unresponsive strains gradually being discovered (ICMR, 2020). Some alternative therapies being researched are nitric oxide (NO) in nanoparticles, normal peptides, and phenolic compounds. Furthermore, reuse of old drugs like miltefosine and iodoquinol is also being explored (Frías-De-León et al., 2020). Early and timely diagnosis of fungal infections, along with susceptibility report-guided treatment and robust infection control practices, is needed to tackle *C. auris* infections in hospitals.

6 Initiatives in India for Containment of AMR

In the recent years, it has been acknowledged in India that AMR alleviation is a nation's main concern. India has announced various approaches, changing from instructive and responsiveness initiatives, infection governing regimes, reconnaissance agendas, and antimicrobial stewardship to govern the calamity of AMR (Fig. 3).

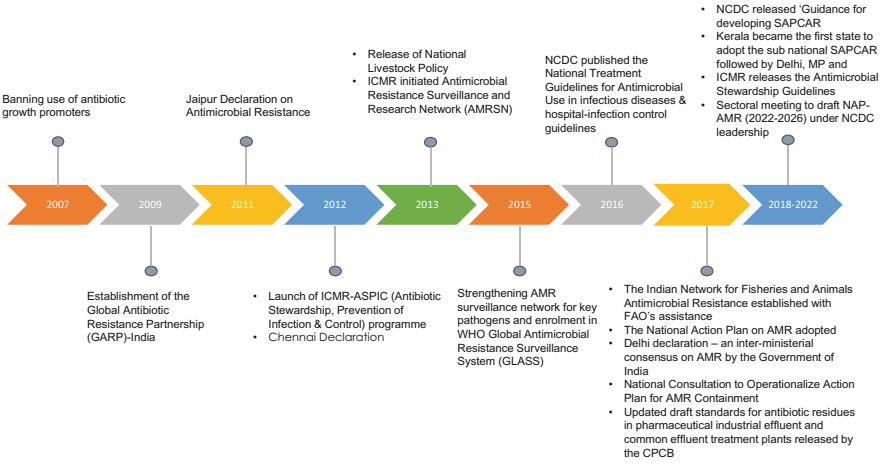


Fig. 3 Initiatives for containment of AMR in India

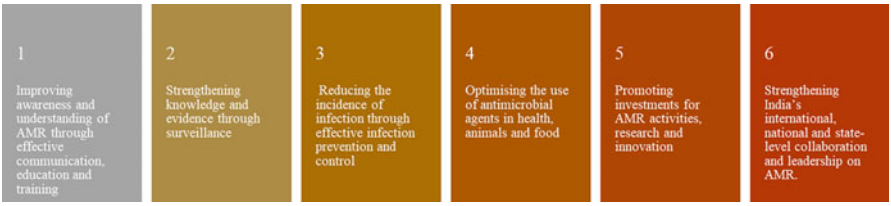


Fig. 4 Strategic priorities of NAP-AMR

6.1 National Action Plan (2017–2021)

NAP-AMR replicates the GAP of WHO and adheres to a One Health concept, including AMR in the perspective of animal, agriculture, environment, and human well-being sectors following the six premeditated primacies (Fig. 4).

Though the NAP-AMR efficaciously emulates WHO's Global Action Plan, inadequate multisectoral coordination, insufficient fiscal support across the nation, poor implementation, and pandemic of COVID-19 have hampered the progress.

6.2 Combating AMR: Concept of "*One Health*"

The concept of "*One Health"* is based on a combined strength of numerous disciplines that join together to make available elucidations for animal, environmental, and human well-being. In the process, the impediments to surpass are the contending benefits of manifold fiscal sectors and organizations mentioned above. Various stakeholders need to agree on key priorities for action, the best ways to monitor AMR and control infections, and the policies that should govern

Fig. 5 Key strategies for addressing AMR from the One Health approach

antimicrobial use. Some of the significant stratagems for tackling the menace of AMR from the "*One Health"* approach are shown in Fig. 5.

7 Conclusion

Though India has announced capable actions for undertaking AMR, there is a long way to go and necessitates noteworthy determinations from all interested parties. Vigorously augmented intersectoral coordination and public–private partnerships will help reinforce the nation's crusade on AMR. To fight drug obduracy menace, it is mandatory to back "One Health" system that includes animal, environment, human, and plant health. A collaborative effort from all sectors, including human, faunal, food, and environment, is obligatory to control the advent and transmission of multidrug-resistant "superbugs" as these pathogens add tremendous health and financial burden by increasing morbidity and mortality.

Acknowledgments The authors thank Moe Moore, Reeti Hobson, Ashima Jain Vidyarthi, Shreeparna Ghosh, and Rajesh Bhatia for their efforts in providing an insightful review and inputs to refine the chapter.

Disclaimer This chapter was created with funding from the USAID, Global Health, under the terms of the Infectious Disease Detection and Surveillance contract GS00Q14OADU119. Opinions stated are not essentially those of USAID or the US government.

References

Ahmed, M. O., & Baptiste, K. E. (2018). Vancomycin-resistant enterococci: A review of antimicrobial resistance mechanisms and perspectives of human and animal health. *Microbial Drug Resistance, 24*(5), 590–606. https://doi.org/10.1089/mdr.2017.0147

Antimicrobial Resistance Collaborators. (2022). Global burden of bacterial antimicrobial resistance in 2019: A systematic analysis. *The Lancet, 399*(10325), 629–655. https://doi.org/10.1016/s0140-6736(21)02724-0

Borren, N. Z., Ghadermarzi, S., Hutfless, S., & Ananthakrishnan, A. N. (2017). The emergence of Clostridium difficile infection in Asia: A systematic review and meta-analysis of incidence and impact. *PLoS One, 12*(5). https://doi.org/10.1371/journal.pone.0176797

Brown, N. M., Brown, E. M., & the Guideline Development Group. (2021). Treatment of methicillin-resistant *Staphylococcus aureus* (MRSA): Updated guidelines from the UK. *Journal of Antimicrobial Chemotherapy, 76*(6), 1377–1378. https://doi.org/10.1093/jac/dkab036

Burnham, C. D., Leeds, J., Nordmann, P., O'Grady, J., & Patel, J. (2017). Diagnosing antimicrobial resistance. *Nature Reviews Microbiology, 15*(11), 697–703. https://doi.org/10.1038/nrmicro.2017.103

Centers for Disease Control and Prevention. (2018). *National antimicrobial resistance monitoring system for enteric bacteria (NARMS): Human isolates surveillance report for 2015 (final report)*. U.S. Department of Health and Human Services, CDC.

Centers for Disease Control and Prevention. (2019). *Antibiotic resistance threats in the United States, 2019*. U.S. Department of Health and Human Services, CDC. https://doi.org/10.15620/cdc:82532

Centers for Disease Control and Prevention. (2021). *2020 national and state healthcare-associated infections progress report*. Retrieved 7 Sept 2022, from https://www.cdc.gov/hai/data/portal/progress-report.html

Cetinkaya, Y., Falk, P., & Mayhall, C. G. (2000). Vancomycin-resistant enterococci. *Clinical Microbiology Reviews, 13*(4), 686–707. https://doi.org/10.1128/cmr.13.4.686

Chey, W. D., Leontiadis, G. I., Howden, C. W., & Moss, S. F. (2017). ACG clinical guideline: Treatment of Helicobacter pylori infection. *American Journal of Gastroenterology, 112*(2), 212–239. https://doi.org/10.1038/ajg.2016.563

Codjoe, F., & Donkor, E. (2017). Carbapenem resistance: A review. *Medical Science, 6*(1), 1. https://doi.org/10.3390/medsci6010001

Correa-Martínez, C. L., Jurke, A., Schmitz, J., Schaumburg, F., Kampmeier, S., & Mellmann, A. (2022). Molecular epidemiology of vancomycin-resistant enterococci bloodstream infections in Germany: A population-based prospective longitudinal study. *Microorganisms, 10*(1), 130. https://doi.org/10.3390/microorganisms10010130

Costa-Lourenço, A. P., Barros dos Santos, K. T., Moreira, B. M., Fracalanzza, S. E., & Bonelli, R. R. (2017). Antimicrobial resistance in *Neisseria gonorrhoeae*: History, molecular mechanisms and epidemiological aspects of an emerging global threat. *Brazilian Journal of Microbiology, 48*(4), 617–628. https://doi.org/10.1016/j.bjm.2017.06.001

Cristillo, A. D., Bristow, C. C., Torrone, E., Dillon, J., Kirkcaldy, R. D., Dong, H., et al. (2019). Antimicrobial resistance in *Neisseria gonorrhoeae*: Proceedings of the Star sexually transmitted infection—Clinical trial group programmatic meeting. *Sexually Transmitted Diseases, 46*(3). https://doi.org/10.1097/olq.0000000000000929

Crump, J. A., Sjölund-Karlsson, M., Gordon, M. A., & Parry, C. M. (2015). Epidemiology, clinical presentation, laboratory diagnosis, antimicrobial resistance, and antimicrobial management of invasive salmonella infections. *Clinical Microbiology Reviews, 28*(4), 901–937. https://doi.org/10.1128/cmr.00002-15

Cuypers, W. L., Jacobs, J., Wong, V., Klemm, E. J., Deborggraeve, S., & Van Puyvelde, S. (2018). Fluoroquinolone resistance in Salmonella: Insights by whole-genome sequencing. *Microbial Genomics, 4*(7). https://doi.org/10.1099/mgen.0.000195

Dadgostar, P. (2019). Antimicrobial resistance: Implications and costs. *Infection and Drug Resistance, 12*, 3903–3910. https://doi.org/10.2147/idr.s234610

Davis, E., Hicks, L., Ali, I., Salzman, E., Wang, J., Snitkin, E., et al. (2020). Epidemiology of vancomycin-resistant *Enterococcus faecium* and *Enterococcus faecalis* colonization in nursing facilities. *Open Forum Infectious Diseases, 7*(1). https://doi.org/10.1093/ofid/ofz553

Dhingra, S., Rahman, N. A., Peile, E., Rahman, M., Sartelli, M., Hassali, M. A., et al. (2020). Microbial resistance movements: An overview of global public health threats posed by antimicrobial resistance, and how best to counter. *Frontiers in Public Health, 8*. https://doi.org/10.3389/fpubh.2020.535668

Dilnessa, T., Getaneh, A., Hailu, W., Moges, F., & Gelaw, B. (2022). Prevalence and antimicrobial resistance pattern of clostridium difficile among hospitalized diarrheal patients: A systematic review and meta-analysis. *PLoS One, 17*(1). https://doi.org/10.1371/journal.pone.0262597

Ding, Y., Wang, Y., Hsia, Y., Sharland, M., & Heath, P. T. (2019). Systematic review of carbapenem-resistant Enterobacteriaceae causing neonatal sepsis in China. *Annals of Clinical Microbiology and Antimicrobials, 18*(1), 36.

Doern, G. V., & Brecher, S. M. (2011). The clinical predictive value (or lack thereof) of the results of in vitro antimicrobial susceptibility tests. *Journal of Clinical Microbiology, 49*(9_Supplement). https://doi.org/10.1128/jcm.00580-11

Doi, Y. (2019). Treatment options for carbapenem-resistant gram-negative bacterial infections. *Clinical Infectious Diseases, 69*(Supplement_7). https://doi.org/10.1093/cid/ciz830

Du, H., Bing, J., Hu, T., Ennis, C. L., Nobile, C. J., & Huang, G. (2020). Candida Auris: Epidemiology, biology, antifungal resistance, and virulence. *PLoS Pathogens, 16*(10). https://doi.org/10.1371/journal.ppat.1008921

Elshamy, A. A., & Aboshanab, K. M. (2020). A review on bacterial resistance to carbapenems: Epidemiology, detection and treatment options. *Future Science OA, 6*(3). https://doi.org/10.2144/fsoa-2019-0098

Eng, S., Pusparajah, P., Ab Mutalib, N., Ser, H., Chan, K., & Lee, L. (2015). Salmonella: A review on pathogenesis, epidemiology and antibiotic resistance. *Frontiers in Life Science, 8*(3), 284–293. https://doi.org/10.1080/21553769.2015.1051243

European Centre for Disease Prevention and Control. (2018). *Surveillance of antimicrobial resistance in Europe – Annual report of the European Antimicrobial Resistance Surveillance Network (EARS-Net) 2017*. ECDC.

European Centre for Disease Prevention and Control. (2020). *Antimicrobial consumption in the EU/EEA – Annual epidemiological report 2019*. ECDC.

Frías-De-León, M. G., Hernández-Castro, R., Vite-Garín, T., Arenas, R., Bonifaz, A., Castañón-Olivares, L., et al. (2020). Antifungal resistance in Candida auris: Molecular determinants. *Antibiotics, 9*(9), 568. https://doi.org/10.3390/antibiotics9090568

Garcia-Bustos, V., Cabanero-Navalon, M. D., Ruiz-Saurí, A., Ruiz-Gaitán, A. C., Salavert, M., Tormo, M. Á., & Pemán, J. (2021). What do we know about Candida auris? State of the art, knowledge gaps, and future directions. *Microorganisms, 9*(10), 2177. https://doi.org/10.3390/microorganisms9102177

Gelband, H. (2016). *CARA: The conscience of antimicrobial resistance accountability, and the next big thing for CDDEP [Web log post]*. Retrieved 2022, from https://cddep.org/blog/posts/cara_conscience_antimicrobial_resistance_accountability_and_next_big_thing_cddep/

Goderska, K., Agudo Pena, S., & Alarcon, T. (2017). Helicobacter pylori treatment: Antibiotics or probiotics. *Applied Microbiology and Biotechnology, 102*(1), 1–7. https://doi.org/10.1007/s00253-017-8535-7

Gong, B., Li, H., Feng, Y., Zeng, S., Zhuo, Z., Luo, J., et al. (2022). Prevalence, serotype distribution and antimicrobial resistance of non-typhoidal salmonella in hospitalized patients in Conghua District of Guangzhou, China. *Frontiers in Cellular and Infection Microbiology, 12*. https://doi.org/10.3389/fcimb.2022.805384

Guo, Y., Song, G., Sun, M., Wang, J., & Wang, Y. (2020). Prevalence and therapies of antibiotic-resistance in *Staphylococcus aureus*. *Frontiers in Cellular and Infection Microbiology, 10*. https://doi.org/10.3389/fcimb.2020.00107

Harnvoravongchai, P., Pipatthana, M., Chankhamhaengdecha, S., & Janvilisri, T. (2017). Insights into drug resistance mechanisms in *Clostridium difficile*. *Essays in Biochemistry, 61*(1), 81–88. https://doi.org/10.1042/ebc20160062

Hay, S. I., Rao, P. C., Dolecek, C., Day, N. P., Stergachis, A., Lopez, A. D., & Murray, C. J. (2018). Measuring and mapping the global burden of antimicrobial resistance. *BMC Medicine, 16*(1). https://doi.org/10.1186/s12916-018-1073-z

Hu, Y., Zhu, Y., & Lu, N. (2017). Novel and effective therapeutic regimens for helicobacter pylori in an era of increasing antibiotic resistance. *Frontiers in Cellular and Infection Microbiology, 7*. https://doi.org/10.3389/fcimb.2017.00168

ICMR. (2019). *Indian Council of medical research treatment guidelines for antimicrobial use in common syndromes* (2nd ed.). ICMR, Division of ECD, Ansari Nagar.

ICMR (2020). *Indian Council of Medical Research Antimicrobial Resistance Research and Surveillance Network (January 2020 to December 2020). Annual report*. Division of Epidemiology and Communicable Diseases, ICMR.

Isler, B., Doi, Y., Bonomo, R. A., & Paterson, D. L. (2019). New treatment options against carbapenem-resistant Acinetobacter baumannii infections. *Antimicrobial Agents and Chemotherapy, 63*(1). https://doi.org/10.1128/aac.01110-18

Jean, S., Harnod, D., & Hsueh, P. (2022). Global threat of carbapenem-resistant gram-negative bacteria. *Frontiers in Cellular and Infection Microbiology, 12*. https://doi.org/10.3389/fcimb.2022.823684

Jevons, M. P. (1961). "Celbenin" – Resistant Staphylococci. *British Medical Journal, 1*(5219), 124–125.

Kaakoush, N. O., Castaño-Rodríguez, N., Mitchell, H. M., & Man, S. M. (2015). Global epidemiology of Campylobacter infection. *Clinical Microbiology Reviews, 28*(3), 687–720. https://doi.org/10.1128/cmr.00006-15

Kayange, N., Kamugisha, E., Mwizamholya, D. L., Jeremiah, S., & Mshana, S. E. (2010). Predictors of positive blood culture and deaths among neonates with suspected neonatal sepsis in a tertiary hospital, Mwanza-Tanzania. *BMC Pediatrics, 10*(1). https://doi.org/10.1186/1471-2431-10-39

Khademi, F., & Sahebkar, A. (2020). Prevalence of fluoroquinolone-resistant Campylobacter species in Iran: A systematic review and meta-analysis. *International Journal of Microbiology, 2020*, 1–14. https://doi.org/10.1155/2020/8868197

Kocsmár, É., Buzás, G. M., Szirtes, I., Kocsmár, I., Kramer, Z., Szijártó, A., et al. (2021). Primary and secondary clarithromycin resistance in helicobacter pylori and mathematical modeling of the role of Macrolides. *Nature Communications, 12*(1). https://doi.org/10.1038/s41467-021-22557-7

Kueakulpattana, N., Wannigama, D. L., Luk-in, S., Hongsing, P., Hurst, C., Badavath, V. N., et al. (2021). Multidrug-resistant *Neisseria gonorrhoeae* infection in heterosexual men with reduced susceptibility to ceftriaxone, first report in Thailand. *Scientific Reports, 11*(1). https://doi.org/10.1038/s41598-021-00675-y

Lakhundi, S., & Zhang, K. (2018). Methicillin-resistant *Staphylococcus aureus*: Molecular characterization, evolution, and epidemiology. *Clinical Microbiology Reviews, 31*(4). https://doi.org/10.1128/cmr.00020-18

Laxminarayan, R., Van Boeckel, T., Frost, I., Kariuki, S., Khan, E. A., Limmathurotsakul, D., et al. (2020). The Lancet Infectious Diseases Commission on Antimicrobial Resistance: 6 years later. *The Lancet Infectious Diseases, 20*(4). https://doi.org/10.1016/s1473-3099(20)30003-7

Leffler, D. A., & Lamont, J. T. (2015). Clostridium difficile infection. *New England Journal of Medicine, 372*(16), 1539–1548. https://doi.org/10.1056/nejmra1403772

Levitus, M., Rewane, A., & Perera, T. B. (2022). *Vancomycin-resistant Enterococci*. Stat Pearls Publishing.

Li, J., Song, X., Yang, T., Chen, Y., Gong, Y., Yin, X., & Lu, Z. (2016). A systematic review of antibiotic prescription associated with upper respiratory tract infections in China. *Medicine, 95*(19). https://doi.org/10.1097/md.0000000000003587

Li, J., Hao, H., Sajid, A., Zhang, H., & Yuan, Z. (2018). Fluoroquinolone resistance in salmonella: Mechanisms, fitness, and virulence. In *Salmonella – A re-emerging pathogen*. https://doi.org/10.5772/intechopen.74699

Lin, J., Michel, L. O., & Zhang, Q. (2002). CMEABC functions as a multidrug efflux system in Campylobacter jejuni. *Antimicrobial Agents and Chemotherapy, 46*(7), 2124–2131. https://doi.org/10.1128/aac.46.7.2124-2131.2002

Losito, A. R., Raffaelli, F., Del Giacomo, P., & Tumbarello, M. (2022). New drugs for the treatment of pseudomonas aeruginosa infections with limited treatment options: A narrative review. *Antibiotics, 11*(5), 579. https://doi.org/10.3390/antibiotics11050579

Majumder, M. A., Rahman, S., Cohall, D., Bharatha, A., Singh, K., Haque, M., & Gittens-St Hilaire, M. (2020). Antimicrobial stewardship: Fighting antimicrobial resistance and protecting Global Public Health. *Infection and Drug Resistance, 13*, 4713–4738. https://doi.org/10.2147/idr.s290835

Miftahussurur, M., Aftab, H., Shrestha, P. K., Sharma, R. P., Subsomwong, P., Waskito, L. A., et al. (2019). Effective therapeutic regimens in two South Asian countries with high resistance to major Helicobacter pylori antibiotics. *Antimicrobial Resistance & Infection Control, 8*(1). https://doi.org/10.1186/s13756-019-0482-x

Młynarczyk-Bonikowska, B., Majewska, A., Malejczyk, M., Młynarczyk, G., & Majewski, S. (2019). Multiresistant Neisseria gonorrhoeae: A new threat in second decade of the XXI century. *Medical Microbiology and Immunology, 209*(2), 95–108. https://doi.org/10.1007/s00430-019-00651-4

Modi, C. M., Singh, S. P., Pandya, Y. G., Patel, C. P., & Patel, R. M. (2021). Prevalence of carbapenem-resistant Enterobacteriaceae in a tertiary care hospital of Gujarat, India. *Journal of Clinical and Diagnostic Research*. https://doi.org/10.7860/jcdr/2021/47332.14627

National Centre for Disease Control. (2016). *National treatment guidelines for antimicrobial use in infectious diseases (Version 1.0)*. NCDC, DGHS, MoHFW, GoI.

National Centre for Disease Control. (2021). (rep.). *National Antimicrobial Resistance Surveillance Network (NARS-Net, India). Reporting period January to December 2020. Annual report 2021*. NCDC, DGHS, MoHFW, GoI.

National Centre for Disease Control & World Health Organization. (2017). *National action plan on antimicrobial resistance (NAP-AMR) 2017–2021 (Rep.)*. NCDC, MoHFW, GoI.

Nichols, W. W., De Jonge, B. L., Kazmierczak, K. M., Karlowsky, J. A., & Sahm, D. F. (2016). In vitro susceptibility of global surveillance isolates of pseudomonas aeruginosa to ceftazidime-avibactam (inform 2012 to 2014). *Antimicrobial Agents and Chemotherapy, 60*(8), 4743–4749. https://doi.org/10.1128/aac.00220-16

O'Neill, J. (2016). *Tackling drug-resistant infections globally: Final report and recommendations. Review on antimicrobial resistance*. Government of the United Kingdom

Peng, Z., Jin, D., Kim, H. B., Stratton, C. W., Wu, B., Tang, Y., & Sun, X. (2017). Update on antimicrobial resistance in clostridium difficile: Resistance mechanisms and antimicrobial

susceptibility testing. *Journal of Clinical Microbiology, 55*(7), 1998–2008. https://doi.org/10.1128/jcm.02250-16

Prestinaci, F., Pezzotti, P., & Pantosti, A. (2015). Antimicrobial resistance: A global multifaceted phenomenon. *Pathogens and Global Health, 109*(7), 309–318. https://doi.org/10.1179/2047773215y.0000000030

Rosales-Reyes, R., Gayosso-Vázquez, C., Fernández-Vázquez, J. L., Jarillo-Quijada, M. D., Rivera-Benítez, C., Santos-Preciado, J. I., & Alcántar-Curiel, M. D. (2017). Virulence profiles and innate immune responses against highly lethal, multidrug-resistant nosocomial isolates of Acinetobacter baumannii from a tertiary care hospital in Mexico. *PLoS One, 12*(8). https://doi.org/10.1371/journal.pone.0182899

Saleem, N., & Howden, C. W. (2020). Update on the management of Helicobacter pylori infection. *Current Treatment Options in Gastroenterology, 18*(3), 476–487. https://doi.org/10.1007/s11938-020-00300-3

Sheu, C., Chang, Y., Lin, S., Chen, Y., & Hsueh, P. (2019). Infections caused by carbapenem-resistant Enterobacteriaceae: An update on therapeutic options. *Frontiers in Microbiology, 10*. https://doi.org/10.3389/fmicb.2019.00080

Sholeh, M., Krutova, M., Forouzesh, M., Mironov, S., Sadeghifard, N., Molaeipour, L., et al. (2020). Antimicrobial resistance in *Clostridioides* (clostridium) *difficile* derived from humans: A systematic review and meta-analysis. *Antimicrobial Resistance & Infection Control, 9*(1). https://doi.org/10.1186/s13756-020-00815-5

Sierra-Arguello, Y. M., Furian, T. Q., Perdoncini, G., Moraes, H. L. S., Salle, C. T. P., Rodrigues, L. B., dos Santos, L. R., Pereira Gomes, M. J., & do Nascimento, V. P. (2018). Fluoroquinolone resistance in Campylobacter jejuni and Campylobacter coli from poultry and human samples assessed by PCR-restriction fragment length polymorphism assay. *PLoS One, 13*(7). https://doi.org/10.1371/journal.pone.0199974

Sproston, E. L., Wimalarathna, H. M., & Sheppard, S. K. (2018). Trends in fluoroquinolone resistance in campylobacter. *Microbial Genomics, 4*(8). https://doi.org/10.1099/mgen.0.000198

St. Cyr, S., Barbee, L., Workowski, K. A., Bachmann, L. H., Pham, C., Schlanger, K., et al. (2020). Update to CDC's treatment guidelines for gonococcal infection, 2020. *MMWR. Morbidity and Mortality Weekly Report, 69*(50), 1911–1916. https://doi.org/10.15585/mmwr.mm6950a6

Taccone, F. S., Rodriguez-Villalobos, H., De Backer, D., De Moor, V., Deviere, J., Vincent, J., & Jacobs, F. (2006). Successful treatment of septic shock due to pan-resistant Acinetobacter baumannii using combined antimicrobial therapy including Tigecycline. *European Journal of Clinical Microbiology and Infectious Diseases, 25*(4), 257–260. https://doi.org/10.1007/s10096-006-0123-1

Taneja, N., & Sharma, M. (2019). Antimicrobial resistance in the environment: The Indian scenario. *Indian Journal of Medical Research, 149*(2), 119. https://doi.org/10.4103/ijmr.ijmr_331_18

Teo, J. Q.-M., Lim, J. C., Tang, C. Y., Lee, S. J.-Y., Tan, S. H., Sim, J. H.-C., Ong, R. T.-H., & Kwa, A. L.-H. (2021). Ceftolozane/tazobactam resistance and mechanisms in carbapenem-non susceptible Pseudomonas aeruginosa. *mSphere, 6*(1). https://doi.org/10.1128/msphere.01026-20

Turner, N. A., & Anderson, D. J. (2020). Hospital infection control: *Clostridiodes difficile*. *Clinics in Colon and Rectal Surgery, 33*(02), 098–108. https://doi.org/10.1055/s-0040-1701234

Unemo, M., Lahra, M. M., Escher, M., Eremin, S., Cole, M. J., Galarza, P., Ndowa, F., Martin, I., Dillon, J. R., Galas, M., Ramon-Pardo, P., Weinstock, H., & Wi, T. (2021). Who global antimicrobial resistance surveillance for Neisseria gonorrhoeae 2017–18: A retrospective observational study. *The Lancet Microbe, 2*(11). https://doi.org/10.1016/s2666-5247(21)00171-3

Vasala, A., Hytönen, V. P., & Laitinen, O. H. (2020). Modern tools for rapid diagnostics of antimicrobial resistance. *Frontiers in Cellular and Infection Microbiology, 10*. https://doi.org/10.3389/fcimb.2020.00308

Veeraraghavan, B., Pragasam, A. K., Ray, P., Kapil, A., Nagaraj, S., Perumal, S. P., et al. (2021). Evaluation of antimicrobial susceptibility profile in Salmonella typhi and Salmonella paratyphi A: Presenting the current scenario in India and strategy for future management. *The Journal of Infectious Diseases, 224*(Supplement_5). https://doi.org/10.1093/infdis/jiab144

Vercelli, C., Gambino, G., Amadori, M., & Re, G. (2022). Implications of veterinary medicine in the comprehension and stewardship of antimicrobial resistance phenomenon. From the origin till nowadays. *Veterinary and Animal Science, 16*, 100249. https://doi.org/10.1016/j.vas.2022.100249

Viehman, J. A., Nguyen, M. H., & Doi, Y. (2014). Treatment options for carbapenem-resistant and extensively drug-resistant Acinetobacter baumannii infections. *Drugs, 74*(12), 1315–1333. https://doi.org/10.1007/s40265-014-0267-8

Whelan, M. V., Ardill, L., Koide, K., Nakajima, C., Suzuki, Y., Simpson, J. C., & Cróinín, Ó. T. (2019). Acquisition of fluoroquinolone resistance leads to increased biofilm formation and pathogenicity in campylobacter jejuni. *Scientific Reports, 9*(1). https://doi.org/10.1038/s41598-019-54620-1

Wieczorek, K., & Osek, J. (2013). Antimicrobial resistance mechanisms among campylobacter. *BioMed Research International, 2013*, 1–12. https://doi.org/10.1155/2013/340605

Wong, D., Nielsen, T. B., Bonomo, R. A., Pantapalangkoor, P., Luna, B., & Spellberg, B. (2017). Clinical and pathophysiological overview of Acinetobacter infections: A century of challenges. *Clinical Microbiology Reviews, 30*(1), 409–447. https://doi.org/10.1128/cmr.00058-16

World Health Organization. (2014). *WHO's first global report on antibiotic resistance reveals serious, worldwide threat to public health. News release*. WHO. Available on https://www.who.int/southeastasia/news/detail/30-04-2014-who-s-first-global-report-on-antibiotic-resistance-reveals-serious-worldwide-threat-to-public-health

World Health Organization. (2017). *WHO publishes list of bacteria for which new antibiotics are urgently needed. News Release*. WHO. Available on https://www.who.int/news/item/27-02-2017-who-publishes-list-of-bacteria-for-which-new-antibiotics-are-urgently-needed

Xu, C., Wang, D., Zhang, X., Liu, H., Zhu, G., Wang, T., et al. (2020). Mechanisms for rapid evolution of carbapenem resistance in a clinical isolate of Pseudomonas aeruginosa. *Frontiers in Microbiology, 11*. https://doi.org/10.3389/fmicb.2020.01390

Antimicrobial Resistance in Food-Borne *Campylobacter* spp.

Manjusha Lekshmi, Sanath H. Kumar, Binaya Bhusan Nayak, and Manuel F. Varela

Contents

Abstract

Campylobacteriosis is one of the important reasons for food-related gastrointestinal infections across the world. The microaerophilic group of *Campylobacteraceae* consists of 32 spp. and 9 subspp. Among these, *Campylobacter jejuni* and *Campylobacter coli* are predominantly involved in human infections. Inadequately cooked poultry and animal meats are commonly responsible for food-borne human infections, although several other food types including contaminated milk, vegetables, and fish can be the sources of infections. Campylobacteriosis is characterized primarily by acute gastroenteritis, and the infection in humans is frequently associated with diverse sequelae such as inflammatory bowel disease, acute appendicitis, cholecystitis, celiac disease, colon cancer, endocarditis, pneumonia, and bloodstream infections. The capacity

M. Lekshmi · S. H. Kumar (✉) · B. B. Nayak
Post-Harvest Technology, ICAR-Central Institute of Fisheries Education, Andheri (W), Mumbai, India
e-mail: sanathkumar@cife.edu.in

M. F. Varela
Department of Biology, Eastern New Mexico University, Portales, NM, USA

M. P. Mothadaka et al. (eds.), *Handbook on Antimicrobial Resistance*,
https://doi.org/10.1007/978-981-19-9279-7_16

to endure temperatures of refrigeration and freezing for prolonged times, together with the low dose of infection, make *C. jejuni* an important food-borne pathogen of public health significance. Additionally, the expanding spectrum of antimicrobial resistance (AMR) in *Campylobacter* spp. has confounded the problem of campylobacteriosis. The unsusceptible condition to fluoroquinolones and macrolide antibiotics which are critically important in treating *Campylobacter* infections is on the upsurge. Campylobacteriosis has emerged as a foremost human health problem which deserves worldwide responsiveness. Some key measures can be the reduction of animal and human carriage of *Campylobacter* spp., exclusion of antibiotics in livestock and poultry, and application of biocontrol measures to reduce food contamination with this pathogen.

Keywords

Campylobacter spp. · Antibiotic resistance · Poultry · Virulence · Gene transfer

1 Introduction

Campylobacter spp. is an important food-borne gastrointestinal pathogen infecting humans of all age groups. The bacteria under the genus *Campylobacter* are Gram-negative with a short, curved morphology, oxidase-positive, and microaerophilic in nature. These bacteria occur naturally as commensal organisms in the gastral tracts of humans, domesticated and untamed faunae, especially of avian spp. (Silva et al., 2011). These microaerophilic bacteria use tricarboxylic acid intermediates and amino acids as the sources of energy, unlike other bacteria that ferment or oxidize carbohydrates (Debruyne et al., 2008). Among 32 and 9 species and subspecies of *Campylobacter* genus, *C. jejuni* and *C. coli* are the key pathogens that are responsible for infections in humans. The remaining *Campylobacter* spp., too, are randomly associated with human and animal infections. For example, *C. fetus* subsp. fetus and *C. fetus* subsp. venerealis have been categorized as important veterinary pathogens (Heredia and Garcia, 2018). Further, *Campylobacter* species are associated with sporadic but diverse contagions in humans and animals such as gastroenteritis, wound infections, Crohn's disease, invasive bloodstream infections/bacteremia, meningitis, ulcerative colitis, periodontal disease, and bone abscess (Costa and Iraola, 2019). *Campylobacter* spp., in increasing numbers in recent period, were identified as the evolving pathogens of zoonotic significance, which included *C. concisus*, *C. fetus*, C. *lari*, *C. lanienae*, *C. hepaticus*, *C. hyointestinalis*, *C. ureolyticus*, and *C. upsaliensis* (Costa & Iraola, 2019). These species have evolved efficiently to colonize a range of higher animals and birds. This expanded host range provides a wider dissemination power to the pathogen, while from zoonotic point of view, forming diverse sources of contamination of food and water with pathogenic *Campylobacter* spp.

In poultry farms, *Campylobacter* from the environment are ingested by the growing birds which get colonized in their gastrointestinal tract and spread further.

Meat and meat products from animals and birds, milk, and water contaminated with *Campylobacter* spp. are some of the important sources of human infections. Chickens are therefore considered as the major reservoirs of *Campylobacter* spp. Poultry meat can easily get contaminated from *Campylobacter* spp. present in the intestinal contents of infected birds, and infection occurs when insufficiently cooked poultry meat is consumed. Ingestion of this microbe in numbers ranging from 500 to 800 is able to infect humans, and this low dose of infection is a grave concern requiring critical management of pasteurization process in the poultry meat industry (Frirdich et al., 2017). Poultry meat alone is responsible for nearly 30% of the human campylobacteriosis cases worldwide (Thames and Sukumaran, 2020). The symptoms of *Campylobacter* spp. infection in humans include watery diarrhea, often characterized by the presence of blood, severe abdominal cramps, fever, vomiting, and nausea, collectively known as campylobacteriosis. The symptoms usually appear within 48–120 h, nevertheless can extend to nearly 240 h (10 days) in certain circumstances. *Campylobacter* infections can also lead to other complications such as bloodstream infections, pneumonia, neonatal sepsis, irritable bowel syndrome (IBS), acute appendicitis, ulcerative colitis, and a neurological condition termed as Guillain-Barré syndrome (GBS). Infections resulting in sudden inflammation of joints are also reported (Skarp et al., 2016).

2 Epidemiology

The role of *Campylobacter* spp. in human gastroenteritis was not known until 1957, although *Campylobacter* infections in farm animals were known in the early twentieth century. Veterinary scientists McFayden and Stockman isolated the causative bacterial agent of bovine abortions which was named as *Vibrio fetus* (now known as *Campylobacter fetus*). Similar bacteria isolated from livestock and swine with diarrhea were nomenclatured to *Vibrio jejuni* and *Vibrio coli*, respectively. In 1973, the *Campylobacter* as new genus came into existence and with effect *Vibrio jejuni* and *Vibrio coli were* cognominated to *Campylobacter jejuni* and *Campylobacter coli* (Zilbauer et al., 2008). With about 96 million cases each year around the world, *Campylobacter* has become one of the predominant bacterial agents responsible for human gastrointestinal infections (Llarena et al., 2017). In the USA, nearly 2% of the food-borne outbreaks every year is contributed by *Campylobacter* spp. (CDC, 2013). Campylobacteriosis is recognized as one of the most recurrently occurring infections originating from animal sources in the EU too. Infections caused by *Salmonella* and *Yersinia* are reported next to campylobacteriosis. Consumption of contaminated and partially cooked chicken constitutes 50–80% of the human infections by *Campylobacter* spp., while fresh milk and its foodstuffs and polluted potable waters have also been connected as the reasons for infection in many of the reported outbreaks. Nearly 20–30% of the human cases of *Campylobacter* infections across the world are attributed to broiler chicken meat as the predominant source (Silva et al., 2011).

3 Virulence

The pathogenesis of *Campylobacter* spp. is attributed to several virulence factors such as its ability to invade and attach to intestinal mucosal cells, iron acquisition capacity, motility facilitated by the presence of flagella, toxin production, and capability to continue in viable but non-culturable (VBNC) state. The adhesion to cells of the intestinal epithelium is the first step among a series of events that culminates with the successful colonization of the host leading to infection. The bacterium colonizes the small intestine with the help of flagella and subsequently translocates to the colon, the ultimate target of infection (Poly and Guerry, 2008). Attachment to intestinal epithelial cells is done with the help of adhesion protein which includes LPS and proteins of outer membranes (OMPs) in addition to the flagella which help to bind to epithelial cells. A protein that binds fibronectin of CadF (*Campylobacter* adhesion protein to fibronectin) plays a pivotal part in the primary contact of *Campylobacter jejuni* with the epithelial cells of the intestine leading to its uptake and internalization (Monteville et al., 2003). Similarly, another protein FlpA (fibronectin-like protein A) also aids in the adhesion and internalization of *C. jejuni* by the epithelial cells, and both CadF and FlpA are responsible for the secretion of effector proteins which participate in signalling pathways leading to successful colonization of host cells. Several other proteins such as the CapA (*Campylobacter* adhesion protein A), Peb (periplasmic binding proteins), and a glycosylated lipoprotein JlpA are also recognized as important virulence factors that are responsible for the capability of *C. jejuni* to adhere to the epithelial cells with persistence in the intestinal environment (Kreling et al., 2020).

The bacterial attachment to host cells triggers a complex signalling cascade involving diverse effector proteins similar to other intracellular pathogens such as *Salmonella* and *Legionella.* Internalization of *Campylobacter* occurs by endocytosis which is preceded by a series of events that induce membrane ruffling involving rearrangement of actin cytoskeleton leading to its internalization. A flagella-associated T3SS (type 3 secretion system) assisted by the Cia (*Campylobacter* invasion antigen) proteins is involved in the invasion and intracellular survival (Eucker and Konkel, 2012).

A protein toxin, namely, cytolethal distending toxin (CDT) with DNAse activity, causes inflammation of the cells resulting in the loss of absorptive potential of the intestine. The toxin is secreted by *C. jejuni* after the bacterium attaches and colonizes the human intestinal cells. The CDT consists of three subunits that interfere with cell division and inhibit mitosis, resulting in cell death (Zilbauer et al., 2008). The flagellum, along with several flagella-secreted factors, protein adhesins, lipooligosaccharide (LOS), and serine protease HtrA, contribute to the pathogenicity of this bacterium and play critical roles in host colonization and progression of disease. Numerous pathogenic characteristics of *Campylobacter* such as motility, attachment, adhesion, invasion, survival, chemotaxis, and cellular translocation are linked to the presence of these virulence factors (Tegtmeyer et al., 2021). With its ability to overcome host immune response and resist gastric acids and bile salts, *Campylobacter* colonizes the host intestine (Dasti et al., 2010). The virulence characteristics

such as adherence, colonization of the small intestine, and invasion of host target cells are coded by the gene *flaA* (Jain et al., 2005). The sialyl transferase activity of the protein coded by *cstII* gene mimics the action of human ganglioside cells causing disruption of intestinal epithelial cells with the help of lipooligosaccharides leading to diarrhea (Pérez-Boto et al., 2010). Unlike other enteric bacterial pathogens, *Campylobacter* harbors a novel protein N-glycosylation (pgl) system that codes for the enzymes required for the production of a conserved heptasaccharide which can modify the periplasmic and membrane-bound proteins (Elmi et al., 2021). Capsular polysaccharides are present in some strains of *Campylobacter*, which effects pathogenicity through colonization, invasion, and host immune response (Kreling et al., 2020). *Campylobacter* can form strong biofilms on surfaces such as steel and plastic, either alone or in combination with other bacteria, which enhances its survival outside the host for a protracted time period (Lehtola et al., 2006).

The whole genome sequences of *Campylobacter* species available in public databases have helped to identify putative virulence factors that might contribute to diverse pathogenicity of emerging pathogenic *Campylobacter* species, for example, the presence of the gene sequences of zonula occludens toxin (*zot*) in the WGS (whole genome sequence) of *Campylobacter concisus*, an evolving pathogen increasingly being concomitant with diarrhea and inflammatory bowel disease (IBD) in all age groups. Zot is a virulence factor associated with *Vibrio cholerae*, and its presence in *C. concisus* might point out at horizontal acquisition. In addition, pathogenicity islands identified in the genome sequences of *C. concisus* encode type IV secretion systems (T4SS) and the associated putative effector proteins with a number of proteins bearing similarities with virulence effector proteins involved in intracellular survival of *Legionella pneumophila* (Chung et al., 2016). Further, a protein resembling cytotoxin-associated protein A (CagA) of *Helicobacter pylori* has also been found, and this protein is predicted to undertake a crucial role in the virulence of *C. concisus* (Chung et al., 2016).

Campylobacter spp. can assume the state of VBNC (viable but non-culturable), a condition that helps this bacterium to persist longer in the environment while maintaining infectious status as shown by their resuscitation in mouse and chicken embryo models (Baffone et al., 2006; Cappelier et al., 1999). *C. jejuni* reacts to extreme temperature, pH, salinity, and desiccation by entering into VBNC state. Clinical strains of *C. jejuni* in VBNC state in artificial seawater at 4 °C could be resuscitated after 152 days (Baffone et al., 2006).

4 Food-Borne Campylobacteriosis

Campylobacteriosis is an important food-associated sickness of worldwide concern with predicted annual causalities of 1,500,000 alone in the USA only and 96 million cases globally involving foods of diverse types, both of plant and animal origins, contaminated with *Campylobacter* spp. (CDC, 2019; Thames & Sukumaran, 2020). Raw milk, chicken, and fresh produce have been implicated in a vast majority of *Campylobacter*-related illnesses in USA, Canada, and EU countries (Gharst et al., 2013). Since

Campylobacter jejuni is an agent of bovine mastitis, milk from infected animals may contain this bacterium, in addition to contamination from cow feces and water (Facciolà et al., 2017). A 2007 outbreak in Kansas City, USA, was attributed to unpasteurized milk and cheese contaminated with *C. jejuni* (CDC, 2009).

In the European Union, grilled chicken is stated to be an important source of *C. jejuni* which is associated with 50–80% of campylobacteriosis in humans, while improper handling, storage, and cross contamination during preparation contributes to 20–30% of the cases (EFSA, 2006). *Campylobacter* prevalence can vary from 22% to 100% of cloacal samples of chicken (broiler) (Baali et al., 2020; Gharbi et al., 2018; Kalupahana et al., 2018). Colonization in flocks begins just 3 weeks after introduction in farms and can reach 100% within 4–6 weeks (Zhang et al., 2017). Several factors such as the age of flocks, weather conditions, etc., influence the colonization of broiler chicken. Contaminated broiler chicken is commonly involved in food-borne infections, contributing to nearly 30–80% of infections in which the causative agent has been identified (Mossong et al., 2016). The contamination of chicken meat occurs primarily from chicken feces and the gut contents during the processing of meat, and the numbers can increase during storage and handling by the consumers. Human infections occur primarily due to the consumption of insufficiently cooked chicken products containing this bacterium in numbers sufficient to cause infection. The low infectious dose of this bacterium (~500 CFU) confounds this problem, as this number can be reached quickly at ambient temperature even when the initial load of the bacterium is too low. Alternatively, foods such as the raw chicken harbor a large number of *C. jejuni*, and even a minute level of contamination involving food handlers or wash water can result in human infections.

Fish-borne campylobacteriosis is rare, although the occurrence of *Campylobacter* spp. has been reported from fish and shellfish. The incidence of *Campylobacter jejuni* is observed in fish and shellfish, ostensibly owing to the contamination of shoreline waters or fish-growing environments such as the aquaculture farms. Studies revealed that the incidence of thermophilic *Campylobacter* is 42% in bivalve mollusks, with urease-positive strains being predominant (Wilson and Moore, 1996). A study reported the occurrence of *Campylobacter jejuni, C. coli*, and *C. lari* in brackish water, freshwater, and shellfish samples in France (Rincé et al., 2018), suggesting resilience of this group to persist in settings of widespread environments. Lacuna on the information on occurrence and distribution of *Campylobacter* spp. in coastal-marine environment is one important aspect to be noted.

Other fresh produces involved in frequent outbreaks of campylobacteriosis include fruits and vegetables. The increasing trend in the consumer preference for raw vegetables such as spinach, lettuce, cucumber, sprouts, and cabbage has increased the risk of *Campylobacter* infections as the bacterium can be introduced into the raw produces at various stages from farm to fork (Mohammadpour et al., 2018). Organically farmed vegetables are attracting increased consumer demand in the recent years owing to the environment-friendly farming practices adopted in this kind of farming. However, organic farming involves the application of poultry or livestock manures, and these are known reservoirs of campylobacters (Facciola et al., 2017). The reported epidemics of campylobacteriosis were linked with the

ingestion of cucumber (Kirk et al., 1997), and food contaminated by an infected food handler (Olsen et al., 2001).

5 Temperature Tolerance

Campylobacter spp. characteristically have a narrow range of temperature for growth, and unlike other food-borne pathogens, *Campylobacter jejuni* requires optimum temperature 42 °C. But, the temperatures beneath 31 °C are not favorable for the growth of this bacterium (Chan et al., 2001). Nevertheless, *Campylobacter jejuni* is adept to survive the refrigeration and freezing temperatures for considerable periods of time. *C. jejuni* can endure for a number of weeks at 4 °C standard temperature of refrigeration. Studies indicate that the bacterium could enter into the state of VBNC and remains active for 4 months at refrigeration temperature (Rollins and Colwell (1986). The bacterium is capable of surviving in chicken meat for 14 days at -20 °C and for 56 days at −70 °C (Chan et al., 2001). However, the survival of this bacterium in different food matrices at low temperatures is not well understood. From the risk assessment point of view, the significant aspect is to comprehend the response of pathogenic *Campylobacter* spp., at different temperatures at which foods are stored. Strain-to-strain variation in temperature tolerance complicates this issue as clinical isolates were shown to survive for longer duration at 4 °C compared to the poultry isolates (Chan et al., 2001).

6 Antibiotic Resistance

The increasing ability of *Campylobacter* spp. to resist antimicrobial treatment is a major concern, as some of the clinically relevant antibiotics are fast losing their potency against this pathogen. The antibiotic-resistant strains of *Campylobacter* that complicate the treatment regime are reported to cause nearly 310,000 infections (Yang et al., 2019). The CDC of the USA testified that 24% isolates of *Campylobacter* were ciprofloxacin or azithromycin resistant. Although infection by *Campylobacter* spp. is an infection of self-limited kind, in some patients the illness can prolong leading to related sequelae such as the GB syndrome, necessitating antibiotic treatment. Infections in children may often require chemotherapeutic intervention to control fecal shedding of the bacterium. When antibiotic treatment is deemed necessary, especially in elderly and immune-compromised individuals, fluoroquinolones and macrolides are administered (Yang et al., 2019). But, the advent of *Campylobacter* spp. resistant to clinically important antibiotics has stifled the treatment regimen in cases of infection associated with resistant strains leading to increased hospital stay, increased cost of treatment, and, at times, treatment failures. Reports from India suggest the involvement of *Campylobacter jejuni* resistant to ampicillin, ciprofloxacin, tetracycline, furazolidone, gentamicin, and erythromycin in clinical settings (Jain et al., 2005; Mukherjee et al., 2014). The predictive study of more than one and half decades revealed an increasing trend in *Campylobacter*

insusceptibility to fluoroquinolones, nalidixic acid, phenicols, tetracycline, and doxycycline (Zhou et al., 2016). The problem is more serious and equally complicated in developing and underdeveloped countries where the inappropriate use of antibiotics occurs due to easy access to antibiotics and lack of stringent laws prohibiting the application of antibiotics in poultry and livestock. Application of antibiotics in livestock for the purposes other than treatment such as the growth promotion and prophylaxis exposes the bacteria to sublethal concentrations of antibiotics. As a result, *Campylobacter* spp. which are naturally associated with gastrointestinal tracts of animals tend to gradually develop resistance to the antibiotics. The application of antibiotics in livestock is often arbitrary, and the application of these compounds through feed and water can also promote the development of resistance. Since poultry is the major meat type involved in *Campylobacter* infections, the occurrence of antibiotic-resistant bacteria in bird and chicken products assumes significance from public health point of view. Campylobacters from broiler chicken flocks can have higher antibiotic resistance than human isolates (Bardon et al., 2009). Table 1 describes the antibiotic resistance exhibited by *Campylobacter* spp. isolated from chicken.

C. jejuni isolates from an outbreak linked to pet store companies involving puppies as sources of infection in the USA exhibited resistance to macrolides and quinolones (Montgomery et al., 2018). A 15-year prospective study from Canada

Table 1 Antimicrobial resistance of *Campylobacter* spp. isolates from broiler chicken

Place of study	Antibiotics	% resistance	Bacterial species	References
North East Tunisia	Macrolide, tetracycline, quinolones, and chloramphenicol	88.6–100%	*Campylobacter* spp.	Gharbi et al. (2018)
Japan	Enrofloxacin	29.5% 41.3%	*C. jejuni* *C. coli*	Haruna et al. (2012)
Czech Republic	Oxolinic acid Ciprofloxacin Ampicillin	77% 72% 26%	*Campylobacter* spp.	Bardon et al. (2009)
China	Quinolones Macrolides Gentamicin Amikacin Multidrug resistance	>89.7% 59.8% 42.7% 36.8% 94.0%	*Campylobacter* spp.	Zhang et al. (2018)
Italy	Quinolones, tetracycline, and combination of sulfamethoxazole and trimethoprim	65–100%	*Campylobacter* spp.	Giacomelli et al. (2014)
Poland	Ciprofloxacin Tetracycline	100% 78.6%	*Campylobacter* spp.	Woźniak-Biel et al. (2018)
Brazil	Quinolone and fluoroquinolone	37–74%	*Campylobacter* spp.	Ramires et al. (2020)

observed increasing rates of resistance against quinolones (nalidixic acid) and fluoroquinolones (ciprofloxacin) in *C. jejuni* recovered from humans, most of which were acquired domestically from cattle and chicken reservoirs (Inglis et al., 2021). Further, a report from the USA associated 18% of the *Campylobacter* infections with international travel, 60% of which involved quinolone-resistant isolates (Ricotta et al., 2014). A comparable investigation from Denmark shows that one-third of cases are travel associated, with children below 10 years of age, are additionally prone to infection that exhibited a seasonal pattern (Kuhn et al., 2018). In an important finding, the higher body temperature (42 °C) of poultry, an ideal temperature for the development of *C. jejuni*, increased the rate of conjugative transmission of resistance marker of tetracycline in *C. jejuni* (Cuevas-Ferrando et al., 2020). Cross-resistance to antibiotics was also described in *Campylobacter* spp. For example, when tylosin is administered to broiler chicken over a period of time, development of resistance to erythromycin has been detected at a higher frequency in *C. coli* in comparison with *C. jejuni* (Ladely et al., 2007). The isolates of *C. coli* from avian and piggery sources can undergo natural transformation with erythromycin-resistant gene (Kim et al., 2006). Figure 1 demonstrates the causes and sources of antibiotic-resistant *Campylobacter* spp. in food chain and their routes of dissemination. The increasing incidence of multidrug-resistant *Campylobacter* spp.,

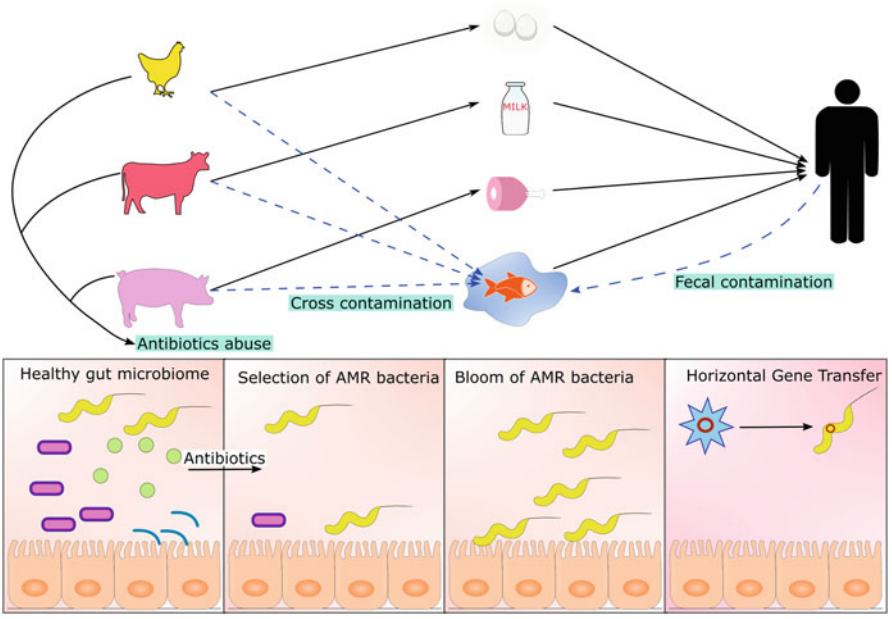


Fig. 1 Foods of animal origin are the major sources of human campylobacteriosis. Emergence of antibiotic-resistant bacteria occurs due to appropriate use of antibiotic in poultry and livestock. The presence of antibiotics in food production systems select for resistant clones. Spread of antibiotic resistance gene occurs through horizontal gene transfer (HGT)

insusceptible to many antibiotics in animal foods, suggests the need for continuous monitoring of antibiotic susceptibility patterns of this pathogen. Rapid evolution of ciprofloxacin-resistant *C. jejuni* in fluoroquinolone-treated poultry birds suggests the importance of antibiotic stewardship, and stringent legal framework to prevent emergence and spread of highly resistant clones in food production facilities.

Studies from several European countries, namely, Denmark, Ireland, Norway, and Iceland, suggest exceptionally high (70–100%) prominently insusceptible nature of *Campylobacter jejuni* isolated from clinical and faunal settings to ciprofloxacin (EFSA and ECDC, 2019). The *Campylobacter* spp. isolates of human origin from Norway and Portugal showed resistance of 24.5% and 96.5% to ciprofloxacin, respectively. Further, erythromycin and tetracycline resistance has been showing an increasing trend in some European countries. Conversely, the general resistance pattern to erythromycin was trivial (2%) in Europe, the highest being 6.3% in Portugal (EFSA and ECDC, 2019). Nevertheless, the *Escherichia coli* insusceptibility to erythromycin resistance is presenting a growing tendency in Europe, ranging from 2.2% to 21.4% among the member states. Moreover, disturbing intensities of insusceptibility to ciprofloxacin are detected in isolates of *C. jejuni* from broiler chicken (66.8%) and turkeys (73.8%). A study from Canada reported lowest (6%) and the highest (35%) ciprofloxacin resistance in *Campylobacter* from Québec and British Columbia, respectively (Deckert et al., 2010).

The intrinsic resistance to antibiotics such bacitracin, novobiocin, trimethoprim, polymyxin/colistin, and vancomycin is characteristic of *Campylobacter* spp., presumably due to poor permeability of antibiotics, lack of antibiotic targets, or variant forms of antibiotic-converting enzymes (dihydrofolate in the case of trimethoprim) (Iovine, 2013) (Table 2).

7 Mechanisms of Antibiotic Resistance

The characteristic feature of *Campylobacter* spp., namely, *C. jejuni*, is its intrinsic insusceptibility to numerous antimicrobials that included trimethoprim, vancomycin, peptide antibiotics colistin and polymyxin, novobiocin, and bacitracin. This bacterium possesses variant dihydrofolate reductase(s) that are not effectively bound by trimethoprim (Gibreel et al., 1998). The genes encoding these variant enzymes are presumably acquired from unknown donor bacterial species. Some of the naturally expressed porins confer enhanced resistance to certain antibiotics either by antibiotic extrusion or by preventing the entry of antimicrobials into the cell. The absence of suitable targets in *C. jejuni* for antibiotics such as colistin, vancomycin, bacitracin, and novobiocin is responsible for the intrinsic resistance against these antibiotics (Iovine, 2013).

Some of the early investigations on process of resistance of fluoroquinolone indicated transformations in the regions of chromosomes that determine resistance to quinolone (QRDR) within the *gyrA* gene (Luangtongkum et al., 2009; Piddock et al., 2003). The mutation Thr-86-Ile which is farthest occurred in *gyrA* is responsible for quinolone and fluoroquinolone resistance, while resistance to nalidixic acid

Table 2 An outline of antibiotic insusceptibility of *Campylobacters* associated with foods from different countries

Country	Food source	Antibiotic resistance	References
USA	Raw meats	TET, NAL, CIP, ERY, DOX	Ge et al. (2003)
Italy	Raw meats	TET, CIP, STR	Pezzotti et al. (2003)
Switzerland	Raw poultry meat	CIP, TET, AMP	Ledergerber et al. (2003)
Denmark	Raw poultry meat	TET, NAL, CHL, CIP	Andersen et al. (2006)
Canada	Chicken, raw milk	ERY, TET	Lévesque et al. (2007)
Korea	Chicken, pork & beef	DOX, CIP, NAL, TET, ENR	Hong et al. (2007)
Japan	Broken liquid egg	TET, NAL, NOR, OFX, CIP	Sato & Sashihara (2010)
Korea	Chicken & pork	TET, NAL, CIP, AZM, AMP, STR	Kim et al. (2010)
Malaysia	Chicken	PEN, VAN, AMP, ERY	Khalid et al. (2015)
India	Milk & milk products	NAL, TET, CIP	Modi et al. (2015)
Tanzania	Beef & raw milk	AMP, CIP, ERY, GEN, STR, AZM, CHL	Kashoma et al. (2016)
China	Chicken	NOR, CIP, TET, AMP,	Zhang et al. (2016)
Philippines	Chicken parts	CLI, ERY, NAL, TET, GEN, CHL	Lim et al. (2017)
Iran	Raw milk, fish, poultry, & red meat	CIP, TET, NAL	Raeisi et al. (2017)
Canada	Retail meats	CIP, NAL, AZT, ERY	Narvaez-Bravo et al. (2017)
Czech Republic	Poultry meat Pork liver	ERY, STR, CIP	Bardon et al. (2009)
India	Poultry meat-related	TMP/SMX, CEF, TET	Khan et al. (2018)
Poland	Raw milk, beef, and pork meat	CIP, TET	Andrzejewska et al. (2019)
South Korea	Raw chicken & duck meat	CIP, NAL, TET	Kim et al. (2019)
Egypt	Raw milk, cheese	CIP, NAL,TET	Zeinhom et al. (2021)

Abbreviations: *TET* tetracycline, *NAL* nalidixic acid, *CIP* ciprofloxacin, *ERY* erythromycin, *DOX* doxycycline, *STR* streptomycin, *AMP* ampicillin, *CHL* chloramphenicol, *ENR* enrofloxacin, *NOR* norfloxacin, *OFX* ofloxacin, *AZM* azithromycin, *PEN* penicillin, *VAN* vancomycin, *GEN* gentamicin, *CLI* colistin, *AZT* aztreonam, *TMP*/*SMX* trimethoprim/sulfamethoxazole, *CEF* cephalothin

alone is mediated by Thr-86-Ala mutation, and interestingly, the latter does not confer resistance to fluoroquinolones (Jesse et al., 2006). Intermediate resistance to fluoroquinolones in *C. jejuni* is attributed to two mutations (Asp-90-Asn and Ala-70-Thr) that occur infrequently. However, no transformations were detected that are accountable for quinolone resistance which has been reported in the *gyrB*, *parC*, and *parE* genes of *C. jejuni*.

The production of penicillinase and OXA-lactamases such as OXA-61 and OXA-184 decreased permeability of antibiotics due to mutations in major outer membrane protein. The efflux pumps, namely, *CmeABC* and analogous proteins disseminated in the genome of *Campylobacter* spp., facilitate resistance to β-lactam antibiotics. The genome of *C. jejuni* has 14 putative efflux pumps, which may have important roles in its antibiotic resistance. The existence of carbapenemase-encoding genes, viz., bla_{IMP}, bla_{VIM}, and bla_{SIM}, in *C. jejuni* (Noreen et al., 2020) is concomitant to carbapenem resistance. The putative beta-lactamase $bla_{OXA-493}$ and $bla_{OXA-576}$ genes in the genome of *Campylobacter* spp. were identified in an in silico study by Rivera-Mendoza and others (2020), in addition to genetic determinants of resistance to different classes of antibiotics, viz., aminoglycosides, β-lactams, fluoroquinolones, lincosamides, macrolides, phenicols, and tetracyclines. The self-transmissible plasmid of 45–58 kb size that harbors lone *tet(O)* gene is responsible for imparting resistance to tetracycline (Wieczorek and Osek, 2013). The gene *fexA* confers florfenicol resistance to *Campylobacter* spp. of animal origin (Liu et al., 2020). The studies of Tenover et al. (1989) revealed that aminoglycoside resistance is due to the presence of a kanamycin phosphotransferase gene, apha-3, positioned on plasmid of 14 kb in kanamycin-resistant *C. jejuni*, whereas *aph(2″)-If* identified as the principal resistance gene is noticed in isolates of gentamycin resistance (Yao et al., 2017).

The amino acid substitution A2075G of the 23S rRNA gene is the main reason for insusceptibility to macrolide in *Campylobacters*. Besides this mutation, presence of *erm(B)* gene that encodes for a ribosomal methylase is responsible for azithromycin resistance (Li et al., 2016). *C. jejuni* isolates carrying *erm*(B) gene harbor multidrug resistance genomic islands (MDRGIs). These genomic islands harbor the resistance-enhancing gene RE-*cmeABC* that codes for a multidrug-resistance-enhancing efflux pump (Liu et al., 2019). MDRGIs carry genes of resistance to multiple antibiotic classes (Wang et al., 2014). Further, Liu and others (2019) reported that resistances to antibiotics, viz., aminoglycosides, tetracyclines, fluoroquinolones, and macrolides, have been exhibited by *C. jejuni* isolates whose genomic island harbors multiple resistance genes, such as *erm*(B), *tet*(O), *cat*, *aadA*, *aadE*, *aad9*, *aph2*, *aphA3*, *aac*, and *aph(2″)-If*.

In-depth studies were carried out on antibiotic efflux pump in *C. jejuni*, namely, Cme (*Campylobacter* multidrug efflux) efflux pump of the resistance nodulation cell division (RND). In the operon *cmeABC*, the three genes, viz., CmeA, CmeB, and CmeC, encrypt for the proteins periplasmic protein, transmembrane efflux protein, and outer membrane protein, respectively. The CmeABC efflux pumps confer resistance to diverse and structurally unrelated antimicrobials that included fluoroquinolones, β-lactams, macrolides, tetracycline, and rifampin, which were well documented by Lin and others (2002). The CmeABC efflux pump stimulates the growth and development of fluoroquinolone transformations in campylobacteria besides conferring insusceptiblity at higher levels to fluoroquinolones that are preferred drugs in treating campylobacteriosis (Yan et al., 2006). The survival of *C. jejuni* in bile salts and successful inhabitation of host intestine are facilitated by CmeABC efflux system. Further, the expression of CmeABC operon is highly upregulated by bile salts leading to elevated resistance to antibiotics.

Few efflux pumps of putative type, namely, CmeDEF and CmeG, have possible vital roles in antimicrobial and biocide resistance of *C. jejuni*, and these pumps remain to be characterized with respect to their substrate profiles.

8 Horizontal Gene Transfer (HGT) Mechanisms in *Campylobacter* spp.

Campylobacter spp. exhibit natural competence and take up DNA from the environment through common mechanisms of transformation, transduction, and conjugation (Wang and Taylor, 1990). This ability of *Campylobacter* helps the bacterium to evolve rapidly to resist host immune response and enhance its fitness to survive in the natural environment including the chicken intestine. Inter- and intraspecies genetic exchange and homologous recombination among large genomic islands are characteristics of *Campylobacter* spp. The HGT of *tet(O)* gene between donor and recipient strains of *Campylobacter jejuni* has been demonstrated in chicken (Avrain et al., 2004). *Campylobacter jejuni* can also acquire a chromosomally encoded streptomycin resistance gene from closely related species such as *Helicobacter pylori* through conjugation (Oyarzabal et al., 2007). HGT in *Campylobacter jejuni* cells resulting in the exchange of chloramphenicol and kanamycin resistance markers has been shown, and the rate of transfer was tenfold higher when the chicken fecal contents were present in the growth medium (Samarth and Kwon, 2020).

9 Conclusions

The success of *Campylobacter* as a food-borne zoonotic pathogen is due to its versatility in terms of host adaption, virulence mechanisms, antibiotic resistance, and persistence in the environment. Since poultry and livestock are the reservoirs of this pathogen, the application of antibiotics in these food production environments can promote the progress and spread of antibiotic insusceptible strains. Biocontrol of *Campylobacter* spp. using bacteriophages is one approach that can potentially reduce the pathogen load in foods and reduce the health risk. The "One Health approach" for control of *Campylobacter* infection encompasses controlling pathogen burden in food animals, regulation of antimicrobial use, adequate thermal treatment of foods, and educating all stakeholders involved.

References

Andersen, S. R., Saadbye, P., Shukri, N. M., Rosenquist, H., Nielsen, N. L., & Boel, J. (2006). Antimicrobial resistance among *Campylobacter jejuni* isolated from raw poultry meat at retail level in Denmark. *International Journal of Food Microbiology, 107*(3), 250–255.

Andrzejewska, M., Szczepańska, B., Śpica, D., & Klawe, J. J. (2019). Prevalence, virulence, and antimicrobial resistance of *Campylobacter* spp. in raw milk, beef, and pork meat in Northern Poland. *Foods (Basel, Switzerland), 8*(9), 420.

Avrain, L., Vernozy-Rozand, C., & Kempf, I. (2004). Evidence for natural horizontal transfer of tetO gene between *Campylobacter jejuni* strains in chickens. *Journal of Applied Microbiology, 97*(1), 134–140.

Baali, M., Lounis, M., Amir, H. L. A., Ayachi, A., Hakem, A., & Kassah-Laouar, A. (2020). Prevalence, seasonality, and antimicrobial resistance of thermotolerant Campylobacter isolated from broiler farms and slaughterhouses in East Algeria. *Veterinary World, 13*(6), 1221–1228.

Baffone, W., Casaroli, A., Citterio, B., Pierfelici, L., Campana, R., Vittoria, E., Guaglianone, E., & Donelli, G. (2006). *Campylobacter jejuni* loss of culturability in aqueous microcosms and ability to resuscitate in a mouse model. *International Journal of Food Microbiology, 107*, 83–91.

Bardon, J., Kolar, M., Cekanova, L., Hejnar, P., & Koukalova, D. (2009). Prevalence of *Campylobacter jejuni* and its resistance to antibiotics in poultry in The Czech Republic. *Zoonoses and Public Health, 56*(3), 111–116.

Cappelier, J. M., Minet, J., Magras, C., Colwell, R. R., & Federighi, M. (1999). Recovery in embryonated eggs of viable but nonculturable *Campylobacter jejuni* cells and maintenance of ability to adhere to HeLa cells after resuscitation. *Applied and Environmental Microbiology, 65*(11), 5154–5157.

CDC (2019) Campylobacter (Campylobacteriosis) | Campylobacter. Center for Disease Control & Prevention (CDC). https://www.cdc.gov/campylobacter/index.html

Center for Disease Control and Prevention (CDC). (2009). *Campylobacter jejuni* infection associated with unpasteurized milk and cheese—Kansas, 2007. *Morbidity and Mortality Weekly Report, 57*(51), 1377–1379.

Center for Disease Control and Prevention (CDC). (2013). Multistate outbreak of *Campylobacter jejuni* infections associated with undercooked chicken livers—Northeastern United States, 2012. *Morbidity and Mortality Weekly Report, 62*(44), 874–876.

Chan, K. F., Le Tran, H., Kanenaka, R. Y., & Kathariou, S. (2001). Survival of clinical and poultry-derived isolates of *Campylobacter jejuni* at a low temperature (4 °C). *Applied and Environmental Microbiology, 67*(9), 4186–4191.

Chung, H. K., Tay, A., Octavia, S., Chen, J., Liu, F., Ma, R., Lan, R., Riordan, S. M., Grimm, M. C., & Zhang, L. (2016). Genome analysis of *Campylobacter concisus* strains from patients with inflammatory bowel disease and gastroenteritis provides new insights into pathogenicity. *Science Reports, 6*, 38442.

Costa, D., & Iraola, G. (2019). Pathogenomics of emerging *Campylobacter* species. *Clinical Microbiology Reviews, 32*(4), e00072–e00018.

Cuevas-Ferrando, E., Guirado, P., Miró, E., Iglesias-Torrens, Y., Navarro, F., Alioto, T. S., Gómez-Garrido, J., Madrid, C., & Balsalobre, C. (2020). Tetracycline resistance transmission in Campylobacter is promoted at temperatures resembling the avian reservoir. *Veterinary Microbiology, 244*, 108652.

Dasti, J. I., Tareen, A. M., Lugert, R., Zautner, A. E., & Gross, U. (2010). *Campylobacter jejuni*: A brief overview on pathogenicity-associated factors and disease-mediating mechanisms. *International Journal of Medical Microbiology, 300*(4), 205–211.

Debruyne, L., Gevers, D., & Vandamme, P. (2008). Taxonomy of the family Campylobacteraceae. In I. Nachamkin, C. Szymanski, & M. Blaser (Eds.), *Campylobacter* (Third ed.). ASM Press.

Deckert, A., Gow, S., Rosengren, L., Léger, D., Avery, B., Daignault, D., Dutil, L., Reid-Smith, R., & Irwin, R. (2010). Canadian integrated program for antimicrobial resistance surveillance (CIPARS) farm program: Results from finisher pig surveillance. *Zoonoses and Public Health, 57*(Suppl. 1), 71–84.

EFSA (European Food Safety Authority). (2006). The community summary report on trends and sources of zoonoses, zoonotic agents, antimicrobial resistance and foodborne outbreaks in the European Union in 2005. *EFSA Journal, 4*(12), 94r.

Elmi, A., Nasher, F., Dorrell, N., Wren, B., & Gundogdu, O. (2021). Revisiting *Campylobacter jejuni* virulence and fitness factors: Role in sensing, adapting, and competing. *Frontiers in Cellular and Infection Microbiology, 10*, 607704.

Eucker, T. P., & Konkel, M. E. (2012). The cooperative action of bacterial fibronectin-binding proteins and secreted proteins promote maximal *Campylobacter jejuni* invasion of host cells by stimulating membrane ruffling. *Cellular Microbiology, 14*, 226–238.

European Food Safety Authority and European Centre for Disease Prevention and Control. (2019). The European Union summary report on antimicrobial resistance in zoonotic and indicator bacteria from humans, animals and food in 2017. *EFSA Journal. European Food Safety Authority, 17*(2), e05598.

Facciolà, A., Riso, R., Avventuroso, E., Visalli, G., Delia, S. A., & Laganà, P. (2017). Campylobacter: From microbiology to prevention. *Journal of Preventive Medicine and Hygiene, 58*(2), E79–E92.

Frirdich, E., Biboy, J., Huynh, S., Parker, C. T., Vollmer, W., & Gaynor, E. C. (2017). Morphology heterogeneity within a *campylobacter jejuni* helical population: The use of calcofluor white to generate rod-shaped *C. jejuni* 81-176 clones and the genetic determinants responsible for differences in morphology within 11168 strains. *Molecular Microbiology, 104*(6), 948–971.

Ge, B., White, D. G., McDermott, P. F., Girard, W., Zhao, S., Hubert, S., & Meng, J. (2003). Antimicrobial-resistant *Campylobacter* species from retail raw meats. *Applied and Environmental Microbiology, 69*(5), 3005–3007.

Gharbi, M., Béjaoui, A., Ben Hamda, C., Jouini, A., Ghedira, K., Zrelli, C., Hamrouni, S., Aouadhi, C., Bessoussa, G., Ghram, A., & Maaroufi, A. (2018). Prevalence and antibiotic resistance patterns of *Campylobacter* spp. isolated from broiler chickens in the north of Tunisia. *BioMed Research International*, 7943786.

Gharst, G., Oyarzabal, O. A., & Hussain, S. K. (2013). Review of current methodologies to isolate and identify *Campylobacter* spp. from foods. *Journal of Microbiological Methods, 95*(1), 84–92.

Giacomelli, M., Salata, C., Martini, M., Montesissa, C., & Piccirillo, A. (2014). Antimicrobial resistance of *campylobacter jejuni* and *campylobacter coli* from poultry in Italy. *Microbial Drug Resistance (Larchmont, N.Y.), 20*(2), 181–188.

Gibreel, A., & Sköld, O. (1998). High-level resistance to trimethoprim in clinical isolates of *Campylobacter jejuni* by acquisition of foreign genes (*dfr1* and *dfr9*) expressing drug-insensitive dihydrofolate reductases. *Antimicrobial Agents and Chemotherapy, 42*, 3059–3064.

Haruna, M., Sasaki, Y., Murakami, M., Ikeda, A., Kusukawa, M., Tsujiyama, Y., Ito, K., Asai, T., & Yamada, Y. (2012). Prevalence and antimicrobial susceptibility of Campylobacter in broiler flocks in Japan. *Zoonoses and Public Health, 59*(4), 241–245.

Heredia, N., & García, S. (2018). Animals as sources of food-borne pathogens: A review. *Animal Nutrition, 4*(3), 250–255.

Hong, J., Kim, J. M., Jung, W. K., Kim, S. H., Bae, W., Koo, H. C., Gil, J., Kim, M., Ser, J., & Park, Y. H. (2007). Prevalence and antibiotic resistance of *Campylobacter* spp. isolated from chicken meat, pork, and beef in Korea, from 2001 to 2006. *Journal of Food Protection, 70*(4), 860–866.

Inglis, G. D., Taboada, E. N., & Boras, V. F. (2021). Rates of fluoroquinolone resistance in domestically acquired *Campylobacter jejuni* are increasing in people living within a model study location in Canada. *Canadian Journal of Microbiology, 67*(1), 37–52.

Iovine, N. M. (2013). Resistance mechanisms in *Campylobacter jejuni*. *Virulence, 4*(3), 230–240.

Jain, D., Sinha, S., Prasad, K. N., & Pandey, C. M. (2005). Campylobacter species and drug resistance in a north Indian rural community. *Transactions of the Royal Society of Tropical Medicine and Hygiene, 99*(3), 207–214.

Jesse, T. W., Englen, M. D., Pittenger-Alley, L. G., & Fedorka-Cray, P. J. (2006). Two distinct mutations in gyrA lead to ciprofloxacin and nalidixic acid resistance in *Campylobacter coli* and *Campylobacter jejuni* isolated from chickens and beef cattle. *Journal of Applied Microbiology, 100*(4), 682–688.

Kalupahana, R. S., Mughini-Gras, L., Kottawatta, S. A., Somarathne, S., Gamage, C., & Wagenaar, J. A. (2018). Weather correlates of Campylobacter prevalence in broilers at slaughter under tropical conditions in Sri Lanka. *Epidemiology and Infection, 146*(8), 972–979.

Kashoma, I. P., Kassem, I. I., John, J., Kessy, B. M., Gebreyes, W., Kazwala, R. R., & Rajashekara, G. (2016). Prevalence and antimicrobial resistance of Campylobacter isolated from dressed beef carcasses and raw milk in Tanzania. *Microbial Drug Resistance (Larchmont, N.Y.), 22*(1), 40–52.

Khalid, M. I., Tang, J. Y. H., Baharuddin, N. H., Rahman, N. S., Rahimi, N. F., & Radu, S. (2015). Prevalence, antibiogram, and cdt genes of toxigenic *Campylobacter jejuni* in salad style vegetables (ulam) at farms and retail outlets in Terengganu. *Journal of Food Protection, 78*(1), 65–71.

Khan, J. A., Rathore, R. S., Abulreesh, H. H., Qais, F. A., & Ahmad, I. (2018). Prevalence and antibiotic resistance profiles of *Campylobacter jejuni* isolated from poultry meat and related samples at retail shops in Northern India. *Foodborne Pathogens and Disease, 15*(4), 218–225.

Kim, J. S., Carver, D. K., & Kathariou, S. (2006). Natural transformation-mediated transfer of erythromycin resistance in *Campylobacter coli* strains from turkeys and swine. *Applied and Environmental Microbiology, 72*(2), 1316–1321.

Kim, J. M., Hong, J., Bae, W., Koo, H. C., Kim, S. H., & Park, Y. H. (2010). Prevalence, antibiograms, and transferable tet(O) plasmid of *campylobacter jejuni* and *Campylobacter coli* isolated from raw chicken, pork, and human clinical cases in Korea. *Journal of Food Protection, 73*(8), 1430–1437.

Kim, J., Park, H., Kim, J., Kim, J. H., Jung, J. I., Cho, S., Ryu, S., & Jeon, B. (2019). Comparative analysis of aerotolerance, antibiotic resistance, and virulence gene prevalence in *Campylobacter jejuni* isolates from retail raw chicken and duck meat in South Korea. *Microorganisms, 7*(10), 433.

Kirk, M., Waddell, R., Dalton, C., Creaser, A., & Rose, N. (1997). A prolonged outbreak of Campylobacter infection at a training facility. *Communicable Diseases Intelligence, 21*(5), 57–61.

Kreling, V., Falcone, F. H., Kehrenberg, C., & Hensel, A. (2020). *Campylobacter* sp.: Pathogenicity factors and prevention methods—New molecular targets for innovative antivirulence drugs? *Applied Microbiology and Biotechnology, 104*, 10409–10436.

Kuhn, K. G., Nielsen, E. M., Mølbak, K., & Ethelberg, S. (2018). Epidemiology of campylobacteriosis in Denmark 2000-2015. *Zoonoses and Public Health, 65*(1), 59–66.

Ladely, S. R., Harrison, M. A., Fedorka-Cray, P. J., Berrang, M. E., Englen, M. D., & Meinersmann, R. J. (2007). Development of macrolide-resistant Campylobacter in broilers administered subtherapeutic or therapeutic concentrations of tylosin. *Journal of Food Protection, 70*(8), 1945–1951.

Ledergerber, U., Regula, G., Stephan, R., Danuser, J., Bissig, B., & Stärk, K. D. C. (2003). Risk factors for antibiotic resistance in *Campylobacter* spp. isolated from raw poultry meat in Switzerland. *BMC Public Health, 3*, 39.

Lehtola, M. J., Pitkänen, T., Miebach, L., & Miettinen, I. T. (2006). Survival of *Campylobacter jejuni* in potable water biofilms: A comparative study with different detection methods. *Water Science and Technology, 54*, 57–61.

Lévesque, S., Frost, E., & Michaud, S. (2007). Comparison of antimicrobial resistance of *Campylobacter jejuni* isolated from humans, chickens, raw milk, and environmental water in Québec. *Journal of Food Protection, 70*(3), 729–735.

Li, B., Ma, L., Li, Y., Jia, H., Wei, J., Shao, D., Liu, K., Shi, Y., Qiu, Y., & Ma, Z. (2016). Antimicrobial resistance of *Campylobacter* species isolated from broilers in live bird markets in Shanghai, China. *Foodborne Pathogens and Disease, 14*(2), 96–102.

Lim, P. W. N., Tiam-Lee, D. C., Paclibare, P. A. P., Subejano, M. S. E., Cabero-Palma, J. A. S., & Penuliar, G. M. (2017). High rates of contamination of poultry meat products with drug-resistant Campylobacter in Metro Manila, Philippines. *Japanese Journal of Infectious Diseases, 70*(3), 311–313.

Lin, J., Michel, L. O. & Zhang, Q. (2002) CmeABC functions as a multidrug efflux system in Campylobacter jejuni. *Antimicrobial Agents and Chemotherapy, 46*, 2124–2131.

Liu, D., Liu, W., Lv, Z., Xia, J., Li, X., Hao, Y., Zhou, Y., Yao, H., Liu, Z., Wang, Y., Shen, J., Ke, Y., & Shen, Z. (2019). Emerging erm(B)-mediated macrolide resistance associated with novel multidrug resistance genomic islands in Campylobacter. *Antimicrobial Agents and Chemotherapy, 63*(7), e00153–e00119.

Liu, D., Li, X., Wang, Y., Schwarz, S., & Shen, J. (2020). Emergence of the phenicol exporter gene fexA in *Campylobacter coli* and *Campylobacter jejuni* of animal origin. *Antimicrobial Agents and Chemotherapy, 64*(6), e00240-20.

Llarena, A. K., Taboada, E., & Rossi, M. (2017). Whole-genome sequencing in epidemiology of *Campylobacter jejuni* infections. *Journal of Clinical Microbiology, 55*(5), 1269–1275.

Luangtongkum, T., Jeon, B., Han, J., Plummer, P., Logue, C. M., & Zhang, Q. (2009). Antibiotic resistance in Campylobacter: Emergence, transmission and persistence. *Future Microbiology, 4*(2), 189–200.

Modi, S., Brahmbhatt, M. N., Chatur, Y. A., & Nayak, J. B. (2015). Prevalence of *Campylobacter* species in milk and milk products, their virulence gene profile and anti-bio gram. *Veterinary World, 8*(1), 1–8.

Mohammadpour, H., Berizi, E., Hosseinzadeh, S., Majlesi, M., & Zare, M. (2018). The prevalence of *Campylobacter* spp. in vegetables, fruits, and fresh produce: A systematic review and meta-analysis. *Gut Pathogens, 10*(1), 41.

Monteville, M. R., Yoon, J. E., & Konkel, M. E. (2003). Maximal adherence and invasion of INT 407 cells by *Campylobacter jejuni* requires the CadF outer-membrane protein and microfilament reorganization. *Microbiology, 149*, 153–165.

Montgomery, M. P., Robertson, S., Koski, L., Salehi, E., Stevenson, L. M., Silver, R., Sundararaman, P., Singh, A., Joseph, L. A., Weisner, M. B., Brandt, E., Prarat, M., Bokanyi, R., Chen, J. C., Folster, J. P., Bennett, C. T., Francois Watkins, L. K., Aubert, R. D., Chu, A., & Laughlin, M. E. (2018). Multidrug-resistant *Campylobacter jejuni* outbreak linked to puppy exposure—United States', 2016-2018. *Morbidity and Mortality Weekly Report, 67*(37), 1032–1035.

Mossong, J., Mughini-Gras, L., Penny, C., Devaux, A., Olinger, C., Losch, S., Cauchie, H. M., van Pelt, W., & Ragimbeau, C. (2016). Human campylobacteriosis in Luxembourg, 2010–2013: A case-control study combined with multilocus sequence typing for source attribution and risk factor analysis. *Scientific Reports, 6*(1), 20939.

Mukherjee, P., Ramamurthy, T., Mitra, U., & Mukhopadhyay, A. K. (2014). Emergence of high-level azithromycin resistance in *Campylobacter jejuni* isolates from pediatric diarrhea patients in Kolkata, India. *Antimicrobial Agents and Chemotherapy, 58*(7), 4248–4248.

Narvaez-Bravo, C., Taboada, E.N., Mutschall, S.K. & Aslam, M. (2017) Epidemiology of antimicrobial resistant Campylobacter spp. isolated from retail meats in Canada. *International Journal of Food Microbiology, 253*, 43–47.

Noreen, Z., Siddiqui, F., Javed, S., Wren, B. W., & Bokhari, H. (2020). Transmission of multidrug-resistant *Campylobacter jejuni* to children from different sources in Pakistan. *Journal of Global Antimicrobial Resistance, 20*, 219–224.

Olsen, S. J., Hansen, G. R., Bartlett, L., Fitzgerald, C., Sonder, A., Manjrekar, R., Riggs, T., Kim, J., Flahart, R., Pezzino, G., & Swerdlow, D. L. (2001). An outbreak of *Campylobacter jejuni* infections associated with food handler contamination: The use of pulsed-field gel electrophoresis. *The Journal of Infectious Diseases, 183*(1), 164–167.

Oyarzabal, O. A., Rad, R., & Backert, S. (2007). Conjugative transfer of chromosomally encoded antibiotic resistance from *Helicobacter pylori* to *Campylobacter jejuni*. *Journal of Clinical Microbiology, 45*(2), 402–408.

Pérez-Boto, D., López-Portolés, J. A., Simón, C., Valdezate, S., & Echeita, M. A. (2010). Study of the molecular mechanisms involved in high-level macrolide resistance of Spanish *Campylobacter jejuni* and *Campylobacter coli* strains. *The Journal of Antimicrobial Chemotherapy, 65*(10), 2083–2088.

Pezzotti, G., Serafin, A., Luzzi, I., Mioni, R., Milan, M., & Perin, R. (2003). Occurrence and resistance to antibiotics of *Campylobacter jejuni* and *Campylobacter coli* in animals and meat in north eastern Italy. *International Journal of Food Microbiology, 82*(3), 281–287.

Piddock, L. J. V., Ricci, V., Pumbwe, L., Everett, M. J., & Griggs, D. J. (2003). Fluoroquinolone resistance in *Campylobacter* species from man and animals: Detection of mutations in topoisomerase genes. *The Journal of Antimicrobial Chemotherapy, 51*(1), 19–26.

Poly, F., & Guerry, P. (2008). Pathogenesis of Campylobacter. *Current Opinion in Gastroenterology, 24*(1), 27–31.

Raeisi, M., Khoshbakht, R., Ghaemi, E. A., Bayani, M., Hashemi, M., Seyedghasemi, N. S., & Shirzad-Aski, H. (2017). Antimicrobial resistance and virulence-associated genes of *Campylobacter* spp. isolated from raw milk, fish, poultry, and red meat. *Microbial Drug Resistance (Larchmont, N.Y.), 23*(7), 925–933.

Ramires, T., de Oliveira, M. G., Kleinubing, N. R., de FátimaRauberWürfel, S., Mata, M. M., Iglesias, M. A., Lopes, G. V., Dellagostin, O. A., & da Silva, W. P. (2020). Genetic diversity, antimicrobial resistance, and virulence genes of thermophilic campylobacter isolated from broiler production chain. *Brazilian Journal of Microbiology: [Publication of the Brazilian Society for Microbiology], 51*(4), 2021–2032.

Ricotta, E. E., Palmer, A., Wymore, K., Clogher, P., Oosmanally, N., Robinson, T., Lathrop, S., Karr, J., Hatch, J., Dunn, J., Ryan, P., & Blythe, D. (2014). Epidemiology and antimicrobial resistance of international travel-associated Campylobacter infections in the United States, 2005-2011. *American Journal of Public Health, 104*(7), e108–e114.

Rincé, A., Balière, C., Hervio-Heath, D., Cozien, J., Lozach, S., Parnaudeau, S., Le Guyader, F. S., Le Hello, S., Giard, J. C., Sauvageot, N., Benachour, A., Strubbia, S., & Gourmelon, M. (2018). Occurrence of bacterial pathogens and human noroviruses in shellfish-harvesting areas and their catchments in France. *Frontiers in Microbiology, 9*, 2443.

Rivera-Mendoza, D., Martínez-Flores, I., Santamaría, R. I., Lozano, L., Bustamante, V. H., & Pérez-Morales, D. (2020). Genomic analysis reveals the genetic determinants associated with antibiotic resistance in the zoonotic pathogen *Campylobacter* spp. distributed globally. *Frontiers in Microbiology, 11*, 513070.

Rollins, D. M., & Colwell, R. R. (1986). Viable but nonculturable stage of *Campylobacter jejuni* and its role in survival in the natural aquatic environment. *Applied and Environmental Microbiology, 52*(3), 531–538.

Samarth, D. P., & Kwon, Y. M. (2020). Horizontal genetic exchange of chromosomally encoded markers between *Campylobacter jejuni* cells. *PLoS One, 15*(10), e0241058.

Sato, M., & Sashihara, N. (2010). Occurrence of Campylobacter in commercially broken liquid egg in Japan. *Journal of Food Protection, 73*(3), 412–417.

Silva, J., Leite, D., Fernandes, M., Mena, C., Gibbs, P. A., & Teixeira, P. (2011). *Campylobacter* spp. as a foodborne pathogen: A review. *Frontiers in Microbiology, 2*, 200.

Skarp, C. P. A., Hänninen, M. L., & Rautelin, H. I. K. (2016). Campylobacteriosis: The role of poultry meat. *Clinical Microbiology and Infection: The Official Publication of the European Society of Clinical Microbiology and Infectious Diseases, 22*(2), 103–109.

Tegtmeyer, N., Sharafutdinov, I., Harrer, A., Soltan Esmaeili, D., Linz, B., & Backert, S. (2021). *Campylobacter* virulence factors and molecular host–pathogen interactions. *Current Topics in Microbiology and Immunology, 431,* 169–202.

Tenover, F. C., Gilbert, T., & O'Hara, P. (1989). Nucleotide sequence of a novel kanamycin resistance gene, aphA-7, from *Campylobacter jejuni* and comparison to other kanamycin phosphotransferase genes. *Plasmid, 22*(1), 52–58.

Thames, H. T., & Sukumaran, A. T. (2020). A Review of *Salmonella* and *Campylobacter* in Broiler Meat: Emerging Challenges and Food Safety Measures. *Foods*, *9*(6), 776.

Wang, Y., & Taylor, D. E. (1990). Natural transformation in *Campylobacter* species. *Journal of Bacteriology, 172*(2), 949–955.

Wang, Y., Zhang, M., Deng, F., Shen, Z., Wu, C., Zhang, J., Zhang, Q., & Shen, J. (2014). Emergence of multidrug-resistant *Campylobacter* species isolates with a horizontally acquired rRNA methylase. *Antimicrobial Agents and Chemotherapy, 58*(9), 5405–5412.

Wieczorek, K., & Osek, J. (2013). Antimicrobial resistance mechanisms among Campylobacter. *BioMed Research International, 2013*, 340605.

Wilson, I. G., & Moore, J. E. (1996). Presence of *Salmonella* spp. and *Campylobacter* spp. in shellfish. *Epidemiology and Infection, 116*(2), 147–153.

Woźniak-Biel, A., Bugla-Płoskońska, G., Kielsznia, A., Korzekwa, K., Tobiasz, A., Korzeniowska-Kowal, A., & Wieliczko, A. (2018). High prevalence of resistance to fluoroquinolones and tetracycline *Campylobacter* spp. isolated from poultry in Poland. *Microbial Drug Resistance (Larchmont, N.Y.), 24*(3), 314–322.

Yan, M., Sahin, O., Lin, J., & Zhang, Q. (2006). Role of the CmeABC efflux pump in the emergence of fluoroquinolone-resistant *Campylobacter* under selection pressure. *Journal of Antimicrobial Chemotherapy, 58*(6), 1154–1159.

Yang, Y., Feye, K. M., Shi, Z., Pavlidis, H. O., Kogut, M., Ashworth, A., & Ricke, S. C. (2019). A historical review on antibiotic resistance of foodborne Campylobacter. *Frontiers in Microbiology, 10*, 1509.

Yao, H., Liu, D., Wang, Y., Zhang, Q., & Shen, Z. (2017). High prevalence and predominance of the aph(2″)-if gene conferring aminoglycoside resistance in Campylobacter. *Antimicrobial Agents and Chemotherapy, 61*(5), e00112–e00117.

Zeinhom, M. M. A., Abdel-Latef, G. K., & Corke, H. (2021). Prevalence, characterization, and control of *Campylobacter jejuni* isolated from raw milk, cheese, and human stool samples in Beni-Suef Governorate, Egypt. *Foodborne Pathogens and Disease, 18*(5), 322–330.

Zhang, T., Luo, Q., Chen, Y., Li, T., Wen, G., Zhang, R., Luo, L., Lu, Q., Ai, D., Wang, H., & Shao, H. (2016). Molecular epidemiology, virulence determinants and antimicrobial resistance of Campylobacter spreading in retail chicken meat in Central China. *Gut Pathogens, 8*, 48.

Zhang, X., Yin, T., Du, X., Yang, W., Huang, J., & Jiao, X. (2017). Occurrence and genotypes of *Campylobacter* species in broilers during the rearing period. *Avian Pathology, 46*(2), 215–223.

Zhang, X., Tang, M., Zhou, Q., Zhang, J., Yang, X., & Gao, Y. (2018). Prevalence and characteristics of Campylobacter throughout the slaughter process of different broiler batches. *Frontiers in Microbiology, 9*, 2092.

Zhou, J., Zhang, M., Yang, W., Fang, Y., Wang, G., & Hou, F. (2016). A seventeen-year observation of the antimicrobial susceptibility of clinical *Campylobacter jejuni* and the molecular mechanisms of erythromycin-resistant isolates in Beijing, China. *International Journal of Infectious Diseases, 42*, 28–33.

Zilbauer, M., Dorrell, N., Wren, B. W., & Bajaj-Elliott, M. (2008). *Campylobacter jejuni*-mediated disease pathogenesis: An update. *Transactions of the Royal Society of Tropical Medicine and Hygiene, 102*(2), 123–129.

Vancomycin and Methicillin Resistance in *Staphylococcus aureus*: What Is the Next?

Anna Sherin Pulithara Sebastian, Murugadas Vaiyapuri, and Madhusudana Rao Badireddy

Contents

A. S. Pulithara Sebastian · M. Vaiyapuri (✉)
Microbiology Fermentation and Biotechnology Division of ICAR-Central Institute of Fisheries Technology (ICAR-CIFT), Cochin, Kerala, India
e-mail: annuanna94@gmail.com; Murugadas.v@icar.gov.in

M. R. Badireddy
Visakhapatnam Research Centre of ICAR-CIFT, ICAR-Central Institute of Fisheries Technology (ICAR-CIFT), Visakhapatnam, Andhra Pradesh, India
e-mail: bmrcift@gmail.com

M. P. Mothadaka et al. (eds.), *Handbook on Antimicrobial Resistance*,
https://doi.org/10.1007/978-981-19-9279-7_17

Abstract

Initially four decades ago, *Staphylococcus aureus* caused infections in clinical and civic situations. This microbe has instigated a significant amount of burden. A subset of antimicrobial-resistant *S. aureus*, viz., methicillin-resistant *S. aureus* (MRSA) and vancomycin-intermediate/vancomycin-resistant *S. aureus* (VISA/VRSA), are prioritized as high-risk pathogens by the WHO for controlling the antimicrobial resistance (AMR). Due to its various virulence weapons and the ongoing evolution of AMR, this pathogen has occupied key significance in the last four decades. These resistant pathogens, which were confined to hospital-acquired infections, are now being found in a growing number of civic surroundings and also in food-producing faunas. The global epidemiology of MRSA and VRSA has been extensively illustrated. This chapter dealt on the evolution of AMR in the pathogen *S. aureus*, as well as the identification of resistance mechanism identified. As a result, the need for novel antimicrobials to treat these AMR bacteria is highlighted along with the future prediction on the development of resistance.

Keywords

MRSA · VRSA · VISA · AMR · Evolution · Resistance mechanism

1 Introduction

Staphylococcus aureus is known to affect health of human, animal, and the food-producing faunas, causing high healthcare expenses, morbidity, and mortality. The organism causes endocarditis, osteoarticular (osteomyelitis, septic arthritis, prosthetic joint infections), dermatological and lenient tissue-related infections (cutaneous blisters, impetigo, cellulitis, and purulent cellulitis), and pneumonia, and biofilm-forming infections in postsurgical conditions (Tong et al., 2015) are just a few of the infections caused by *S. aureus* in humans. In the animal healthcare system, it causes domestic infections in wild and laboratory animals, such as mastitis in cows and goats, bumble foot in chicken, and so on (Smith, 2015; Haag et al., 2019). Furthermore, *S. aureus* is a recognized food pathogen that has generated significant apprehensions in the food safety community due to its ability to cause food poisoning to all walks of food production and consumption (Vaiyapuri et al., 2019). Humans and many warm-blooded animals, including wild animals, have *S. aureus* in their respiratory systems. MSSA (methicillin-susceptible) and MRSA (methicillin-resistant) strains of *S. aureus* are both responsible for hospital-acquired or nosocomial infections and community-acquired infections (Haag et al., 2019). *S. aureus* has emerged as one of the most difficult pathogens to treat in clinical settings, owing to three factors: multi-armory pathogenicity, antibiotic resistance, and biofilm development (Tong et al., 2015; Thomer et al., 2016). The occurrence or absence of these variables determines the pathophysiology of *S. aureus* infections.

For these distinct pathophysiological situations, over 30 potential virulence factors have been identified, which are either synthesized intracellularly or expelled outside the bacterial cells to produce the effect. Coagulase, nuclease, clumping factor, protein A, leukocidins, fibronectins, staphylococcal complement inhibitors, aureolysins, hemolysin, epidermolytic toxins, staphylococcal enterotoxins/superantigens, hyaluronidase, enterotoxins, enterotoxins, kinases, fibrinogen, and proteases (Thomer et al., 2016).

Furthermore, throughout time, *S. aureus* evolved as the prime Gram-positive bacterium for antibiotic resistance (Padera, 2006). Virulence factors are mainly found in their accessory genome's MGEs (mobile genetic elements), such as SCCs (staphylococcal cassette chromosomes), transposons, plasmids, bacteriophages, genomic islands, pathogenicity islands, and insertion sequences (Lindsay and Holden, 2006; Malachowa and Deleo, 2010; Weidenmaier et al., 2012; Vaiyapuri et al., 2019). These factors are responsible for the diversity and spread of various virulence and resistance attributes which includes antimicrobial resistance, metal resistance, etc.

In the era of antibiotic resistance, *S. aureus* exhibits different strain emergence events. *S. aureus* developed various characteristics during this developing process, the most powerful of which is resistance to methicillin and vancomycin, owing to selection pressure in clinical healthcare with consumption of antibiotics (Bronner et al., 2004). *S. aureus* became methicillin-resistant after obtaining a movable genetic material called "staphylococcal cassette chromosome *mec* (SCC*mec*)." This MGE cassette encoded the *mecA* gene, responsible for production of altered penicillin-binding protein, PBP2, a moiety of low lure for penicillin medicines (Ito et al., 1999; Gill et al., 2005; Plata et al., 2009), and the transmission process continued with this MGE. The acquisition of van plasmids bearing *van* genes for vancomycin resistance resulted in the evolution of vancomycin resistance. Methicillin resistance mediated by the *mecC* element has just been discovered.

In this current chapter, the readers are introduced to the expansion of AMR in *S. aureus* after the discovery and introduction of antimicrobials into the healthcare systems including their possible mechanism of resistance and future predicted trend in the spread of AMR.

2 Road Map of Resistance Development in *S. aureus*

S. aureus was originally discovered in 1880 in Aberdeen, Scotland. Bacterial illnesses were on the rise in the 1920s, throughout World War II. The first antibiotic, penicillin, was discovered in 1928, and it was used extensively during the period of discovery to control the bacterial infections. AMR has been evolving since the first discovery of antimicrobials and the subsequent discoveries of newer and synthetic antimicrobials. Four decades' trend in the discovery of antimicrobials is depicted in Fig. 1.

Golden age of antimicrobial discoveries and development included the following: 1928, penicillin discovery by Alexander Fleming; 1935, Domagk synthetically

Fig. 1 Road map of antimicrobial discovery and trend of resistance development

developed sulfonamides; 1944, streptomycin discovery from *Streptomyces griseus* by Selman Waksman, Albert Schatz, and Elizabeth Bugie; 1945–1955, chloramphenicol was discovered from *Streptomyces venezuelae* by Parke–Davis team; tetracycline was discovered from *Streptomyces rimosus* by Alexander Finlay and colleagues; macrolide was discovered from *Streptomyces erythreus* by Brockmann and Hekel; 1956, the glycopeptide, vancomycin, was discovered from *Streptomyces orientalis* (currently *Nocardia orientalis*) by Eli Lilly; 1960, methicillin was synthesized; 1962, nalidixic acid and quinolone were synthesized; 1960–1986, cephems and other generation of cephalosporins were synthesized (Tomoo Saga and Keizo Yamaguchi, 2009); 1984, norfloxacin was synthesized; and 1998, another glycopeptide, teicoplanin, was discovered from *Actinoplanes teichomyceticus*. However, since the last three decades, there has been only modification of already existing antibiotic molecules happening, and no new class of antibiotic has been discovered. The last antibiotic to be discovered was daptomycin in 1987 (Foster, 2017).

The foremost objective of antibiotics used for controlling *S. aureus* is targeting cell wall; ribosomes or nucleic acid mainly and other antibiotics with other targets have also been used (Foster, 2017). The resistance development and transfer occur by either horizontal or vertical gene transfer mechanisms, and resistance in the *S. aureus* for various antibiotics occurs due to the acquiring of genetic determinants by MGEs (Jensen and Lyon 2009). The AMR in *S. aureus* occurs mainly for the reasons, viz., modification in the outer membrane permeability, operation of drug

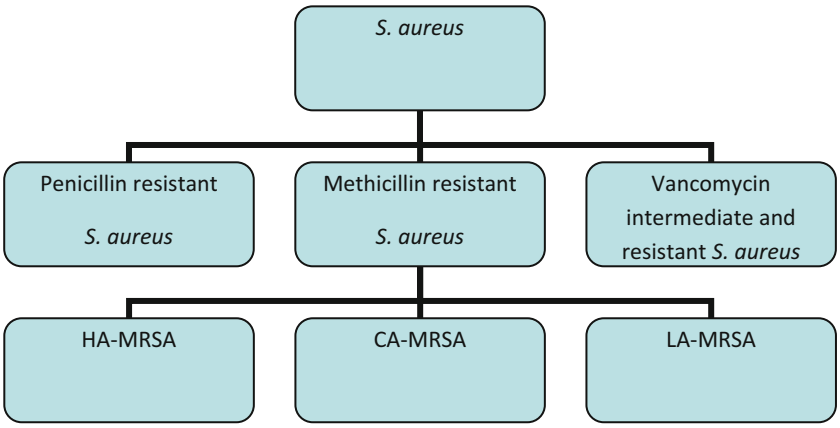


Fig. 2 Dimensions of resistance phenotype developed in *S. aureus*

efflux pump mechanism, excessive production of hydrolytic enzymes, mutation occurring at the drug target site, acquiring of resistance genes, biofilm formation, etc. (Foster, 2017; Guo et al., 2020). The major three important AMR developments in *S. aureus* were the initial emergence of penicillin resistance, development of methicillin resistance, and vancomycin or glycopeptide resistance. The subset of events that occurred within the MRSA development were HA-MRSA (hospital associated), CA-MRSA (community associated), and LA-MRSA (livestock associated). These three forms of resistance made the *S. aureus* to the tip of the iceberg in the realm of control of AMR.

There are different dimensions for development of antimicrobial resistance in *Staphylococcus aureus* (Fig. 2)

2.1 *S. aureus*-Resistant to Penicillin

The discovery of penicillin by Alexander Fleming in 1928 subsequently leads to its employment vigorously in the 1940s for the treatment of clinical infections. In 1942, soon after its use, penicillin-resistant *S. aureus* (PRSA) was detected. The resistance level rose to 80% in 1957, and the trend of its resistance increased to 90% level in *S. aureus* (Gootz, 1990; Schito, 2006). The penicillinase or β-lactamase enzyme produced by *S. aureus* is responsible for resistance to penicillin by means of destruction of the β-lactam ring of the antibiotics encrypted by *blazA* in the plasmids. Subsequently, the same plasmids were detected with genes responsible for erythromycin and gentamicin resistance (Lowy, 2003; Schito, 2006). The DDA (disk diffusion assay) and MIC (minimum inhibitory concentration) are methods of choice for determination of penicillin resistance in *S. aureus*. MICs $\leq$0.12 μg/mL and or $\geq$29 mm zone diameters in DDA should be subjected to test for β-lactamase production (CLSI, 2022; ED32).

2.2 *S. aureus*-Resistant to Methicillin

The first incidence of MRSA confirmation from a hospital (Jevons, 1961; Appelbaum, 2006) and since the 1990s is termed as HA-MRSA (hospital-associated MRSA (HA-MRSA) (Wendlandt et al., 2013). Further spread of the community setting is termed as CA-MRSA (community-associated MRSA) (Healy et al., 2004; Kazakova et al., 2005; Song et al., 2011) with SCCmec type IV and V cassette and additional resistant determinant for β-lactams and macrolides and Panton-Valentine leukocidin (PVL) for soft tissue infections with increased virulence. Now, MRSA has been identified with host association in livestock called as livestock-associated MRSA (LA-MRSA) (David and Daum, 2010; Vaiyapuri et al., 2019).

As a measure to fight the penicillinase enzyme, penicillinase-stable penicillin was presented. In this direction methicillin and oxacillin acid-stable β-lactam antibiotics were introduced. However, resistance developed quickly due to the development of a novel penicillin-binding protein (PBP2a) with a lower binding affinity for methicillin, resulting in overall resistance to β-lactam antibiotics, including cephalosporins. *mecA*, which is located in the staphylococcal chromosomal cassette *mec* (SCC*mec*) transferred from *Staphylococcus sciuri*, is responsible for PBP2a production (Jevons, 1961; Woodford, 2005; Schito, 2006). Worldwide spread of MRSA began during 1970–1980. The prevalence rate in hospitals has risen dramatically, and the global epidemiology has shifted over time. Determination of MIC with oxacillin ($\geq$4 μg/mL) and/or cefoxitin ($\geq$8 μg/mL) and DDA with cefoxitin ($\leq$21 mm) or spot inoculation in oxacillin salt agar are the methods of choice for the determination of resistance of methicillin or oxacillin in *S. aureus* (CLSI, 2022; ED32).

Several semisynthetic cephalosporins were developed to counter the MRSA; however, the fifth-generation cephalosporins, viz., ceftobiprole and ceftaroline, showed significant activity, and thus ceftaroline was allowed for therapy against MRSA (Saravolatz et al., 2011). Soon after the release of these antibiotics in the 2010s, the reduced susceptibility was noted (Mendes et al., 2012).

2.3 Vancomycin- or Glycopeptide-Resistant *S. aureus*

Glycopeptides include teicoplanin and vancomycin, which are the last resort antibiotics against MRSA and Gram+ve cocci infections. Vancomycin was introduced for treatment in 1958 (Micek, 2007). VISA strain of *S. aureus* was identified in Japan in 1996. VRSA strains were discovered in Japan in 1997 and in the United States in 2002 (Appelbaum, 2007). Genetic determinant for vancomycin resistance is encoded in *vanA* operon transferred from vancomycin-resistant *Enterococci*. Heterogeneous vancomycin-intermediate *S. aureus* (hVISA) were also reported (Amberpet et al., 2019). VISA strains have a MIC of 4–8 μg/mL), and VRSA have a MIC more or equal 16 μg/mL. *S. aureus* isolates having condensed sensitivity to teicoplanin, a structural relative of vancomycin, appeared in Europe shortly after 1992 (Manquat et al., 1992). Thickened cell wall peptidoglycan or aberrantly cross-linked

peptidoglycans are the main reason for VISA isolates (McCallum et al., 2010). MIC is the method of choice for determination of VISA or VRSA in the *S. aureus* which includes MRSA too. The MIC of more or equal to 16 μg/mL and more or equal to 32 μg/mL for vancomycin and teicoplanin is considered resistant for vancomycin or glycopeptide (CLSI, 2022; ED32). Of late, lipoglycopeptides such as dalbavancin, oritavancin, and telavancin were also employed in dealing with infections of S. *aureus* that included MRSA.

2.4 Daptomycin-Resistant *S. aureus*

The lipopeptide medication daptomycin consequent of *Streptomyces roseosporus* was first used in clinical trials in 2003 (Arbeit et al., 2004; Heidary et al., 2018). This antibiotic requires calcium for its activity; hence, the testing procedure also requires optimization. Vancomycin, linezolid, and quinupristin/dalofopine all have a slower bactericidal action than daptomycin (Stefani et al., 2015). Daptomycin has the versatility of resisting many clinically important Gram− + ve coccal contagions that are not susceptible to antibiotics, viz., MRSA, MR-CoNS (methicillin-resistant-coagulase-negative staphylococci), PRSP (penicillin-resistant *Streptococcus pneumoniae*), and VRE (vancomycin-resistant enterococci) (Chuang et al., 2016). MIC is the method of choice for determination of daptomycin resistance, and MIC of $\leq$1 μg/mL is considered susceptible and not to be taken into consideration for respiratory tract isolates (CLSI, 2022; ED32).

2.5 Linezolid-Resistant *S. aureus*

Linezolid (oxazolidinone) is a newly created antibiotic class that works against glycopeptide and β-lactam-resistant Gram-positive bacteria, MRSA, VRSA, *Enterococcus*, and *Streptococcus*, and was first approved for clinical use in 2001 (Bain and Wittbrodt, 2001; Pillai et al., 2002). This antibiotic was expected to control *S. aureus*, which was resistant to several medicines as well as other older antibiotics (Krueger and Unertl, 2002). During the year 2001, linezolid-resistant *S. aureus* (LRSA) was discovered (Tsiodras et al., 2001), and soon after that MRSA also become unsusceptible for linezolid due to mutations in the domain variable region of 23s rRNA and *cfr*-mediated resistance. Other compounds, such as tedizolid and radezolid, were synthesized to control the development of resistance to linezolid. *cfr*, *cfr(B)*, *cfr(C)*, *cfr(D)*, *cfr(E)*, *optrA*, and *poxtA* are seven MGEs which carried oxazolidinone resistance genes. The non-susceptibility to oxazolidinones and phenicols is due to *optrA* gene, while *poxtA* gene is responsible for the state of non-susceptibility to oxazolidinones, phenicols, and tetracyclines (Schwarz et al., 2021). According to a global surveillance study, almost 1% of *S. aureus* are LRSA (Gu et al., 2013). DDA and MIC values of $\leq$20 mm and $\geq$8 μg/mL are considered resistant (CLSI, 2022; ED32).

2.6 Macrolide-, Lincosamide-, and Streptogramin-Resistant *S. aureus*

The MLS group of antibiotics includes macrolides, lincosamides, and streptogramins. This group of antibiotics was introduced during the 1950s (Nicola et al., 1998; Maranan et al., 1997; Schito, 2006). In *S. aureus*, there are two phenotypes of MLS resistance. The first is due to antimicrobial drugs failing to connect to their ribosomal target site as a result of ribosomal methylation by 23S rRNA methylases. Resistance for this type is facilitated by the genes *ermA*, *ermB*, and *ermC*, which are located on plasmids or chromosomes. The second kind of resistance is facilitated by *msrA* that comprises the dynamic efflux of the MLS with the energy dependent (ATP-dependent) which keeps the concentration of the antibiotic below the threshold level to reach the ribosomal binding site. Both MIC and DDA are recommended for the method of choice; DDA value of less than or equal to 13 mm and MIC value of more than or equal to 8 μg/mL are reported to be non-susceptible for macrolides (CLSI, 2022; ED32). For the streptogramin resistance, less than or equal to 1 mm and more than or equal to 4 μg/mL values in DDA and MIC are considered resistance.

2.7 Aminoglycoside-Resistant *S. aureus*

Aminoglycosides were initially put into use in 1944, and the aminoglycoside-resistant *S. aureus* (ARSA) was detected during the 1950s (Gootz, 1990; Schito, 2006). Resistance development in staphylococci attributed to three types of events such as mutation occurring in the chromosome which alters the binding ability of the aminoglycosides to the ribosome; enzymatic modification of the antibiotic aminoglycoside or altering the transport of aminoglycoside; and reducing the concentration of antibiotic available to the ribosomal binding region. The genes involved in the modification of aminoglycoside are *ant*, *aph*, and *acc* coded for the enzyme production aminoglycoside acetyltransferases, phosphotransferases, and adenyl transferases, respectively. The functioning of three efflux pumping systems (*QacA*, *NorA*, and *Smr*) of *S. aureus* for the lowering of outer membrane permeability in aminoglycoside resistance (Foster, 2017; Guo et al., 2020) and excessive production of β-lactamase enzymes in *S. aureus* (Khan et al., 2014; Foster, 2017; Guo et al., 2020). MIC and DDA are recommended for the determination of resistance to gentamicin; less than or equal (≤) 12 mm in DDA and more than or equal (≥) 16 μg/mL are considered resistant (CLSI, 2022; ED32).

2.8 Quinolone-Resistant *S. aureus*

Quinolone-resistant MRSA strains developed due to the gradual accumulation of chromosomal changes, despite the fact that fluoroquinolones were initially

marketed in the 1980s. Changes in the enzyme–DNA (nucleic acid) development's QRDR (quinolone resistance-determining region) reduced the quinolone affinity to its targets, namely, the DNA gyrase and the topoisomerase IV. MIC and DDA are endorsed for the determination of resistance to quinolones. In general, less than or equal (≤) 15 mm in DDA and more than or equal (≥) 4 μg/mL are resistant with minor variations exist to the antibiotics with the class (CLSI, 2022; ED32).

2.9 Tetracycline-Resistant *S. aureus*

Tetracyclines attach to the 30S subunit near the area where the anticodon in incoming amino acyl (aa) tRNA recognizes the codon in mRNA, and hence ribosomal protection confers resistance to tetracyclines in *S. aureus* (Wilson, 2016). Ribosomal protection mediated by TetO/M is the most prevalent. The efflux process, which is mediated by *TetK* and *TetA*, is another mechanism of tetracycline resistance (L). Tetracycline is a third-generation semisynthetic tetracycline that is far more effective than tigecycline (glycylcyclines). It was developed in 2005 and has very little resistance to *TetK* and *TetL*-mediated resistance (Foster, 2017). Subinhibitory tetracycline doses induce both efflux and ribosome protection in *S. aureus* (Trzcinski et al., 2000; McCallum et al., 2010). The MIC and DDA are employed for resistance determinations; values of less than or equal to 14 mm in DDA and more than or equal to16 μg/mL in MIC are considered resistant (CLSI, 2022; ED32).

2.10 Clindamycin-Resistant *S. aureus*

Point mutations in the ribosomal RNA methylase generate clindamycin and erythromycin resistance (Martinez et al., 2018). Clindamycin was the antibiotic of choice for CA-MRSA-related epidermal and malleable-tissue ailments owing to its greater oral availability, skin penetration, and low cost (Stevens et al., 1988). Later, inducible resistance to clindamycin created a bottleneck to this mainstay therapy for CA-MRSA (McGehee et al., 1968). When tested with erythromycin, the D-zone screening in general is endorsed for confirming induced clindamycin non-susceptibility in *S. aureus* (Lewis and Jorgensen, 2005). Clindamycin binds to the same place as the macrolide 23S rRNA subunit, and the experimental resistance is triggered by mutations, substitutions, or deletions in the region upstream to the *ermC* methylase open reading frame (ORF) (Woods, 2009). Both MIC and DDA are recommended for determining clindamycin resistance; less than or equal to 14 mm and more than or equal to 4 μg/mL in DDA and MIC, respectively, are considered resistant. However, erythromycin susceptibility screening is mandatory before proceeding to clindamycin susceptibility screening (CLSI, 2022; ED32).

2.11 Mupirocin-Resistant *S. aureus*

In the treatment for nasal decolonization of MRSA or MSSA in community and public health personnel, the only antibiotic permitted is mupirocin (pseudomonic acid A) (Caffrey et al., 2010). The resistant strain of *S. aureus* was observed in 1987 for mupirocin in the UK (Rahman et al., 1987), and has since been found in a number of countries throughout the world (Shittu et al., 2018). Mupirocin is classified as high-level or low-level based on the *mupA* gene and chromosomal point mutation. Other classifications of mupirocin resistance among the MSSA and MRSA strains of MRSA are MuRSA and MuR-MRSA. The high- and low-level MuR-MRSA are further classified as HL-MuR-MRSA and LL-MuR-MRSA (Dadashi et al., 2020). MuRSA (7.6%), MuR-MRSA (13.8%), HL-MuRSA (8.5%), and HL-MuR-MRSA (13.8%) were all shown to be prevalent at 8.1% level. HL-MuRSA accounted for 60.1% of MuRSA, while HL-MuR-MRSA accounted for 44.4% of MuR-MRSA (Dadashi et al., 2020).

In Asia, the frequency of HL-MuR-MRSA is higher than in Europe and the Americas. The Americas had the highest combined prevalence of MuRSA strains followed by Europe and Asia. There is a significant enhancement in general infections associated with *S. aureus* with a positive correlation essentiality for antibiotics, and apropos Africa it is at 14% (Shittu et al., 2018).

2.12 Sulfonamide and Trimethoprim Resistance in *S. aureus*

Sulfonamide is the first drug tried in the mouse model in the 1930s; the drug interferes in the folic acid synthesis of the bacteria. Despite the fact more than 150 promising derivatives were industrialized from sulfonamides, the sulfonamide group gained lesser importance to other new-fangled molecules discovered due to accompanying side effects, and the very important reason is the development of mutation and by this means AMR (Sköld, 2000). The resistance development in the sulfonamide occurs due to chromosomal mutation in the *dhps* (*folP*) gene for dihydropteroate synthetase. Plasmid-mediated sulfonamide resistance occurs due to sul1 and sul2 genes which are linked to the presence of integron *Tn21* and *IncQ* plasmids in Gram -ve bacteria, respectively. Recently *sul3* and *sul4* are detected, all encrypting for mutant *dhps* that do not attach to the sulfonamides (Razavi et al., 2017; Sánchez-Osuna et al., 2019). Resistance to trimethoprim occurs due to mutation in the *dhfr* (dihydrofolate reductase enzyme). Trimethoprim-sulfamethoxazole less than or equal to 10 mm and more than or equal to 4/76 μg/mL in DDA and MIC, respectively, are considered resistant (CLSI, 2022; ED32).

2.13 Phenicol Resistance in *S. aureus*

Chloramphenicol (Cm) also known as chloromycetin, a broad-spectrum antibiotic, was detected from *Streptomyces venezuelae* in 1947. Chloramphenicol acetyltransferase (CAT) inactivates the chloramphenicol which is the focal tool of

resistance (Schwarz et al., 2004). This inactivation process occurs separately by two types of enzymes A and B along with subclasses. Other mechanisms of resistance in phenicols are efflux pump mediated (*fexA*), mutation in the 23rRNA region, 23rRNA methylase target site modification, and phosphotransferase-mediated inactivation (*cfr*) (Van Duijkeren et al., 2018). DDA of less than or equal to 12 mm and more than or equal to 32 μg/mL MIC are considered resistant to chloramphenicol non-susceptibility in *S. aureus* (CLSI, 2022; ED32).

2.14 Ansamycin Resistance *in S. aureus*

Professor Piero Sensi discovered the antibiotic rifampicin or also called as rifampin. This is the drug of first choice for tuberculosis; however, it was used for other infections like staphylococcal type. The focal mechanism of resistance to rifampin occurs due to mutation affecting DNA-dependent RNA polymerase submit β. Few other rare mechanisms observed are permeability or efflux/influx (Goldstein, 2014). DDA of less than or equal to 16 mm and more than or equal to 4 μg/mL MIC are considered resistant to chloramphenicol resistance in *S. aureus* (CLSI, 2022; ED32) (Fig. 3).

3 Current Status Athwart the Globe in AMR in *S. aureus* Across the Sectors

The occasions of distinct antibiotic resistance strain emergence in the *S. aureus* history are resistance to penicillin, methicillin, and vancomycin. Later on, the resistance emerged toward daptomycin, teicoplanin, and linezolids. The development of AMR occasions in *S. aureus* occurred from strain level within species level

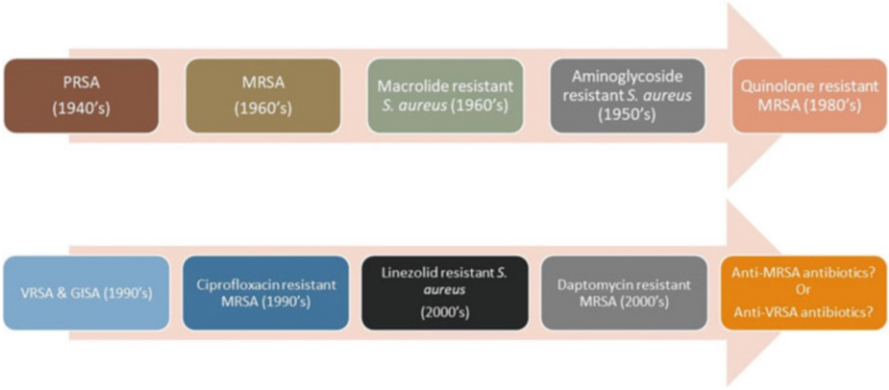


Fig. 3 Trends in recognizing the *S. aureus* drug resistance (PRSA penicillin-resistant *S. aureus*, MRSA methicillin-resistant *S. aureus,* ARSA aminoglycoside-resistant *S. aureus,* VRSA vancomycin-resistant *S. aureus,* and GISA glycopeptide-intermediate *S. aureus*)

or from different genera. Plasmids, lysogenic phages, and SCC*mec* elements played a crucial part in the incidence of AMR in *S. aureus* (Haaber et al., 2017). Diverse ARGs were carried over few hundreds of MGEs. The MGEs, viz., staphylococcal pathogenicity island (SaPI), integrative conjugative elements (ICE), staphylococcal chromosome cassettes (SCCs), and integron, contribute lesser to the diversity compared to the plasmids, and transposons. Over 25 plasmids were documented for carrying ARGs in the *S. aureus* (Shearer et al., 2011; McCarthy and Lindsay, 2012; Haaber et al., 2017).

Plasmids reported for harboring various classes of ARGs are pI258, pMS97, pKKS825, pE194, and pIP524 for macrolide resistances; pUB110, SAP049A, and pS194 for aminoglycoside resistance; SAP0882A for glycopeptide resistance; pKH21 for oxazolidone resistances; pSCFS1, pC221, pC223, pUB112, and pC194 for phenicol resistance, pS194, pSK1, pSK16, and pKKS825 for sulfonamide resistance; pMW2, pSAS, pI147, and pI258 for β-lactam resistance; and pKK825 for tetracycline resistance (Shearer et al., 2011; Haaber et al., 2017). Next to the plasmid mediation, transposon mediation resistance was prominent. The transposons, namely, Tn4001, Tn552, Tn554/Tn551, and Tn558, are mediated for aminoglycoside, β-lactam, macrolide, and phenicol resistance, respectively. SCC-mediated resistance was observed for aminoglycoside, tetracycline, and macrolide resistance (McCarthy and Lindsay, 2012). These MGEs responsible for the AMR-carried ARGs are sometimes presented in MRSA isolates or sometimes in MSSA or in both groups (Turner et al., 2019).

Predominant clonal complexes of *S. aureus* reported in the clinical and epidemiologically linked system are CC5, CC8, CC398, and CC30. Other clones which are reported across the globes are CC1, CC15, CC80, CC72, CC9, CC97, CC25, CC45, CC59, CC121, and CC22 (Planet et al., 2017).

PRSA was mostly associated with MLST type ST 30 belonging to the clonal complex 30 (CC30) (Robinson et al., 2005; McGuinness et al., 2017). The frequency of development and transmission of more forms of antimicrobial resistant *S. aureus* is due to the accessory genome diversity which carries diverse MGEs (Haaber et al., 2017). Generally smaller plasmids carried resistance for erythromycin or phenicol or tetracyclines, whereas the bigger plasmids carried resistance genes for macrolides, aminoglycosides, and β-lactams. SCCmec carried β-lactams, macrolides, streptomycin, or spectinomycin resistance in addition to the *mec* elements (Haaber et al., 2017).

4 AMR in *Staphylococcus aureus*: Future Scenario

Development of AMR in *Staphylococcus aureus* is complex in nature; additionally the complexity increases with the MRSA, because the pathogen can co-harbor determinants for additional resistance to other antibiotics (Vestergaard et al., 2019). MRSA is prevalent in many areas of the globe including developed and developing nations. In some of the developed countries, MRSA prevalence has reached to more than 50% level. In many instances, the additional resistance was

attributed to *Smr*, *NorA*, and *QacA* for multidrug efflux pump and cell membrane modifications. The MRSA strains are dominating in the global epidemiology in the circulation of *S. aureus* resistant to antibiotics; albeit, spread of vancomycin resistance is restrained. The antibiotics, viz., vancomycin, norvancomycin, teicoplanin, linezolid, quinupristin, daptomycin, tigecycline, ceftobiprole, oritavancin, and dalbavancin are developed for anti-MRSA activity, but their usage pattern varied across the globe (Guo et al., 2020). The tendency in the increase of novel resistance strains depends on the antibiotics being used in the treatment of MRSA in the hospitals, viz., daptomycin, oxazolidone, and mupirocin. Even though VRSA is not spreading to many parts of the world and it is under control, the development of VISA is slightly on the increase (Haaber et al., 2017). There are newer promising antibiotics that are being developed for the MRSA, viz., anti-MRSA quinolones (delafloxacin, levonadifloxacin, acorafloxacin) and pyrazolylpyridine analogues (Chao et al., 2022; Nanjundaswamy et al., 2022). In the wake of antimicrobial resistance, there is a switch over to alternatives to antibiotics, and several molecules are in the pipeline. Phytochemicals, quorum sensing inhibitors (liposome based), lectin inhibition agents, iron chelation agents, phages, nanoparticles, and combinations such as superoxide radical-mediated self-synthesized Au/MoO_{3-x} and novel Schiff-bases are some of the alternative molecules under development (Li et al., 2017; Haseeb et al., 2019; Zeng et al., 2022; Cao et al., 2022; Bendre et al., 2022; Sinsinwar et al., 2022). Renewed interest being generated on the bacteriophages against MRSA and several products is being explored for the phage therapy either as single or in combination with antibiotics (Abd-Allah et al., 2022; Doub et al., 2022; Kebriaei et al., 2022). Other novel antibacterial targets are curtailing the fatty acid biosynthesis of *S. aureus* (Debio1452); multicellular biocide, triclosan; molecules targeting cell division (FtsZ protein); molecules targeting the killing of persister *S. aureus* cells (acylpepsipeptide antibiotic ADEP4); novel lipid II inhibitor (teixobactin); and teichoic acid biosynthesis inhibitors (Foster, 2017).

In an effort to control the emergence and dissemination of resistance to new antibiotics, there need to be a testing strategy before resorting to antibiotics as evidenced by the Gram Stain-Guided Antibiotics Choice for VAP group in Japan for the control of MRSA-associated pneumonia (Yoshimura et al., 2022). The infection control program should be implemented at region, state, and national level; antibiotic stewardship awareness and education among the stakeholders, and systematic surveillance program on the development of resistance in superbugs are all very critical in successfully reducing mupirocin resistance, particularly in MRSA isolates (Dadashi et al., 2020). Considerable variation in the AMR in MRSA and MSSA may occur in response to the change in the selection pressure due to disinfectants and heavy metal usage (Turner et al., 2019). In the process of the evolution, there could be a shift in the clonal complex associated with the *S. aureus* infections along with subsequent changes in the virulence and AMR pattern. In order to curtail the infectious conditions due to MRSA/MSSA, there require an understanding of genomic data, metabolomic analysis, and correlating to the clinical outcome.

5 Conclusion

S. aureus has demonstrated extraordinary adaptability to antimicrobial resistance, allowing it to survive in the community even when antibiotics of last resort are used. In order to survive in hospital, community, and livestock populations, the pathogen has grown substantially. MRSA has been showing considerable threat to human health. There is no significant increase in the vancomycin resistance. In the current era of AMR, the development of resistance cannot be completely avoided; however, it should be managed adequately. A proactive plan has to be devised for each and every antibiotic with a time line to develop resistance phenomenon and strategize a rotation plan for use of antibiotics. The restricted use of critically important antimicrobials has been notified for the sectors with adequate prejudice in order to control further advancement of resistance to antimicrobials. Limiting the use of antibiotics pan-sectors, viz., human healthcare, animal healthcare, and animal agriculture, thereby reducing the resistance simultaneously developing in all the sectors through a one health approach is need of the hour. Promoting the drug discovery for the AMR superbugs with newer targets or modified targets combats the risk pathogens identified by the WHO and also ESKAPE pathogens, as the nature provided with plethora of novel antimicrobial molecules. To limit the spread of resistance, human and animal healthcare systems must adhere to strong infection prevention control strategies. To manage staphylococcal infections, it is also necessary to develop a unique drug target that belongs to a new class or an appropriate modification of an existing antibiotic targeting specifically to the species level.

6 Cross-References

► Avenues in the Determination of AMR in Human Health
► Evolution and Milestones in the Development of AMR in Bacteria

References

Abd-Allah, I. M., El-Housseiny, G. S., Alshahrani, M. Y., El-Masry, S. S., Aboshanab, K. M., & Hassouna, N. A. (2022). An anti-MRSA phage from raw fish rinse: Stability evaluation and production optimization. *Frontiers in Cellular and Infection Microbiology, 563*.

Amberpet, R., Sistla, S., Sugumar, M., Nagasundaram, N., Manoharan, M., & Parija, S. C. (2019). Detection of heterogeneous vancomycin-intermediate Staphylococcus aureus: A preliminary report from South India. *The Indian Journal of Medical Research, 150*, 194–198. https://doi.org/10.4103/ijmr.IJMR_1976_17

Appelbaum, P. C. (2006). MRSA—The tip of the iceberg. *Clinical Microbiology and Infection, 12*(Suppl 2), 3–10.

Appelbaum, P. C. (2007). Reduced glycopeptide susceptibility in methicillin-resistant Staphylococcus aureus (MRSA). *International Journal of Antimicrobial Agents, 30*(5), 398–408.

Arbeit, R. D., Maki, D., Tally, F. P., Campanaro, E., Eisenstein, B. I., & Daptomycin 98-01 and 99-01 Investigators. (2004). The safety and efficacy of daptomycin for the treatment of

complicated skin and skin-structure infections. *Clinical Infectious Diseases, 38*(12), 1673–1681.

Bain, K. T., & Wittbrodt, E. T. (2001). Linezolid for the treatment of resistant gram-positive cocci. *The Annals of Pharmacotherapy, 35*, 566–575.

Bendre, R. S., Patil, R. D., Patil, P. N., Patel, H. M., & Sancheti, R. S. (2022). Synthesis and characterization of new Schiff-bases as Methicillin resistant Staphylococcus aureus (MRSA) inhibitors. *Journal of Molecular Structure, 1252*, 132152.

Bronner, S., Monteil, H., & Prévost, G. (2004). Regulation of virulence determinants in Staphylococcus aureus: Complexity and applications. *FEMS Microbiology Reviews, 28*(2), 183–200.

Caffrey, A. R., Quilliam, B. J., & LaPlante, K. L. (2010). Risk factors associated with mupirocin resistance in methicillin-resistant Staphylococcus aureus. *Journal of Hospital Infection, 76*(3), 206–210.

Cao, M., Chang, Z., Tan, J., Wang, X., Zhang, P., Lin, S., et al. (2022). Superoxide radical-mediated self-synthesized Au/MoO3–x hybrids with enhanced peroxidase-like activity and photothermal effect for anti-MRSA therapy. *ACS Applied Materials & Interfaces, 14*(11), 13025–13037.

Chao, C. M., Weng, T. S., Chen, Y. H., Lai, C. C., & Lin, W. T. (2022). Anti-MRSA quinolones for acute bacterial skin and skin structure infection: A systematic review and meta-analysis of randomized controlled trials. *Expert Review of Anti-Infective Therapy, 20*(5), 733–739.

Chuang, Y. C., Lin, H. Y., Chen, P. Y., Lin, C. Y., Wang, J. T., & Chang, S. C. (2016). Daptomycin versus linezolid for the treatment of vancomycin-resistant enterococcal bacteraemia: Implications of daptomycin dose. *Clinical Microbiology and Infection, 22*(10), 890–8e1.

Clinical and Laboratory Standards Institute (CLSI). *Performance standards for antimicrobial susceptibility testing*. 32nd ed. CLSI supplement M100 ISBN 978-1-68440-135-2 [Electronic]). Clinical and Laboratory Standards Institute, USA, 2022. (E version accessed ED32 electronic in 03 March 2022).

Dadashi, M., Hajikhani, B., Darban-Sarokhalil, D., van Belkum, A., & Goudarzi, M. (2020). Mupirocin resistance in Staphylococcus aureus: A systematic review and meta-analysis. *Journal of Global Antimicrobial Resistance, 20*, 238–247.

David, M. Z., & Daum, R. S. (2010). Community-associated methicillin-resistant Staphylococcus aureus: Epidemiology and clinical consequences of an emerging epidemic. *Clinical Microbiology Reviews, 23*(3), 616–687.

Doub, J. B., Ng, V. Y., Lee, M., Chi, A., Lee, A., Würstle, S., & Chan, B. (2022). Salphage: Salvage bacteriophage therapy for recalcitrant MRSA prosthetic joint infection. *Antibiotics, 11*(5), 616.

Foster, T. J. (2017). Antibiotic resistance in Staphylococcus aureus. Current status and future prospects. *FEMS Microbiology Reviews, 41*(3), 430–449.

Gill, S. R., Fouts, D. E., Archer, G. L., Mongodin, E. F., DeBoy, R. T., Ravel, J., et al. (2005). Insights on evolution of virulence and resistance from the complete genome analysis of an early methicillin-resistant Staphylococcus aureus strain and a biofilm-producing methicillin-resistant Staphylococcus epidermidis strain. *Journal of Bacteriology, 187*(7), 2426–2438.

Goldstein, B. P. (2014). Resistance to rifampicin: a review. *The Journal of Antibiotics, 67*(9), 625–630.

Gootz, T. D. (1990). Discovery and development of new antimicrobial agents. *Clinical Microbiology Reviews, 3*, 13–31.

Gu, B., Kelesidis, T., Tsiodras, S., Hindler, J., & Humphries, R. M. (2013). The emerging problem of linezolid-resistant Staphylococcus. *The Journal of Antimicrobial Chemotherapy, 68*(1), 4–11. https://doi.org/10.1093/jac/dks354

Guo, Y., Song, G., Sun, M., Wang, J., & Wang, Y. (2020). Prevalence and therapies of antibiotic-resistance in Staphylococcus aureus. *Frontiers in Cellular and Infection Microbiology, 10*, 107. https://doi.org/10.3389/fcimb.2020.00107

Haaber, J., Penadés, J. R., & Ingmer, H. (2017). Transfer of antibiotic resistance in Staphylococcus aureus. *Trends in Microbiology, 25*(11), 893–905.

Haag, A. F., Fitzgerald, J. R., & Penadés, J. R. (2019). Staphylococcus aureus in animals. *Microbiology Spectrum, 7*(3), 7–3.

Haseeb, A., Ajit Singh, V., Teh, C. S. J., & Loke, M. F. (2019). Addition of ceftaroline fosamil or vancomycin to PMMA: An in vitro comparison of biomechanical properties and anti-MRSA efficacy. *Journal of Orthopaedic Surgery, 27*, 2309499019850324. https://doi.org/10.1177/2309499019850324

Healy, C. M., Hulten, K. G., Palazzi, D. L., Campbell, J. R., & Baker, C. J. (2004). Emergence of new strains of methicillin-resistant *Staphylococcus aureus* in a neonatal intensive care unit. *Clinical Infectious Diseases, 39*, 1460–1466.

Heidary, M., Khosravi, A. D., Khoshnood, S., Nasiri, M. J., Soleimani, S., & Goudarzi, M. (2018). Daptomycin. *Journal of Antimicrobial Chemotherapy, 73*(1), 1–11.

Ito, T., Katayama, Y., & Hiramatsu, K. (1999). Cloning and nucleotide sequence determination of the entire mec DNA of pre-methicillin-resistant Staphylococcus aureus N315. *Antimicrobial Agents and Chemotherapy, 43*(6), 1449–1458.

Jensen, S. O., & Lyon, B. R. (2009). Genetics of antimicrobial resistance in Staphylococcus aureus. *Future Microbiology, 4*(5), 565–582.

Jevons, M. P. (1961). 'Celbenin'-resistant staphylococci. *BMJ, 1*, 124–125.

Kazakova, S. V., Hageman, J. C., Matava, M., Srinivasan, A., Phelan, L., Garfinkel, B., Boo, T., McAllister, S., Anderson, J., Jensen, B., Dodson, D., Lonsway, D., McDougal, L. K., Arduino, M., Fraser, V. J., Killgore, G., Tenover, F. C., Cody, S., & Jernigan, D. B. (2005). A clone of methicillin resistant Staphylococcus aureus among professional football players. *The New England Journal of Medicine, 2005*(352), 468–475.

Kebriaei, R., Lev, K. L., Shah, R. M., Stamper, K. C., Holger, D. J., Morrisette, T., et al. (2022). Eradication of biofilm-mediated methicillin-resistant *Staphylococcus aureus* infections in vitro: Bacteriophage-antibiotic combination. *Microbiology Spectrum, 10*(2), e00411–e00422.

Khan, S., Sallum, U. W., Zheng, X., Nau, G. J., & Hasan, T. (2014). Rapid optical determination of beta-lactamase and antibiotic activity. *BMC Microbiology, 14*, 84. https://doi.org/10.1186/1471-2180-14-84

Krueger, W. A., & Unertl, K. E. (2002). New treatment option for gram-positive infections in critically ill patients-overview over linezolid. *Anasthesiologie, Intensivmedizin, Notfallmedizin, Schmerztherapie: AINS, 37*(4), 199–204.

Lewis, J. S., & Jorgensen, J. H. (2005). Inducible clindamycin resistance in staphylococci: Should clinicians and microbiologists be concerned? *Clinical Infectious Diseases, 40*(2), 280–285.

Li, M., Zou, P., Tyner, K., & Lee, S. (2017). Physiologically based pharmacokinetic (PBPK) modelling of pharmaceutical nanoparticles. *The AAPS Journal, 19*, 26–42. https://doi.org/10.1208/s12248-016-0010-3

Lindsay, J. A., & Holden, M. T. (2006). Understanding the rise of the superbug: Investigation of the evolution and genomic variation of Staphylococcus aureus. *Functional & Integrative Genomics, 6*(3), 186–201.

Lowy, F. D. (2003). Antimicrobial resistance: The example of Staphylococcus aureus. *The Journal of Clinical Investigation, 111*, 1265–1212.

Malachowa, N., & DeLeo, F. R. (2010). Mobile genetic elements of Staphylococcus aureus. *Cellular and Molecular Life Sciences, 67*(18), 3057–3071.

Manquat, G., Croize, J., Stahl, J. P., Meyran, M., Hirtz, P., & Micoud, M. (1992). Failure of teicoplanin treatment associated with an increase in MIC during therapy of Staphylococcus aureus septicaemia. *Journal of Antimicrobial Chemotherapy, 29*(6), 731–732.

Maranan, M. C., Moreira, B., Boyle-Vavra, S., & Daum, R. S. (1997). Antimicrobial resistance in staphylococci. Epidemiology, molecular mechanisms, and clinical relevance. *Infectious Disease Clinics of North America, 11*, 813–849.

Martínez, N., Luque, R., Milani, C., Ventura, M., Bañuelos, O., & Margolles, A. (2018). A gene homologous to rRNA methylase genes confers erythromycin and clindamycin resistance in bifidobacterium breve. *Applied and Environmental Microbiology, 84*(10), e02888–e02817.

McCallum, N., Berger-Bächi, B., & Senn, M. M. (2010). Regulation of antibiotic resistance in Staphylococcus aureus. *International Journal of Medical Microbiology, 300*(2–3), 118–129.

McCarthy, A. J., & Lindsay, J. A. (2012). The distribution of plasmids that carry virulence and resistance genes in Staphylococcus aureus is lineage associated. *BMC Microbiology, 12*(1), 1–8.

McGehee, R. F., Barrett, F. F., & Finland, F. (1968). Resistance of Staphylococcus aureus to lincomycin, clindamycin and erythromycin. *Antimicrobial Agents and Chemotherapy, 13*, pg. 392-7.

McGuinness, W. A., Malachowa, N., & DeLeo, F. R. (2017). Vancomycin resistance in *Staphylococcus aureus*. *The Yale Journal of Biology and Medicine, 90*(2), 269–281.

Mendes, R. E., Tsakris, A., Sader, H. S., Jones, R. N., Biek, D., McGhee, P., et al. (2012). Characterization of methicillin-resistant Staphylococcus aureus displaying increased MICs of ceftaroline. *Journal of Antimicrobial Chemotherapy, 67*(6), 1321–1324.

Micek, S. T. (2007). Alternatives to vancomycin for the treatment of methicillin resistant Staphylococcus aureus infections. *Clinical Infectious Diseases, 45*(Suppl 3), S184–S190. https://doi.org/10.1086/519471

Nanjundaswamy, S., Jayashankar, J., Chethana, M. H., Renganathan, R. A., Karthik, C. S., Ananda, A. P., et al. (2022). Design, synthesis, and in-silico studies of pyrazolylpyridine analogues: A futuristic antibacterial contender against coagulase positive superbug-MRSA. *Journal of Molecular Structure, 132400*, 132400.

Nicola, F. G., McDougal, L. K., Biddle, J. W., & Tenover, F. C. (1998). Characterization of erythromycin-resistant isolates of Staphylococcus aureus recovered in the United States from 1958 through 1969. *Antimicrobial Agents and Chemotherapy, 42*, 3024–3027.

Padera, R. F. (2006). Infection in ventricular assist devices: The role of biofilm. *Cardiovascular Pathology, 15*, 264–270. https://doi.org/10.1016/j.carpath.2006.04.008

Pillai, S. K., Sakoulas, G., Wennersten, C., Eliopoulos, G. M., Moellering, R. C., Jr., Ferraro, M. J., & Gold, H. S. (2002). Linezolid resistance in Staphylococcus aureus: Characterization and stability of resistant phenotype. *The Journal of Infectious Diseases, 186*(11), 1603–1607.

Planet, P. J., Narechania, A., Chen, L., Mathema, B., Boundy, S., Archer, G., & Kreiswirth, B. (2017). Architecture of a species: Phylogenomics of Staphylococcus aureus. *Trends in Microbiology, 25*(2), 153–166.

Plata, K., Rosato, A. E., & Wegrzyn, G. (2009). Staphylococcus aureus as an infectious agent: Overview of biochemistry and molecular genetics of its pathogenicity. *Acta Biochimica Polonica, 56*(4), 597–612.

Rahman, M., Noble, W. C., & Cookson, B. (1987). Mupirocin resistant *Staphylococcus aureus*. *Lancet, 330*, 387–388. https://doi.org/10.1016/S0140-6736(87)92398-1

Razavi, M., Marathe, N. P., Gillings, M. R., Flach, C. F., Kristiansson, E., & Joakim Larsson, D. G. (2017). Discovery of the fourth mobile sulfonamide resistance gene. *Microbiome, 5*(1), 1–12.

Robinson, D. A., Kearns, A. M., Holmes, A., Morrison, D., Grundmann, H., Edwards, G., et al. (2005). Re-emergence of early pandemic Staphylococcus aureus as a community-acquired methicillin-resistant clone. *The Lancet, 365*(9466), 1256–1258.

Saga, T., & Yamaguchi, K. (2009). History of antimicrobial agents and resistant bacteria. *Japan Medical Association Journal, 52*(2), 103–108.

Saravolatz, L. D., Stein, G. E., & Johnson, L. B. (2011). Ceftaroline: a novel cephalosporin with activity against methicillin-resistant Staphylococcus aureus. *Clinical Infectious Diseases, 52*(9), 11567–1163.

Sánchez-Osuna, M., Cortés, P., Barbé, J., & Erill, I. (2019). Origin of the mobile di-hydro-pteroate synthase gene determining sulfonamide resistance in clinical isolates. *Frontiers in Microbiology, 9*, 3332.

Schito, G. C. (2006). The importance of the development of antibiotic resistance in Staphylococcus aureus. *Clinical Microbiology and Infection, 12*(Suppl. 1), 3–8.

Schwarz, S., Kehrenberg, C., Doublet, B., & Cloeckaert, A. (2004). Molecular basis of bacterial resistance to chloramphenicol and florfenicol. *FEMS Microbiology Reviews, 28*(5), 519–542.

Schwarz, S., Zhang, W., Du, X. D., Krüger, H., Feßler, A. T., Ma, S., et al. (2021). Mobile oxazolidinone resistance genes in gram-positive and gram-negative bacteria. *Clinical Microbiology Reviews, 34*(3), e00188–e00120.

Shearer, J. E., Wireman, J., Hostetler, J., Forberger, H., Borman, J., Gill, J., et al. (2011). Major families of multiresistant plasmids from geographically and epidemiologically diverse staphylococci. *G3: Genes|Genomes|Genetics, 1*(7), 581–591.

Shittu, A. O., Kaba, M., Abdulgader, S. M., et al. (2018). Mupirocin-resistant *Staphylococcus aureus* in Africa: A systematic review and meta-analysis. *Antimicrobial Resistance and Infection Control, 7*, 101. https://doi.org/10.1186/s13756-018-0382-5

Sinsinwar, S., Jayaraman, A., Mahapatra, S. K., & Vellingiri, V. (2022). Anti-virulence properties of catechin-in-cyclodextrin-in-phospholipid liposome through down-regulation of gene expression in MRSA strains. *Microbial Pathogenesis, 105585*.

Sköld, O. (2000). Sulfonamide resistance: Mechanisms and trends. *Drug Resistance Updates, 3*(3), 155–160.

Smith, T. C. (2015). Livestock-associated Staphylococcus aureus: The United States experience. *PLoS Pathogens, 11*(2), e1004564.

Song, J. H., Hsueh, P. R., Chung, D. R., Ko, K. S., Kang, C. I., Peck, K. R., et al. (2011). Spread of methicillin-resistant *Staphylococcus aureus* between the community and the hospitals in Asian countries: An ANSORP study. *Journal of Antimicrobial Chemotherapy, 66*(5), 1061–1069.

Stefani, S., Campanile, F., Santagati, M., Mezzatesta, M. L., Cafiso, V., & Pacini, G. (2015). Insights and clinical perspectives of daptomycin resistance in Staphylococcus aureus: A review of the available evidence. *International Journal of Antimicrobial Agents, 46*(3), 278–289.

Stevens, D. L., Gibbons, A. E., Bergstrom, R., & Winn, V. (1988). The eagle effect revisited: Efficacy of clindamycin, erythromycin, and penicillin in the treatment of streptococcal myositis. *Journal of Infectious Diseases, 158*(1), 23–28.

Thomer, L., Schneewind, O., & Missiakas, D. (2016). Pathogenesis of Staphylococcus aureus bloodstream infections. *Annual Review of Pathology, 11*, 343.

Tong, S. Y., Davis, J. S., Eichenberger, E., Holland, T. L., & Fowler, V. G., Jr. (2015). Staphylococcus aureus infections: Epidemiology, pathophysiology, clinical manifestations, and management. *Clinical Microbiology Reviews, 28*(3), 603–661.

Trzcinski, K., Cooper, B. S., Hryniewicz, W., & Dowson, C. G. (2000). Expression of resistance to tetracyclines in strains of methicillin-resistant Staphylococcus aureus. *Journal of Antimicrobial Chemotherapy, 45*(6), 763–770.

Tsiodras, S., Gold, H. S., Sakoulas, G., Eliopoulos, G. M., Wennersten, C., Venkataraman, L., Moellering, R. C., & Ferraro, M. J. (2001). Linezolid resistance in a clinical isolate of Staphylococcus aureus. *Lancet, 358*(9277), 207–208. https://doi.org/10.1016/S0140-6736(01)05410-1

Turner, N. A., Sharma-Kuinkel, B. K., Maskarinec, S. A., et al. (2019). Methicillin-resistant *Staphylococcus aureus*: An overview of basic and clinical research. *Nature Reviews. Microbiology, 17*, 203–218. https://doi.org/10.1038/s41579-018-0147-4

Vaiyapuri, M., Joseph, T. C., Rao, B. M., Lalitha, K. V., & Prasad, M. M. (2019). Methicillin-resistant Staphylococcus aureus in seafood: Prevalence, laboratory detection, clonal nature, and control in seafood chain. *Journal of Food Science, 84*(12), 3341–3351.

Van Duijkeren, E., Schink, A. K., Roberts, M. C., Wang, Y., & Schwarz, S. (2018). Mechanisms of bacterial resistance to antimicrobial agents. *Microbiology Spectrum, 6*(2), 6–2.

Vestergaard, M., Frees, D., & Ingmer, H. (2019). Antibiotic resistance and the MRSA problem. *Microbiology Spectrum, 7*, GPP3-0057-2018. https://doi.org/10.1128/microbiolspec.GPP3-0057-2018

Weidenmaier, C., Goerke, C., & Wolz, C. (2012). Staphylococcus aureus determinants for nasal colonization. *Trends in Microbiology, 20*(5), 243–250.

Wendlandt, S., Schwarz, S., & Silley, P. (2013). Methicillin-resistant *Staphylococcus aureus*: A food-borne pathogen? *Annual Review of Food Science and Technology, 4*, 117–139.

Wilson, D. N. (2016). The ABC of ribosome-related antibiotic resistance. *MBio, 7*(3), e00598–e00516.

Woodford, N. (2005). Biological counterstrike: antibiotic resistance mechanisms of gram-positive cocci. *Clinical Microbiology and Infection, 11*(suppl 3), 2–21.

Woods, C. R. (2009). Macrolide-inducible resistance to clindamycin and the D-test. *The Pediatric Infectious Disease Journal, 28*(12), 1115–1118.

Yoshimura, J., Yamakawa, K., Ohta, Y., Nakamura, K., Hashimoto, H., Kawada, M., et al. (2022). Effect of gram stain–guided initial antibiotic therapy on clinical response in patients with ventilator-associated pneumonia: The GRACE-VAP randomized clinical trial. *JAMA Network Open, 5*(4), e226136.

Zeng, Q., Wang, Z. J., Chen, S., Wang, H., Xie, T. Z., Xu, X. J., et al. (2022). Phytochemical and anti-MRSA constituents of Zanthoxylum nitidum. *Biomedicine & Pharmacotherapy, 148*, 112758.

Antibiotic Resistance of *Arcobacter* Species: An Emerging Pathogen

Ranjit Kumar Nadella, V. A. Minimol, and T. Muthulakshmi

Contents

Abstract

The *Arcobacter* species are regarded as an emerging bacterial pathogen mainly responsible for infection such as enteritis, septicemia, diarrhea, and bacteremia in human populations and enteritis and abortion in farm-reared animals. For the past several years, an increase in the frequency of antibiotic-resistant strains of *Arcobacter* has been observed due to disproportionate use of veterinary drugs including antibiotics in the treatment process for controlling infections in food production avenues and human populations. Among different species of *Arcobacter*, presently three species of *Arcobacter*, namely, *A. butzleri*, *A. cryaerophilus*, and *A. skirrowii*, are well established as human pathogens and are associated with clinical manifestations. Drug-resistant *Arcobacter* were

R. K. Nadella (✉) · V. A. Minimol · T. Muthulakshmi
MFB Division, ICAR-Central Institute of Fisheries Technology, Willingdon Island, Cochin, Kerala, India
e-mail: nranjeetkumar@gmail.com; Ranjit.Nadella@icar.gov.in; minimol.a@icar.gov.in; Muthulakshmi.T@icar.gov.in

M. P. Mothadaka et al. (eds.), *Handbook on Antimicrobial Resistance*,
https://doi.org/10.1007/978-981-19-9279-7_18

frequently isolated from different food products, and the mechanisms of resistance are being studied for better understanding. However, limitation for studying antimicrobial susceptibility is lack of specific breakpoints for *Arcobacter* species. This chapter describes the antibiotic resistance pattern in *Arcobacter* with reference to three dominant species from this genus.

Keywords

Arcobacter · Antibiotic resistance · Emerging pathogens · Disc diffusion · Foods

1 Introduction

Arcobacter species belongs to a wide and diverse category of bacteria which has the ability to grow in different environmental habitats and hosts. They belonged to rod-shaped bacterium having no spores, Gram-negative, aerotolerant, and motile of the family *Campylobacteraceae*. The main difference between the genus *Arcobacter* and the *Campylobacter* is their ability to survive and multiply in low oxygen levels at lower temperatures. The name "Arcobacter" to this genus was suggested by Vandamme and others in 1991 initially with the species, namely, *Arcobacter nitrofigilis* and *A. cryaerophilus*. Later, this group was upgraded to four species in 1992 by including *A. butzleri* and *A. skirrowii* isolated from diarrheic fecal material, bulls' preputial fluid, and ovine aborted fetuses (Vandamme et al., 1992). Recently, based on the validated taxonomy, it has been suggested that *Arcobacter* group should be placed separately in the *Arcobacteraceae* family within the genus *Arcobacter*, under *Campylobacterales* order belonging to the class *Campylobacteria* (Waite et al., 2017, 2018). As on date, there are 29 identified species in the genus *Arcobacter* with the number increasing as novel species are being isolated from various sources (Ferreira et al., 2019). Based on 16S rRNA similarity, the present genus *Arcobacter* can be divided into seven different groups: (1) *Arcobacter*, (2) *Pseudoarcobacter*, (3) *Aliiarcobacter*, (4) *Malacobacter*, (5) *Poseidonibacter*, (6) *Haloarcobacter*, and (7) *Arcomarinus* (Diéguez et al., 2017). Further, the genus *Aliiarcobacter* gen. nov. included the most important eight emerging pathogens such as *Aliiarcobacter cryaerophilus*, *A. butzleri*, *A. skirrowii*, *A. cibarius*, *A. thereius*, *A. trophiarum*, *A. lanthieri*, and *A. faecis* (Pérez-Cataluña et al., 2019a, b).

2 Distribution of *Arcobacter* Species

As there are a wide number of species in the genus *Arcobacter*, the information pertaining to distribution is not available for all the species. Mostly, the data is available only for three species, viz., *A. butzleri*, *A. skirrowii*, and *A. cryaerophilus,* as they are pathogenic to humans. Other than these, *A. cibarius* was found in effluents from pig farm (Chinivasagam et al., 2007); *A. thereius* was isolated from

stool samples of humans (Van den Abeele et al., 2013), and *A. mytili*, *A. nitrofigilis*, *A. defluvii*, *A. ellisii*, *A. molluscorum*, *A. venerupis*, and *A. bivalviorum* were found prevalent in shellfish of coastal environment (Levican et al., 2014). Apart from shellfish samples, *Arcobacter* species were also widely distributed in water samples associated with fecal contamination or sewage pollution. Although the species were widely distributed, the data on prevalence of these species were less studied. Some earlier studies suggested that consumption of raw vegetables was one of the potential reasons for the distribution of this pathogen through food cycle.

3 Transmission of *Arcobacter* Species

Similar to the data on distribution, transmission routes of this emerging bacterial pathogen were also less studied. The routes of transmission were not only limited to be associated through the disease occurrence in human, but also they are transmitted by the intake of contaminated food or water with of *Arcobacter*. Several reports are available on the transmission of *Arcobacter* in the natural environment by means of food- and water-related outbreak aids in strengthening the role of *Arcobacter* dissemination routes (Lappi et al., 2013). Apart from food, water, and disease routes of transmission, *Arcobacter* also transmits by the rearing pet animals such as dogs and cats. Fera et al. (2009) have also suggested the role of pet animals in the transmission of *Arcobacter* species as one of the main routes in the occurrence of disease in humans. There are also reports available on the transmission of the *Arcobacter* through personal contact especially in the schoolchildren where there was severe abdominal cramp noticed in an outbreak related to *Arcobacter* (Vandamme et al., 1992) and also some reports suggested that in neonatals, the source of infection might by due to the contraction in the uterus (On et al., 1995).

4 Pathogenicity and Virulence Capacity of *Arcobacter* Species

Arcobacter species are responsible for several infections in humans as well as domestic-reared animals. In humans they cause infections such as gastric enteritis and bacterial septicemia in the intestine, whereas, in veterinary animals they are responsible for abortions and inflammation of the intestine. Several studies have been carried out to understand the mechanisms for the mode of entry of bacteria and pathogenesis in both in vitro and in vivo cell culture models. Sufficient progress has been achieved which proves that *Arcobacter* can invade the cells, produce the toxins, and can damage the tissues. It also harbors several virulent determinants that are responsible for pathogenicity in cells. The virulent determinants studied mostly were *cadF*, *ciaB*, *cj1349*, *pldA*, *mviN*, *tlyA*, *hecA*, *hecB*, and *irgA*. These nine genes are responsible for causing different infections which are the main drivers of pathogenicity in *Arcobacter* species. Two genes (*cj1349* and *cadF*) encode the outer membrane proteins and are responsible for bacterial adherence through fibronectin

of the cell membrane (Flanagan et al., 2009). *ciaB* gene is mainly involved in the invasion of the host cell (Konkel et al., 1999). *mviN* gene encodes for the protein need for peptidoglycan synthesis (Inoue et al., 2008). *pldA* gene is responsible for encoding the phospholipase A that was required for hemolytic activity on the host cell membrane (Grant et al., 1997). *tlyA* gene encodes for hemolysin activity and to adhere to Caco-2 cell membranes (Salamaszynska-Guz & Klimuszko, 2008). *hecA* gene codes for a protein that belongs to adhesin class and is responsible for adhesion to the host cells (Rojas et al., 2002). Another gene called *hecB* encodes for a hemolysin-related protein and plays an important role in its activation (Miller et al., 2007). *irgA* gene of *Arcobacter* codes for iron-regulated protein that is necessary for attachment in the outer host cell membrane (Mey et al., 2002). All the virulence genes that are responsible for pathogenicity can be detected by using PCR assay.

5 Antimicrobial Susceptibility of *Arcobacter*

Different antibiotics were used for treating Arcobacter infections in humans and reared veterinary animals. At present, limited literature is available on Arcobacter antimicrobial susceptibility studies, mostly limited to three species (*A. butzleri*, *A. skirrowii*, and *A. cryaerophilus*). Assessing the recent trends of antibiotic resistance pattern in *Arcobacter* is improper due to lack of proper disease outbreak surveillance programs, thereby hampering the estimation of the prevalence of this bacterial pathogen on a global scale.

Limited studies are available on antibiotic susceptibility of mainly three pathogenic species of *Arcobacter*, i.e., *A. butzleri*, *A. cryaerophilus*, and *A. skirrowii*, although the other species from this genus may also require susceptibility studies to assess their role in acquiring resistance to different antibiotics. The major hindrances associated with the assessment of antibiotic resistance pattern in *Arcobacter* species are the lack of systematic surveillance programs on occurrence and the wide distribution of this pathogen in all habitats. At present there is no standard protocol available exclusively for *Arcobacter* genus to carry out the antimicrobial susceptibility testing (Fanelli et al., 2019) and also no interpretive criteria for the comparison of inhibition zones with the quality control strains which hampers the evaluation of antibiotic testing (Ferreira et al., 2016). The established breakpoints were not defined properly for *Arcobacter* which resulted in resistance misclassification (Brückner et al., 2020). As a result, many authors are reporting antibiotic susceptibility of *Arcobacter* species against different antibiotics by employing the disc diffusion method of the standard described in Clinical and Laboratory Standards Institute (CLSI) by comparing the data with *Enterobacteriaceae*, *Campylobacter* sp., and *Staphylococcus* spp. (Vandenberg et al., 2006; Son et al., 2007a). The variations observed in the antimicrobial susceptibility patterns were mainly due to the dosage and application of antibiotics in prophylaxis and curing infections in animals or humans (Brückner et al., 2020) and the dearth for standard antimicrobial sensitivity

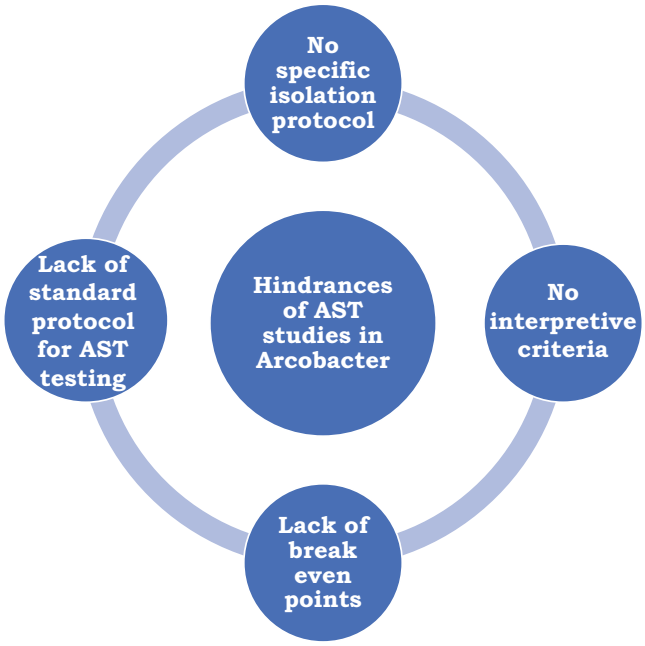


Fig. 1 Hindrances in antimicrobial susceptibility studies (AST) of *Arcobacter* species

procedures and break-even points. In nutshell, lack of standard sensitivity methods and breakpoints creates greater difficulties in the evaluation of antibiotic resistance profiles or the analysis of results of resistance patterns of *Arcobacter* spp. which lead to chaos in classification of sensitivity studies (Fig. 1). The increased prevalence of antibiotic resistance in *Arcobacter* was due to the selective pressure exerted on the bacterial population by the antibiotics along with excessive use by humans for treating gastrointestinal infections. The contamination of food with drug-resistant microorganisms possibly contributes to the dissemination of resistance to other pathogens (Brückner et al., 2020).

Case study reports indicate wide use of antibiotics in treatment of *Arcobacter* infections. However, the choice of drug for bacteremia treatment is administration of cephalosporins singly or in combination with other class of antibiotics (Lau et al., 2002). In the treatment of gastrointestinal illness, the application of quinolones, macrolide, tetracycline, and β-lactam antibiotics has been proposed by Kayman et al. (2012), Fernández et al. (2004), Lerner et al. (1994), and Figueras et al. (2014), respectively. In addition to the above drugs, Vandenberg et al. (2006) have suggested fluoroquinolone treatment for severe *Arcobacter enteritis* since low resistance rate was found for this drug. The mechanism described for antibiotic resistance in *Arcobacter* was mainly acquired through extrachromosomal gene transfer (Miller et al., 2007; Abdelbaqi et al., 2007) with the resistant genes identified in plasmids of bacterial cells (Douidah et al., 2014).

6 Mechanism of Resistance

Bacterial resistance to antibiotics was developed through the transfer of antibiotic resistance genes to bacteria that ultimately result in the change of resistant phenotype. This type of transfer was mediated by the extrachromosomal genetic material present in the bacterial cells which are termed as "plasmids" and other mobile genetic elements such as integrons, transposons, gene cassettes, and insertion sequences. Among them, most commonly seen is through plasmid which are circular extrachromosomal elements (size 1–100 kbp) and self-replicating carrying the resistant genes generation after generation. Plasmids are regarded as an important element of pathogenicity to a particular bacterium carrying the virulent genes, antibiotic-resistant genes, resistance genes to heavy metals, genes encoding for toxicity in the host cells, and genes necessary for attachment and replication in the host cells. Transfer of the particular genes by plasmid-mediated gene transfer to the new bacterium results in the confer of additional features that helps in survival of bacteria in new environs which are acquired through horizontal gene transfer influencing the diversity and evolution of resistant bacterial phenotypes (Slater et al., 2008).

The prevalence of antibiotic-resistant plasmids was reported in very few studies in *Arcobacter* species (Douidah et al., 2014; Toh et al., 2011). In a study by Harrass et al. (1998), the prevalence of plasmids (size ranged from 2 to 5 kbp) was detected in *A. butzleri* isolates (24%) which are isolated from slaughtered broiler hens where the *Arcobacter* isolates did not harbor more than one type of plasmid and showing identical pattern in the restriction analysis. Similarly, Toh et al. (2011) reported the presence of small plasmid harboring replication and hypothetical protein coding genes in *Arcobacter* species isolated from microbial fuel cell. In addition, the presence of plasmid in *A. butzleri* strain NCTC 12481 was sequenced and submitted in the public domain with the reference number NC_012733.1). There are reports that the presence of plasmids in *Arcobacter* species (*A. butzleri*, *A. skirrowii*, and *A. cryaerophilus*) isolated from rearing animals such as pigs, cattle, and chicken but not in the isolates from humans, horse, and sheep. The analysis of sequencing results of plasmids isolated from *A. butzleri* (three isolates) and *A. cryaerophilus* revealed the presence of genes encoding for proteins involved in the replication of the bacterial plasmid and their transfer into new bacterial cells. The presence of these small plasmids in the bacterial species can be considered as good candidates of bacterial vectors needed for genotypic and phenotypic characterization of *Arcobacter* species through the presence of mobilization protein prevalent in the plasmid (Douidah et al., 2014). *A. butzleri* isolated from poultry source also harbored larger plasmid of the size of 27.5 kbp (Douidah et al., 2014), and the sequencing results showed that it harbored genes encoding for type IV secretion system (TSS IV) which mediates the transfer of the plasmid DNA, replication proteins, and toxin secretion. Thus, the presence of plasmids of varied sizes confers their resistance to antibiotics and metals as well as toxicity in the host cells.

Apart from the plasmid-mediated gene transfer of antibiotic-resistant genes, presence of antibiotic-resistant genes on chromosomes was also reported by few

studies (Miller et al., 2007; Douidah et al., 2014). In *Campylobacter* and *Arcobacter*, fluoroquinolone resistance mechanism described was related to the alterations of the gyrA gene or change in the efflux pump for the antibiotics (Iovine, 2013). Sometimes, fluoroquinolone resistance mechanism in *Arcobacter* was related to specific mutations in position 254 of the subunit of DNA gyrase of QRDR (quinolone resistance-determining region) in gyrA gene which results in transition of cytosine to thymine. Abdelbaqi et al. (2007) studied this kind of mutations in *A. butzleri* and *A. cryaerophilus* bacterial strains which are resistant to ciprofloxacin. In addition, Arcobacter strains isolated from poultry birds and poultry slaughter environment from Portugal also showed mutation in gyrA gene. The DNA sequence result suggesting the absence of point mutations in gyrA of *A. butzleri* strain RM4018 points out that the resistance mechanism toward quinolone antibiotics was at uptake level, thereby increasing the impermeability of antibiotic and also through efflux pump (Miller et al., 2007). In case of chloramphenicol resistance, it was suggested that *Arcobacter* isolates harboring cat gene which encodes for chloramphenicol O-acetyltransferase were responsible for the development of resistance (Miller et al., 2007). The mechanism for β-lactam resistance pattern in Arcobacter RM4018 bacterial genome identified was the presence of lrgAB operon which is associated with the production of putative β-lactamases. Similarly, in *A. butzleri* strain RM4018, the resistance toward 5-fluorouracil is due to the lack of *upp* gene that encodes for uracil phosphoribosyl transferase in the drug metabolism pathway. The main reason for the development of antibiotic resistance in *Arcobacter* like other bacterial pathogens is due to the exertion of selection pressure on the bacteria by the application of the drugs at subtherapeutic levels for treatment of infections in veterinary animals, humans, aquaculture, and anthropogenic activities. Contamination of food with drug-resistant bacteria is also responsible for the dissemination of resistant determinants to other pathogenic or commensal bacteria (Rahimi, 2014).

7 Antibiotic Susceptibility Studies in *A. butzleri*

A. butzleri is the most studied species for antimicrobial susceptibility toward various antibiotics among all the pathogenic species. The treatment for the infections caused by this bacterium is comprised of tetracyclines, fluoroquinolone, ciprofloxacin, and aminoglycosides. These antibiotics were found to be more susceptible for *A. butzleri* in general and mostly recommended for curing the gastrointestinal infections caused by the bacterium (Collado et al., 2014; Zacharow et al., 2015; Ferreira et al., 2016; Rathlavath et al., 2017). The highest antibiotic resistance toward tetracycline has been identified in many studies (Yesilmen et al., 2014; Vicente-Martins et al., 2018) which is a major concern. Similarly, for ciprofloxacin, resistance against *A. butzleri* has been reported (Ferreira et al., 2013; Rathlavath et al., 2017; Dekker et al., 2019; Isidro et al., 2020). According to Ferreira et al. (2019) based on pooled estimate analysis, *A. butzleri* was highly resistant to ampicillin, azithromycin, cefalotin, amoxicillin/clavulanic acid (AMC), and levofloxacin, while most sensitive toward gentamicin, erythromycin, enrofloxacin, tetracycline, streptomycin, and

doxycycline. On the contrary, percent resistance for erythromycin, nalidixic acid, and chloramphenicol ranged from 0% to 100% possibly due to variations in the dosage recommended locally for treatment to these antibiotics (Otth et al., 2004) which is seen more frequently in case of farm-reared animals that might later spread to humans (Tang et al., 2017). In some cases, intrinsic antibiotic resistance commonly observed in several Gram-negative bacterial strains toward glycopeptides (vancomycin) has also been studied as in *A. butzleri* (Fanelli et al., 2019) in addition to the resistance against β-lactam group of antibiotics (ampicillin and cefotaxime) (Nicolosi et al., 2010).

In recent investigations, genetic determinants which were responsible for antibiotic resistance have been described in detail by performing a comprehensive study against 49 *A. butzleri* isolates in terms of resistance genes data as exploited with the databases such as CARD (Comprehensive Antibiotic Resistance Database) and ResFinder (Isidro et al., 2020). The findings included three *bla* genes, 1 *cat* gene, 19 efflux pump system genes, and 1 Type 1 secretory system gene with the conclusion that extrusion mechanisms might involve in the development of antimicrobial resistance in *A. butzleri*. Further, it was observed that a truncated *tetR* protein regulates the resistance to erythromycin due to insertion sequence element in efflux pump 16 system in the same study. Abdelbaqi et al. (2007) described the mechanism of quinolone antibiotic resistance in *A. butzleri* due to the Thr-85-Ile substitutions in *GyrA* region. In addition to these studies, Fanelli et al. (2019, 2020) have performed genomic characterization studies of *A. butzleri* strains and found several putative genes which are responsible for antibiotic resistance. To mitigate the antibiotic resistance problem in *A. butzleri,* Sousa et al. (2019) have tried polyphenols as a modulator and found that pinosylvin and resveratrol can act as inhibitors of efflux pump systems. Several studies reported multidrug resistance pattern (MDR) in *A. butzleri* strains which are resistant to at least three or more classes of antimicrobials (Magiorakos et al., 2012; Zacharow et al., 2015; Šilha et al., 2017; Shah et al., 2017; Fanelli et al., 2020). In addition to MDR, resistance to heavy metals also coexisted in *A. butzleri* which may be possibly due to selective pressure exerted both by the antimicrobial compounds and the heavy metals on the bacteria along with anthropogenic activities (Xavier et al., 2019). The mechanism is that the genetic determinants associated with co-resistance are found on the same mobile genetic element and may be transferred through horizontal gene transfer to other bacteria (Baker-Austin et al., 2006) which in turn develops resistance to both heavy metals and antibiotics. The data on the studies conducted on antibiotic resistance pattern in *A. butzleri* from different samples is shown in Table 1.

8 Antibiotic Susceptibility Studies in *A. cryaerophilus*

Prior to 2012, the resistance of *A. cryaerophilus* for ciprofloxacin was rarely seen (Rahimi, 2014). However, some reports suggest that *Arcobacter* bacterial isolates from farm-reared animals, water samples, and different environments showed higher

Table 1 Antibiotic resistance reported in *Arcobacter butzleri* from different sources

S No.	Source of isolation	Resistant to antibiotic	References
1	Meat products from retail shops	Clm, NA, Van	Kabeya et al. (2004)
2	Patient stool samples	Amp, NA, Tet,	Kayman et al. (2012)
3	Poultry slaughter House waste	Amp, Clm, Cip, Van	Ferreira et al. (2013)
4	Seafood (bivalve mollusks)	Amp, Cip, NA,	Collado et al. (2014)
5	Poultry meat samples	Amp, Clm, Cip, NA, Van	Rahimi (2014)
6	Milk and milk products	Amp, Erm, NA, Str, Tet, Van	Yesilmen et al. (2014)
7	Different meat products	Amp, Cef, Cip, Erm, Gen, Tet	Zacharow et al. (2015)
8	Domestic cow and sheep feces	Amp, Clm, Gen, NA, Van	Aski et al. (2016)
9	Patient stool samples	Amp, Cip, Erm, Tet,	Van den Abeele et al. (2016)
10	Dairy unit	Amp, Cef, Cip, NA,	Ferreira et al. (2017)
11	Meat, slaughter house swab, and water samples	Amp, Erm, NA,	Elmali and Can (2017)
12	Vegetables	Cip	González et al. (2017)
13	Seafood and water samples	Amp, Cef, Clm, Cip, Erm, NA, Van	Rathlavath et al. (2017)
14	Cattle meat, milk, and animal-rearing environment	Amp, Cef, Cip, Erm, Gen, Tet,	Shah et al. (2017)
15	Meat, water, and human cases	Amp, Clm, Cip, Erm, Gen, NA, Str, Tet	Šilha et al. (2017)
16	Animal food and humans	Clm, Erm, Gen, Kan, NA, Van	Soma et al. (2017)
17	Ready-to-eat products	Amp, Cef, Clm, Cip, Erm, NA, Tet	Vicente-Martins et al. (2018)
18	Poultry meat (fresh and frozen)	Cip	Dekker et al. (2019)
19	Shellfish	Amp, Cef, Clm, Erm, NA, Tet, Van	Fanelli et al. (2019)
20	Ready-to-consume vegetable products	Amp, Cef, Clm, Erm, Van	Fanelli et al. (2020)
21	Meat, slaughter house, milk, dairy unit, river water, and human cases	Amp, Cef, Cip, Erm, NA	Isidro et al. (2020)

Table 2 Antibiotic resistance reported in *Arcobacter cryaerophilus* from different sources

S No.	Source of isolation	Resistant to antibiotic	References
1	Poultry meat	Erm, NA	Houf et al. (2004)
2	Retail meat products	NA, Met, Cet, SMZ/TMP	Kabeya et al. (2004)
3	Broiler carcasses	Cip, NA, AZT, CLI,	Son et al. (2007a)
4	In-line milk filters	Amp	Serraino et al. (2013)
5	Porcine samples	Erm, NA, Gen, Kan	Scanlon et al. (2013)
6	Domestic geese cloacal swab samples	Amp, Cet,	Unver et al. (2013)
7	Chicken viscera	Erm, Cip, Gen	Villalobos et al. (2013)
8	Poultry meat	Amp, NA, AZT, CLI, Met, Clm	Rahimi (2014)
9	Shellfish	Amp, Cip, NA	Collado et al. (2014)
10	Ready-to-eat products	Amp, Cef, Clm, Tet, Lev, Cip, NA	Vicente-Martins et al. (2018)

resistance toward ciprofloxacin ranging from 12.5% to 55.8% for *A. cryaerophilus* (Ferreira et al., 2013; Villalobos et al., 2013). *A. cryaerophilus* showed higher susceptibility for ciprofloxacin and other fluoroquinolones, namely, norfloxacin, ofloxacin, and enoxacin (Kiehlbauch et al., 1992). Similarly, in antibiotic susceptibility studies, it was found that levofloxacin is the best drug in the class of fluoroquinolones against *A. cryaerophilus* isolated from brackish water samples (Fera et al., 2003). Very low resistance was observed for *A. cryaerophilus* against ampicillin in 78 isolates from humans and farmed animals (Kiehlbauch et al., 1992). Multiple antibiotic resistance (MAR) in *A. cryaerophilus* isolates (71.8%) from broiler carcasses (Son et al., 2007b) and 9.1% of the isolates of *A. cryaerophilus* showing multidrug resistance are also reported (Kabeya et al., 2004). *A. cryaerophilus* from Belgian patients showed higher susceptibility to gentamicin (99%) and tetracycline (89%), respectively, while resistance was noticed higher for erythromycin (78%), doxycycline (76%), and ciprofloxacin (72%) (Van den Abeele et al., 2016). Mutation in *GyrA* of ciprofloxacin-resistant isolates was also seen in the study. Ferreira et al. (2019) conducted pooled analysis (PE) and found that *A. cryaerophilus* was more resistant for ampicillin and AMC. The studies conducted on antibiotic resistance pattern in *A. cryaerophilus* from different samples are shown in Table 2.

9 Antibiotic Susceptibility Studies in *A. skirrowii*

As on date very few studies on antimicrobial susceptibility are available on *A. skirrowii* when compared to *A. butzleri* and *A. cryaerophilus*. *A. skirrowii* was most susceptible to several antibiotics studied (Houf et al., 2001). Similarly, Kabeya et al. (2004) have reported that 13.3% isolates of *A. skirrowii* were multidrug resistant. In another study, all the *A. skirrowii* isolates were seen more susceptible

Table 3 Antibiotic resistance reported in *Arcobacter skirrowii* from different sources

S No.	Source of isolation	Resistant to antibiotic	References
1	Retail meat products	NA, Met, Cet, SMZ/TMP, Clm	Kabeya et al. (2004)
2	Domestic geese cloacal swab samples	Amp, Clm	Unver et al. (2013)
3	Poultry meat	Amp, NA, Met, Cet	Rahimi (2014)
4	Shellfish	Amp, NA	Collado et al. (2014)
5	Poultry fecal samples	Str	Hänel et al. (2018)
6	Ready-to-eat products	Cef, Tet, NA	Vicente-Martins et al. (2018)

to amoxicillin/clavulanic acid and resistant to cloxacillin, optochin, vancomycin, cefazolin, and fusidic acid, while intermediate resistance was observed to oxytetracycline, amikacin, ofloxacin, enrofloxacin, ampicillin, chloramphenicol, erythromycin, nitrofurantoin, and amoxicillin (Unver et al., 2013). Based on the pooled estimate analysis, *A. skirrowii* was found to be highly susceptible toward penicillins and cephalosporins, resistant to tetracyclines and macrolides (Ferreira et al., 2019). Table 3 depicts the studies conducted on antibiotic resistance pattern in *A. cryaerophilus* from different samples.

10 Survival of *Arcobacter* in Food and Water

Several reports are available on the ability of *Arcobacter* to survive in food production systems including animals. D'Sa and Harrison (2005) demonstrated *A. butzleri* can survive in ground pork in a strain-dependent way without increase in cell density. Similarly, the ability of three *Arcobacter* species (*A. butzleri, A. skirrowii*, and *A. cryaerophilus*) to survive during scalding processing at 52 °C for 3 min may led to cross contamination in processing of poultry birds (Ho et al., 2008). Also the capability of survival of *Arcobacter* in processed milk as well as raw milk was also reported in a study especially in the milk stored at 4 °C, 10 °C, and even at 20 °C. But in case of raw milk kept at 20 °C, there were significant low numbers of *A. butzleri* and *A. cryaerophilus* recorded because of rapid division of competitive microflora. Apart from this, factors such as lack of proper facilities for storing, unhygienic post-processing conditions, and contamination from humans are also responsible for the survival of *Arcobacter*, and it was associated with several human health implications due to consumption of milk (Giacometti et al., 2014). Prevalence of *Arcobacter* has also been reported from several conventional and industrial dairy units in Italy from the surfaces which are in contact, processed milk products and in raw milk (Serraino & Giacometti, 2014). Survival of *A. butzleri* in buffalo mozzarella cheese samples collected from different points during processing has been reported by Serraino et al. (2013). *A. butzleri* was able to survive in freshly prepared fruit purees, but upon

storage the bacterial count significantly decreased due to more sugar content, acidic pH, low water activity, and presence of polyphenols (Lee et al., 2012). Survival of *A. butzleri* has in seawater in viable condition but non-culturable state for 270 days has been reported by Fera et al. (2008). These bacterial cells although lost its culturable state but are able to survive because of starvation until the attainment of favorable conditions. Presence of *Arcobacter* in water samples collected from river and drinking water was reported by Collado et al. (2010), but in case of chlorinated water, *Arcobacter* was not detected as it is sensitive and also lost its membrane integrity (Moreno et al., 2004). This suggests that chlorination of drinking water need to be carried out at regular intervals to contain the spread of *Arcobacter*. Presence of *Arcobacter* in drinking water samples and its ability to survive at low temperatures and in environs with low nutrients suggested that *Arcobacter* is a potential waterborne pathogen.

11 Control Measures

Resistance of *Arcobacter* toward commonly used coupled with its ability to survive and multiply under different conditions makes it a potential emerging pathogen for humans (D'Sa & Harrison, 2005). Implementation of strict sanitation and hygienic practices, following proper disinfection protocols, and reinforcement of decontamination techniques are the main prerequisites for minimizing both *Arcobacter* infections and dissemination of resistance determinants. Antibiotic resistance can be minimized by judicious use of drugs which are recommended for the treatment of *Arcobacter* infection. The frequency and required dose should be administered to the patients who are under chemotherapy. Research has been carried on the use of several natural compounds with antimicrobial activity for controlling *Arcobacter*. Cervenka et al. (2006) reported the application of extracts of cinnamon, chamomile, bearberry, and rosemary has been successfully used for treatment of gastrointestinal enteritis caused by *Arcobacter* species. Ferreira et al. (2014) found that "resveratrol" a phytoalexin which is synthesized in plants can act as an inhibitor of efflux pump against the *Arcobacter* species that results in death of bacterial cells. Knowledge on antimicrobial resistance mechanisms, adaptive mechanism to specific stress, and immune evasion mechanisms should be considered before developing mitigation measures for infections with *Arcobacter*. Control measures should also focus on implementation of proper surveillance programs to enumerate the prevalence of *Arcobacter* species at national and international levels.

12 Conclusion

In conclusion, *Arcobacter* species show higher resistance to commonly used antibiotics as well as multidrug resistance. As there is a wide diversity of *Arcobacter* species, antibiotic susceptibility tests should be carried out for other emerging species other than *A. butzleri*, *A. cryaerophilus*, and *A. skirrowii* for better

understanding of resistance mechanisms. The lack of standardized methods for screening antibiotic susceptibility and non-description of specific break-even points for this bacterium hamper the determination of the resistance pattern. The resistance is attributed to the capability of *Arcobacter* to persist in food processing and storage conditions, as well as the wide distribution in food chain and natural environment. In the era of developing and disseminating the antimicrobial resistance toward different commonly employed antibiotics, there is a great demand for discovering alternate and novel treatment strategies for controlling infections with *Arcobacter* in an improved manner, as adopted for other food-borne pathogens. In addition, there is a need for deeper understanding of the mechanisms involved in the development of drug resistance to new category of antibiotics to formulate mitigation strategies.

References

Abdelbaqi, K., Menard, A., Prouzet-Mauleon, V., Bringaud, F., Lehours, P., & Megraud, F. (2007). Nucleotide sequence of the gyrA gene of Arcobacter species and characterization of human ciprofloxacin-resistant clinical isolates. *FEMS Immunology and Medical Microbiology, 49*(3), 337–345. https://doi.org/10.1111/j.1574-695X.2006.00208.x

Aski, H. S., Tabatabaei, M., Khoshbakht, R., & Raeisi, M. (2016). Occurrence and antimicrobial resistance of emergent *Arcobacter* spp. isolated from cattle and sheep in Iran. *Comparative Immunology, Microbiology and Infectious Diseases, 44*, 37–40. https://doi.org/10.1016/j.cimid.2015.12.002

Baker-Austin, C., Wright, M. S., Stepanauskas, R., & McArthur, J. V. (2006). Co-selection of antibiotic and metal resistance. *Trends in Microbiology, 14*(4), 176–182. https://doi.org/10.1016/j.tim.2006.02.006

Brückner, V., Fiebiger, U., Ignatius, R., Friesen, J., Eisenblätter, M., Höck, M., Alter, T., Bereswill, S., Gölz, G., & Heimesaat, M. M. (2020). Prevalence and antimicrobial susceptibility of Arcobacter species in human stool samples derived from out-and inpatients: The prospective German Arcobacter prevalence study Arcopath. *Gut Pathogens, 12*, 1–8.

Cervenka, L., Peskova, I., Foltynova, E., Pejchalova, M., Brozkova, I., & Vytrasova, J. (2006). Inhibitory effects of some spice and herb extracts against *Arcobacter butzleri*, *A. cryaerophilus*, and *A. skirrowii*. *Current Microbiology, 53*, 435–439.

Chinivasagam, H. N., Corney, B. G., Wright, L. L., Diallo, I. S., & Blackall, P. J. (2007). Detection of Arcobacter spp. in piggery effluent and effluent-irrigated soils in Southeast Queensland. *Journal of Applied Microbiology, 103*(2), 418–426.

Collado, L., Kasimir, G., Perez, U., Bosch, A., Pinto, R., Saucedo, G., Huguet, J. M., & Figueras, M. J. (2010). Occurrence and diversity of *Arcobacter* spp. along the Llobregat River catchment, at sewage effluents and in a drinking water treatment plant. *Water Research, 44*(12), 3696–3702.

Collado, L., Jara, R., Vásquez, N., & Telsaint, C. (2014). Antimicrobial resistance and virulence genes of Arcobacter isolates recovered from edible bivalve molluscs. *Food Control, 46*, 508–512.

D'Sa, E. M., & Harrison, M. A. (2005). Effect of pH, NaCl content, and temperature on growth and survival of *Arcobacter* spp. *Journal of Food Protection, 68*, 18–25.

Dekker, D., Eibach, D., Boahen, K. G., Akenten, C. W., Pfeifer, Y., Zautner, A. E., Mertens, E., Krumkamp, R., Jaeger, A., Flieger, A., & Owusu-Dabo, E. (2019). Fluoroquinolone-resistant *Salmonella enterica*, *Campylobacter* spp., and *Arcobacter butzleri* from local and imported poultry meat in Kumasi, Ghana. *Foodborne Pathogens and Disease, 16*(5), 352–358. https://doi.org/10.1089/fpd.2018.2562

Diéguez, A. L., Balboa, S., Magnesen, T., & Romalde, J. L. (2017). *Arcobacter lekithochrous* sp. nov., isolated from a molluscan hatchery. *International Journal of Systematic and Evolutionary Microbiology, 67*(5), 1327–1332. https://doi.org/10.1099/ijsem.0.001809

Douidah, L., De Zutter, L., Van Nieuwerburgh, F., Deforce, D., Ingmer, H., Vandenberg, O., Van den Abeele, A. M., & Houf, K. (2014). Presence and analysis of plasmids in human and animal associated Arcobacter species. *PLoS One, 9*(1), e85487.

Elmali, M., & Can, H. Y. (2017). Occurrence and antimicrobial resistance of Arcobacter species in food and slaughterhouse samples. *Food Science and Technology, 37*(2), 280–285. https://doi.org/10.1590/1678-457x.19516

Fanelli, F., Di Pinto, A., Mottola, A., Mule, G., Chieffi, D., Baruzzi, F., Tantillo, G., & Fusco, V. (2019). Genomic characterization of *Arcobacter butzleri* isolated from shellfish: Novel insight into antibiotic resistance and virulence determinants. *Frontiers in Microbiology, 10*, 670. https://doi.org/10.3389/fmicb.2019.00670

Fanelli, F., Chieffi, D., Di Pinto, A., Mottola, A., Baruzzi, F., & Fusco, V. (2020). Phenotype and genomic background of *Arcobacter butzleri* strains and taxogenomic assessment of the species. *Food Microbiology, 89*, 103416. https://doi.org/10.1016/j.fm.2020.103416

Fera, M. T., Maugeri, T. L., Giannone, M., Gugliandolo, C., La Camera, E., Blandino, G., & Carbone, M. (2003). *In vitro* susceptibility of *Arcobacter butzleri* and *Arcobacter cryaerophilus* to different antimicrobial agents. *International Journal of Antimicrobial Agents, 21*(5), 488–491.

Fera, M. T., Maugeri, T. L., Gugliandolo, C., La Camera, E., Lentini, V., Favaloro, A., Bonanno, D., & Carbone, M. (2008). Induction and resuscitation of viable nonculturable *Arcobacter butzleri* cells. *Applied and Environmental Microbiology, 74*(10), 3266–3268.

Fera, M. T., La Camera, E., Carbone, M., Malara, D., & Pennisi, M. G. (2009). Pet cats as carriers of *Arcobacter* spp. in southern Italy. *Journal of Applied Microbiology, 106*(5), 1661–1666.

Fernández, H., Krause, S., & Paz Villanueva, M. (2004). *Arcobacter butzleri* an emerging enteropathogen: Communication of two cases with chronic diarrhea. *Brazilian Journal of Microbiology, 35*(3), 216–218.

Ferreira, S., Fraqueza, M. J., Queiroz, J. A., Domingues, F. C., & Oleastro, M. (2013). Genetic diversity, antibiotic resistance and biofilm-forming ability of *Arcobacter butzleri* isolated from poultry and environment from a Portuguese slaughterhouse. *International Journal of Food Microbiology, 162*(1), 82–88. https://doi.org/10.1016/j.ijfoodmicro.2013.01.003

Ferreira, S., Silva, F., Queiroz, J. A., Oleastro, M., & Domingues, F. C. (2014). Resveratrol against *Arcobacter butzleri* and *Arcobacter cryaerophilus*: Activity and effect on cellular functions. *International Journal of Food Microbiology, 180*, 62–68.

Ferreira, S., Queiroz, J. A., Oleastro, M., & Domingues, F. C. (2016). Insights in the pathogenesis and resistance of Arcobacter: A review. *Critical Reviews in Microbiology, 42*(3), 364–383. https://doi.org/10.3109/1040841X.2014.954523

Ferreira, S., Oleastro, M., & Domingues, F. C. (2017). Occurrence, genetic diversity and antibiotic resistance of *Arcobacter* sp. in a dairy plant. *Journal of Applied Microbiology, 123*(4), 1019–1026. https://doi.org/10.1111/jam.13538

Ferreira, S., Luis, A., Oleastro, M., Pereira, L., & Domingues, F. C. (2019). A meta-analytic perspective on *Arcobacter* spp. antibiotic resistance. *Journal of Global Antimicrobial Resistance, 16*, 130–139.

Figueras, M. J., Levican, A., Pujol, I., Ballester, F., Quilez, M. R., & Gomez-Bertomeu, F. (2014). A severe case of persistent diarrhoea associated with *Arcobacter cryaerophilus* but attributed to *campylobacter* sp. and a review of the clinical incidence of *Arcobacter* spp. *New Microbes and New Infections, 2*(2), 31–37.

Flanagan, R. C., Neal-McKinney, J. M., Dhillon, A. S., Miller, W. G., & Konkel, M. E. (2009). Examination of Campylobacter jejuni putative adhesins leads to the identification of a new protein, designated FlpA, required for chicken colonization. *Infection and Immunity, 77*, 2399–2407.

Giacometti, F., Serraino, A., Pasquali, F., De Cesare, A., Bonerba, E., & Rosmini, R. (2014). Behavior of *Arcobacter butzleri* and *Arcobacter cryaerophilus* in ultrahigh-temperature, pasteurized, and raw cow's milk under different temperature conditions. *Foodborne Pathogens and Disease, 11*(1), 15–20.

González, A., Morejón, I. F. B., & Ferrús, M. A. (2017). Isolation, molecular identification and quinolone-susceptibility testing of *Arcobacter* spp. isolated from fresh vegetables in Spain. *Food Microbiology, 65*, 279–283. https://doi.org/10.1016/j.fm.2017.02.011

Grant, K. A., Belandia, I. U., Dekker, N., Richardson, P. T., & Park, S. F. (1997). Molecular characterization of pldA, the structural gene for a phospholipase A from *Campylobacter coli*, and its contribution to cell-associated hemolysis. *Infection and Immunity, 65*, 1172–1180.

Hänel, I., Hotzel, H., Tomaso, H., & Busch, A. (2018). Antimicrobial susceptibility and genomic structure of *Arcobacter skirrowii* isolates. *Frontiers in Microbiology, 9*, 3067.

Harrass, B., Schwarz, S., & Wenzel, S. (1998). Identification and characterization of Arcobacter isolates from broilers by biochemical tests, antimicrobial resistance patterns and plasmid analysis. *Journal of Veterinary Medicine, Series B, 45*(1–10), 87–94.

Ho, H. T. K., Lipman, L. J. A., & Gaastra, W. (2008). The introduction of *Arcobacter* spp. in poultry slaughterhouses. *International Journal of Food Microbiology, 125*, 223–229.

Houf, K., Devriese, L. A., Van Hoof, J., & Vandamme, P. (2001). Susceptibility of *Arcobacter butzleri, Arcobacter cryaerophilus*, and *Arcobacter skirrowii* to antimicrobial agents used in selective media. *Journal of Clinical Microbiology, 39*(4), 1654–1656. https://doi.org/10.1128/JCM.39.4.1654-1656.2001

Houf, K., Devriese, L. A., Haesebrouck, F., Vandenberg, O., Butzler, J. P., Hoof, J. V., & Vandamme, P. (2004). Antimicrobial susceptibility patterns of *Arcobacter butzleri* and *Arcobacter cryaerophilus* strains isolated from humans and broilers. *Microbial Drug Resistance, 10*(3), 243–247.

Inoue, A., Murata, Y., Takahashi, H., Tsuji, N., Fujisaki, S., & Kato, J. (2008). Involvement of an essential gene, *mviN*, in Murein synthesis in *Escherichia coli*. *Journal of Bacteriology, 190*, 7298–7301.

Iovine, N. M. (2013). Resistance mechanisms in *Campylobacter jejuni*. *Virulence, 4*, 230–240.

Isidro, J., Ferreira, S., Pinto, M., Domingues, F., Oleastro, M., Gomes, J. P., & Borges, V. (2020). Virulence and antibiotic resistance plasticity of *Arcobacter butzleri*: Insights on the genomic diversity of an emerging human pathogen. *Infection, Genetics and Evolution, 80*, 104213. https://doi.org/10.1016/j.meegid.2020.104213

Kabeya, H., Maruyama, S., Morita, Y., Ohsuga, T., Ozawa, S., Kobayashi, Y., Abe, M., Katsube, Y., & Mikami, T. (2004). Prevalence of Arcobacter species in retail meats and antimicrobial susceptibility of the isolates in Japan. *International Journal of Food Microbiology, 90*(3), 303–308. https://doi.org/10.1016/S0168-1605(03)00322-2

Kayman, T., Atabay, H. I., & Abay, S. (2012). Human acute gastroenteritis associated with *Arcobacter butzleri*. *Clinical Microbiology Newsletter, 34*, 197–199.

Kiehlbauch, J. A., Baker, C. N., & Wachsmuth, I. K. (1992). *In vitro* susceptibilities of aerotolerant Campylobacter isolates to 22 antimicrobial agents. *Antimicrobial Agents and Chemotherapy, 36*(4), 717–722.

Konkel, M. E., Kim, B. J., Rivera-Amill, V., & Garvis, S. G. (1999). Bacterial secreted proteins are required for the internalization of *Campylobacter jejuni* into cultured mammalian cells. *Molecular Microbiology, 32*, 691–701.

Lappi, V., Archer, J. R., Cebelinski, E., Leano, F., Besser, J. M., Klos, R. F., Medus, C., Smith, K. E., Fitzgerald, C., & Davis, J. P. (2013). An outbreak of foodborne illness among attendees of a wedding reception in Wisconsin likely caused by *Arcobacter butzleri*. *Foodborne Pathogens and Disease, 10*(3), 250–255.

Lau, S. K. P., Woo, P. C. Y., Teng, J. L. L., Leung, K. W., & Yuen, K. Y. (2002). Identification by 16S ribosomal RNA gene sequencing of *Arcobacter butzleri* bacteraemia in a patient with acute gangrenous appendicitis. *Molecular Pathology, 55*(3), 182.

Lee, C., Agidi, S., Marion, J. W., & Lee, J. (2012). Arcobacter in Lake Erie beach waters: An emerging gastrointestinal pathogen linked with human-associated fecal contamination. *Applied and Environmental Microbiology, 78*, 5511–5519.

Lerner, J., Brumberger, V., & Preac-Mursic, V. (1994). Severe diarrhea associated with *Arcobacter butzleri*. *European Journal of Clinical Microbiology and Infectious Diseases, 13*(8), 660–662.

Levican, A., Collado, L., Yustes, C., Aguilar, C., & Figueras, M. J. (2014). Higher water temperature and incubation under aerobic and microaerobic conditions increase the recovery and diversity of *Arcobacter* spp. from shellfish. *Applied and Environmental Microbiology, 80*(1), 385–391.

Magiorakos, A. P., Srinivasan, A., Carey, R. T., Carmeli, Y., Falagas, M. T., Giske, C. T., Harbarth, S., Hindler, J. T., Kahlmeter, G., Olsson-Liljequist, B., & Paterson, D. T. (2012). Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: An international expert proposal for interim standard definitions for acquired resistance. *Clinical Microbiology and Infection, 18*(3), 268–281. https://doi.org/10.1111/j.1469-0691.2011.03570.x

Mey, A. R., Wyckoff, E. E., Oglesby, A. G., Rab, E., Taylor, R. K., & Payne, S. M. (2002). Identification of the *Vibrio cholerae* enterobactin receptors VctA and IrgA: IrgA is not required for virulence. *Infection and Immunity, 70*, 3419–3426.

Miller, W. G., Parker, C. T., Rubenfield, M., Mendz, G. L., Wösten, M. M., Ussery, D. W., Stolz, J. F., Binnewies, T. T., Hallin, P. F., Wang, G., & Malek, J. A. (2007). The complete genome sequence and analysis of the epsilon proteobacterium *Arcobacter butzleri*. *PLoS One, 2*(12), e1358.

Moreno, Y., Alonso, J. L., Botella, S., Ferrús, M. A., & Hernández, J. (2004). Survival and injury of *Arcobacter* after artificial inoculation into drinking water. *Research in Microbiology, 155*(9), 726–730.

Nicolosi, D., Scalia, M., Nicolosi, V. M., & Pignatello, R. (2010). Encapsulation in fusogenic liposomes broadens the spectrum of action of vancomycin against Gram-negative bacteria. *International Journal of Antimicrobial Agents, 35*(6), 553–558. https://doi.org/10.1016/j.ijantimicag.2010.01.015

On, S. L. W., Stacey, A., & Smyth, J. (1995). Isolation of Arcobacter-Butzleri from a neonate with bacteremia. *Journal of Infection, 31*, 225–227.

Otth, L., Wilson, M., Cancino, R., & Fernández, H. (2004). *In vitro* susceptibility of *Arcobacter butzleri* to six antimicrobial drugs. *Archivos de Medicina Veterinaria, 36*(2), 207–210. https://doi.org/10.4067/S0301-732X2004000200012

Pérez-Cataluña, A., Salas-Massó, N., Diéguez, A. L., Balboa, S., Lema, A., Romalde, J. L., & Figueras, M. J. (2019a). Erratum: Corrigendum: Revisiting the taxonomy of the genus Arcobacter: Getting order from the chaos. *Frontiers in Microbiology, 10*, 121. https://doi.org/10.3389/fmicb.2019.00121

Pérez-Cataluña, A., Salas-Massó, N., Diéguez, A. L., Balboa, S., Lema, A., Romalde, J. L., & Figueras, M. J. (2019b). Corrigendum (2): Revisiting the taxonomy of the genus Arcobacter: Getting order from the chaos. *Frontiers in Microbiology, 10*, 2253. https://doi.org/10.3389/fmicb.2019.02253

Rahimi, E. (2014). Prevalence and antimicrobial resistance of Arcobacter species isolated from poultry meat in Iran. *British Poultry Science, 55*(2), 174–180. https://doi.org/10.1080/00071668.2013.878787

Rathlavath, S., Kohli, V., Singh, A. S., Lekshmi, M., Tripathi, G., Kumar, S., & Nayak, B. B. (2017). Virulence genotypes and antimicrobial susceptibility patterns of *Arcobacter butzleri* isolated from seafood and its environment. *International Journal of Food Microbiology, 263*, 32–37. https://doi.org/10.1016/j.ijfoodmicro.2017.10.005

Rojas, C. M., Ham, J. H., Deng, W. L., Doyle, J. J., & Collmer, A. (2002). *HecA*, a member of a class of adhesins produced by diverse pathogenic bacteria, contributes to the attachment, aggregation, epidermal cell killing, and virulence phenotypes of *Erwinia chrysanthemi* EC16 on *Nicotiana clevelandii* seedlings. *Proceedings of the National Academy of Sciences of the United States of America, 99*, 13142–13147.

Salamaszynska-Guz, A., & Klimuszko, D. (2008). Functional analysis of the *Campylobacter jejuni* cj0183 and cj0588 genes. *Current Microbiology, 56*, 592–596.

Scanlon, K. A., Cagney, C., Walsh, D., McNulty, D., Carroll, A., McNamara, E. B., McDowell, D. A., & Duffy, G. (2013). Occurrence and characteristics of fastidious Campylobacteraceae species in porcine samples. *International Journal of Food Microbiology, 163*(1), 6–13.

Serraino, A., & Giacometti, F. (2014). Short communication: Occurrence of Arcobacter species in industrial dairy plants. *Journal of Dairy Science, 97*, 2061–2065.

Serraino, A., Florio, D., Giacometti, F., Piva, S., Mion, D., & Zanoni, R. G. (2013). Presence of Campylobacter and Arcobacter species in in-line milk filters of farms authorized to produce and sell raw milk and of a water buffalo dairy farm in Italy. *Journal of Dairy Science, 96*(5), 2801–2807.

Shah, A. H., Barham, G. S., Khaskheli, G. B., Jamali, M. A., Korejo, N. A., Jalbani, Y. M., & Tunio, S. G. (2017). Minimum inhibitory concentration evaluator and disc diffusion testing techniques to profile antimicrobial resistance in *Arcobacter butzleri*. *Sindh University Research Journal-SURJ (Science Series), 49*(3), 649–654.

Šilha, D., Pejchalová, M., & Šilhová, L. (2017). Susceptibility to 18 drugs and multidrug resistance of Arcobacter isolates from different sources within the Czech Republic. *Journal of Global Antimicrobial Resistance, 9*, 74–77. https://doi.org/10.1016/j.jgar.2017.01.006

Slater, F. R., Bailey, M. J., Tett, A. J., & Turner, S. L. (2008). Progress towards understanding the fate of plasmids in bacterial communities. *FEMS Microbiology Ecology, 66*(1), 3–13.

Soma, S. M., Srinivasa, R. T., Bindu, K. C., Subramanyam, K. V., & Mohammad, S. N. (2017). Antibiogram of Arcobacter species isolated from animals, foods of animal origin and humans in Andhra Pradesh, India. *International Journal of Science, Environment and Technology, 6*(2), 1260–1269.

Son, I., Englen, M. D., Berrang, M. E., Fedorka-Cray, P. J., & Harrison, M. A. (2007a). Antimicrobial resistance of Arcobacter and Campylobacter from broiler carcasses. *International Journal of Antimicrobial Agents, 29*(4), 451–455.

Son, I., Englen, M. D., Berrang, M. E., Fedorka-Cray, P. J., & Harrison, M. A. (2007b). Prevalence of Arcobacter and Campylobacter on broiler carcasses during processing. *International Journal of Food Microbiology, 113*(1), 16–22.

Sousa, V., Luís, Â., Oleastro, M., Domingues, F., & Ferreira, S. (2019). Polyphenols as resistance modulators in *Arcobacter butzleri*. *Folia Microbiologica, 64*(4), 547–554. https://doi.org/10.1007/s12223-019-00678-3

Tang, K. L., Caffrey, N. P., Nóbrega, D. B., Cork, S. C., Ronksley, P. E., Barkema, H. W., & Ghali, W. A. (2017). Restricting the use of antibiotics in food-producing animals and its associations with antibiotic resistance in food-producing animals and human beings: A systematic review and meta-analysis. *The Lancet Planetary Health, 1*(8), e316–e327. https://doi.org/10.1016/S2542-5196(17)30141-9

Toh, H., Sharma, V. K., Oshima, K., Kondo, S., Hattori, M., Ward, F. B., Free, A., & Taylor, T. D. (2011). Complete genome sequences of *Arcobacter butzleri* ED-1 and *Arcobacter* sp. strain L, both isolated from a microbial fuel cell. *Journal of Bacteriology, 193*(22), 6411–6412.

Unver, A., Atabay, H. I., Sahin, M., & Celebi, O. (2013). Antimicrobial susceptibilities of various Arcobacter species. *Turkish Journal of Medical Sciences, 43*(4), 548–552.

Van den Abeele, A. M., Vogelaers, D., & Houf, K. (2013) *Arcobacter thereius* isolations from human stool: Another Arcobacter queuing for a role as zoonotic pathogen? In *17th international workshop on campylobacter, helicobacter and related organisms*. Aberdeen, Scotland, UK.

Van den Abeele, A. M., Vogelaers, D., Vanlaere, E., & Houf, K. (2016). Antimicrobial susceptibility testing of *Arcobacter butzleri* and *Arcobacter cryaerophilus* strains isolated from Belgian patients. *Journal of Antimicrobial Chemotherapy, 71*(5), 1241–1244. https://doi.org/10.1093/jac/dkv483

Vandamme, P., Pugina, P., Benzi, G., Van Etterijck, R., Vlaes, L., Kersters, K., Butzler, J. P., Lior, H., & Lauwers, S. (1992). Outbreak of recurrent abdominal cramps associated with *Arcobacter butzleri* in an Italian school. *Journal of Clinical Microbiology, 30*(9), 2335–2337.

Vandenberg, O., Houf, K., Douat, N., Vlaes, L., Retore, P., Butzler, J. P., & Dediste, A. (2006). Antimicrobial susceptibility of clinical isolates of non-jejuni/coli campylobacters and arcobacters from Belgium. *Journal of Antimicrobial Chemotherapy, 57*(5), 908–913. https://doi.org/10.1093/jac/dkl080

Vicente-Martins, S., Oleastro, M., Domingues, F. C., & Ferreira, S. (2018). *Arcobacter* spp. at retail food from Portugal: Prevalence, genotyping and antibiotics resistance. *Food Control, 85*, 107–112. https://doi.org/10.1016/j.foodcont.2017.09.024

Villalobos, E. G., Jaramillo, H. F., Ulate, C. C., & Echandi, M. L. A. (2013). Isolation and identification of zoonotic species of genus Arcobacter from chicken viscera obtained from retail distributors of the metropolitan area of San José, Costa Rica. *Journal of Food Protection, 76*(5), 879–882.

Waite, D. W., Vanwonterghem, I., Rinke, C., Parks, D. H., Zhang, Y., Takai, K., Sievert, S. M., Simon, J., Campbell, B. J., Hanson, T. E., & Woyke, T. (2017). Comparative genomic analysis of the class Epsilonproteobacteria and proposed reclassification to Epsilonbacteraeota (phyl. nov.). *Frontiers in Microbiology, 8*, 682. https://doi.org/10.3389/fmicb.2017.00682

Waite, D. W., Vanwonterghem, I., Rinke, C., Parks, D. H., Zhang, Y., Takai, K., & Hugenholtz, P. (2018). Addendum: Comparative genomic analysis of the class Epsilonproteobacteria and proposed reclassification to Epsilonbacteraeota (phyl. nov.). *Frontiers in Microbiology, 9*, 772. https://doi.org/10.3389/fmicb.2018.00772

Xavier, J. C., Costa, P. E. S., Hissa, D. C., Melo, V. M. M., Falcão, R. M., Balbino, V. Q., Mendonça, L. A. R., Lima, M. G. S., Coutinho, H. D. M., & Verde, L. C. L. (2019). Evaluation of the microbial diversity and heavy metal resistance genes of a microbial community on contaminated environment. *Applied Geochemistry, 105*, 1–6. https://doi.org/10.1016/j.apgeochem.2019.04.012

Yesilmen, S., Vural, A., Erkan, M. E., & Yildirim, I. H. (2014). Prevalence and antimicrobial susceptibility of Arcobacter species in cow milk, water buffalo milk and fresh village cheese. *International Journal of Food Microbiology, 188*, 11–14. https://doi.org/10.1016/j.ijfoodmicro.2014.07.006

Zacharow, I., Bystroń, J., Wałecka-Zacharska, E., Podkowik, M., & Bania, J. (2015). Prevalence and antimicrobial resistance of *Arcobacter butzleri* and *Arcobacter cryaerophilus* isolates from retail meat in lower Silesia region, Poland. *Polish Journal of Veterinary Sciences, 18*(1), 63–69.

Multidrug Resistance in *Salmonella* Serotypes Across the Globe: Alarming Rate of Spread

S. S. Greeshma, Devika Pillai, and Toms C. Joseph

Contents

Abstract

The presence of foodborne pathogens in food items and their related supply chain is a serious matter of public health concern worldwide. *Salmonella*, a member of Enterobacteriaceae family with more than 2500 serotypes, is considered as zero-tolerant organism in food safety aspect. Mere presence of *Salmonella* in food items results in import-export rejection; and consumption of food items contaminated with *Salmonella* causes mild to severe typhoidal infections and salmonellosis in children, older population, and immunocompromised adults all over the world. The spread of antimicrobial resistance among *Salmonella* serotypes across

S. S. Greeshma (✉) · T. C. Joseph
Microbiology Fermentation and Biotechnology Division, ICAR-Central Institute of Fisheries Technology (ICAR-CIFT), Kochi, Kerala, India
e-mail: Greeshma.SS@icar.gov.in

D. Pillai
Kerala University of Fisheries and Ocean Studies, Panangad, Kerala, India

M. P. Mothadaka et al. (eds.), *Handbook on Antimicrobial Resistance*,
https://doi.org/10.1007/978-981-19-9279-7_20

the globe aggravates the situation with increased mortality, morbidity, treatment failure, and cost. This chapter overviews the alarming spread of antimicrobial resistance towards clinically important antibiotics among *Salmonella* serotypes from different sectors, namely, healthcare settings, environment, livestock, fresh produce, aquaculture, and seafood etc.

Keywords

Salmonella · Antimicrobial resistance

1 Introduction

The rapid emergence of multidrug resistance in foodborne pathogens such as *Salmonella* in the last few decades is a multifaceted problem that drew global attention. *Salmonella* is a facultative Gram-negative anaerobic bacillus of Enterobacteriaceae family. According to 16S rRNA sequence analysis, the genus *Salmonella* is classified into two species, *Salmonella bongori* and *Salmonella enterica* (type species). Further, based on the biochemical properties and genomic relatedness, *S. enterica* is classified into six subspecies and is denoted with roman numerals: I, *S. enterica subsp. enterica*; II, *S. enterica subsp. salamae*; IIIa, *S. enterica subsp. arizonae*; IIIb, *S. enterica subsp. diarizonae*; IV, *S. enterica subsp. houtenae*; and VI, *S. enterica subsp. indica*. Chattaway et al. (2021) proposed a new form of nomenclature scheme for *Salmonella* based on the data generated by whole genome sequencing and sequence types, and their relation with the serotypes. *S. enterica subsp. enterica* is found predominant among the other *Salmonella* subspecies and accounted for 99% of salmonellosis in warm-blooded animals and humans. But, the other five *Salmonella* subspecies along with *S. bongori* were found mainly in cold-blooded animals and in the environment, and are rarely reported from human beings. Clinical symptoms of salmonellosis range from systemic illness that can lead to fatality to mild fever with gastroenteritis (abdominal cramps, diarrhea, and fever), enteric fevers (including typhoid fever), focal infections, septicemia, and an asymptomatic carrier state. Globally, it was estimated nearly 22 million cases of illness and 200,000 deaths were due to enteric fever by typhoidal strains, predominantly from underdeveloped countries. CDC reports that 93.8 million cases of non-typhoidal *Salmonella* (NTS) (strains other than S. Typhi and S. Paratyphi) infection annually are associated with a high death toll as 150,000 (Dong et al., 2020). Severity of salmonellosis varies depending on the serotype involved, namely, typhoidal strains such as *S.* Typhi, *S*. Paratyphi A and B produce enteric fever; *S. choleraesuis* causes focal infections or septicemia; NTS serovars *S.* Typhimurium and *S.* Enteritidis cause self-limiting gastroenteritis, etc. Mostly infants, older adults, and immunocompromised individuals are susceptible to salmonellosis. Typhoidal strains generally lack a significant animal reservoir in the transmission cycle and spreads from person to person through water contaminated with human excreta. But, NTS has several animal reservoirs and causes zoonotic infections transmitted through contaminated food. Recently, invasive infections with NTS acquired from reservoir food

animals are considered more serious than with typhoidal and paratyphoidal serotypes (Ke et al., 2020). Among NTS infections, a wide host range of S. Typhimurium makes them the "common serotype" in livestock and associated food commodities and accounts for 60% of human salmonellosis (Simpson et al., 2018).

Salmonella is considered as zero-tolerant organism from food safety point of view. However, epidemiologic trace back studies from 2006 to 2018 frequently identified food items such as cereals, cantaloupes, vegetables, pistachios, fruit/fruit pulp, dried/shredded coconut, tomatoes, fresh produce, alfalfa sprouts, ground beef, turkeys, chicken meat, pork, seafood, and other related processed items as vehicles of *Salmonella.* Introduction of antibiotics-based therapy against *Salmonella* greatly minimized the mortality due to salmonellosis from around 30% to less than 10% (Djeghout et al., 2018), but resulted in emergence of resistance towards such clinically important antibiotics. The occurrence of drug-resistant clones of *Salmonella* in food items, supply chains, and in extraintestinal environments raises concerns about food safety and hence aggravates the situation. The emergence of resistance is theoretically possible in the carrier state also which makes the situation more challenging. Frequent reports of houseflies as mechanical vector in the spread of MDR *Salmonella* from swine farms and retail markets raised more worries in terms of public health and food safety. Better understanding on the *Salmonella* antimicrobial resistance trends and their impact in healthcare system, environment, livestock, fresh produce, aquaculture, seafood etc. is to be considered as the need of the hour. Studies on drug-resistant mechanisms and alternative interventions against emerging multidrug-resistant strains (MDR) of *Salmonella* may help us to tackle the situation more efficiently in future through a holistic approach.

2 Antimicrobial Resistance in *Salmonella* Serotypes: Trends and Threat (Table 1)

2.1 Healthcare System

Usage of antibiotics as therapeutics is inevitable in healthcare systems. Poor regulation together with excessive usage of hospital-based antibiotics remains as the key force for hospital-acquired infections (nosocomial infections) due to drug-resistant bacteria. Trans-boundary human movement facilitates easy spread of multidrug resistance worldwide. Infrequent scattered nature of disease outbreaks due to superbugs makes it more difficult to quantify the exact amount of mortality, morbidity, and related socioeconomic loss in both developed and developing countries. Based on predictions, by 2050, a total GDP loss of $100.2 trillion and nearly 10 million deaths annually happen due to antimicrobial-resistant infections, if appropriate actions are not taken (O'Neill, 2014). *Salmonella* strains resistant to clinically important antibiotics may lead to treatment failures and increase the treatment cost in healthcare systems. There have been reports of emergence of *S.* Typhi which are extensively drug resistant (XDR) (Klemm et al., 2018).

Table 1 Infections reported due to antibiotic-resistant Salmonella from different countries

Sl. no	Antibiotics to which *Salmonella* is resistant	*Salmonella* serotype	Country	References
1	Chloramphenicol	*S. Typhi*	Kerala, India	Paniker and Vimala (1972)
			Mexico	Olarte and Galindo (1973)
			Vietnam, Indonesia, Korea, Chile, Bangladesh	Threlfall et al. (1992)
			South Asia	Thanh et al. (2016), Levine and Simon (2018)
2	Fluoroquinolone	*S. Typhi*	Asia and Africa	Wong et al. (2015), Park et al. (2018)
			China, India, Nepal, and Bangladesh	Qian et al. (2020)
		S. Paratyphi A	Pakistan	Klemm et al. (2018)
3	Ceftriaxone	S. Typhi	India	Sharma et al. (2018)
4	Extended spectrum β-lactamases (ESBL) (resistance to third- and fourth-generation cephalosporins)	*Salmonella* sp	Philippines	Naiemi et al. (2008)
			India	Uma et al. (2010)
			Bangladesh	Elumalai et al. (2014)
			Nepal	Pokharel et al. (2016)
			Nigeria	Oghenevo et al. (2016), Narasanna et al. (2018)
			Sri Lanka	Ahamed Riyaaz et al. (2018), Prabhurajan et al. (2019)
			Peru	Garcia et al. (2019)
			Europe	Coipan et al. (2020)
			Sub-Saharan Africa	Moirongo et al. (2020)
			Pakistan	Molloy et al. (2010), Iqbal et al. (2020)
5	Azithromycin	S. Paratyphi A	Cambodia	Vileghe et al. (2012)
		Salmonella sp	Netherlands	Hassing et al. (2014)
			China	Wong et al. (2014)
			Singapore	Phoon et al. (2015)

(continued)

Table 1 (continued)

Sl. no	Antibiotics to which *Salmonella* is resistant	*Salmonella* serotype	Country	References
			South Asia	Levine and Simon (2018)
			Bangladesh	Hooda et al. (2019), Ahsan and Rahman (2019)
			Nepal	Duy et al. (2020)
			Taiwan	Liu et al. (2020)
			India	Carey et al. (2020)
			Europe Denmark	Gebreyes and Altier (2002)
6	Ampicillin, chloramphenicol, streptomycin, sulfonamides, tetracycline	S. Typhimurium DT 104	Europe Denmark	Angulo and Mølbak (2005)

During the 1950s to the 1960s, chloramphenicol was the first drug of choice against *Salmonella* infections soon after its introduction in 1948 and was replaced by co-trimoxazole and ampicillin after the emergence of *S.* Typhi strains resistant to chloramphenicol. Sporadic occurrence of chloramphenicol resistant typhoidal infections was reported in Kuwait, Chile, and Aden in the 1970s. By early 1970s, multidrug resistance (MDR) was identified in *S.* Typhi strains against first-line antibiotics used in treatment such as ampicillin, chloramphenicol, co-trimoxazole (ACCoT). In 1972, MDR typhoid epidemic was reported in Mexico City and other parts of Mexico with more than 10,000 cases (Olarte & Galindo, 1973). Simultaneously, outbreak with *S*. Typhi resistant to chloramphenicol was reported in Kerala, India, and mortality was high in both the places (Paniker & Vimala, 1972). Succeeding 5 years, similar outbreaks due to chloramphenicol-resistant typhoid infections was reported from many countries, notably, Vietnam, Korea, Indonesia, Bangladesh, and Chile (Threlfall et al., 1992). In Vietnam, 90% of typhoid cases were associated with MDR strains. Among *Salmonella* isolates, a different MDR pattern in "ACCoT" with additional resistance to tetracycline was reported among the floating population of Punjab, India.

Third-generation cephalosporins (ceftriaxone) or fluoroquinolones (ciprofloxacin, oflaxacin) were employed as first-line antibiotic therapy for *Salmonella* infections due to the spread and persistence of ACCoT MDR phenotype (Crump et al., 2015). During the late 1990s itself, South Asia has witnessed the enhanced fluoroquinolone (FQ) resistance with the emergence of H58 clade (genotype 4.3.1) of S. Typhi (Thanh et al., 2016), and this clade is rapidly displacing other lineages. Dissemination of (H58) clone, a specific MDR lineage across Africa and Asia also catalyzed the global spread of *S*. Typhi strains with reduced FQ susceptibility (Wong et al., 2015). In addition, Thanh et al. (2016) reported treatment failures in healthcare systems of Nepal due to gatifloxacin-resistant H58 subclade which was most likely introduced from India. In contrast, MDR strains of S. Typhi in West Africa belonged

mostly to 4.3.1 and 3.1.1. genotypes (Park et al., 2018). Rise in typhoid infections with reduced fluoroquinolones susceptibility with point mutations in quinolone resistance determining region (QRDR) of gyrA and parC was also reported in South Asia (Levine & Simon, 2018). From 1999 to 2017, ciprofloxacin resistance among *S.* Typhi increased from 22% to 74% (CDC, 2020). The occurrence of fluoroquinolones-resistant typhoidal fever was observed in Africa. High prevalence of fluoroquinolones resistance is reported among *S.* Paratyphi A strains from China, India, Nepal, and Bangladesh (Qian et al., 2020).

The entry of third-generation cephalosporin in the healthcare systems replaced the penicillin and FQ usage; extended spectrum β-lactamase (ESBL) *Salmonella* was very soon reported as the cause of many treatment failures from many countries, including India. Klemm et al. (2018) reported the existence of XDR H58 subclade of *S.* Typhi with ceftriaxone resistance in Pakistan that affected more than 5000 people and 11 children in the USA who travelled to or from Pakistan. In a study from a North India tertiary care hospital, the minimum inhibitory concentration (MIC) values of ceftriaxone were found to be increasing towards resistance during the last decade and poor clinical response was noticed when treated with ceftriaxone (Sharma et al., 2018). The frequency of occurrence of ESBL *Salmonella* strains from pediatric patients of Iran was found to be 29% (Ranjbar et al., 2018). Similarly, infections due to ESBL *Salmonella* was reported from different countries including UAE, Philippines (Naiemi et al., 2008), India (Uma et al., 2010), Bangladesh (Elumalai et al., 2014), Nepal (Pokharel et al., 2016), Nigeria (Oghenevo et al., 2016; Narasanna et al., 2018), Sri Lanka (Ahamed Riyaaz et al., 2018; Prabhurajan et al., 2019), Peru (Garcia et al., 2019), Europe (Coipan et al., 2020), sub-Saharan Africa (Moirongo et al., 2020) leading to treatment failures in healthcare systems worldwide.

Azithromycin is recommended for treatment of salmonellosis, and is being used for typhoid fever treatment. The first report of azithromycin-resistant *S.* Paratyphi A infection was reported from Pakistan that resulted in treatment failure of a 48-year-old doctor (Molloy et al., 2010). Hassing et al. (2014) reported *Salmonella* subpopulation with increased MIC for azithromycin among travelers back to Netherlands. Sporadic resistance towards azithromycin was reported from many parts of the world such as China (Wong et al., 2014), Singapore (Phoon et al., 2015), South Asia (Levine & Simon, 2018), and Bangladesh (Ahsan & Rahman, 2019*).* Very recently, *S.* Typhi with reduced susceptibility to azithromycin was reported from Pakistan (Iqbal et al., 2020), Nepal (Duy et al., 2020), Taiwan (Liu et al., 2020), and India (Carey et al., 2020) which raises the concerns for new treatment options.

Multidrug resistance in NTS is continuously emerging and has turned out to be of more serious concern than typhoidal strains. Soon after the first identification of an epidemic MDR strain of S. Typhimurium, that is, definitive phage type 104 (DT104) during the 1990s, antimicrobial resistance in NTS turned out as a major concern globally. Usually, S. Typhimurium strain definitive phage type 104 (DT104) has gene cassette either with a tetra ASSuT (ampicillin, streptomycin, sulfonamides, and tetracycline) or penta ACSSuT (ampicillin, chloramphenicol, streptomycin, sulfonamides, and tetracycline) pattern (Thung et al., 2016; Wang et al., 2019; Mellor et al., 2019). In Denmark, 25 patients were infected with DT 104 strain with penta ACSSuT pattern

along with nalidixic acid resistance (Angulo & Mølbak, 2005). *S.* Typhimurium DT193 is another MDR strain in contaminated pork products of Europe responsible for outbreaks in humans in the late 1980s and the early 1990s (Gebreyes & Altier, 2002). According to CDC, 2020, 16% of NTS were resistant to at least one essential antibiotic and causes nearly 212,500 infections and 70 deaths per year, while 2% of NTS were resistant to more than three essential antibiotics accounting for 20,800 infections and 10 deaths. Decreased susceptibility to azithromycin is also reported in 0.5% of NTS and causes 7400 infections and less than five deaths among US population.

2.2 Livestock

In addition to therapeutic use, antibiotics are widely used prophylactically to prevent infections and nontherapeutically to promote the growth rate in farmed animals. Antibiotics usage in food animals have direct as well as indirect implications to human health as it results in emergence of superbugs which easily pass between the animals, environment, and human beings (Landers et al., 2012). FDA states an increase of 26% in sales of antimicrobials used in animal production industry from 2009 to 2015 (FDA, 2017). Based on an estimate, livestock sector consumes 50–80% of the antibiotics produced in developed countries (Cully, 2014). Antimicrobial usage (AMU) was reported as highest in poultry, trailed by pork and dairy at 138, 40 and 10 doses/1000 animal days, respectively. The Inter Quartile Range (IQR) was 91.1–438.3, 8.5–120.4, and 5.5–13.6 for poultry, pork, and dairy animals. However, in terms of meat production per kg, AMU was maximum in pork, tailed by poultry and diary (Cuong et al., 2018). Such overuse of antibiotics and their complex interactions between different components of farming makes the pattern and evolution of drug resistance more complicated. The MDR *Salmonella* can spread in farming systems both vertically and horizontally as well as between the species. For example, DT104 and DT204 emerged in dairy cattle initially, but later on spread to poultry and pigs. In contrast, *S.* 4,[5],12:i:- DT193/DT120 strains emerged initially in pork and very recently transmitted to cattle and poultry in UK where they remain as minority types (Mueller-Doblies et al., 2018).

2.3 Poultry

Frequent recovery of *Salmonella* with resistance to clinically important antibiotics from poultry farming environments, slaughter houses, meat, manure, and eggs is extremely worrying as these medicines are crucial in the treatment of salmonellosis. CDC (2019) reported one death, 129 infections, and 25 hospitalizations in 39 states of the USA due to MDR *Salmonella* from raw chicken products. In Europe, broilers and turkeys harbored 56% and 73% of MDR *Salmonella.* High level of resistance in *Salmonella* was also reported from broiler meat (10.1% cefotaxime resistance and 68% ciprofloxacin) and turkey meat (4.7% cefotaxime and 73.4% ciprofloxacin) which adds up the AMR burden (EFSA, 2018). CDC reported that the derived meat

of chicken and turkey in the USA carries about 6% and 39.6% of MDR *Salmonella* respectively (CDC, 2018). In addition, it was reported that *S*. Enteritidis, *S*. Kentucky, and *S*. Infantis from poultry shows high-level resistance to ciprofloxacin. In India, it was reported that *Salmonella* isolated from country yard chicken have high resistance towards β-lactam and macrolide antibiotics and were susceptible to co-trimoxazole and ciprofloxacin. Poultry-associated serotype S. Heidelberg was found to have high ceftriaxone resistance in turkey (9%) and retail chicken (20%). High prevalence of MDR (54.8%) and XDR (20%) were reported in S. Enteritidis from poultry of Pakistan (Asif et al., 2017). Salmonella Infantis from poultry carcasses of Serbia were reported as resistant to sulfamethoxazole and ceftazidime (Nikolić et al., 2017). High prevalence (81.1%) of ESBL *Salmonella* was reported in poultry meat of China (Zhang et al., 2018). MDR S. Infantis isolated from poultry of Slovenia exhibited CipNxSSuT- (65.5%) and CipNxSuT-resistant pattern (Pate et al., 2019). S. Enterica (76.7%) isolated from broiler chicken and chicken carcass were found to be MDR with multiple antibiotic resistance index (MARI) of 0.2–0.6 (Elkenany et al., 2019). MDR *Salmonella* in poultry was reported from India (Meenakshi et al., 2019), Bangladesh (Alam et al., 2020), Brazil (Souza et al., 2020; Castro-Vargas et al., 2020), Ethiopia (Asfaw Ali et al., 2020), Zambia (Phiri et al., 2020). According to CIDRAP (2019), chicken products labelled as "antibiotic free," "organic," and "no antibiotics ever" are also likely to carry MDR *Salmonella* at lower levels compared to conventional poultry in USA.

2.4 Cattle, Beef, and Dairy Products

Dairy cattle and derived products are likely carriers of *Salmonella*, including slaughtering areas, ground beef, feed, milk and its products, bedding, cattle teat, milk container, milk parlor, shed soil, potable water, associated equipment, and personnel and can act as vehicles in the spread of AMR *Salmonella*. Ground beef contaminated with MDR *Salmonella* in the USA is an emerging health concern which warrants strict surveillance. During slaughtering, intestinal contents of even one MDR *Salmonella*-infected animal may get mixed up with large batches of final products and may result in potential contamination of many tons of meat from a single carcass which is contaminated with MDR *Salmonella* (Angulo & Mølbak, 2005). During 1985, MDR *S*. Newport from California with chloramphenicol resistance pattern was reported to be involved 45 infections. Later in 2002, *S*. Newport with additional kanamycin and ampicillin resistance resulted in 47 ground beef-associated infections. Two MDR outbreaks caused by *S*. Typhimurium DT 104 infection were linked to cheese in Northern California and Washington State of USA. During 2003–2004, consumption of contaminated ground beef with MDR S. Newport and S. Typhimurium resulted in 118 infections with ACSSuT as the pattern of resistance. In 2016, a multistate MDR *S*. Heidelberg infection from cattle was reported by CDC with 56 cases, 17 hospitalizations, and zero death. During 2019, unusual 255 outbreaks of *Salmonella* Newport infections in 32 states linked to beef and soft cheese and showing resistance to multiple antibiotics in the USA. *S*. Newport strains exhibited

nonsusceptibility to ciprofloxacin and decreased susceptibility to azithromycin (Plumb et al., 2019). MDR infections through raw milk were reported with S. Typhimurium having AKSSuT resistance pattern during 1985 and 2000 but S. Typhimurium with ACSSuT was reported as the cause of milk-related salmonellosis in the USA during 1997. *Salmonella* resistant to erythromycin, doxycycline, and amoxicillin were isolated from raw milk of Bangladesh (Rahman et al., 2018). Simultaneously, MDR *Salmonella* was isolated from milk and its produce across the globe, namely, India (Singh et al., 2018), Canada (Mangat et al., 2019), Iraq (Abdallah & Ahmed, 2020), and Pakistan (Qamar et al., 2020).

2.5 Pork and Derivatives

Pig and pork meat also have potential role in persistence and dissemination of clinically relevant MDR *Salmonella* infections. Multistate outbreaks of MDR *Salmonella* 14, (5), 12: i: and S. Infantis with 192 cases, 32 hospitalizations with zero death due to contaminated pork consumption was reported. Gebreyes and Altier (2002) reported the existence of MDR *S.* Typhimurium DT104 phenotype with ACSSuT resistance pattern in pork meat. Along with *S.* Typhimurium DT 193 with usual penta-resistance (AKSSuT), an unusual hexa-resistance strain with additional resistance to gentamicin was also reported. Lesser likelihood of MDR *Salmonella* occurrence with ACSSuT R-type was reported in "antibiotic-free" swine herds. *S*. Rissen and *S*. Typhimurium DT104 were recognized in the areas of pig production and their yields. From England and Wales, and Germany, the epidemiological record of *S*. Typhimurium infections during 1970–2010 in livestock was categorized by the series of prevailing MDR S. Typhimurium clones, namely, DT104, DT204, and the current *S*. 4,[5],12:i:- DT193/DT120 strains. The frequent presence of S. Typhimurium DT104 in pigs, pork meat and carcasses, and supervisors in slaughterhouses of Portugal were mostly associated with the resistant phenotype ASSuT. The emergence of rare R-type with ASSuT pattern in S. Typhimurium DT104 of swine and meat handlers at the abattoir was reported (Gomes-Neves et al., 2014). S. Typhimurium with resistance to norfloxacin, sulfamethoxazole-trimethoprim, and gentamicin was isolated from pig feces of Ghana (Osei Sekyere & Adu, 2015) and Thailand (Love et al., 2015). Iwu et al. (2016) reported the occurrence of MDR *Salmonella* in swine from Eastern Cape Province of South Africa with ampicillin, tetracycline, and streptomycin resistance. MDR S. Enterica with tetra ASSuT was reported from pig farms of Spain (Cameron-Veas et al., 2018). Infrequent occurrence of *S.* Mbandaka and *S*. Enteritidis with R-type ANSSuT in swine production was also reported (Campos et al., 2019). The incidence of two epidemics of MDR *Salmonella* I 4,[5],12:i: were associated with products of pork, including whole roaster pigs sold raw from a single slaughter and processing facility of Washington (Kawakami et al., 2019). S. Choleraesuis and S. Rissen with AMP-CAZ-CRO-CTX-NA-C-CN-TE-AX resistance profile was reported in Thailand (Kongsoi et al., 2020).

2.6 Fresh Produce

Fresh produce in the form of fresh vegetables, fruits are often reported as either reservoir or vehicles for major *Salmonella* outbreaks in humans. During 2015, 40 states involving multistate outbreak resulted in 204 hospitalizations and six deaths was reported by CDC due to the consumption of cucumber contaminated with *S. Poona* resistant to tetracycline, ciprofloxacin, and nalidixic acid. The spread of *Salmonella* in fresh produce is facilitated through irrigation with contaminated water (Gu et al., 2018) and usage of untreated animal excreta as manure in agricultural fields. The domestic as well as intruding wild fauna, also responsible for transmission of drug-unresponsive phenotypes of *Salmonella* through polluted environs, may consequently cause contamination in vegetables also. Many previous studies confirmed the persistence of *Salmonella* with MDR for extended periods of time in the farm, and hence, fresh produce commonly eaten as raw is a major concern for food safety (Machado-Moreira et al., 2019). The most common MDR phenotype of *Salmonella* from vegetables confirms resistance to streptomycin and ampicillin. *Salmonella* with resistance to ampicillin, vancomycin, erythromycin, and penicillin are also often isolated from vegetables. MDR-linked *S.* Urbana disease outbreak through papayas was reported in the USA with complete streptomycin resistance and intermediate tetracycline resistance (CDC, 2017). MDR *Salmonella* were recovered from fresh vegetables at retail level with erythromycin-kanamycin-ampicillin-(EKA) and vancomycin-streptomycin resistance patterns. MDR *S.* Enterica isolated from the cabbage and lettuce of Ghana exhibited high resistance to ofloxacin and erythromycin. The resistance pattern from lettuce and cabbage of Ghana with AmpOfxE was the commonest among the *S.* Enterica isolates and the reported MAR index ranged from 0.22 to 0.78 (Adzitey, 2018).

2.7 Aquaculture and Seafood

Aquaculture products as well as seafood can become MDR *Salmonella* sources by misuse of antibiotics, through processing practices, contact with polluted water or, therefore signifying a serious risk to public well-being. Aquaculture and seafood also transfer the risk of antibiotic obdurate microbes to public either by means of ingestion or unintended genetic transmission. The outbreak of MDR S. Typhimurium, DT104L linked to dried anchovy in Singapore. Recovery of *Salmonella* serotypes with similar resistance pattern from aquaculture and terrestrial agriculture indicates water sources from the farmland as a possible cradle of contamination. The frequent occurrence of plasmid-mediated quinolone-resistant (PMQR) genes which are similar to terrestrial MDR *Salmonella* were reported from fish farms or their surrounding environments from China and Egypt. Antunes et al. (2018) reported the incidence of *S.* Enterica with PMQR in trout farms and suggested the possible contamination through inflow water. *Salmonella* with high insensitivity to tetracycline, ampicillin, and amoxicillin-clavulanic acid at 90.71%, 70%, and 45%, respectively, in sea foods was recorded in Saudi Arabia, and it was also reported that the spread of MDR *Salmonella* between

countries occurs mainly through export and import (Elhadi, 2014). Recently, ready-to-eat (RTE) shrimp from Nigeria was reported as contaminated with MDR S. Enteritidis and S. Typhimurium with MAR index 0.21 and 0.46, respectively (Beshiru et al., 2019). From Nigeria, Ahmed et al. (2019) reported the occurrence of MDR *Salmonella* in catfish with unresponsiveness in descending order of 43.5%, 34.8%, and 21.7% to streptomycin, sulfamethoxazole, and trimethoprim, respectively, that poses a grim well-being hazard for humans. From Taiwan, Chiou et al. (2019) reported the occurrence of a multidrug-resistant *Salmonella enterica* serotype Anatum strain, and this was subsequently reported by Karp et al. (2020) in seafood imported from Asia.

3 Conclusion

Overall, it is evident that MDR in *Salmonella* is a multifaceted problem that increases the global burden in terms of mortality, morbidity, and treatment cost in healthcare settings. Lack of proper surveillance, clinical misuse, ease of availability, and substandard quality of antibiotics, transboundary human movements worldwide remain as the driving forces behind emergence and spread of antibiotic resistance. The determinants that impart obduracy to clinically important β-lactams, aminoglycosides, extended spectrum β-lactams, tetracyclines, chloramphenicol, and fluoroquinolones have been reported from different serotypes of *Salmonella* in the supply chain of various food items. The data on MDR *Salmonella* clearly shows that the spread is taking place at a fast phase and the existing linkages of spread between environment, livestock, fresh produce, pets, aquaculture products, seafood etc., is a matter of serious concern. Through resistance genotyping and Whole Genome Sequencing (WGS), it is possible to add more data to global framework to confirm and characterize the drug-resistant subpopulation of Salmonella, especially in travel-associated spread within the region and international spread. To combat this emerging problem of antibiotic resistance in *Salmonella,* proper regulations have to be framed and enforced for responsible use of antibiotics in various fields such as healthcare systems, veterinary sector, agriculture, aquaculture etc. at the global level. Usage of probiotics, prebiotics, plant-based derivatives, essential oils, organic acids etc. can be promoted as a safe alternative for antibiotics in various fields. Collaborative research involving multiple sectors are most warranted to boost the existing slowdown phase in the antibiotic discovery and also to fill the knowledge gap in the evolving genetic elements of drug resistance mechanism.

References

Abdallah, N. M., & Ahmed, S. T. (2020). Prevalence of multidrug-resistant Salmonella and Brucella from minced meat, chicken meat, cheese and raw milk samples in Baghdad. *Biochemical and Cellular Archives, 20*(2), 4189–4194.

Adzitey, F. (2018). Antibiotic resistance of Escherichia coli and Salmonella enterica isolated from cabbage and lettuce samples in Tamale metropolis of Ghana. *International Journal of Food Contamination, 5*(1), 1–7.

Ahamed Riyaaz, A. A., Perera, V., Sivakumaran, S., & de Silva, N. (2018). Typhoid fever due to extended spectrum β-lactamase-producing Salmonella enterica serovar Typhi: A case report and literature review. *Case Reports in Infectious Diseases, 2018*, 4610246. https://doi.org/10.1155/2018/4610246

Ahmed, A. O., Raji, M. A., Mamman, P. H., Kwanashie, C. N., Raufu, I. A., Aremu, A., & Akorede, G. J. (2019). Salmonellosis: Serotypes, prevalence and multi-drug resistant profiles of Salmonella enterica in selected poultry farms, Kwara State, North Central Nigeria. *Onderstepoort Journal of Veterinary Research, 86*(1), 1–8.

Ahsan, S., & Rahman, S. (2019). Azithromycin resistance in clinical isolates of Salmonella enterica serovars Typhi and Paratyphi in Bangladesh. *Microbial Drug Resistance, 25*(1), 8–13. https://doi.org/10.1089/mdr.2018.0109

Alam, S. B., Mahmud, M., Akter, R., Hasan, M., Sobur, A., Nazir, K. H. M., Noreddin, A., Rahman, T., El Zowalaty, M. E., & Rahman, M. (2020). Molecular detection of multidrug resistant Salmonella species isolated from broiler farm in Bangladesh. *Pathogens, 9*(3), 201. https://doi.org/10.3390/pathogens9030201

Angulo, F. J., & Mølbak, K. (2005). Human health consequences of antimicrobial drug—Resistant Salmonella and other foodborne pathogens. *Clinical Infectious Diseases, 41*(11), 1613–1620. https://doi.org/10.1086/497599

Antunes, P., Campos, J., Mourão, J., Pereira, J., Novais, C., & Peixe, L. (2018). Inflow water is a major source of trout farming contamination with Salmonella and multidrug resistant bacteria. *Science of the Total Environment, 642*, 1163–1171. https://doi.org/10.1016/j.scitotenv.2018.06.143

Asfaw Ali, D., Tadesse, B., & Ebabu, A. (2020). Prevalence and antibiotic resistance pattern of Salmonella isolated from caecal contents of exotic chicken in Debre Zeit and Modjo, Ethiopia. *International Journal of Microbiology, 2020*, 1910630. https://doi.org/10.1155/2020/1910630

Asif, M., Rahman, H., Qasim, M., Khan, T. A., Ullah, W., & Jie, Y. (2017). Molecular detection and antimicrobial resistance profile of zoonotic Salmonella enteritidis isolated from broiler chickens in Kohat, Pakistan. *Journal of the Chinese Medical Association, 80*(5), 303–306. https://doi.org/10.1016/j.jcma.2016.11.007

Beshiru, A., Igbinosa, I. H., & Igbinosa, E. O. (2019). Prevalence of antimicrobial resistance and virulence gene elements of Salmonella serovars from ready-to-eat (RTE) shrimps. *Frontiers in Microbiology, 10*, 1613. https://doi.org/10.3389/fmicb.2019.01613

Cameron-Veas, K., Fraile, L., Napp, S., Garrido, V., Grilló, M. J., & Migura-Garcia, L. (2018). Multidrug resistant Salmonella enterica isolated from conventional pig farms using antimicrobial agents in preventative medicine programmes. *The Veterinary Journal, 234*, 36–42. https://doi.org/10.1016/j.tvjl.2018.02.002

Campos, J., Mourão, J., Peixe, L., & Antunes, P. (2019). Non-typhoidal Salmonella in the pig production chain: A comprehensive analysis of its impact on human health. *Pathogens, 8*(1), 19. https://doi.org/10.3390/pathogens8010019

Carey, M. E., Jain, R., Yousuf, M., Maes, M., Dyson, Z. A., Thu, T. N. H., Nguyen, T. N. T., Dan, T. H. N., Nguyen, Q. N. P., Mahindroo, J., & Pham, D. T. (2020). Spontaneous emergence of azithromycin resistance in independent lineages of Salmonella Typhi in Northern India. *bioRxiv*. https://doi.org/10.1101/2020.10.23.351957

Castro-Vargas, R. E., Herrera-Sánchez, M. P., Rodríguez-Hernández, R., & Rondón-Barragán, I. S. (2020). Antibiotic resistance in Salmonella spp. isolated from poultry: A global overview. *Veterinary World, 13*(10), 2070. http://www.veterinaryworld.org/

Centers for Disease Control and Prevention (CDC). (2017). *Salmonella Urbana infections linked to imported Maradol papayas*. Available online at: https://www.cdc.gov/salmonella/urbana-09-17/index.html

Centers for Disease Control and Prevention (CDC). (2018). *Outbreak of multidrug-resistant Salmonella infections linked to raw Turkey products*. Available online at: https://www.cdc.gov/salmonella/reading-07-18/index.html

Centers for Disease Control and Prevention (CDC). (2019). *Outbreak of multidrug-resistant Salmonella infections linked to raw chicken products*. Available online at: https://www.cdc.gov/salmonella/infantis-10-18/index.html

Centers for Disease Control and Prevention (CDC). (2020). *NARMS now: Human data*. US Department of Health and Human Services. https://wwwn.cdc.gov/narmsnow

Chattaway, M. A., Langridge, G. C., & Wain, J. (2021). *Salmonella* nomenclature in the genomic era: A time for change. *Nature Scientific Report, 11*, 7494. https://doi.org/10.1038/s41598-021-86243-w

Chiou, C. S., Hong, Y. P., Liao, Y. S., Wang, Y. W., Tu, Y. H., Chen, B. H., & Chen, Y. S. (2019). New multidrug-resistant Salmonella enterica serovar Anatum clone, Taiwan, 2015–2017. *Emerging Infectious Diseases, 25*(1), 144. https://doi.org/10.3201/eid2501.181103

CIDRAP (Centre for Infectious Disease Research and Policy). (2019). *Study: Antibiotic-free poultry meat less likely to harbor multidrug-resistant Salmonella*. https://www.cidrap.umn.edu/news-perspective/2019/10

Coipan, C. E., Westrell, T., van Hoek, A. H., Alm, E., Kotila, S., Berbers, B., De Keersmaecker, S. C., Ceyssens, P. J., Borg, M. L., Chattaway, M., & McCormick, J. (2020). Genomic epidemiology of emerging ESBL-producing Salmonella Kentucky bla CTX-M-14b in Europe. *Emerging Microbes & Infections, 9*(1), 2124–2135. https://doi.org/10.1080/22221751.2020.1821582

Crump, J. A., Sjölund-Karlsson, M., Gordon, M. A., & Parry, C. M. (2015). Epidemiology, clinical presentation, laboratory diagnosis, antimicrobial resistance, and antimicrobial management of invasive Salmonella infections. *Clinical Microbiology Reviews, 28*(4), 901–937. https://doi.org/10.1128/CMR.00002-15

Cully, M. (2014). Public health: The politics of antibiotics. *Nature, 509*(7498), S16–S17.

Cuong, N. V., Padungtod, P., Thwaites, G., & Carrique-Mas, J. J. (2018). Antimicrobial usage in animal production: A review of the literature with a focus on low-and middle-income countries. *Antibiotics, 7*(3), 75. https://doi.org/10.3390/antibiotics7030075

Djeghout, B., Saha, S., Sajib, M. S. I., Tanmoy, A. M., Islam, M., Kay, G. L., Langridge, G. C., Endtz, H. P., Wain, J., & Saha, S. K. (2018). Ceftriaxone-resistant Salmonella Typhi carries an IncI1-ST31 plasmid encoding CTX-M-15. *Journal of Medical Microbiology, 67*(5), 620–627. https://doi.org/10.1099/jmm.0.000727

Dong, N., Li, Y., Zhao, J., Ma, H., Wang, J., Liang, B., Du, X., Wu, F., Xia, S., Yang, X., & Liu, H. (2020). The phenotypic and molecular characteristics of antimicrobial resistance of Salmonella enterica subsp. enterica serovar Typhimurium in Henan Province, China. *BMC Infectious Diseases, 20*(1), 1–11.

Duy, P. T., Dongol, S., Giri, A., Nguyen To, N. T., Dan Thanh, H. N., Nhu Quynh, N. P., Duc Trung, P., Thwaites, G. E., Basnyat, B., Baker, S., & Rabaa, M. A. (2020). The emergence of azithromycin-resistant Salmonella Typhi in Nepal. *JAC-Antimicrobial Resistance, 2*(4), dlaa109. https://doi.org/10.1093/jacamr/dlaa109

Elhadi, N. (2014). Prevalence and antimicrobial resistance of Salmonella spp. in raw retail frozen imported freshwater fish to Eastern Province of Saudi Arabia. *Asian Pacific Journal of Tropical Biomedicine, 4*(3), 234–238. https://doi.org/10.1016/S2221-1691(14)60237-9

Elkenany, R., Elsayed, M. M., Zakaria, A. I., El-Sayed, S. A. E. S., & Rizk, M. A. (2019). Antimicrobial resistance profiles and virulence genotyping of Salmonella enterica serovars recovered from broiler chickens and chicken carcasses in Egypt. *BMC Veterinary Research, 15*(1), 1–9.

Elumalai, S., Muthu, G., Selvam, R. E. M., & Ramesh, S. (2014). Detection of TEM-, SHV-and CTX-M-type β-lactamase production among clinical isolates of Salmonella species. *Journal of Medical Microbiology, 63*(7), 962–967. https://doi.org/10.1099/jmm.0.068486-0

European Food Safety Authority (EFSA). (2018). The European Union summary report on antimicrobial resistance in zoonotic and indicator bacteria from humans, animals and food in 2016. *EFSA Journal, 16*(2), 5182. https://doi.org/10.2903/j.efsa.2018.5182

FDA. (2017). Available online at: https://www.fda.gov/files/about%20fda/published/2016-Summary-Report-on-Antimicrobials-Sold-or-Distributed-for-Use-in-Food Producing-Animals.pdf

Garcia, C., Hinostroza, N., Astocondor, L., Ochoa, T., & Jacobs, J. (2019). Characterization of ESBL-producing Salmonella enterica serovar infantis infection in humans, Lima, Peru. *The American Journal of Tropical Medicine and Hygiene, 101*(4), 746–748. https://doi.org/10.4269/ajtmh.19-0213

Gebreyes, W. A., & Altier, C. (2002). Molecular characterization of multidrug-resistant Salmonella enterica subsp. enterica serovar Typhimurium isolates from swine. *Journal of Clinical Microbiology, 40*(8), 2813–2822. https://doi.org/10.1128/JCM.40.8.2813-2822.2002

Gomes-Neves, E., Antunes, P., Manageiro, V., Gärtner, F., Caniça, M., da Costa, J. M. C., & Peixe, L. (2014). Clinically relevant multidrug resistant Salmonella enterica in swine and meat handlers at the abattoir. *Veterinary Microbiology, 168*(1), 229–233. https://doi.org/10.1016/j.vetmic.2013.10.017

Gu, G., Strawn, L. K., Oryang, D. O., Zheng, J., Reed, E. A., Ottesen, A. R., Bell, R. L., Chen, Y., Duret, S., Ingram, D. T., & Reiter, M. S. (2018). Agricultural practices influence Salmonella contamination and survival in pre-harvest tomato production. *Frontiers in Microbiology, 9*, 2451. https://doi.org/10.3389/fmicb.2018.02451

Hassing, R. J., Goessens, W. H., van Pelt, W., Mevius, D. J., Stricker, B. H., Molhoek, N., Verbon, A., & van Genderen, P. J. (2014). Salmonella subtypes with increased MICs for azithromycin in travelers returned to The Netherlands. *Emerging Infectious Diseases, 20*(4), 705. https://doi.org/10.3201/eid2004.131536

Hooda, Y., Sajib, M.S., Rahman, H., Luby, S. P., Bondy-Denomy, J., Santosham, M., Andrews, J. R., Saha, S. K., & Saha, S. (2019). Molecular mechanism of azithromycin resistance among typhoidal Salmonella strains in Bangladesh identified through passive pediatric surveillance. *PLoS Neglected Tropical Diseases, 13*(11), p.e0007868. https://doi.org/10.1371/journal.pntd.0007868

Iqbal, J., Dehraj, I. F., Carey, M. E., Dyson, Z. A., Garrett, D., Seidman, J. C., Kabir, F., Saha, S., Baker, S., & Qamar, F. N. (2020). A Race against Time: Reduced Azithromycin Susceptibility in Salmonella enterica Serovar Typhi in Pakistan. *mSphere, 5*(4), e00215-20. https://doi.org/10.1128/mSphere.00215-20

Iwu, C. J., Iweriebor, B. C., Obi, L. C., Basson, A. K., & Okoh, A. I. (2016). Multidrug-resistant Salmonella isolates from swine in the Eastern Cape province, South Africa. *Journal of Food Protection, 79*(7), 1234–1239. https://doi.org/10.4315/0362-028X.JFP-15-224

Karp, B. E., Leeper, M. M., Chen, J. C., Tagg, K. A., Watkins, L. K. F., & Friedman, C. R. (2020). Multidrug-resistant Salmonella serotype Anatum in travelers and seafood from Asia, United States. *Emerging Infectious Diseases, 26*(5), 1030. https://doi.org/10.3201/eid2605.190992

Kawakami, V., Bottichio, L., Lloyd, J., Carleton, H., Leeper, M., Olson, G., Li, Z., Kissler, B., Angelo, K. M., Whitlock, L., & Sinatra, J. (2019). Multidrug-resistant Salmonella I 4,[5],12:i:- and Salmonella Infantis infections linked to whole roasted pigs from a single slaughter and processing facility. *Journal of Food Protection, 82*(9), 1615–1624. https://doi.org/10.4315/0362-028X.JFP-19-048

Ke, Y., Lu, W., Liu, W., Zhu, P., Chen, Q., & Zhu, Z. (2020). Non-typhoidal Salmonella infections among children in a tertiary hospital in Ningbo, Zhejiang, China, 2012–2019. *PLoS Neglected Tropical Diseases, 14*(10), e0008732. https://doi.org/10.1371/journal.pntd.0008732

Klemm, E. J., Shakoor, S., Page, A. J., Qamar, F. N., Judge, K., Saeed, D. K., Wong, V. K., Dallman, T. J., Nair, S., Baker, S., & Shaheen, G. (2018). Emergence of an extensively drug-resistant Salmonella enterica serovar Typhi clone harboring a promiscuous plasmid encoding resistance to fluoroquinolones and third-generation cephalosporins. *mBio, 9*(1), e00105-18. https://doi.org/10.1128/mBio.00105-18

Kongsoi, S., Chumsing, S., Satorn, D., & Noourai, P. (2020). Serotypes and antimicrobial resistance profiles of Salmonella enterica recovered from clinical swine samples. *Veterinary World, 13*(11), 2312. https://doi.org/10.14202/vetworld.2020.2312-2318

Landers, T. F., Cohen, B., Wittum, T. E., Larson, E. L. (2012). A review of antibiotic use in food animals: perspective, policy, and potential. *Public health reports, 127*(1), 4–22. https://doi.org/10.1177%2F003335491212700103

Levine, M. M., & Simon, R. (2018). The gathering storm: Is untreatable typhoid fever on the way? *mBio, 9*(2), e00482-1. https://doi.org/10.1128/mBio.00482-1

Liu, P. Y., Wang, K. C., Hong, Y. P., Chen, B. H., Shi, Z. Y., & Chiou, C. S. (2020). The first imported case of extensively drug-resistant Salmonella enterica serotype Typhi infection in Taiwan and the antimicrobial therapy. *Journal of Microbiology, Immunology and Infection, 54*, 740–744. https://doi.org/10.1016/j.jmii.2020.03.017

Love, D. C., Tharavichitkul, P., Arjkumpa, O., Imanishi, M., Hinjoy, S., Nelson, K., & Nachman, K. E. (2015). Antimicrobial use and multidrug-resistant Salmonella spp., Escherichia coli, and Enterococcus faecalis in swine from northern Thailand. *The Thai Journal of Veterinary Medicine, 45*(1), 43–53.

Machado-Moreira, B., Richards, K., Brennan, F., Abram, F., & Burgess, C. M. (2019). Microbial contamination of fresh produce: What, where, and how? *Comprehensive Reviews in Food Science and Food Safety, 18*(6), 1727–1750. https://doi.org/10.1111/1541-4337.12487

Mangat, C. S., Bekal, S., Avery, B. P., Côté, G., Daignault, D., Doualla-Bell, F., Finley, R., Lefebvre, B., Bharat, A., Parmley, E. J., & Reid-Smith, R. J. (2019). Genomic investigation of the emergence of invasive multidrug-resistant Salmonella enterica serovar Dublin in humans and animals in Canada. *Antimicrobial Agents and Chemotherapy, 63*(6), e00108-19. https://doi.org/10.1128/AAC.00108-19

Meenakshi, B., Jha, V., Ajitkumar, B. S., & Jhangiani, A. R. (2019). Prevalence study and resistance profiles of multi-drug resistant Salmonella obtained from poultry across Mumbai Region. *Acta Scientific Microbiology, 2*(5), 36–41.

Mellor, K. C., Petrovska, L., Thomson, N. R., Harris, K., Reid, S. W., & Mather, A. E. (2019). Antimicrobial resistance diversity suggestive of distinct Salmonella Typhimurium sources or selective pressures in food-production animals. *Frontiers in Microbiology, 10*, 708. https://doi.org/10.3389/fmicb.2019.00708

Moirongo, R. M., Lorenz, E., Ntinginya, N. E., Dekker, D., Fernandes, J., Held, J., & Lamshöft, M. (2020). Regional variation of extended-spectrum beta-lactamase (ESBL)-producing Enterobacterales, fluoroquinolone-resistant Salmonella enterica and methicillin-resistant Staphylococcus aureus among febrile patients in sub-Saharan Africa. *Frontiers in Microbiology, 11*, 2408. https://doi.org/10.3389/fmicb.2020.567235

Molloy, A., Nair, S., Cooke, F. J., Wain, J., Farrington, M., Lehner, P. J., & Torok, M. E. (2010). First report of Salmonella enterica serotype paratyphi A azithromycin resistance leading to treatment failure. *Journal of Clinical Microbiology, 48*(12), 4655–4657. https://doi.org/10.1128/JCM.00648-10

Mueller-Doblies, D., Speed, K. C. R., Kidd, S., & Davies, R. H. (2018). Salmonella Typhimurium in livestock in Great Britain–trends observed over a 32-year period. *Epidemiology & Infection, 146*(4), 409–422. https://doi.org/10.1017/S095026881800002X

Naiemi, N., Zwart, B., Rijnsburger, M. C., Roosendaal, R., Debets-Ossenkopp, Y. J., Mulder, J. A., Fijen, C. A., Maten, W., Vandenbroucke-Grauls, C. M., & Savelkoul, P. H. (2008). Extended-spectrum-beta-lactamase production in a Salmonella enterica serotype Typhi strain from the Philippines. *Journal of Clinical Microbiology, 46*(8), 2794–2795. https://doi.org/10.1128/JCM.00676-08

Narasanna, R., Chavadi, M., & Chandrakanth, K. (2018). Prevalence of multidrug-resistant Salmonella typhi in typhoid patients and detection of blaCTX-M2 and blaCTX-M9 genes in cefetoxime-mediated extended spectrum β-lactamase-producing Salmonella typhi isolates. *Biomedical Research, 29*(14), 3015–3021. https://doi.org/10.4066/biomedicalresearch.29-18-794

Nikolić, A., Baltić, T., Velebit, B., Babić, M., Milojević, L., & Đorđević, V. (2017). Antimicrobial resistance among Salmonella enterica serovar Infantis from broiler carcasses in Serbia. *IOP Conference Series: Earth and Environmental Science, 85*(1), 012077. IOP Publishing. https://doi.org/10.1088/1755-1315/85/1/012077

O'Neill, J. (2014). *Antimicrobial resistance: Tackling a crisis for health and wealth of nations*. https://amr-review.org/sites/default/files/AMR%20Review%20Paper%20-%20Tackling%20a%20crisis%20for%20the%20health%20and%20wealth%20of%20nations_1.pdf

Oghenevo, O., Bassey, B. E., Yhiler, N. Y., Francis, U. M., & Angela, O. (2016). Antibiotic resistance in extended spectrum beta-lactamases (ESBLs) Salmonella species isolated from patients with diarrhoea in Calabar, Nigeria. *Journal of Clinical Infectious Diseases, 1*, 107.

Olarte, J., & Galindo, E. (1973). Salmonella typhi resistant to chloramphenicol, ampicillin, and other antimicrobial agents: Strains isolated during an extensive typhoid fever epidemic in Mexico. *Antimicrobial Agents and Chemotherapy, 4*(6), 597–601. https://doi.org/10.1128/AAC.4.6.597

Osei Sekyere, J., & Adu, F. (2015). Prevalence of multidrug resistance among Salmonella enterica serovar Typhimurium isolated from pig faeces in Ashanti Region, Ghana. *International Journal of Antibiotics, 2015*, 1–4.

Paniker, C. K. J., & Vimala, K. N. (1972). Transferable chloramphenicol resistance in Salmonella typhi. *Nature, 239*(5367), 109–110.

Park, S. E., Pham, D. T., Boinett, C., Wong, V. K., Pak, G. D., Panzner, U., Espinoza, L. M. C., von Kalckreuth, V., Im, J., Schütt-Gerowitt, H., & Crump, J. A. (2018). The phylogeography and incidence of multi-drug resistant typhoid fever in sub-Saharan Africa. *Nature Communications, 9*(1), 1–10.

Pate, M., Mičunovič, J., Golob, M., Vestby, L. K., & Ocepek, M. (2019). Salmonella Infantis in broiler flocks in Slovenia: The prevalence of multidrug resistant strains with high genetic homogeneity and low biofilm-forming ability. *Biomedical Research International, 2019*, 4981463. https://doi.org/10.1155/2019/4981463

Phiri, N., Mainda, G., Mukuma, M., Sinyangwe, N. N., Banda, L. J., Kwenda, G., Muonga, E. M., Flavien, B. N., Mwansa, M., Munyeme, M., & Muma, J. B. (2020). Antibiotic-resistant Salmonella species and Escherichia coli in broiler chickens from farms, abattoirs and open markets in selected districts of Zambia. *bioRxiv*. https://doi.org/10.1101/2020.04.20.050914

Phoon, Y. W., Chan, Y. Y. C., & Koh, T. H. (2015). Isolation of multidrug-resistant Salmonella in Singapore. *Singapore Medical Journal, 56*(8), e142. https://doi.org/10.11622/smedj.2015129

Plumb, I. D., Schwensohn, C. A., Gieraltowski, L., Tecle, S., Schneider, Z. D., Freiman, J., Cote, A., Noveroske, D., Kolsin, J., Brandenburg, J., & Chen, J. C. (2019). Outbreak of Salmonella newport infections with decreased susceptibility to azithromycin linked to beef obtained in the United States and soft cheese obtained in Mexico—United States, 2018–2019. *Morbidity and Mortality Weekly Report, 68*(33), 713. https://doi.org/10.15585/mmwr.mm6833a1

Pokharel, P., Lekhak, B., Amatya, R., Bhattarai, S., & Pokharel, P. (2016). Enteric fever caused by Salmonella enterica serovar paratyphi A: An emerging health concern in Nepal. *African Journal of Microbiology Research, 10*(42), 1784–1791.

Prabhurajan, R., Kiran, R., & Padmavathy, K. (2019). Extended spectrum beta-lactamase producing Salmonella Lindenberg gastroenteritis. *The Journal of Infection in Developing Countries, 13*(09), 851–853. https://doi.org/10.3855/jidc.11409

Qamar, A., Ismail, T., & Akhtar, S. (2020). Prevalence and antibiotic resistance of Salmonella spp. in South Punjab-Pakistan. *PLoS One, 15*(11), e0232382. https://doi.org/10.1371/journal.pone.0232382

Qian, H., Cheng, S., Liu, G., Tan, Z., Dong, C., Bao, J., Hong, J., Jin, D., Bao, C., & Gu, B. (2020). Discovery of seven novel mutations of gyrB, parC and parE in Salmonella Typhi and Paratyphi strains from Jiangsu Province of China. *Scientific Reports, 10*(1), 1–8.

Rahman, M. A., Rahman, A. K. M. A., Islam, M. A., & Alam, M. M. (2018). Detection of multi–drug resistant Salmonella from milk and meat in Bangladesh. *Bangladesh Journal of Veterinary Medicine, 16*(1), 115–120. https://doi.org/10.3329/bjvm.v16i1.37388

Ranjbar, R., Ardashiri, M., Samadi, S., & Afshar, D. (2018). Distribution of extended-spectrum β-lactamases (ESBLs) among Salmonella serogroups isolated from pediatric patients. *Iranian Journal of Microbiology, 10*(5), 294.

Sharma, P., Dahiya, S., Manral, N., Kumari, B., Kumar, S., Pandey, S., Sood, S., Das, B. K., & Kapil, A. (2018). Changing trends of culture-positive typhoid fever and antimicrobial susceptibility in a tertiary care North Indian Hospital over the last decade. *Indian Journal of Medical Microbiology, 36*(1), 70–76. https://doi.org/10.4103/ijmm.IJMM_17_412

Simpson, K. M., Hill-Cawthorne, G. A., Ward, M. P., & Mor, S. M. (2018). Diversity of Salmonella serotypes from humans, food, domestic animals and wildlife in New South Wales, Australia. *BMC Infectious Diseases, 18*(1), 1–11. https://doi.org/10.1186/s12879-018-3563-1

Singh, P., Singh, R. V., Gupta, B., Tripathi, S. S., Tomar, K. S., Jain, S., & Sahni, Y. P. (2018). Prevalence study of Salmonella spp. in milk and milk products. *Asian Journal of Dairy and Food Research, 37*(1), 7–12. https://doi.org/10.18805/ajdfr.DR-1252

Souza, A. I., Saraiva, M. M., Casas, M. R., Oliveira, G. M., Cardozo, M. V., Benevides, V. P., Barbosa, F. O., Freitas Neto, O. C., Almeida, A. M., & Berchieri, A. (2020). High occurrence of β-lactamase-producing Salmonella Heidelberg from poultry origin. *PLoS One, 15*(3), e0230676. https://doi.org/10.1371/journal.pone.0230676

Thanh, D. P., Karkey, A., Dongol, S., Thi, N. H., Thompson, C. N., Rabaa, M. A., Arjyal, A., Holt, K. E., Wong, V., Thieu, N. T. V., & Vinh, P. V. (2016). A novel ciprofloxacin-resistant subclade of H58 Salmonella Typhi is associated with fluoroquinolone treatment failure. *eLife, 5*, e14003. https://doi.org/10.7554/eLife.14003.001

Threlfall, E. J., Ward, L. R., Rowe, B., Raghupathi, S., Chandrasekaran, V., Vandepitte, J., & Lemmens, P. (1992). Widespread occurrence of multiple drug-resistant Salmonella typhi in India. *European Journal of Clinical Microbiology and Infectious Diseases, 11*(11), 990–993.

Thung, T. Y., Mahyudin, N. A., Basri, D. F., Radzi, C. W. M., Nakaguchi, Y., Nishibuchi, M., & Radu, S. (2016). Prevalence and antibiotic resistance of Salmonella enteritidis and Salmonella Typhimurium in raw chicken meat at retail markets in Malaysia. *Poultry Science, 95*(8), 1888–1893. https://doi.org/10.3382/ps/pew144

Uma, B., Prabhakar, K., Rajendran, S., & Sarayu, Y. L. (2010). Prevalence of extended spectrum beta lactamases in Salmonella species isolated from patients with acute gastroenteritis. *Indian Journal of Gastroenterology, 29*(5), 201–204.

Vlieghe, E. R., Phe, T., De Smet, B., Veng, C. H., Kham, C., Bertrand, S., Vanhoof, R., Lynen, L., Peetermans, W. E., & Jacobs, J. A. (2012). Azithromycin and ciprofloxacin resistance in Salmonella bloodstream infections in Cambodian adults. *PLoS Neglecting Tropical Diseases, 6*(12), 1933. https://doi.org/10.1371/journal.pntd.0001933

Wang, X., Biswas, S., Paudyal, N., Pan, H., Li, X., Fang, W., & Yue, M. (2019). Antibiotic resistance in Salmonella Typhimurium isolates recovered from the food chain through national antimicrobial resistance monitoring system between 1996 and 2016. *Frontiers in Microbiology, 10*, 985. https://doi.org/10.3389/fmicb.2019.00985

Wong, M. H. Y., Yan, M., Chan, E. W. C., Biao, K., & Chen, S. (2014). Emergence of clinical Salmonella enterica serovar Typhimurium isolates with concurrent resistance to ciprofloxacin, ceftriaxone, and azithromycin. *Antimicrobial Agents and Chemotherapy, 58*(7), 3752–3756. https://doi.org/10.1128/AAC.02770-13

Wong, V. K., Baker, S., Pickard, D. J., Parkhill, J., Page, A. J., Feasey, N. A., Kingsley, R. A., Thomson, N. R., Keane, J. A., Weill, F. X., & Edwards, D. J. (2015). Phylogeographical analysis of the dominant multidrug-resistant H58 clade of Salmonella Typhi identifies inter-and intracontinental transmission events. *Nature Genetics, 47*(6), 632–639.

Zhang, L., Fu, Y., Xiong, Z., Ma, Y., Wei, Y., Qu, X., Zhang, H., Zhang, J., & Liao, M. (2018). Highly prevalent multidrug-resistant Salmonella from chicken and pork meat at retail markets in Guangdong, China. *Frontiers in Microbiology, 9*, 2104. https://doi.org/10.3389/fmicb.2018.02104

Emergence of Antimicrobial Resistance in Seafood-Borne *Listeria monocytogenes*: An Overview

Ahamed Basha Kusunur, Murugadas Vaiyapuri, and Madhusudana Rao Badireddy

Contents

Abstract

Listeria monocytogenes is an important opportunistic foodborne pathogen linked with rare outbreaks but often leading to fatal illness. Antibiotics are used as immediate therapeutic agents for its control and to reduce infection. Ampicillin

A. B. Kusunur (✉)
Visakhapatnam Research Centre of ICAR-Central Institute of Fisheries Technology (ICAR-CIFT), Visakhapatnam, Andhra Pradesh, India
e-mail: kusunur.ahamedbasha@icar.gov.in

M. Vaiyapuri
Microbiology Fermentation and Biotechnology Division of ICAR-Central Institute of Fisheries Technology (ICAR-CIFT), Cochin, Kerala, India

M. R. Badireddy
Visakhapatnam Research Centre of ICAR-CIFT, ICAR-Central Institute of Fisheries Technology (ICAR-CIFT), Visakhapatnam, Andhra Pradesh, India

M. P. Mothadaka et al. (eds.), *Handbook on Antimicrobial Resistance*,
https://doi.org/10.1007/978-981-19-9279-7_21

along with aminoglycoside, trimethoprim in combination with sulfa drug and erythromycin were the common drugs used to treat patients suffering from listeriosis. However, incidence of resistant strains in clinical and food isolates against the previously susceptible antibiotics is an alarming issue that poses threat to public health. This chapter highlights the occurrence and the response of *L. monocytogenes* isolates from seafood environment to different antibiotics. The most widespread mechanism of acquiring resistance in these isolates is through the attainment of mobile genetic determinants such as plasmids and transposons through conjugation, and the most effective mechanism of resistance is through efflux pumps. Furthermore, the biofilm-producing ability of *L. monocytogenes* and control options to be implemented in seafood processing plants is discussed.

Keywords

Listeria monocytogenes · Seafood · Antimicrobial resistance · Biofilm

1 Introduction

Seafood is a vital reservoir of proteins, vitamin D, omega-3 fatty acids, iodine, selenium, and other beneficial nutrients. High seafood consumption has significant effect on human health and is associated with reduction of cardiovascular diseases, elevated blood pressure, stroke, and other inflammatory diseases. Increase in the seafood consumption across the globe has resulted in the enhanced movement of fish and fishery products across the world. Although seafood is desirable in meeting the demands of food for rising population, contamination of seafood with human pathogenic organisms is of major food safety concern. Seafood contamination occurs possibly due to fish caught from overboard sewage discharge, sewage-contaminated environments, and from inland runoff areas due to abundant rains or flooding (Iwamoto et al., 2010), and as a consequence, aquatic animals harvested from these sources harbor enteric pathogens. Furthermore, seafood contamination takes place at some stage in handling and processing, and other possible routes of contamination include deviation in temperature during storage and transportation of fish products.

The incidence of human pathogenic bacteria in fish and shellfish directly reflects the safety of the aquatic environment from which they are harvested. It is a widely known fact that cooking is a processing step that kills majority of pathogens. Nevertheless, in few parts of the world, seafood is consumed as raw or lightly cooked unlike meat and poultry, which are universally consumed in cooked form. Generally, microorganisms such as bacteria, fungi, viruses, and parasites are the major pathogens linked with foodborne infections with mild gastroenteritis to serious syndromes. Seafood is accountable for a noteworthy proportion of foodborne illness in several countries worldwide. An epidemiological survey conducted in the United States indicated that major proportion of the seafood-borne illness was due to bacteria (76%), followed by viruses (21%) and parasites (2.6%) (Iwamoto et al., 2010). According to the same author, among various seafood species consumed,

most of the outbreaks are associated with molluscan shellfish (45%), followed by finfish (39%) and crustaceans (16%). *Listeria monocytogenes*, *Escherichia coli*, *Salmonella enterica*, and *Vibrio* spp. are the significant pathogenic bacteria observed in seafood (Amagliani et al., 2012).

2 Incidence of *Listeria* Spp. in Fish and Fishery Environments

Listeria monocytogenes is considered as human pathogenic bacteria but is reported as native microflora in aquatic environments due to its ability to survive in external environment for relatively long duration. *L. monocytogenes* is a foodborne pathogen that leads to listeriosis in humans. Even though its incidence is low, the organism has reported mortality rate of 20–40% in susceptible population group and also in immunocompromised individuals. *L. monocytogenes* is omnipresent in nature and has been reported from animal feces, silage, soil from fresh and marine aquatic waters, and sediments. The pathogen prevalence is reported in fish smokehouses (68%), fish slaughter units (16%), freshwater fish culture systems (10%), and seawater fish farms (2%) (Hansen et al., 2006). *Listeria* spp. is a major hazard in seafood processing plants due to its ability to survive and multiply at very low temperatures, even at 4 °C (Budzinska et al., 2012). The formation of biofilm is a defining feature of *L. monocytogenes* survival, as biofilm is believed to resist the antibiotics and disinfectants. *L. monocytogenes* presence is seen not only in fishery environment (Basha et al., 2019) but also in processed fish products, namely frozen seafood, fish salads, cold-smoked salmon, fermented fish, marinated fish, hot-smoked salmon, etc. (Papadopoulos et al., 2010). Various studies on the occurrence conducted for this bacterium reported to be 4–12% in the temperate areas. However, low percentage (0–2%) of *Listeria* genus was reported in the tropical climatic zones. Dhanashree et al. (2003) reported that 0.95% incidence of *L. monocytogenes* in seafood samples, and their presence was not observed in other food samples, such as milk, milk products, meat, and vegetables, and hence stressed that contaminated seafood pose a health threat to consumers. The entry of *L. monocytogenes* into fish products takes place at some stage in the processing of raw meat and meat products. The other possible spread of this pathogen occurs due to cross infection via utensils, personnel, water, ice, and environment (Papadopoulos et al., 2010). Another study demonstrated that *L. monocytogenes* could be transmitted from flesh to utensil and equipment surfaces during the preparation of salmon fillets (Duffes, 1999). Basha et al. (2019) identified that ice might be a vehicle of transmission for the contamination of *L. monocytogenes* in fish from retail fish markets in Kerala, India. Despite their high incidence in fish and fishery environments, hygienic handling and effective sanitation programs might be a useful control regime to restrict this bacterium in retail fish markets and processing facilities (Miettinen & Wirtanen, 2005).

L. monocytogenes is characterized as a significant public health hazard causing serious consequences in immunocompromised humans such as meningoencephalitis, meningitis, and septicemia. Furthermore, serious complications are produced by *L. monocytogenes* in newborns, elderly population, and pregnant women with

mortality rate ranging between 20% and 30% (Swaminathan & Gerner-Smidt, 2007). Consequently, antibiotics are used as protective therapy for the management of the infection reported by this bacterium. Generally, *L. monocytogenes* is susceptible to a broad spectrum of antibiotics effective towards Gram-positive bacteria, namely erythromycin, tetracyclines, gentamicin, and ampicillin (Teuber, 1999). Conversely, *L. monocytogenes* showed innate resistance towards oxacillin, fosfomycin, cefotaxime, cefepime, and lincosamides (CA-SFM, 2010). The increased detection of antibiotic-resistant *L. monocytogenes* strains in seafood and its ecosystems is a growing concern influencing listeriosis treatment.

3 Use of Antibiotics as a Therapy to Control Listeriosis

Antibiotics are the agents of natural, semisynthetic, or synthetic in origin that are employed to treat or control bacterial infections in animals and humans. Antibiotics with bacteriostatic and bactericidal effects are widely used as therapeutic agents in humans experiencing listeriosis. Early diagnosis and treatment of listeriosis are crucial in preventing mortality since commercial vaccine against this pathogen is not available. Source tracking of listeriosis is complex due to the relatively longer incubation period (up to 70 days) of *L. monocytogenes* (Rhoades et al., 2009). β-lactam antibiotics, namely penicillin and ampicillin, in combination with aminoglycoside (gentamicin), are the choice of treatment for human listeriosis treatment (Ramaswamy et al., 2007; Alonso-Hernando et al., 2012). However, for patients exhibiting allergic response to penicillin, a second choice of treatment is co-trimoxazole, which is a combination of trimethoprim along with sulfamethoxazole (Alonso-Hernando et al., 2012). Antibiotics, namely chloramphenicol, rifampicin, fluoroquinolones, and tetracyclines, are also the selected agents of treatment for listeriosis.

4 Rise of Antimicrobial-Resistant Strains of *L. monocytogenes* in Fish and Shellfish Environment

Antimicrobial resistance (AMR) occurs due to the ability of the organism to withstand the effect of antibiotics in response to the inappropriate application of the drugs. AMR is a growing global threat affecting public health, making the disease complicated to treat, ultimately leading to an increase in mortality rate and medical expenditure. Antibiotics are basically used in the animal sector to treat and control bacterial infections but their usage is observed as growth-promoting agents in some countries. However, their usage as feed additive is banned in the European Union in 2006. The major driving feature for the emergence of antibiotic resistance is mainly through the over-usage of antibiotics. Epidemiological studies showed that a direct relationship exists between antibiotic consumption and the emergence of resistant bacteria strains (Read & Woods, 2014). The possible routes of AMR emergence are through horizontal gene transfer (HGT) between the different species of bacteria and through spontaneous mutation (Read & Woods, 2014). It is believed that

L. monocytogenes strains are susceptible to antibacterial agents that are effective against Gram-positive organisms. However, *L. monocytogenes* displays native resistance to the cephalosporins of third generation (Charpentier & Courvalin, 1999; Teuber, 1999). Ampicillin, in combination with aminoglycosides (gentamicin), was the primary choice for listeriosis treatment (Hof et al., 1997). An association of trimethoprim with sulfonamide was considered to be a second choice of treatment against listeriosis. Clinical strains of *L. monocytogenes* showing resistance to gentamicin were also reported (Charpentier & Courvalin, 1999). The earliest multidrug (tetracycline, erythromycin, chloramphenicol, and streptomycin)-resistant *L. monocytogenes* strain was reported in 1988 from a patient suffering from meningoencephalitis in France (Poyart-Salmeron et al., 1990). Consequently, the prevalence of *L. monocytogenes* strains showing resistance to more than single antibiotic has been reported from various sources, namely environmental, food, and clinical samples. Chen et al. (2018) investigated the antibiotic susceptibility of 72 *L. monocytogenes* isolates from fresh aquatic products in China and observed that majority of the isolates have low antibiotic resistance. Fallah et al. (2013) observed resistance to one or more antibiotics in 225 out of 278 *L. monocytogenes* screened from raw, seafood, market, and processing environment of Iran. The results highlighted that *L. monocytogenes* reported high resistance against tetracycline, ampicillin, penicillin, and vancomycin. Obaidat et al. (2015) recovered 104 *L. monocytogenes* from fresh fish samples imported by Jordan from three countries, namely India, Egypt, and Yemen, and observed that 99% of isolates showed resistance to penicillin, 71% were inhibited by rifampicin, 66% were resistant to clindamycin, 65% were resistant to erythromycin, and 64% of the isolates were resistant to tetracycline. The study concluded that 73.1% of the *L. monocytogenes* recovered from three distinct countries displayed resistance to higher than three antibiotics. The high levels of resistance in *L. monocytogenes* to cefotaxime, ampicillin, and penicillin in the clinical and seafood isolates, and 100% resistant cefotaxime isolates was observed in the clinical and seafood isolates (Abdollahzadeh et al., 2016). A high penicillin resistance rate of 71.4% was noted in the clinical and 57% in the seafood isolates (Abdollahzadeh et al., 2016). In Iran, Rezai et al. (2018) reported that 36% samples were infected with *Listeria* spp., and the antibiotic sensitivity test results of 86 *Listeria* spp. isolates indicated highest rates of antibiotic resistance against tetracycline (63%), enrofloxacin (57%), ciprofloxacin (38%), penicillin (36%), and ampicillin (35%). The antibiotic susceptibility of 43 *L. monocytogenes* isolates from fish and fishery environment was investigated by Jamali et al. (2015), and they found that 6.4% of the isolates showed resistance to two or more antimicrobial agents. Their results showed that 27.9% of *L. monocytogenes* were resistant to tetracycline, ampicillin, penicillin G, cephalothin, and streptomycin. The evaluation of antibiotic resistance pattern in 57 *L. monocytogenes* screened from fresh and smoked fish of Poland revealed that 58% of the tested isolates were resistant to oxacillin, 31.6% to ceftriaxone, and 68% of the isolates showed intermediate susceptibility to clindamycin (Wieczorek & Osek, 2017) evaluated. Furthermore, 2 out of the 57 isolates recovered from cod and smoked salmon belonged to the serogroup 1/2a and 3a, and they were multidrug

resistant to penicillins, cephalosporins, and lincosamides. The incidence of *L. monocytogenes* was noted in 38.5% (237/614) samples during different stages of production cycle from raw material to finished product storage of a fish processing plant located in Poland (Skowron et al., 2018). After identifying genetic similarities through pulsed field gel electrophoresis (PFGE), antibiotic susceptibility testing for 70 isolates of *L. monocytogenes* strains against erythromycin, ampicillin, penicillin, meropenem, and trimethoprim/sulfamethoxazole revealed that 7% of the isolates were resistant to the entire set of antibiotics examined. Moreover, 47% of the isolates tested were resistant to erythromycin and trimethoprim/sulfamethoxazole. The study concluded that among the various stages of the production procedures, most of the resistant strains were screened from the raw material (Skowron et al., 2018). In a clinical study, 8 antimicrobial agents were evaluated against the 14 *L. monocytogenes* isolates (7 each from seafood and human patient origin) collected previously from Tehran and Karaj areas of Iran (Abdollahzadeh et al. (2016). Among the antibiotics (chloramphenicol, penicillin, streptomycin, tetracycline, ampicillin, gentamicin, trimethoprim-sulfamethoxazole, and cefotaxime) tested, all the 14 isolates showed resistance to ampicillin and cefotaxime. It was observed that 57% of the *L. monocytogenes* isolates from seafood were resistant to penicillin. The study concluded that *L. monocytogenes* showed 100% resistance to the antibiotics for treating listeriosis, which is a potential threat to consumers. Antibiotic susceptibility test was carried out against 15 antibiotics in *Listeria* spp. (221), namely *L. welshimeri, L. seeligeri*, *L. ivanovii* (94), *L. innocua* (41), and *L. monocytogenes* (86), recovered from catfish and processing surroundings (Chen et al., 2010). Further, the findings showed that all the 86 *L. monocytogenes* isolates have resistance or intermediate susceptibility to clindamycin and cefotaxime. Fifteen out of 86 isolates showed sensitivity to penicillin, and the remaining isolates were resistant to penicillin. Sixty-nine percent of *L. monocytogenes* exhibited resistance to clindamycin. However, no *L. monocytogenes* isolate showed multidrug resistance. Rodas-Suarez et al. (2006) evaluated the resistance of 15 *L. monocytogenes* (4.5% in fish and 8.3% in seawater) to 12 antibiotics. These isolates exhibited maximum resistance to pefloxacin (29%) followed by cephalothin, ampicillin, penicillin, and ceftazidime. Notably, 6% of *L. monocytogenes* isolates exhibited multidrug resistance to dicloxacillin, erythromycin, ampicillin, tetracycline, and trimethoprim-sulfamethoxazole. The study on drug resistance pattern of *L. monocytogenes* isolates contaminated in fish and tools used in fish processing units revealed nearly 70% of the isolates were inhibited by all the antibiotics exposed, namely ampicillin, penicillin, meropenem, co-trimoxazole, and erythromycin. However, the remaining 11 isolates (30.6%) exhibited resistance to at least one of the antibiotics investigated and 2 isolates (5.6%) were resistant to 3 antibiotics, namely erythromycin, penicillin, and ampicillin (Skowron et al., 2018); clinical samples from Kashmir, India, against 15 antibiotics revealed that all the isolates from fish showed maximum resistance to cefpodoxime (100%), cefotaxime (80%), streptomycin (80%), and intermediate susceptibility to cephalexin (60%) and norfloxacin (80%). The study also observed high sensitivity to doxycycline, amoxicillin/clavulanic acid, ampicillin/cloxacillin, ciprofloxacin, ceftriaxone, enrofloxacin, and gentamicin (Bhat et al., 2013).

L. monocytogenes recovered from Peter the Great Bay (Sea of Japan) were minimally resistant to penicillin, ampicillin, gentamicin, lincomycin, and rifampicin. However, 19% strains were multidrug resistant and all of these strains originated from seafood (Beleneva, 2011). Seven *L. monocytogenes* strains from Nha Trang Bay (South China Sea) were sensitive to cefotaxime, ceftazidime, lincomycin, rifampicin, gentamicin, and ciprofloxacin. However, high resistance to tetracycline, penicillins, and chloramphenicol was reported in all the strains tested. Interestingly, the study found that the number of multidrug-resistant strains was greater in tropical zone compared to the temperate zone. Five out of seven strains from tropical region, namely sea urchins (2), shrimps (2), and fish (1), showed resistance to greater than two antibiotics (Beleneva, 2011). In a study, Gawade et al. (2010) reported that 5 out of 111 marine fish and shellfish processed samples were positive for *L. monocytogenes*, and the results from antibiotic sensitivity tests showed that all the isolates of the positive samples were susceptible to the antibiotics in the ascending order, ciprofloxacin > sulfafurazole > norfloxacin > ampicillin > gentamicin. Basha et al. (2019) observed that 2.7% of samples from fish retail markets of Kerala, India, were contaminated with *L. monocytogenes.* Antibiotic sensitivity test results for all the strains showed multidrug resistance to tetracycline, erythromycin, clindamycin, ampicillin, and penicillin. In Iran, 6.6% of common carp samples were contaminated with *L. monocytogenes*, and the antimicrobial susceptibility test revealed that out of 12 antibacterial agents tested, 7 agents, namely tetracycline, cefixime, cefotaxime, methicillin, oxacillin, ampicillin, and penicillin G, were least susceptible (Al-Gburi, 2020). Antibiotic resistance profiles of *L. monocytogenes* from fish and fishery environment are represented in Table 1.

5 Antibiotic Resistance Mechanisms in *L. Monocytogenes*

Listeriosis outbreak in humans is mainly attributed to the consumption of food infected with *L. monocytogenes*. The acquisition of various types of mobile genetic determinants such as plasmids (self-transferable, mobile) and transposons is the contributing factor for the emergence of antibiotic resistance in *L. monocytogenes* (Poyart-Salmeron et al., 1990; Charpentier & Courvalin, 1999). The resistant strains spread these mobile genetic elements to the susceptible strains and thereby make them prone to resistance. Another prominent strategy of antibiotic resistance is efflux pump mechanism that was found to be associated with macrolide, cefotaxime, and fluoroquinolone resistance in *L. monocytogenes* (Mata et al., 2000; Godreuil et al., 2003).

6 Antibiotic Resistance Intervened Through Conjugation

It has been demonstrated that conjugation is the chief process of acquiring resistance in *Listeria* spp. The spread of plasmids through conjugation process has been described in *Enterococcus* and *Streptococcus* and is believed that these bacteria

Table 1 Antibiotic resistance profiles reported in *L. monocytogenes* of fish and fishery settings

Sample details	Resistant antibiotics	Multidrug resistance (MDR) (%)	References
Fish and seawater	Ampicillin, dicloxacillin, erythromycin, tetracycline, and trimethoprim-sulfamethoxazole	6%	Rodas-Suarez et al. (2006)
Catfish fillets and processing environment	Penicillin (82%) Clindamycin (69%)	Nil	Chen et al. (2010)
Sea water and marine organisms (Great Bay)	Amoxicillin and cefotaxime Ceftazidime and erythromycin	19%	Beleneva (2011)
Sea water and marine organisms (Nha Trang Bay)	Chloramphenicol and penicillin	28.5%	Beleneva (2011)
Fish samples	Cefpodoxime, cefotaxime, and streptomycin	100% (n = 2)	Bhat et al. (2013)
Seafood, market, processing environments	Ampicillin, penicillin, tetracycline, and vancomycin	8.9%	Fallah et al. (2013)
Fish and open air fish market	Ampicillin (20.9%), cephalothin (16.3%), penicillin G (16.3%), streptomycin (16.3%), and tetracycline (27.9%)	6.8% of *Listeria* spp.	Jamali et al. (2015)
Imported raw fish	Clindamycin (66.3%), erythromycin (65.4%), penicillin (99%), rifampicin (71.2%), and tetracycline (64.4%)	73.1% (n = 104)	Obaidat et al. (2015)
Seafood and clinical isolates	Ampicillin and cefotaxime	No information	Abdollahzadeh et al. (2016)
Fresh and smoked fish	Ceftriaxone (31.6%), clindamycin (8.8%), and oxacillin (57.9%)	3.5%	Wieczorek and Osek (2017)
Rainbow trout of farms and retail outlets	Ciprofloxacin (38.37%), enrofloxacin (56.97%), and tetracycline (62.79%)	No information	Rezai et al. (2018)
Fish processing plant (raw material to finished product)	Ampicillin, erythromycin, meropenem, penicillin, trimethoprim/sulfamethoxazole	5.6%	Skowron et al. (2018)
Fish and fish processing plants	Ampicillin, erythromycin, and penicillin	5.6%	Skowron et al. (2019)
Freshwater fish (common carp)	Ampicillin, cefotaxime, cefixime, methicillin, penicillin G, oxacillin, and tetracycline	6.66%	Al-Gburi (2020)
Fish and fishery environment	Ampicillin, clindamycin, erythromycin, penicillin, and tetracycline	2.7%	Basha et al. (2019, 2020)

transfer resistance genes to *L. monocytogenes* and other *Listeria* spp., Charpentier and Courvalin (1999) and White et al. (2002) reported that mobile genetic determinants such as plasmids and transposons in *Enterococcus* spp. and *Streptococcus* spp. are accountable for the increase of antibiotic resistance in *Listeria* spp. Studies on the conjugative process of pIP501, a plasmid first reported in *Streptococcus agalactiae* which encodes resistance to different category of antibiotics, noted that pIP501 is able to promote its spread from *S. agalactiae* to *L. monocytogenes* and was able to revert back from *L. monocytogenes* to *S. agalactiae*. Transposon Tn916 identified in *Enterococcus faecalis* showed successful transfer of this broad-host-range transposon between *E. faecalis* and *L. monocytogenes* at a high frequency of 10^{-6} (Charpentier & Courvalin, 1999). Flamm et al. (1984) identified a plasmid pAMβ1 that encodes resistance to erythromycin and was capable of transferring resistance between *L. monocytogenes* and reverting back to *E. faecalis*. A similar result of acquiring resistance through the transfer of plasmid *vanA*, *Cat* determinants was reported by Charpentier and Courvalin (1999).

Resistance to tetracycline is the most prevalent trait reported in *L. monocytogenes* of food and clinical systems (Charpentier & Courvalin, 1999). Tetracycline resistance determinants have been well documented in Gram-positive organisms (*tetK*, *tetL*, *tetM*, *tetO*, *tetP*, and *tetS*), and the presence of *tetL, tetM*, and *tetS* have been found in *L. monocytogenes* strains (Charpentier & Courvalin, 1999; Granier et al., 2011; Escolar et al., 2017). Jamali et al. (2015) studied the antimicrobial resistance genes and found that 6 out of 43 *L. monocytogenes* strains (14%) carried more than one resistance determinants. It is estimated that 83.3% and 91.7% tetracycline resistant isolates harbored *tetA* and *tetM* genes, respectively. Similarly, the presence of *penA* gene was reported in 71% of the penicillin-resistant isolates. Among the seven isolates showing phenotypic resistance to streptomycin, three harbored *strA* and one carried *strB*. In addition, 66.7% of the isolates contained *ampC* resistance gene and *vanA* was observed in 33.3% of vancomycin-resistant isolates. However, in the same study, resistance genes such as *cmlA*, *folR*, *vanB*, *tetB*, *tetC*, *tetL*, and *tetS* were not harbored in *L. monocytogenes* isolates. However, nonconformity between the phenotypic resistance pattern and detection of genes encoding to resistance in these isolates was observed. This variation is possibly due to the gene mutation associated with ribosomal protein or reduced permeability of the outer membrane proteins leading to this inconsistency. In *L. monocytogenes,* the overall development and increase of resistance to tetracycline were commonly reported due to the transfer of mobile genetic elements, namely transposons and plasmids, from *Enterococcus* or *Streptococcus*.

7 Antibiotic Resistance Through Efflux Pumps

Three efflux pumps operate in *L. monocytogenes*: the first pump participates in extruding antibiotics, ethidium bromide, and heavy metals (Mata et al., 2000); the other pump plays a vital role in extruding fluoroquinolone and, to some extent of

ethidium bromide and acridine orange (Godreuil et al., 2003); and the third pump expels fluoroquinolones (Guerin et al., 2014). The protein string of the multidrug efflux transporter of *Listeria* (*MdrL*) was found to have a high extent of similarity to the protein sequence of multidrug efflux transporter of *Bacillus subtilis* (*YfmO*) (Mata et al., 2000). An allele-alternated mutant of *MdrL* in *L. monocytogenes* was unsuccessful to drain ethidium bromide and showed higher sensitivity to heavy metals, cefotaxime, and macrolides. The active efflux pumps are involved in the adaptation of *L. monocytogenes* to the variable conditions of the environment (Mata et al., 2000).

Drug efflux is carried out by five families: (a) the major facilitator superfamily (MFS), (b) the resistance nodulation-cell division (RND), (c) the multidrug and toxic compound extrusion (MATE) families, (d) the small multidrug resistance, and (e) the ATP-binding cassette (ABC) family (Piddock, 2006). Godreuil et al. (2003) found that gene *Lde* (12 transmembrane domain) act as a membrane pump leading to the increased resistance to ciprofloxacin. The *Lde* protein shares 44% identity with *PmrA* from *S. pneumonia* and renders inactive transport leading to the efflux of ciprofloxacin from the cell (Gill et al., 1999). The insertional deactivation of *Lde* in *L. monocytogenes* results in increased sensitivity to fluoroquinolone (Godreuil et al., 2003). However, Jiang et al. (2012) in a study demonstrated that only the overexpression of *Lde* acts as the prime reason for ciprofloxacin resistance in two resistant strains of *L. monocytogenes*. Guerin et al. (2014) studied the novel mechanism of the MATE efflux pump involved in fluoroquinolone resistance in *L. monocytogenes*. Whole genome sequencing and evaluation of resistant strains revealed that overexpression of *FepA* (a new MATE efflux pump) is accountable for fluoroquinolone resistance in *L. monocytogenes*, and the other reason is the inactivation of local repressor (*FepR*). Genomic study (using Illumina HiSeq, 2000 of two *L. monocytogenes* strains) isolated from fried fish and salad revealed that efflux pump determinants, such as *mdrL* and *lde*, conferred resistance to macrolides and quinolone (Lim et al., 2016).

8 Biofilm Formation Ability of *L. monocytogenes*

Biofilm is defined as the association of different microbial communities anchored permanently to biotic or abiotic surrounding areas and is embedded in self-created extracellular component matrix (ECM) showing an altered phenotype in concern its growth rate and transcription of genes (Ripolles-Avila et al., 2018). Although *L. monocytogenes* survival and growth are reported in extreme conditions, this pathogen has the competence to form biofilms in food processing areas and contact surfaces, which subsequently leads to persistence of the pathogen and ensuing contamination of the product. *L. monocytogenes* contamination in food processing settings is encountered in various equipment and its accessories, namely packaging, slicing and dicing machines, gaskets, conveyor belts, containers and utensils such as

knives, tables, environment such as drains, and surface areas like walls and floors. Once this pathogen adheres and produces biofilms, it creates a reservoir of bacterium in processing areas showing increased resistance to sanitizers and disinfectants. Sources of *L. monocytogenes* presence in food-processing units could be mostly from raw materials, ingredients, water, human handling, and utensils. Several studies indicated that *L. monocytogenes* persists on the equipment like conveyer belts' surfaces which are difficult to sanitize and act as the contamination hot spots in the processing industries (Autio et al., 1999; Johansson et al., 1999; Miettinen et al., 1999; Tompkin, 2002). The probable explanations for the biofilm-based resistance in bacteria might be the decreased diffusion via extracellular matrix, modification of antibacterial drug through chemical or enzymatic activity, physiological alterations of the microorganisms due to their delay in growth rate, as well as the triggering of adhesion-specific antibiotic-resistant processes (Gilbert et al., 2002). Doijad et al. (2015) observed that among 32 of 1/2a serotype, 20 of 1/2b serotype, and 46 of 4b serotype, isolated from food and clinical settings, 6 strains belonging to serotype 1/2a and 3 strains of serotype 1/2b were strong biofilm formers, and none of the serotype belonging to 4b produced biofilms.

8.1 Nature of Biofilm Produced by *L. Monocytogenes* and Their Adherence to Various Surface Materials

A study on three-dimensional (3D) structure of biofilm produced by *L. monocytogenes* revealed that it consists of cells overlapped together with extracellular material attaching the cells in their positions (Marsh et al., 2003). However, in relation to other biofilm-forming bacteria, the cells associated with this biofilm are not embedded in extracellular matrix but instead connected with net-like extracellular matrix (Marsh et al., 2003). Analyses on the effect of various disinfectants against *L. monocytogenes* showed that the disinfectants were less effective on adherent bacteria compared to those in suspension (Stopforth et al., 2002). In addition, thick biofilms of *L. monocytogenes* are more resistant than thin biofilms when exposed to dodecylbenzene sulfonic acid and benzalkonium chloride (Frank & Koffi, 1990). Furthermore, *L. monocytogenes* in multispecies combination biofilm were more resistant than the biofilm consisting only *L. monocytogenes* (Norwood & Gilmour, 2000).

The hypothesis from the studies conducted in various laboratories on the effect of surface materials, namely stainless steel, rubber, glass, and polymers, on the attachment and development of biofilm in *L. monocytogenes* showed contradictory results when compared with the same surface materials used in the food industry. This might be due to the varying culturing conditions and/or differences in strains during the experimental period. Chavant et al. (2002) showed that stainless steel surface has high colonizing ability compared to polytetrafluoroethylene (PTFE), and poor adhesion of the pathogen was observed under low temperature (8 °C). In addition to hydrophobic property of the surface material, physicochemical properties of the organism also play vital role in adhesion process (Moretro & Langsrud, 2004).

8.2 Molecular Mechanisms Involved in the Biofilm Formation and Their Control Strategies

Studies at molecular level play an important role in understanding the mechanisms of the attachment and formation of biofilm in *L. monocytogenes*. The presence of flagella is considered as a vital factor in the initial adhesion of *L. monocytogenes* on the glass surfaces and stainless steel in relation to nonmotile cells. Studies on variations in protein patterns conducted by Tremoulet et al. (2002) showed that a total of 31 proteins differed between the biofilm raised and planktonic *L. monocytogenes*. Specifically, among the low expressed proteins at the time of biofilm growth, flagellin was one within the eight proteins. Taylor et al. (2002) described *RelA* and *Htp* proteins as engaged mainly against starvation and nutrient constraints in *L. monocytogenes*. It is observed that *relA* and *htp* mutants and transposon insertion mutants diminished the growth after surface attachment. Few factors such as *LMO1288*, which is a homologous protein similar to *LuxS* and participates in biofilm regulation, and *LMO0842*, similar to *Esp* protein of *Enterococcus faecalis*, were involved in the biofilm development (Chen et al., 2002). Popowska et al. (2017) observed that class I internalin (*InlL*) was potentially involved in the attachment process and was described to be a new molecular element assisting in colonization. Genetic studies demonstrated that *InlL* was involved in initial bacterial anchorage, besides sedentary development in *L. monocytogenes*. Physical methods such as electric field, pulsed electric field with 40 KV cm^{-1}, and ultrasound treatment cannot reduce sessile cells completely (Del Pozo et al., 2008). However, physical methods in association with chemical or biocides will assist in preventing biofilm formation. Chlorine, hydrogen peroxide, peroctanoic acid, titanium dioxide, UV light irradiation, etc., showed significant decrease in sessile cells of *L. monocytogenes* (Fatemi & Frank, 1999; Robbins et al., 2005). Hydrogen peroxide, iodophor, sodium hypochlorite, and benzalkonium chloride coupled with steam were proven as biofilm inhibitors of *L. monocytogenes* (Ban & Kang, 2016). Plant-based extracts such as eugenol, cinnamaldehyde, carvacrol, and thymol inactivated biofilm synthesis on polystyrene plates and steel strips at 4 °C, 25 °C, and 37 °C. Microbial-based products such as bacteriocins derived from *Enterococcus casseliflavus*, *Lactobacillus plantarum*, and *Lactococcus lactis* had the ability to control biofilms generated by *L. monocytogenes* (Minei et al., 2008; Garcia-Almendarez et al., 2008). Among the various approaches tested to control biofilms, sodium hypochlorite and peroxyacetic acid are being used under good manufacturing practices (GMP) in the food processing industry to reduce and discourage biofilm formation (Winkelstroter et al., 2014).

9 Conclusion

L. monocytogenes is a serious foodborne microbe posing risk to public health and causing economic concern. Over the years, *L. monocytogenes* resistance to the first choice of treatment option, namely tetracycline, ampicillin, gentamicin, streptomycin, and trimethoprim, has become a serious concern to public health. Therefore,

tracking the antibiotic resistance pattern and developing therapeutic alternatives is crucial in treating listeriosis. Active surveillance on the antibiotic susceptibility and resistant profiles of *L. monocytogenes* in fish and fishery environment needs to be focused for generating baseline data in the fisheries sector. Novel approaches like the development of phage therapy to control multidrug resistant *L. monocytogenes* in fish and fishery environment need to be explored.

References

Abdollahzadeh, E., Ojagh, S. M., Hosseini, H., Ghaemi, E. A., Irajian, G., & Heidarlo, M. N. (2016). Antimicrobial resistance of *listeria monocytogenes* isolated from seafood and humans in Iran. *Microbial Pathogenesis, 100*, 70–74.

Al-Gburi, N. M. (2020). Detection and pathogenicity of *listeria monocytogenes* in common carp (*Cyprinus carpio*) fish in Baghdad. *Iraqi Journal of Veterinary Sciences, 34*(2), 311–316.

Alonso-Hernando, A., Prieto, M., García-Fernández, C., Alonso-Calleja, C., & Capita, R. (2012). Increase over time in the prevalence of multiple antibiotic resistance among isolates of *listeria monocytogenes* from poultry in Spain. *Food Control, 23*(1), 37–41.

Amagliani, G., Brandi, G., & Schiavano, G. F. (2012). Incidence and role of *salmonella* in seafood safety. *Food Research International, 45*(2), 780–788.

Autio, T., Hielm, S., Miettinen, M., Sjöberg, A. M., Aarnisalo, K., Björkroth, J., Mattila-Sandholm, T., & Korkeala, H. (1999). Sources of *listeria monocytogenes* contamination in a cold-smoked rainbow trout processing plant detected by pulsed-field gel electrophoresis typing. *Applied and Environmental Microbiology, 65*(1), 150–155.

Ban, G. H., & Kang, D. H. (2016). Effect of sanitizer combined with steam heating on the inactivation of food-borne pathogens in a biofilm on stainless steel. *Food Microbiology, 55*, 47–54.

Basha, K. A., Kumar, N. R., Das, V., Reshmi, K., Rao, B. M., Lalitha, K. V., & Joseph, T. C. (2019). Prevalence, molecular characterization, genetic heterogeneity and antimicrobial resistance of *listeria monocytogenes* associated with fish and fishery environment in Kerala, India. *Letters in Applied Microbiology, 69*(4), 286–293.

Basha, K. A., Nadella, R. K., Murugadas, V., Joseph, T. C., & Rao, B. M. (2020). Molecular detection of antibiotic resistance genes in multidrug resistant *listeria monocytogenes* isolated from fish retail markets. *Fishtech Reporter, 6*(2), 5–8.

Beleneva, I. A. (2011). Incidence and characteristics of *Staphylococcus aureus* and *listeria monocytogenes* from the Japan and South China seas. *Marine Pollution Bulletin, 62*(2), 382–387.

Bhat, S. A., Willayat, M. M., Roy, S. S., Bhat, M. A., Shah, S. N., Ahmed, A., Maqbool, S., & Ganayi, B. A. (2013). Isolation, molecular detection and antibiogram of *listeria monocytogenes* from human clinical cases and fish of Kashmir, India. *Comparative Clinical Pathology, 22*(4), 661–665.

Budzińska, K., Wroński, G., & Szejniuk, B. (2012). Survival time of bacteria *listeria monocytogenes* in water environment and sewage. *Polish Journal of Environmental Studies, 21*(1).

Charpentier, E., & Courvalin, P. (1999). Antibiotic resistance in *Listeria* spp. *Antimicrobial Agents and Chemotherapy, 43*(9), 2103–2108.

Chavant, P., Martinie, B., Meylheuc, T., Bellon-Fontaine, M. N., & Hebraud, M. (2002). *Listeria monocytogenes* LO28: Surface physico-chemical properties and ability to form biofilms at different temperatures and growth phases. *Applied and Environmental Microbiology, 68*(2), 728–737.

Chen, X., Schauder, S., Potier, N., Van Dorsselaer, A., Pelczer, I., Bassler, B. L., & Hughson, F. M. (2002). Structural identification of a bacterial quorum-sensing signal containing boron. *Nature, 415*(6871), 545–549.

Chen, B. Y., Pyla, R., Kim, T. J., Silva, J. L., & Jung, Y. S. (2010). Antibiotic resistance in *listeria* species isolated from catfish fillets and processing environment. *Letters in Applied Microbiology, 50*(6), 626–632.

Chen, M., Cheng, J., Wu, Q., Zhang, J., Chen, Y., Xue, L., Lei, T., Zeng, H., Wu, S., Ye, Q., & Bai, J. (2018). Occurrence, antibiotic resistance, and population diversity of *listeria monocytogenes* isolated from fresh aquatic products in China. *Frontiers in Microbiology, 9*, 2215.

Committee of the Antibiotics of the French Society of Microbiology (CA-SFM). (2010). https://www.sfm-microbiologie.org/UserFiles/file/CASFM/casfm_2010.pdf. Accessed 1 Mar 2021.

Del Pozo, J. L., Rouse, M. S., & Patel, R. (2008). Bioelectric effect and bacterial biofilms. A systematic review. *The International Journal of Artificial Organs, 31*(9), 786–795.

Dhanashree, B., Otta, S. K., Karunasagar, I., & Goebel, W. (2003). Incidence of *listeria* spp. in clinical and food samples in Mangalore, India. *Food Microbiology, 20*(4), 447–453.

Doijad, S. P., Barbuddhe, S. B., Garg, S., Poharkar, K. V., Kalorey, D. R., Kurkure, N. V., Rawool, D. B., & Chakraborty, T. (2015). Biofilm-forming abilities of *listeria monocytogenes* serotypes isolated from different sources. *PLoS One, 10*(9), e0137046.

Duffes, F. (1999). Improving the control of *listeria monocytogenes* in cold smoked salmon. *Trends in Food Science and Technology, 10*(6–7), 211–216.

Escolar, C., Gómez, D., del Carmen Rota García, M., Conchello, P., & Herrera, A. (2017). Antimicrobial resistance profiles of *listeria monocytogenes* and *listeria innocua* isolated from ready-to-eat products of animal origin in Spain. *Foodborne Pathogens and Disease, 14*(6), 357–363.

Fallah, A. A., Saei-Dehkordi, S. S., & Mahzounieh, M. (2013). Occurrence and antibiotic resistance profiles of *listeria monocytogenes* isolated from seafood products and market and processing environments in Iran. *Food Control, 34*(2), 630–636.

Fatemi, P., & Frank, J. F. (1999). Inactivation of *listeria monocytogenes*/*pseudomonas* biofilms by peracid sanitizers. *Journal of Food Protection, 62*(7), 761–765.

Flamm, R. K., Hinrichs, D. J., & Thomashow, M. F. (1984). Introduction of pAM beta 1 into *listeria monocytogenes* by conjugation and homology between native *L. monocytogenes* plasmids. *Infection and Immunity, 44*(1), 157–161.

Frank, J. F., & Koffi, R. A. (1990). Surface-adherent growth of *listeria monocytogenes* is associated with increased resistance to surfactant sanitizers and heat. *Journal of Food Protection, 53*, 550–554.

García-Almendárez, B. E., Cann, I. K., Martin, S. E., Guerrero-Legarreta, I., & Regalado, C. (2008). Effect of *Lactococcus lactis* UQ2 and its bacteriocin on *listeria monocytogenes* biofilms. *Food Control, 19*(7), 670–680.

Gawade, L., Barbuddhe, S. B., & Bhosle, S. (2010). Isolation and confirmation of *listeria* species from seafood off Goa region by polymerase chain reaction. *Indian Journal of Microbiology, 50*(4), 385–389.

Gilbert, P., Allison, D. G., & McBain, A. J. (2002). Biofilms *in vitro* and *in vivo*: Do singular mechanisms imply cross-resistance? *Journal of Applied Microbiology, 92*, 98S–110S.

Gill, M. J., Brenwald, N. P., & Wise, R. (1999). Identification of an efflux pump gene, pmrA, associated with fluoroquinolone resistance in *Streptococcus pneumoniae*. *Antimicrobial Agents and Chemotherapy, 43*(1), 187–189.

Godreuil, S., Galimand, M., Gerbaud, G., Jacquet, C., & Courvalin, P. (2003). Efflux pump Lde is associated with fluoroquinolone resistance in *listeria monocytogenes*. *Antimicrobial Agents and Chemotherapy, 47*(2), 704–708.

Granier, S. A., Moubareck, C., Colaneri, C., Lemire, A., Roussel, S., Dao, T. T., Courvalin, P., & Brisabois, A. (2011). Antimicrobial resistance of *listeria monocytogenes* isolates from food and the environment in France over a 10-year period. *Applied and Environmental Microbiology, 77*(8), 2788–2790.

Guerin, F., Galimand, M., Tuambilangana, F., Courvalin, P., & Cattoir, V. (2014). Over-expression of the novel MATE fluoroquinolone efflux pump FepA in *listeria monocytogenes* is driven by inactivation of its local repressor FepR. *PLoS One, 9*, e106340.

Hansen, C. H., Vogel, B. F., & Gram, L. (2006). Prevalence and survival of *listeria monocytogenes* in Danish aquatic and fish-processing environments. *Journal of Food Protection, 69*(9), 2113–2122.

Hof, H., Nichterlein, T., & Kretschmar, M. (1997). Management of listeriosis. *Clinical Microbiology Reviews, 10*(2), 345–357.

Iwamoto, M., Ayers, T., Mahon, B. E., & Swerdlow, D. L. (2010). Epidemiology of seafood-associated infections in the United States. *Clinical Microbiology Reviews, 23*(2), 399–411.

Jamali, H., Paydar, M., Ismail, S., Looi, C. Y., Wong, W. F., Radmehr, B., & Abedini, A. (2015). Prevalence, antimicrobial susceptibility and virulotyping of *listeria* species and *listeria monocytogenes* isolated from open-air fish markets. *BMC Microbiology, 15*(1), 1–7.

Jiang, X., Zhou, L., Gao, D., Wang, Y., Wang, D., Zhang, Z., Chen, M., Su, Y., Li, L., Yan, H., & Shi, L. (2012). Expression of efflux pump gene lde in ciprofloxacin resistant food-borne isolates of *listeria monocytogenes*. *Microbiology and Immunology, 56*(12), 843–846.

Johansson, T., Rantala, L., Palmu, L., & Honkanen-Buzalski, T. (1999). Occurrence and typing of *listeria monocytogenes* strains in retail vacuum-packed fish products and in a production plant. *International Journal of Food Microbiology, 47*, 111–119.

Lim, S. Y., Yap, K. P., & Thong, K. L. (2016). Comparative genomics analyses revealed two virulent *listeria monocytogenes* strains isolated from ready-to-eat food. *Gut Pathogens, 8*(1), 1–8.

Marsh, E. J., Luo, H., & Wang, H. (2003). A three-tiered approach to differentiate *listeria monocytogenes* biofilm-forming abilities. *FEMS Microbiology Letters, 228*(2), 203–210.

Mata, M. T., Baquero, F., & Perez-Diaz, J. C. (2000). A multidrug efflux transporter in *Listeria monocytogenes*. *FEMS Microbiology Letters, 187*(2), 185–188.

Miettinen, H., & Wirtanen, G. (2005). Prevalence and location of *listeria monocytogenes* in farmed rainbow trout. *International Journal of Food Microbiology, 104*(2), 135–143.

Miettinen, M. K., Bjorkroth, K. J., & Korkeala, H. J. (1999). Characterization of *listeria monocytogenes* from an ice cream plant by serotyping and pulsed-field gel electrophoresis. *International Journal of Food Microbiology, 46*(3), 187–192.

Minei, C. C., Gomes, B. C., Ratti, R. P., D'angelis, C. E., & De Martinis, E. C. (2008). Influence of peroxyacetic acid and nisin and coculture with *enterococcus faecium* on *listeria monocytogenes* biofilm formation. *Journal of Food Protection, 71*(3), 634–638.

Moretro, T., & Langsrud, S. (2004). *Listeria monocytogenes*: Biofilm formation and persistence in food-processing environments. *Biofilms, 1*(2), 107.

Norwood, D. E., & Gilmour, A. (2000). The growth and resistance to sodium hypochlorite of *listeria monocytogenes* in a steady-state multispecies biofilm. *Journal of Applied Microbiology, 88*(3), 512–520.

Obaidat, M. M., Bani Salman, A. E., Lafi, S. Q., & Al-Abboodi, A. R. (2015). Characterization of *listeria monocytogenes* from three countries and antibiotic resistance differences among countries and *listeria monocytogenes* serogroups. *Letters in Applied Microbiology, 60*(6), 609–614.

Papadopoulos, T., Abrahim, A., Sergelidis, D., Kirkoudis, I., & Bitchava, K. (2010). Prevalence of *listeria* spp. in freshwater T\s\v (*Oncorhynchus my kiss* and *Carassius gibelio*) and the environment of fish markets in northern Greece. *Journal of the Hellenic Veterinary Medical Society, 61*(1), 15–22.

Piddock, L. J. V. (2006). Clinically relevant chromosomally encoded multidrug resistance efflux pumps in bacteria. *Clinical Microbiology Reviews, 19*(2), 382–402.

Popowska, M., Krawczyk-Balska, A., Ostrowski, R., & Desvaux, M. (2017). InlL from *listeria monocytogenes* is involved in biofilm formation and adhesion to mucin. *Frontiers in Microbiology, 8*, 660.

Poyart-Salmeron, C., Carlier, C., Trieu-Cuot, P., Courvalin, P., & Courtieu, A. L. (1990). Transferable plasmid-mediated antibiotic resistance in *listeria monocytogenes*. *The Lancet, 335*(8703), 1422–1426.

Ramaswamy, V., Cresence, V. M., Rejitha, J. S., Lekshmi, M. U., Dharsana, K. S., Prasad, S. P., & Vijila, H. M. (2007). *Listeria* – Review of epidemiology and pathogenesis. *Journal of Microbiology Immunology and Infection, 40*(1), 4.

Read, A. F., & Woods, R. J. (2014). Antibiotic resistance management. *Evolution, Medicine and Public Health, 1*, 147.

Rezai, R., Ahmadi, E., & Salimi, B. (2018). Prevalence and antimicrobial resistance profile of *listeria* species isolated from farmed and on-sale rainbow trout (*Oncorhynchus mykiss*) in Western Iran. *Journal of Food Protection, 81*(6), 886–891.

Rhoades, J. R., Duffy, G., & Koutsoumanis, K. (2009). Prevalence and concentration of verocytotoxigenic *Escherichia coli*, *salmonella enterica* and *listeria monocytogenes* in the beef production chain: A review. *Food Microbiology, 26*(4), 357–376.

Ripolles-Avila, C., Hascoët, A. S., Guerrero-Navarro, A. E., & Rodríguez-Jerez, J. J. (2018). Establishment of incubation conditions to optimize the *in vitro* formation of mature *listeria monocytogenes* biofilms on food-contact surfaces. *Food Control, 92*, 240–248.

Robbins, J. B., Fisher, C. W., Moltz, A. G., & Martin, S. E. (2005). Elimination of *listeria monocytogenes* biofilms by ozone, chlorine, and hydrogen peroxide. *Journal of Food Protection, 68*(3), 494–498.

Rodas-Suárez, O. R., Flores-Pedroche, J. F., Betancourt-Rule, J. M., Quiñones-Ramirez, E. I., & Vázquez-Salinas, C. (2006). Occurrence and antibiotic sensitivity of *listeria monocytogenes* strains isolated from oysters, fish, and estuarine water. *Applied and Environmental Microbiology, 72*(11), 7410–7412.

Skowron, K., Kwiecińska-Piróg, J., Grudlewska, K., Świeca, A., Paluszak, Z., Bauza-Kaszewska, J., Wałecka-Zacharska, E., & Gospodarek-Komkowska, E. (2018). The occurrence, transmission, virulence and antibiotic resistance of *listeria monocytogenes* in fish processing plant. *International Journal of Food Microbiology, 282*, 71–83.

Skowron, K., Wiktorczyk, N., Grudlewska, K., Wałecka-Zacharska, E., Paluszak, Z., Kruszewski, S., & Gospodarek-Komkowska, E. (2019). Phenotypic and genotypic evaluation of *listeria monocytogenes* strains isolated from fish and fish processing plants. *Annals of Microbiology, 69*(5), 469–482.

Stopforth, J. D., Samelis, J., Sofos, J. N., Kendall, P. A., & Smith, G. C. (2002). Biofilm formation by acid-adapted and nonadapted *listeria monocytogenes* in fresh beef decontamination washings and its subsequent inactivation with sanitizers. *Journal of Food Protection, 65*(11), 1717–1727.

Swaminathan, B., & Gerner-Smidt, P. (2007). The epidemiology of human listeriosis. *Microbes and Infection, 9*(10), 1236–1243.

Taylor, C. M., Beresford, M., Epton, H. A., Sigee, D. C., Shama, G., Andrew, P. W., & Roberts, I. S. (2002). *Listeria monocytogenes* relA and hpt mutants are impaired in surface-attached growth and virulence. *Journal of Bacteriology, 184*(3), 621–628.

Teuber, M. (1999). Spread of antibiotic resistance with food-borne pathogens. *Cellular and Molecular Life Sciences, 56*, 755–763.

Tompkin, R. B. (2002). Control of *listeria monocytogenes* in the food-processing environment. *Journal of Food Protection, 65*(4), 709–725.

Tremoulet, F., Duche, O., Namane, A., Martinie, B., European Listeria Genome Consortium, & Labadie, J. C. (2002). Comparison of protein patterns of *listeria monocytogenes* grown in biofilm or in planktonic mode by proteomic analysis. *FEMS Microbiology Letters, 210*(1), 25–31.

White, D. G., Zhao, S., Simjee, S., Wagner, D. D., & McDermott, P. F. (2002). Antimicrobial resistance of food-borne pathogens. *Microbes and Infection, 4*(4), 405–412.

Wieczorek, K., & Osek, J. (2017). Prevalence, genetic diversity and antimicrobial resistance of *listeria monocytogenes* isolated from fresh and smoked fish in Poland. *Food Microbiology, 64*, 164–171.

Winkelstroter, L. K., dos Reis Teixeira, F. B., Silva, E. P., Alves, V. F., & De Martinis, E. C. P. (2014). Unraveling microbial biofilms of importance for food microbiology. *Microbial Ecology, 68*(1), 35–46.

Evolution and the Role of SXT-Related Integrative Conjugative Elements in Multidrug-Resistant *Vibrio cholerae*

V. A. Minimol, Pankaj Kishore, and Mukteswar Prasad Mothadaka

Contents

Abstract

The emergence of various classes of antibiotics over the years and their widespread use in clinical and food sectors resulted in antimicrobial resistance (AR) among several food-borne pathogens. Studies have shown that both chromosomal and extrachromosomal factors were responsible for the antimicrobial resistance in these pathogenic bacteria. The MGEs (mobile genetic elements) of integrative conjugative elements (ICEs) play an important role in the gene

V. A. Minimol (✉) · M. P. Mothadaka
Microbiology, Fermentation and Biotechnology Division, ICAR-Central Institute of Fisheries Technology (ICAR-CIFT), Cochin, Kerala, India
e-mail: minimol.a@icar.gov.in; prasadmm@hotmail.com

P. Kishore
ICAR-Central Institute of Fisheries Technology, Cochin, Kerala, India
e-mail: Pankaj.Kishore@icar.gov.in

M. P. Mothadaka et al. (eds.), *Handbook on Antimicrobial Resistance*,
https://doi.org/10.1007/978-981-19-9279-7_22

transfer through horizontal means for the distribution of antibiotic resistance determinants between different pathogens including *Vibrio cholerae*. It was reported that sulfamethoxazole-trimethoprim (SXT) and SXT-related ICEs provide resistance to multiple antibiotics in both clinical and environmental isolates of *V. cholerae.* This chapter describes the evolution and mechanism of SXT and related ICEs in antibiotic resistance of *V. cholerae.*

Keywords

V. cholerae · Antibiotic resistance · Diarrhea · Integrative conjugative elements · SXT element

1 Introduction

The human bacterial pathogen *Vibrio cholerae* is one of the major significant public health bacteria causing acute diarrheal disease named cholera, which is commonly distributed in different aquatic environments (Finkelstein, 1996). The virulence nature in *V. cholerae* is contributed by genomic determinants, viz., CT (cholera toxin) and TCP (toxin-coregulated pilus) of VPI I (Vibrio pathogenicity island I) (Faruque et al., 2003). The pathogenic strains of *V. cholerae* of load about 10^6–10^8 CFU/g invade and colonize inside the small intestine and produce toxin named cholera toxin (Chen, 2017). TCP helps in the aggregation and attachment of bacterial cells in the smaller intestine of humans. The secretion of cholera toxin in the intestine allows a massive fluid efflux resulting in dehydration, watery diarrhea, and vomiting ultimately causing mortality in the untreated people. Among the 20 serotypes of *V. cholerae*, the epidemic and pandemic outbreak was caused by two main serogroups: *V. cholerae* O1 and *V. cholerae* O139. The last (seventh) pandemic outbreak was due to the El Tor biotype of *V. cholerae* serogroup O1 (Chowdhury et al., 2017).

Generally, antibiotics are not required for the treatment of gastroenteritis, whereas it is required in case of systemic infections. The treatment of intestinal infection of *V. cholerae* is by oral rehydration therapy by taking good amount of drinking water, juices, and oral rehydration salt solutions. However, the combined treatment of intravenous administration of antibiotics and oral therapy can be more effective in reducing the symptoms (Thiagarajah & Verkman, 2005).

2 Antibiotic Therapy and Evolution of Antimicrobial Resistance in *Vibrio cholerae*

Antibiotics which are commonly used for the clinical treatment of cholera include tetracycline, azithromycin, and fluoroquinolones (Saha et al., 2006). However, increased drug resistance in this bacterium failed the antibiotic therapy to cure the diarrheal diseases in cholera patients. In the 1960s, streptomycin and tetracycline were proven as helping in achieving a higher recovery rate in the treatment of cholera

in adults, and furazolidone was used in treating cholera in children (Kitaoka et al., 2011). In the 1970s, the candidate of choice was sulfamethoxazole-trimethoprim (SXT). The other antibiotics of choice were chloramphenicol, azithromycin, and ciprofloxacin (Waldor et al., 1996).

It was suggested that the mega plasmid of incompatibility complex C (incC) was liable for tetracycline-resistant pattern in *V. cholerae* (Das et al., 2020). *V. cholerae* serotypes (El Tor biotype and serogroup O139) resistant to tetracycline were isolated thereafter. Since the 1990s, ampicillin, nalidixic acid, chloramphenicol, and tetracycline resistance has been reported more from clinical pathogenic isolates of *V. cholerae* (Das et al., 2020). Later in the 2000s, the insertion sequences facilitated antimicrobial resistance dissemination in *V. cholerae*, and the antibiotic resistance to rifampicin, carbenicillin, colistin, and ceftriaxone was frequently reported across different parts of the world (Hochhut et al., 2001a). The development of resistance towards tetracycline started with the presence of resistance gene *tetG* in the 1990s, and several other resistance genes encoding functions such as *tetA*, *teR*, etc. have emerged thereafter resulting in increased fatality rates in cholera patients (Roychowdhury et al., 2008). From 2010 onwards, the plasmid-mediated resistance was reported in *V. cholerae*, and the major antibiotics of this group includes macrolides, polymyxin, aminoglycosides, aztreonam, etc. (Heidelberg et al., 2000; Carraro et al., 2016).

Multidrug-resistant (MDR) *V. cholerae* strains were reported since the 1980s, and a majority of them have been isolated from Asian countries, viz., India, Indonesia, Vietnam, Bangladesh, etc. (Barua, 1992). The MDR *V. cholerae* exhibited a higher resistance towards streptomycin, chloramphenicol, and tetracycline (Verma et al., 2019). Drug resistance was also reported in O1 classical and El Tor, non-O1, O139, and non-O139 bacterial strains (Garg et al., 2000). Kitaoka et al. (2011) have isolated *V. cholerae* from different sources and studied the antimicrobial resistance pattern to 23 different antibiotics and reported multidrug-resistant pattern in these isolates. Among the *V. cholerae* serogroup O139, the MDR strain was first reported in 1996 (Yu et al., 2012). The isolation rate of MDR *V. cholerae* varied from 6.4% to 100%, and this has been reported from different parts of the world including Pakistan, Nepal, and Bangladesh (Chandrasekhar et al., 2008). Recently isolates belonging to *V. cholerae* serogroups O96, O1, O139, and O4 were also reported for extremely drug-resistant (XDR) and MDR category (Kaushik et al., 2019). The resistance factors in MDR and XDR *V. cholerae* are mobile in nature, and it accounts for 4–5% of the genomic content. It is reported that several β-lactamases, encoded genes, and multiple resistance traits against aminoglycoside antibiotics are detected in the genetic elements of both XDR and MDR *V. cholerae* (Verma et al., 2019).

The resistance pattern to multiple antibiotic classes in *V. cholerae* reported since the 1990s is ascribed towards the harboring of SXT (sulfamethoxazole-trimethoprim) element that carries antibiotic resistance to sulfamethoxazole, trimethoprim, and streptomycin (Hochhut & Waldor, 1999). Subsequently, several isolates of environmental origin were also found to possess SXT element and exhibited

resistance against routinely used antibiotics (ampicillin, streptomycin, SXT, and furazolidone) (Opintan et al., 2008). The discovery of integrative and conjugative elements (ICEs) has given more insights to the antimicrobial resistance mechanism in *V. cholerae*.

3 Antimicrobial Resistance Mechanisms in *Vibrio cholerae*

V. cholerae genome is alienated into small- and large-sized circular chromosomes of approximately 1070 and 3000 bp, and it contains approximately 4000 ORFs to perform all the metabolic and cellular functions (Lin et al., 2018). The flexible gene pool of *V. cholerae* acquired by HGT (horizontal gene transfer) constituted around 5–10% of its genome which enables its survival in adverse environmental conditions (Verma et al., 2019). The flexible pool consisted of plasmids, insertion sequences, transposons, and ICEs (integrative conjugative elements) (Davies & Davies, 2010). The genomic islands present in Chromosome 1 of *V. cholerae* are responsible for colonization, virulence, and toxin production, while the genomic islands in Chromosome 2 are involved in antimicrobial resistance in *V. cholerae* (Pant et al., 2020). Molecular studies have shown that acquisition of AMR is independent of its pathogenicity, virulence, and geographical location. Generally, a single isolate can possess resistance traits up to 40 genes in their genome and can produce resistance against different classes of antibiotics.

The chemical modification via phosphorylation, acetylation, and glycosylation of antibiotics leading to their inactivation is reported in *V. cholerae* (Das et al., 2020). Antibiotics of choice for chemical modification include chloramphenicol, fosfomycin, fluoroquinolone, streptomycin, and aminoglycosides. The point mutation in the target molecule can alter the specificity of interaction of antibiotics with the target site which leads to resistance by changing the composition of target site. For example, quinolone resistance is acquired through point mutation in *parC* and *gyrB* genes which inhibit the DNA replication (Jacoby, 2005); rifampicin resistance by changing *rpoB*-encoded protein structure (Alifano et al., 2015); β-lactam resistance by altering the *pbp* gene function (Spratt, 1994); and SXT resistance by structural changes in the *akatG*, *embB*, and *mshA* region (Das et al., 2020). It is reported that the mutation rate in *V. cholerae* is found higher when compared to the same in *E. coli* genetic material (Towner et al., 1980). The mutation in the genes of *V. cholerae* causes resistance towards quinolones by two different mechanisms, viz., inhibition of cell wall synthesis and inhibition of DNA replication (Kim et al., 2010). The studies have shown that the drug resistance developed in *V. cholerae* isolates is also due to the mutation of target molecules in enzymes (*RpoB*, DNA gyrase, topoisomerase) and ribosomal proteins (Alekshun & Levy, 2007). The inactivation of antibiotics can also be attributed to the hydrolysis of active molecule including penicillins, cephalosporins, carbapenems, and monobactams by beta-lactamase enzyme (Bush et al., 1995). Inhibition of macrolide macrocycle lactone ring, fosfomycin epoxide, and bacitracin undecaprenyl pyrophosphate by hydrolysis enzymes was also reported in drug resistance (Das et al., 2020). ATP-binding efflux

cassette provides resistance to different antimicrobials such as ciprofloxacin, tetracycline, doxorubicin, and norfloxacin (Huda et al., 2003). Most of *V. cholerae* possess mobile integrons for the rapid transfer of antibiotic resistance cassettes (Kitaoka et al., 2011). Integrons are associated with transposon, for example, Class 1 integrons and Class 2 integrons which are commonly seen paired with Tn402 transposon and transposon Tn2, respectively (Hall & Collis, 1998). The conjugative plasmids are also able to transfer the resistance traits during conjugation between bacterial cells. Studies have shown that tetracycline-resistant *V. cholerae* strains are transferable to K-12 bacterial strain of *E. coli*, through the process of conjugative transfer of plasmids, and the *E. coli* K-12 strain carries a single plasmid (Hedges & Jacob, 1975). It is reported that, a single plasmid can hold resistant traits for more than six antibiotics that include ampicillin, chloramphenicol, gentamicin, streptomycin, and tetracycline in *V. cholerae* (Das et al., 2020).

4 The Evolution of Integrative Conjugative (IC) Elements in *Vibrio cholerae*

ICEs are the integral part of MGEs which transfer the resistance elements both vertically and horizontally to the host bacteria via a unique integration, maintenance, and transfer mechanism through site-specific recombination event of the Type 4 secretion system (Brown-Jaque et al., 2015). These ICEs also have the ability to carry virulence-related markers and other metabolic factors among the bacteria (Juhas et al., 2009). The evolution and the functional role of ICEs were revealed recently with the advent of whole genome sequencing. Unlike conjugative plasmids, ICEs are protected from segregational loss during cell division and often possess the characteristics of both transposons and prophages. Prior to ICEs, the major mobile genetic elements were plasmids and bacteriophages. ICEs have the characteristics of plasmids, bacteriophages, and transposons. They are similar to transposons as both elements possess unique site for integration in the host cell (Johnson & Grossman, 2015). Both plasmids and ICEs are self-stimulating in nature during conjugation process (Bañuelos-Vazquez et al., 2017). ICEs also possess phage-like regulatory genes (Burrus & Waldor, 2004). The term ICE came to existence in 2002 (Burrus et al., 2002). Before the introduction of ICEs, the conjugative plasmids were described, and the first was isolated from *Enterococcus faecalis* in 1980 (Tomita et al., 1996). Before 2002, these classes of mobile genetic elements were limited to eubacterial subdivisions. However, several ICEs have been emerged from other bacterial subdivisions, including α-proteobacteria, β-proteobacteria, and γ-proteobacteria (Burrus et al., 2006b). About 500 ICEs evolved till date which were categorized into different families based on their specificity of integration site and similarities in their integrase enzyme. Among these, SXT/R391 forms the largest group comprising 89 ICEs. The prototype of ICEs found in *V. cholerae* is SXT/R391 family. The other prototype of ICEs includes Tn*916*, ICE*clc*, ICE*St1*/ICE*St3*, and ICE*Bs1* family which are found in various Gram-negative bacteria (Burrus et al., 2006b).

ICEs were first described during the evolution of the seventh pandemic outbreak in the 1980s and were designated as ICE*Vch*Ind5, which is found in all the seventh pandemic clones of serogroup O1 El Tor *V. cholerae* (Ramamurthy et al., 2019). The SXT element was the second ICEs which was evolved in the year 1992 in pathogenic *V. cholerae* O139 isolate (Taviani et al., 2009). It is believed that both CE*Vch*Ind5 and SXT evolved from environmental vibrios. Later several other Gram-negative species including *Proteus* and *Shewanella* were found to carry SXT/R391 ICEs (Li et al., 2016). However, the natural spread of these ICEs was not possible due to the limited ability of horizontal gene transfer. It was observed that periplasmic fractions of DNA endonuclease *IdeA* gene encode the resistance genes in the ICEs of these strains.

5 SXT (Sulfamethoxazole-Trimethoprim)-Related ICEs in Multidrug-Resistant *Vibrio cholerae*

These are conjugable, self-transmissible, integrating elements which are also called SXT constins. The SXT-resistant element of *V. cholerae* is responsible for the drug resistance to three antibiotics, namely, streptomycin, sulfamethoxazole, and trimethoprim (Iwanaga et al., 2004). It was reported first in *V. cholerae* strain O139 MO10 serogroup in 1992 in Chennai (Wozniak et al., 2009). The ICEs carrying the resistance gene in this strain were denoted as SXT-resistant MO10, and the variant of SXT-resistant MO10 was found in many *V. cholerae* O1 strains called SXTET which showed drug resistance towards sulfamethoxazole, trimethoprim, chloramphenicol, and streptomycin (Saha & Singh, 2020). However, the resistance determinant for tetracycline was also located within the SXT element. Further, the SXT-related ICEs were reported in *Vibrio* species isolated from Africa even before the occurrence of SXTMO10 (Burrus et al., 2006b). The extensive distribution of SXT elements in isolates of *V. cholerae* from both clinical and non-toxigenic environmental isolates of Asian and African countries was reported recently. ICE*Vch*Ind1 ICE in *V. cholerae* El Tor O1 was identified, and later the presence of the same was reported in *V. cholerae* O139 isolate by Hochhut et al. (2001b), and both possessed a common precursor. The antibiotic-sensitive O139 isolate harbored a new ICE derived from SXTMO10 called ICEVchHKo1, and the antibiotic resistance gene of SXTMO10 was absent.

Among the 89 SXT-related ICEs identified, 30 were isolated from both clinical and environmental *V. cholerae* strains. ICEs sequence data related to six SXT isolates (SXTMO10, ICE*Vch*Ind (4, 5), ICE*Vch*Ban (9, 10), and ICE*Vch*Ind5) are available in the public domain out of 15 SXT-related ICEs identified from India during the period 1992 to 2001 (Siriphap et al., 2017). Apart from these, SXT-related ICEs were also found in other bacteria. For example, ICE*Pal*Ban1, a ICE*Vch*Ind1-related element, was present in *Providencia alcalifaciens* of clinical origin in Bangladesh in 1999 (Wozniak et al., 2009). Interestingly, Burrus et al. (2006b) identified a new SXT-related ICE which does not have the gene for conferring antibiotic resistance from *V. cholerae* isolate of environmental origin from Mexico.

This new ICE is termed as ICE*Vch*Mex1, which is quite diverse from SXTMO10 and ICE*Vch*Ind1.

The R391 called conjugative element originally described as R plasmid was first isolated from *Providencia rettgeri* in 1970 and was assigned to a new plasmid incompatibility group *IncJ* (Hochhut et al., 2001a). This confers resistance to kanamycin and mercury. The other members that belong to IncJ group which mediate resistance to kanamycin and mercury include R392, R397, R705, R706, R748, and R749 (Hochhut et al., 2001b). However, R997 group of *Proteus mirabilis* confers resistance to ampicillin (Ryan et al., 2017), pMERPH of *Shewanella putrefaciens* confers resistance to mercury (Böltner & Osborn, 2004), and pJY1 of *V. cholerae* El Tor confers resistance to sulfonamide, chloramphenicol, and streptomycin (Nugent, 1981). The presence of R391 in the extrachromosomal DNA of *E. coli* chromosome with a unique integration site between uxuA and serB was reclassified from R plasmid category to conjugative transposon (Hochhut et al., 2001a). Later, in a study of chromosomal tandem array of R391 and SXT elements in *V. cholerae*, Hochhut et al. (2001b) revealed that both R391 and SXT element share a unique attachment site in the chromosomal substance of *E. coli* within the 5′ end of *prfC*. They found that both SXT and R391 are closely connected and offer stability to each other, similar integrases at the *prfC* locus, and possess a conserved set of genetic materials for transfer of DNA and similar mechanism for integration, development, and dissemination of antibiotic resistance to the host cells. From then, R391 and SXT were assembled into a family of R391/SXT under ICEs.

6 R391/SXT Family of Integrative Conjugative (IC) Elements

The R391/SXT family is a prototype of conjugative elements which are integrated type found widely in antibiotic-resistant *V. cholerae* strains. The basic feature of R391/SXT family is the existence of integrase gene. *Int,* a tyrosine recombinase called intSXT/R391, and the drug-resistant elements are integrated into the host chromosome, viz., *prfC* gene (Hochhut et al., 2001b), i.e., it should integrate into the integration site of size 17 bp which is specific for the integration of antibiotic-resistant element to the host genome. This integration site was generally found in *prfC* gene at the 5′ end. However, recently Bioteau et al. (2018) reported another integration site called 3′ end of tRNA-Ser encoding gene for the site-specific integration of antibiotic-resistant element. The other significant feature is the presence of transfer gene called *tra* gene for the transfer of DNA during conjugation (Burrus et al., 2006b). The *tra* genes of both R391 and SXTMO10 were closely identical in nature (Burrus, 2011). The similar features in both SXT element of *V. cholerae* and R391 element of *Providencia rettgeri* are the presence of three hot spots viz., H1 in between *sO43* and *traL*, H2 in between *trA* and *sO54*, and H3 in between *sO73* and *traF* for the insertion of new sequences, *rumAB* operon, and kanamycin resistance gene (Beaber et al., 2002b).

In order to name the members in the R391/SXT family, first the ICEs are written as prefix, then abbreviation of species, and country of origin by three letters; for

example, the expansion of ICEVchMex1 is ICE *V. cholerae* Mexico (Wozniak et al., 2009). The R391/SXT family consists of two sets of genes, namely, core gene group and accessory gene group. The core gene group is well maintained and stable enough for the transfer of resistance determinants. The ICE SXT and R391 are integrated through this core gene and accessory gene group through common insertion points called hot spots (Wozniak et al., 2009). There are five consistent hot spot regions (H1, H2, H3, H4, and H5), three variable hot spot regions (I, II, and III), and one putative hot spot region (IV) which helps in the insertion of variable gene in to ICE SXT/R391 (Wozniak et al., 2009). Core genome is responsible for the attachment (*attP* and attB), excision and integration (*xis, int*), conjugative transfer-related genes (*tra C*, *tra D*), DNA repair (*rumBA*), exclusion (*eex*), and regulation of ICEs (*orf96*) (Taviani, 2010) (Fig. 1). The maintenance module in the core genome helps in the excision and integration *(xis and int*) of an ICE into the host. The *Int* helps in the recombination of attachment site or specific recognition site of the host genome (*attB*) into an ICE element (*attP*). The genes involved in conjugative transfer module (*traI*, *traC*, *traD*) aid in the dissemination of ICE elements among bacteria. It mediates the transfer of mobilizable genomic elements of which *traI* is responsible for processing of DNA and mobilization of SS DNA to the host bacteria. Genes such

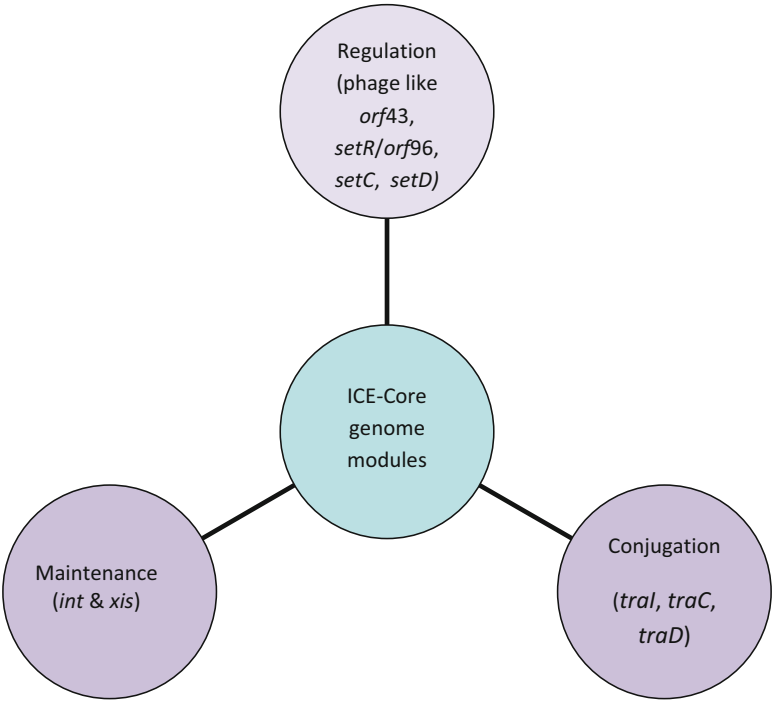


Fig. 1 Modules in ICE core genome and their major genes

as *traC*, *traD*, and *traF* are the putative coupling proteins in the transfer of ICE elements. Genes in regulatory module regulate the excision and integration of ICE into the host genome in which the transcriptional activators (*setC* and *setD*) are expressed and integrate the ICE elements into the recipient host genome.

Accessory genome is responsible for the antibiotic resistance determinants (*merRTPCA, floR, sulII*) (Daccord et al., 2010). Several putative genes of known and unknown functions are also included in this group. The possible reason for the attainment of accessory genes is due to the process of transposons, integrons, insertion sequences, and specific recombinase enzyme in the genome of ICEs (Wozniak et al., 2009). Metabolic-related function and toxin regulation are the other functions of accessory genes. When an R391/SXT ICE is excised from the chromosomal material (*xis* gene), *int* gene mediates the recombination via *attR* and *attL* gene and reconstitutes the attachment site (*attP* and *attB*) (Wozniak & Waldor, 2010). The SXT forms as a circular intermediate during the excision process from the host chromosome and integrates into the 5′ end of *prfC* of the new chromosome via *recA*-independent process and site-specific recombination of individual element and chromosome sequence (Hochhut & Waldor, 1999). The integration process is similar to those in lambda phages. The process of transfer of circular SXT element is comparable to the transfer happening in plasmids, and a pair of transfer genes assists the operation of transfer initiation, translation, and coding of protein (Carraro et al., 2015). The two groups found in the R391/SXT family were R and S groups, respectively (Marrero & Waldor, 2007). The exclusion of the second copy of SXT or R391 during the process of conjugative transfer by the cell already containing SXT or R391 is dependent upon the specific exclusion elements (*EexS, EexR*, exclusion genes) in the S and R exclusion groups. The regulation and transfer of SXT elements are done by a set of phages like regulatory genes (*setR* and *setC/D*) (Wozniak & Waldor, 2010).

Among the 89 SXT/R391 family identified, the sequenced ICE genomes are very few. The first SXT identified in *V. cholerae*, O139, with a size of 100 kb, and 89 kb R391 identified in the pathogenic bacteria *Providencia rettgeri* in 1972 were sequenced (Waldor et al., 1996). SXT/R391 ICE in *Shewanella putrefaciens* W3-18-1 was ICESpuPO1, which is the first to be reported among the bacteria *Shewanella* from the USA in 2000 (Pembroke & Piterina, 2006). The core gene groups of both SXTMO10 which is in the size of 99.5 kb and R391 which is in the size of 89 kb share 95% sequence identity (Ceccarelli et al., 2008). The variants of SXT/R391 ICEs were also reported in several *Vibrio* species. In ICEVchInd1, the trimethoprim resistance is mediated by *dfrA1*, whereas *dfr18* was responsible for those in SXTMO10 (Sarkar et al., 2019). Correspondingly, dissimilarity in sequences was reported in Hot spot 3 of ICEVchLao1 and SXTMO10 (Taviani et al., 2009). Ahmed and Shimamoto (2015) have reported a variant SXT element in *Vibrio fluvialis* with 99% identity in SXT integrase gene of *V. cholerae*. The lists of sequenced SXT/R39l family and its antibiotic resistance characteristics are given in Table 1.

Table 1 List of sequenced SXT/R391 ICEs in *V. cholerae* and its characteristic features

ICEs	Strain/ serogroup	Antibiotic resistance	Reference
ICE SXT (MO10)	O139 MO10	Chloramphenicol, sulfamethoxazole, trimethoprim, streptomycin	Beaber et al. (2002a)
ICE*Vch*Ban5	O1 El LTor	Glyoxalase, trimethoprim, streptomycin, chloramphenicol, sulfamethoxazole	Wozniak et al. (2009)
ICE*Vch*Ban8	O37	Acriflavine	Wozniak et al. (2009)
ICE*Vch*Ban9	MJ-1236	Chloramphenicol, sulfamethoxazole, trimethoprim, streptomycin, glyoxalase, tetracycline, cobalt-zinc-cadmium	Wozniak et al. (2009)
ICE*Vch*Hai1	VC1786	Sulfamethoxazole, trimethoprim, streptomycin, glyoxalase	Sjolund-Karlsson et al. (2011)
ICE*Vch*Mex1	Non-O1-0139	Aminoglycoside	Burrus et al. (2006a)
ICE*Vch*Ind4	0139	Chloramphenicol, sulfamethoxazole, streptomycin	Wozniak et al. (2009)
ICE*Vch*Ind5	O1	Chloramphenicol, glyoxalase, sulfamethoxazole, trimethoprim, streptomycin	Wozniak et al. (2009)
ICE*Vch*CHN2605	ICDC-2605	Sulfamethoxazole, chloramphenicol, streptomycin, trimethoprim	Wang et al. (2016)
ICE*Vch*CHN4210	CDC-4210	Sulfamethoxazole, chloramphenicol, streptomycin, trimethoprim, tetracycline	Wang et al. (2016)

7 Antimicrobial Resistance Determinants in SXT (Sulfamethoxazole-Trimethoprim) Elements of *Vibrio cholerae*

The SXT elements possess a group of genes which causes resistance to different antibiotics such as trimethoprim-sulfamethoxazole, tetracycline, erythromycin, nalidixic acid, chloramphenicol, and streptomycin (Das et al., 2020). The antibiotic resistance gene present in SXT element is found in transposon-like element which has the ability to disturb the *rumAB* operon of SXT element (Carraro & Burrus, 2015). The major antibiotic determinants are *sulI*, encoding sulfamethoxazole resistance, and *strAB*, encoding streptomycin resistance (Hochhut et al., 2001a). Generally, the antibiotic genes are present in three different insertion regions separated by transposase (Herrero et al., 1990). The gene cluster of *tetAR*, *merR*, and *strB* is found in insertion 1 region which confers resistance to tetracycline, mercury, and sulfamethoxazole (Wang et al., 2016). The largest resistance genes are found in insertion 2 region where the *floR* gene and *dhfR* gene offer resistance to chloramphenicol and trimethoprim, respectively (Hochhut et al., 2001a). However, some SXT variants are devoid of the genes in the insertion 2 region, for

example, ICEVchCHN2255, ICEVchCHN57, and AHV1003 (Wang et al., 2016). Further, these ICEs possess another gene called *dfrA1* which is identical to *dfrA1* gene of strain B-1104 of *Klebsiella pneumoniae*. Wang et al. (2016) also found that it possesses another gene called *tetAR* gene encoding efflux protein of Class A tetracycline. Antimicrobial resistance genes in insertion 3 contain *mphR*, *mrx*, and *mphK* gene encoding erythromycin resistance (Wang et al., 2016). In spite of the emergence of several variants of SXT type in terms of conserved backbone assembly, and accessory genes, it has been reported that the SXT types were found to carry multiple drug resistance (MDR) genes in O1 serogroups of *V. cholerae* (Bhardwaj et al., 2014).

8 Persistence and Survival of R391/SXT ICE in Aquatic Environment

The major environmental microbial reservoir of R391/SXT family of conjugative elements of *V. cholerae* is brackish/marine water habitat that provides conducive environments for the persistence of these mobile genetic elements, thereby enabling its transmission to other host bacteria (Roman et al., 2021). The persistence of *Vibrio cholerae* in an aquatic environs depends upon many factors via the ability to produce biofilm, pathogenicity, motility, etc. These phenotypical characteristics are regulated through the production of a second messenger called c-di-GMP (bis (3′-5′) cyclic dimeric guanosine monophosphate) which comprises about 60 genes including diguanylate cyclases (DGCs). Bordeleau et al. (2010) reported that the gene sequences of DGCs such as DgcK and DGcL have enhanced the survival of SXT/R391 ICE group of vibrios in the aquatic environment. Mala et al. (2017) demonstrated that the conjugative mode of horizontal gene transmission of SXT element in both environmental and clinical isolates of *V. cholerae* and the cross dispersion of drug resistance genes between different bacteria might be possible via conjugative plasmid. It has been reported that SXT-related elements in some of the O139 *V. cholerae* isolates lack resistant genes. The rapid spread in SXT-related elements in *Vibrio* population may not always explain the reason for increased antimicrobial resistance; instead, several other factors such as antibiotic use, fitness cost, etc. need to be taken care of for the management of resistance spread. The acquisition of ICEs in several sub-lineages of the seventh pandemic *V. cholerae* strains might be due to the outcome of a series of recombination events via the exchange of large DNA fragments mediated by *recA* gene of the host as well as *bet* and *exo* genes of the ICEs (Spagnoletti et al., 2014). It has been reported that the presence of SXT resistance clusters in ICEs constituting transposases and insertion sequence common region (ISCR) plays an important role in acquiring and organizing new genes in the ICE scaffold genome. It was reported that the relative prevalence of SXT/R391 ICE in aquatic environments by quantitative PCR targeting traB, from North Eastern France and Finland has a wide host range of unsuspected bacteria that belong to Campylobacteraceae, Oceanospirillaceae, etc., suggesting several other bacteria other than *V. cholerae* and Proteus species form a major

reservoir of antimicrobial resistance element and play a vital role in the dissemination of ARGs among bacterial isolates (Roman et al., 2021).

9 Implications of SXT/R391 ICEs in Infection Control

The major challenging situation in the case of global healthcare system is the uncontrollable development and dissemination of drug-resistant bacterial infections. World Health Organization (WHO) in its report suggested that patients may die due to post-antibiotic risk arising due to common infections and minor injuries (WHO, 2014). The treatment options such as tetracycline, fluoroquinolones, and macrolides for *V. Cholerae* now become obsolete due to the acquisition of resistance over these antibiotics. Presently, a single dose of azithromycin is usually given for cholera infections, and the WHO-recommended antibiotics are doxycycline, azithromycin, and ciprofloxacin. However, the majority of the *V. cholerae* isolates of clinical origin harbors SXT/R391 ICEs. The major antibiotic-resistant genes found in *V. Cholerae* strains are *sul2*, *floR*, *strBA*, *dfrA1*, etc. The presence of these genes hampers the antibiotic treatment options for cholera outbreak in both human and animal sectors.

10 Conclusion

The SXT-related ICEs in antibiotic resistance of *V. cholerae* are posing a new challenging situation in antibiotic research. The diversity of SXT in SXT/R391 family shows the flexibility for rapid adaptation of the isolates to the changing environment. Understanding the unknown functions of the R391/SXT ICEs provides more insight into the mechanism of antibiotic resistance in diverse pathogenic bacterial species. The emergence of variants and the lack of efficient approaches to control the dissemination of SXT in multidrug pathogens are the major tasks to reckon with in the present-day scenario.

References

Ahmed, A. M., & Shimamoto, T. (2015). Molecular analysis of multidrug resistance in Shiga toxin-producing *Escherichia coli* O157: H7 isolated from meat and dairy products. *International Journal of Food Microbiology, 193*, 68–73. https://doi.org/10.1016/j.ijfoodmicro.2014.10.014

Alekshun, M. N., & Levy, S. B. (2007). Molecular mechanisms of antibacterial multidrug resistance. *Cell, 128*(6), 1037–1050. https://doi.org/10.1016/j.cell.2007.03.004

Alifano, P., Palumbo, C., Pasanisi, D., & Talà, A. (2015). Rifampicin-resistance, rpoB polymorphism and RNA polymerase genetic engineering. *Journal of Biotechnology, 202*, 60–77. https://doi.org/10.1016/j.jbiotec.2014.11.024

Bañuelos-Vazquez, L. A., Tejerizo, G. T., & Brom, S. (2017). Regulation of conjugative transfer of plasmids and integrative conjugative elements. *Plasmid, 91*, 82–89. https://doi.org/10.3389/fmicb.2019.01134

Barua, D. (1992). *History of cholera*. Springer. https://doi.org/10.1007/978-1-4757-9688-9_1

Beaber, J. W., Burrus, V., Hochhut, B., & Waldor, M. K. (2002a). Comparison of SXT and R391, two conjugative integrating elements: Definition of a genetic backbone for the mobilization of resistance determinants. *Cellular and Molecular Life Sciences CMLS, 59*(12), 2065–2070. https://doi.org/10.1007/s000180200006

Beaber, J. W., Hochhut, B., & Waldor, M. K. (2002b). Genomic and functional analyses of SXT, an integrating antibiotic resistance gene transfer element derived from *Vibrio cholerae*. *Journal of Bacteriology, 184*, 4259–4269. https://doi.org/10.1128/JB.184.15.4259-4269.2002

Bhardwaj, A. K., Vinothkumar, K., Rajpara, N., Mohanty, P., & Kutar, B. M. (2014). Therapeutic limitations due to antibiotic drug resistance: Road to alternate therapies. *Frontiers in Anti-infective Drug Discovery, 3*, 72–141. https://doi.org/10.2174/9781608059126114030004

Bioteau, A., Durand, R., & Burrus, V. (2018). Redefinition and unification of the SXT/R391 family of integrative and conjugative elements. *Applied Environmental Microbiology, 84*(13). https://doi.org/10.1128/AEM.00485-18

Böltner, D., & Osborn, A. M. (2004). Structural comparison of the integrative and conjugative elements R391, pMERPH, R997, and SXT. *Plasmid, 51*(1), 12–23. https://doi.org/10.1016/j.plasmid.2003.10.003

Bordeleau, E., Brouillette, E., Robichaud, N., & Burrus, V. (2010). Beyond antibiotic resistance: Integrating conjugative elements of the SXT/R391 family that encode novel diguanylate cyclases participate to c-di-GMP signalling in *Vibrio cholerae*. *Environmental Microbiology, 12*(2), 510–523. https://doi.org/10.1111/j.1462-2920.2009.02094.x. Epub 2009 Nov 3.

Brown-Jaque, M., Calero-Cáceres, W., & Muniesa, M. (2015). Transfer of antibiotic-resistance genes via phage-related mobile elements. *Plasmid, 79*, 1–7. https://doi.org/10.1016/j.plasmid.2015.01.001

Burrus, V. (2011). *Significance of the SXT/R391 family of integrating conjugative elements in Vibrio cholerae*. Springer. https://doi.org/10.1007/978-1-60327-265-0_9

Burrus, V., & Waldor, M. K. (2004). Shaping bacterial genomes with integrative and conjugative elements. *Research in Microbiology, 155*(5), 376–386. https://doi.org/10.1016/j.resmic.2004.01.012

Burrus, V., Marrero, J., & Waldor, M. K. (2006a). The current ICE age: Biology and evolution of SXT-related integrating conjugative elements. *Plasmid, 55*(3), 173–183. https://doi.org/10.1016/j.plasmid.2006.01.001

Burrus, V., Pavlovic, G., Decaris, B., & Guédon, G. (2002). Conjugative transposons: The tip of the iceberg. *Molecular Microbiology, 46*(3), 601–610. https://doi.org/10.1046/j.1365-2958.2002.03191.x

Burrus, V., Quezada-Calvillo, R., Marrero, J., & Waldor, M. K. (2006b). SXT-related integrating conjugative element in New World *Vibrio cholerae*. *Applied and Environmental Microbiology, 72*(4), 3054–3057. https://doi.org/10.1128/AEM.72.4.3054-3057.2006

Bush, K., Jacoby, G. A., & Medeiros, A. A. (1995). A functional classification scheme for beta-lactamases and its correlation with molecular structure. *Antimicrobial Agents and Chemotherapy, 39*(6), 1211–1233. https://doi.org/10.1128/aac.39.6

Carraro, N., & Burrus, V. (2015). The dualistic nature of integrative and conjugative elements. *Mobile Genetic Elements, 5*(6), 98–102. https://doi.org/10.1080/2159256X.2015.1102796

Carraro, N., Poulin, D., & Burrus, V. (2015). Replication and active partition of integrative and conjugative elements (ICEs) of the SXT/R391 family: The line between ICEs and conjugative plasmids is getting thinner. *PLoS Genet, 11*(6), e1005298. https://doi.org/10.1371/journal.pgen.1005298

Carraro, N., Rivard, N., Ceccarelli, D., Colwell, R. R., & Burrus, V. (2016). IncA/C conjugative plasmids mobilize a new family of multidrug resistance islands in clinical Vibrio cholerae non-O1/non-O139 isolates from Haiti. *MBio, 7*(4). https://doi.org/10.1128/mBio.00509-16

Ceccarelli, D., Daccord, A., René, M., & Burrus, V. (2008). Identification of the origin of transfer (oriT) and a new gene required for mobilization of the SXT/R391 family of integrating conjugative elements. *Journal of Bacteriology, 190*(15), 5328–5338. https://doi.org/10.1128/JB.00150-08

Chandrasekhar, M. R., Krishna, B. V., & Patil, A. B. (2008). Changing charsacteristics of *Vibrio cholerae*: Emergence of multidrug resistance and non-O1, non-O139 serogroups. *Southeast Asian Journal of Tropical Medicine and Public Health, 39*(6), 1092–1097. PMID: 19062701.

Chen, A. (2017). Vibrios in the environment: An investigation of environmental *Vibrio vulnificus*, *Vibrio parahaemolyticus*, and *Vibrio cholerae* (Doctoral dissertation). http://hdl.handle.net/1903/20367

Chowdhury, F. R., Nur, Z., Hassan, N., von Seidlein, L., & Dunachie, S. (2017). Pandemics, pathogenicity and changing molecular epidemiology of cholera in the era of global warming. *Annals of Clinical Microbiology and Antimicrobials, 16*(1), 1–6. https://doi.org/10.1186/s12941-017-0185-1

Daccord, A., Ceccarelli, D., & Burrus, V. (2010). Integrating conjugative elements of the SXT/R391 family trigger the excision and drive the mobilization of a new class of Vibrio genomic islands. *Molecular Microbiology, 78*(3), 576–588. https://doi.org/10.1111/j.1365-2958.2010.07364.x

Das, B., Verma, J., Kumar, P., Ghosh, A., & Ramamurthy, T. (2020). Antibiotic resistance in *Vibrio cholerae*: Understanding the ecology of resistance genes and mechanisms. *Vaccine, 38*, A83–A92. https://doi.org/10.1016/j.vaccine.2019.06.031

Davies, J., & Davies, D. (2010). Origins and evolution of antibiotic resistance. *Microbiology and Molecular Biology Reviews, 74*(3), 417–433. https://doi.org/10.1128/MMBR.00016-10

Faruque, S. M., Kamruzzaman, M., Meraj, I. M., Chowdhury, N., Nair, G. B., Sack, R. B., Colwell, R. R., & Sack, D. A. (2003). Pathogenic potential of environmental *Vibrio cholerae* strains carrying genetic variants of the toxin-coregulated pilus pathogenicity Island. *Infection and Immunity, 71*(2), 1020–1025. https://doi.org/10.1128/iai.71.2.1020-1025.2003

Finkelstein, R. A. (1996). *Cholera, Vibrio cholerae O1 and O139, and other pathogenic Vibrios* (4th ed.). The University of Texas Medical Branch at Galveston. PMID: 21413330. Medical microbiology., 4th.

Garg, P., Chakraborty, S., Basu, I., Datta, S., Rajendran, K., Bhattacharya, T., Yamasaki, S., Bhattacharya, S. K., Takeda, Y., Nair, G. B., & Ramamurthy, T. (2000). Expanding multiple antibiotic resistance among clinical strains of *Vibrio cholerae* isolated from 1992–7 in Calcutta, India. *Epidemiology and Infection, 124*(3), 393–399. https://doi.org/10.1017/s0950268899003957

Hall, R. M., & Collis, C. M. (1998). Antibiotic resistance in gram-negative bacteria: The role of gene cassettes and integrons. *Drug Resistance Updates, 1*(2), 109–119. https://doi.org/10.1016/s1368-7646(98)80026-5

Hedges, R. W., & Jacob, A. E. (1975). A 98 megadalton R factor of compatibility group C in a *Vibrio cholerae* El Tor isolate from southern USSR. *Microbiology, 89*(2), 383–386. https://doi.org/10.1099/00221287-89-2-383

Heidelberg, J. F., Eisen, J. A., Nelson, W. C., Clayton, R. A., Gwinn, M. L., Dodson, R. J., Haft, D. H., Hickey, E. K., Peterson, J. D., Umayam, L., Gill, S. R., Nelson, K. E., Read, T. D., Tettelin, H., Richardson, D., Ermolaeva, M. D., Vamathevan, J., Bass, S., Qin, H., Dragoi, I., Sellers, P., McDonald, L., Utterback, T., Fleishmann, R. D., Nierman, W. C., White, O., Salzberg, S. L., Smith, H. O., Colwell, R. R., Mekalanos, J. J., Venter, J. C., & Fraser, C. M. (2000). DNA sequence of both chromosomes of the cholera pathogen Vibrio cholerae. *Nature, 406*(6795), 477–483. https://doi.org/10.1038/35020000

Herrero, M., de Lorenzo, V., & Timmis, K. N. (1990). Transposon vectors containing non-antibiotic resistance selection markers for cloning and stable chromosomal insertion of foreign genes in gram-negative bacteria. *Journal of Bacteriology, 172*(11), 6557–6567. https://doi.org/10.1128/jb.172.11.6557-6567.1990

Hochhut, B., & Waldor, M. K. (1999). Site specific integration of the conjugal *Vibrio cholerae* SXT element into prfC. *Molecular Microbiology, 32*(1), 99–110. https://doi.org/10.1046/j.1365-2958.1999.01330.x

Hochhut, B., Beaber, J. W., Woodgate, R., & Waldor, M. K. (2001a). Formation of chromosomal tandem arrays of the SXT element and R391, two conjugative chromosomally integrating

elements that share an attachment site. *Journal of Bacteriology, 183*(4), 1124–1132. https://doi.org/10.1128/JB.183.4.1124-1132.2001

Hochhut, B., Lotfi, Y., Mazel, D., Faruque, S. M., Woodgate, R., & Waldor, M. K. (2001b). Molecular analysis of antibiotic resistance gene clusters in *Vibrio cholerae* O139 and O1 SXT constins. *Antimicrobial Agents and Chemotherapy, 45*(11), 2991–3000. https://doi.org/10.1128/AAC.45.11.2991-3000.2001

Huda, N., Lee, E. W., Chen, J., Morita, Y., Kuroda, T., Mizushima, T., & Tsuchiya, T. (2003). Molecular cloning and characterization of an ABC multidrug efflux pump, VcaM, in non-O1 *Vibrio cholerae*. *Antimicrobial Agents and Chemotherapy, 47*(8), 2413–2417. https://doi.org/10.1128/aac.47.8.2413-2417.2003

Iwanaga, M., Toma, C., Miyazato, T., Insisiengmay, S., Nakasone, N., & Ehara, M. (2004). Antibiotic resistance conferred by a Class I integron and SXT constin in *Vibrio cholerae* O1 strains isolated in Laos. *Antimicrobial Agents and Chemotherapy, 48*(7), 2364–2369. https://doi.org/10.1128/AAC.48.7.2364-2369.2004

Jacoby, G. A. (2005). Mechanisms of resistance to quinolones. *Clinical Infectious Diseases, 41*(Supplement_2), S120–S126. https://doi.org/10.1086/428052

Johnson, C. M., & Grossman, A. D. (2015). Integrative and conjugative elements (ICEs): What they do and how they work. *Annual Review of Genetics, 49*, 577–601. https://doi.org/10.1146/annurev-genet-112414-055018

Juhas, M., Van Der Meer, J. R., Gaillard, M., Harding, R. M., Hood, D. W., & Crook, D. W. (2009). Genomic islands: Tools of bacterial horizontal gene transfer and evolution. *FEMS Microbiology Reviews, 33*(2), 376–393. https://doi.org/10.1111/j.1574-6976.2008.00136.x

Kaushik, M., Kumar, S., Kapoor, R. K., & Gulati, P. (2019). Integrons and antibiotic resistance genes in water-borne pathogens: Threat detection and risk assessment. *Journal of Medical Microbiology, 68*(5), 679–692. https://doi.org/10.1099/jmm.0.000972

Kim, H. B., Wang, M., Ahmed, S., Park, C. H., LaRocque, R. C., Faruque, A. S., Salam, M. A., Khan, W. A., Qadri, F., Calderwood, S. B., & Jacoby, G. A. (2010). Transferable quinolone resistance in *Vibrio cholerae*. *Antimicrobial Agents and Chemotherapy, 54*(2), 799–803. https://doi.org/10.1128/AAC.01045-09

Kitaoka, M., Miyata, S. T., Unterweger, D., & Pukatzki, S. (2011). Antibiotic resistance mechanisms of *Vibrio cholerae*. *Journal of Medical Microbiology, 60*(4), 397–407. https://doi.org/10.1099/jmm.0.023051-0

Li, X., Du, Y., Du, P., Dai, H., Fang, Y., Li, Z., Lv, N., Zhu, B., Kan, B., & Wang, D. (2016). SXT/R391 integrative and conjugative elements in *Proteus* species reveal abundant genetic diversity and multidrug resistance. *Scientific Reports, 6*(1), 1–9. https://doi.org/10.1038/srep37372

Lin, H., Yu, M., Wang, X., & Zhang, X. H. (2018). Comparative genomic analysis reveals the evolution and environmental adaptation strategies of Vibrios. *BMC Genomics, 19*(1), 1–14. https://doi.org/10.1186/s12864-018-4531-2

Mala, W., Faksri, K., Samerpitak, K., Yordpratum, U., Kaewkes, W., Tattawasart, U., & Chomvarin, C. (2017). Antimicrobial resistance and genetic diversity of the SXT element in *Vibrio cholerae* from clinical and environmental water samples in northeastern Thailand. *Infection, Genetics and Evolution, 52*, 89–95. https://doi.org/10.1016/j.meegid.2017.04.013

Marrero, J., & Waldor, M. K. (2007). The SXT/R391 family of integrative conjugative elements is composed of two exclusion groups. *Journal of Bacteriology, 189*(8), 3302–3305. https://doi.org/10.1128/JB.01902-06

Nugent, M. E. (1981). A conjugative plasmid lacking autonomous replication. *Microbiology, 126*(2), 305–310. https://doi.org/10.1099/00221287-126-2-305

Opintan, J. A., Newman, M. J., Nsiah-Poodoh, O. A., & Okeke, I. N. (2008). *Vibrio cholerae* O1 from Accra, Ghana carrying a Class 2 integron and the SXT element. *Journal of Antimicrobial Chemotherapy, 62*(5), 929–933. https://doi.org/10.1093/jac/dkn334

Pant, A., Bag, S., Saha, B., Verma, J., Kumar, P., Banerjee, S., Kumar, B., Kumar, Y., Desigamani, A., Maiti, S., & Maiti, T. K. (2020). Molecular insights into the genome dynamics and

interactions between core and acquired genomes of *Vibrio cholerae*. *Proceedings of the National Academy of Sciences, 117*(38), 23762–23773. https://doi.org/10.1073/pnas.2006283117

Pembroke, J. T., & Piterina, A. V. (2006). A novel ICE in the genome of *Shewanella putrefaciens* W3-18-1: Comparison with the SXT/R391 ICE-like elements. *FEMS Microbiology Letters, 264*(1), 80–88. https://doi.org/10.1111/j.1574-6968.2006.00452.x

Ramamurthy, T., Mutreja, A., Weill, F. X., Das, B., Ghosh, A., & Nair, G. B. (2019). Revisiting the global epidemiology of cholera in conjunction with the genomics of *Vibrio cholerae*. *Frontiers in Public Health, 7*, 203. https://doi.org/10.3389/fpubh.2019.00203

Roman, V. L., Merlin, C., & Baron, S. (2021). Abundance and environmental host range of the SXT/R391 ICEs in aquatic environmental communities. *Environmental Pollution, 288*, 117673. https://doi.org/10.1016/J.ENVPOL.2021.117673

Roychowdhury, A., Pan, A., Dutta, D., Mukhopadhyay, A. K., Ramamurthy, T., Nandy, R. K., Bhattacharya, S. K., & Bhattacharya, M. K. (2008). Emergence of tetracycline-resistant *Vibrio cholerae* O1 serotype Inaba, in Kolkata, India. *Japanese Journal of Infectious Diseases, 61*(2), 128. PMID: 18362401.

Ryan, M. P., Armshaw, P., O'Halloran, J. A., & Pembroke, J. T. (2017). Analysis and comparative genomics of R997, the first SXT/R391 integrative and conjugative element (ICE) of the Indian sub-continent. *Scientific Reports, 7*(1), 1–5. https://doi.org/10.1038/s41598-017-08735-y

Saha, D., Karim, M. M., Khan, W. A., Ahmed, S., Salam, M. A., & Bennish, M. L. (2006). Single-dose azithromycin for the treatment of cholera in adults. *New England Journal of Medicine, 354*(23), 2452–2462. https://doi.org/10.1056/NEJMoa054493

Saha, S., & Singh, D. V. (2020). *Mechanism of antibiotic resistance and pathogenicity of Vibrio cholerae*. Springer. https://doi.org/10.1007/978-981-15-1695-5_15

Sarkar, A., Morita, D., Ghosh, A., Chowdhury, G., Mukhopadhyay, A. K., Okamoto, K., & Ramamurthy, T. (2019). Altered integrative and conjugative elements (ICEs) in recent *Vibrio cholerae* O1 isolated from cholera cases, Kolkata, India. *Frontiers in Microbiology, 10*, 2072. https://doi.org/10.3389/fmicb.2019.02072

Siriphap, A., Leekitcharoenphon, P., Kaas, R. S., Theethakaew, C., Aarestrup, F. M., Sutheinkul, O., & Hendriksen, R. S. (2017). Characterization and genetic variation of *Vibrio cholerae* isolated from clinical and environmental sources in Thailand. *PLoS One, 12*(1), e0169324. https://doi.org/10.1371/journal.pone.0169324

Sjolund-Karlsson, M., Reimer, A., & Folster, J. P. (2011). Drug-resistance mechanisms in *Vibrio cholerae* O1 outbreak in Strain, Haiti. *Emerging Infectious Diseases, 17*, 2151–2154. https://doi.org/10.3201/eid1711.110720

Spagnoletti, M., Ceccarelli, D., Rieux, A., Fondi, M., Taviani, E., Fani, R., Colombo, M. M., Colwell, R. R., & Balloux, F. (2014). Acquisition and evolution of SXT-R391 integrative conjugative elements in the seventh-pandemic *Vibrio cholerae* lineage. *MBio, 5*(4), e01356–e01314. https://doi.org/10.1128/mBio.01356-14

Spratt, B. G. (1994). Resistance to antibiotics mediated by target alterations. *Science, 264*(5157), 388–393. https://doi.org/10.1126/science.8153626

Taviani, E. (2010). Genomics and ecology of Integrative conjugative elements (ICE) in *Vibrio cholerae* (Doctoral dissertation). http://hdl.handle.net/1903/11450

Taviani, E., Grim, C. J., Chun, J., Huq, A., & Colwell, R. R. (2009). Genomic analysis of a novel integrative conjugative element in *Vibrio cholerae*. *FEBS Letters, 583*(22), 3630–3636. https://doi.org/10.1016/j.febslet.2009.10.041

Thiagarajah, J. R., & Verkman, A. S. (2005). New drug targets for cholera therapy. *Trends in Pharmacological Sciences, 26*(4), 172–175. https://doi.org/10.1016/j.tips.2005.02.004

Tomita, H., Fujimoto, S., Tanimoto, K., & Ike, Y. (1996). Cloning and genetic organization of the bacteriocin 31 determinant encoded on the *Enterococcus faecalis* pheromone-responsive conjugative plasmid pYI17. *Journal of Bacteriology, 178*(12), 3585–3593. https://doi.org/10.1128/jb.178.12.3585-3593.1996

Towner, K. J., Pearson, N. J., Mhalu, F. S., & O'grady, F. (1980). Resistance to antimicrobial agents of *Vibrio cholerae* El Tor strains isolated during the fourth cholera epidemic in the United Republic of Tanzania. *Bulletin of the World Health Organization, 58*(5), 747. https://apps.who.int/iris/handle/10665/262073

Verma, J., Bag, S., Saha, B., Kumar, P., Ghosh, T. S., Dayal, M., Senapati, T., Mehra, S., Dey, P., Desigamani, A., & Kumar, D. (2019). Genomic plasticity associated with antimicrobial resistance in *Vibrio cholerae*. *Proceedings of the National Academy of Sciences, 116*(13), 6226–6231. https://doi.org/10.1073/pnas.1900141116

Waldor, M. K., Tschäpe, H. E. L. M. U. T., & Mekalanos, J. J. (1996). A new type of conjugative transposon encodes resistance to sulfamethoxazole, trimethoprim, and streptomycin in *Vibrio cholerae* O139. *Journal of Bacteriology, 178*(14), 4157–4165. https://doi.org/10.1128/jb.178.14.4157-4165.1996

Wang, R., Yu, D., Yue, J., & Kan, B. (2016). Variations in SXT elements in epidemic *Vibrio cholerae* O1 El Tor strains in China. *Science Reports, 6*, 22733. https://doi.org/10.1038/srep22733

WHO. (2014). *Antimicrobial resistance: Global report on surveillance*. Available at: http://www.who.int/drugresistance/documents/surveillancereport/en/

Wozniak, R. A., & Waldor, M. K. (2010). Integrative and conjugative elements: Mosaic mobile genetic elements enabling dynamic lateral gene flow. *Nature Reviews Microbiology, 8*(8), 552–563. https://doi.org/10.1038/nrmicro2382

Wozniak, R. A., Fouts, D. E., Spagnoletti, M., Colombo, M. M., Ceccarelli, D., Garriss, G., Déry, C., Burrus, V., & Waldor, M. K. (2009). Comparative ICE genomics: Insights into the evolution of the SXT/R391 family of ICEs. *PLoS Genetics, 5*(12), e1000786. https://doi.org/10.1371/journal.pgen.1000786

Yu, L., Zhou, Y., Wang, R., Lou, J., Zhang, L., Li, J., Bi, Z., & Kan, B. (2012). Multiple antibiotic resistance of *Vibrio cholerae* serogroup O139 in China from 1993 to 2009. *PloS One, 7*(6), e38633. https://doi.org/10.1371/journal.pone.0038633

Evolution, Characteristics, and Clonal Expansion of Livestock-Associated Methicillin-Resistant *Staphylococcus aureus* (LA-MRSA): Global Perspectives

Muneeb Hamza, G. K. Sivaraman, and Mukteswar Prasad Mothadaka

Contents

Abstract

Epidemiological classification of methicillin-resistant *Staphylococcus aureus* (MRSA) as hospital-associated MRSA (HA-MRSA), community-associated MRSA (CA-MRSA), and livestock-associated MRSA (LA-MRSA) has become more complex and unclear. Nevertheless, the global burden of LA-MRSA and its clinical complications have been increased to unprecedented levels. The improper

M. Hamza (✉)
Microbiology, Fermentation and Biotechnology Division, ICAR-Central Institute of Fisheries Technology, Cochin, Kerala, India

Faculty of Sciences, Cochin University of Science and Technology, Cochin, Kerala, India
e-mail: muneebhamzakh@gmail.com

G. K. Sivaraman · M. P. Mothadaka
Microbiology, Fermentation and Biotechnology Division, ICAR-Central Institute of Fisheries Technology (ICAR-CIFT), Cochin, Kerala, India

M. P. Mothadaka et al. (eds.), *Handbook on Antimicrobial Resistance*,
https://doi.org/10.1007/978-981-19-9279-7_23

use of antibiotics contributed to the emergence of MRSA in the livestock sector. Apart from infections, asymptomatic colonization of MRSA in humans and animals can result in the humanosis and/or zoonosis. The LA-MRSA studies drew attention because of its unique properties of harboring the *mecC* locus (an analogue of *mecA*) as the methicillin resistance determinant and varying staphylococcal cassette chromosome *mec* (SCC*mec*) elements. The peculiar feature of LA-MRSA which is non-typeable by *SmaI*-pulsed-field gel electrophoresis (*SmaI*-PFGE) has opened different vistas in LA-MRSA research. The present chapter focused on the prevalence, history of evolution, clonal expansion, and epidemiological characteristics of LA-MRSA from global perspectives. Concerted efforts were made to encompass the major aspects of LA-MRSA by analyzing the related studies. Over the years, LA-MRSA gained special attention owing to its widespread and zoonotic potential.

Keywords

MRSA · LA-MRSA · Zoonosis · Livestock · Clonal complex · CC398

1 Introduction

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a global challenge with an ever-increasing prevalence. Earlier, MRSA infections were common in hospitals and healthcare settings, and the lineage of MRSA that caused widespread infections in clinical settings is known as hospital-associated MRSA (HA-MRSA). Besides, *S. aureus* that were prevalent in the community acquired the methicillin resistance determinant (*mecA* gene) and emerged as community-associated MRSA (CA-MRSA) (DeLeo et al., 2010). The distinction between HA-MRSA and CA-MRSA was more or less obvious since these two lineages displayed distinguishable molecular epidemiology and clonality. In addition to HA- and CA-MRSA, another lineage, namely, livestock-associated MRSA (LA-MRSA), causing infections in animals has been described (Chuang & Huang, 2015). The LA-MRSA has also demonstrated distinct characteristics that separate it from other MRSA lineages. Despite this, recent studies suggested that epidemiological distinction between HA-, CA-, and LA-MRSA has become blurred owing to the overlapping of "the unique" characteristics of each lineage. For instance, detection of the *mecC* gene served as a molecular marker for the easy identification of animal-sourced MRSA. Nonetheless, the first case of "clinical MRSA" harboring *mecC* and *mecC* + *mecA* combination has been described which presented a discrepancy in the epidemiological distinction of MRSA (Khan et al., 2020).

The antibiotic paradox may substantially be accountable for the prevalence and persistence of LA-MRSA in livestock farming (Chen & Wu, 2020). The amount and the frequency of antibiotic use depend on how farmers choose to deal with the bacterial infections. Similarly, farmer's perceptions of the risks associated with the misuse and overuse of antibiotics are also very important. It has been proved that

livestock workers are more prone to LA-MRSA infections owing to their regular, direct occupational contact with the animals. However, "where does the MRSA in livestock sector come from" remains to be a critical question to be addressed. Pig farms have been recognized as a major industry greatly affected by LA-MRSA. The other farm animals, such as cattle, sheep, goats, etc., are also plagued by LA-MRSA infections. The LA-MRSA research is very nascent in many countries as exemplified by the Polish scenario, which reported the first case of LA-MRSA in 2020 (Krukowski et al., 2020). However, the LA-MRSA research eventually received much attention over the other lineages due to their zoonotic transmission. Consequently, research investigations pertaining to the characterization have been escalated; however, there are still certain gaps in LA-MRSA biology to be filled. This chapter focused majorly on the molecular epidemiology, magnitude, and the increasing complexity of LA-MRSA.

2 LA-MRSA: History of Evolution

Reporting the first case of LA-MRSA dated back to 2005 (Voss et al., 2005). A case study by Dr. Voss, a consultant microbiologist from Canisius Wilhelmina Hospital, Netherlands, observed the MRSA colonization in a 6-month-old girl who was hospitalized for thoracic surgery. Surprisingly, neither the girl nor her family had prior chances of having contact with a sick patient or a history of travel or hospitalization in the past few months. After several episodes of surveillance, Dr. Voss identified a pig farm owned by the family as the source of MRSA infection. Through a mass screening of pigs, pig farms, and the farmers, new cases of MRSA were encountered in 2005; one in a pig farmer but from a different location and another in the son of a veterinary doctor whose job is always associated with diseased pigs. Two more cases have been reported from a hospital where the aforementioned infected son was admitted. It was important to be noted that all of the reported MRSA patients had no epidemiological links and were unrelated in terms of time and location of hospitalization. On the contrary, the MRSA isolates recovered from each patient shared certain characteristics in common. Subsequently, the family members of the infected patients also tested MRSA-positive, and decolonization of the strains in these patients was merely impossible. Since then, the magnitude of MRSA infections has reached unprecedented levels, particularly in the Netherlands, and has spread to other parts of Europe. As a result, there has been a surge in research interest on the evolution of LA-MRSA. Initially, clonality of LA-MRSA was limited to the clonal complex 398 (CC398), but the emergence of new clones belonging to CC1, CC9, CC97, etc. was also reported in the later years (this area has been detailed under section "Clonal Expansion in LA-MRSA") (Butaye et al., 2016). A phylogenetic analysis of an international collection of methicillin-sensitive *S. aureus* (MSSA) and MRSA suggested that the MSSA belonging to CC398 could be the potential ancestor of LA-MRSA (Price et al., 2012). Surprisingly, MSSA CC398 was first reported in France in the same year when the first case of LA-MRSA was reported (Armand-Lefevre et al., 2005).

3 Evidence of LA-MRSA

3.1 Pig and Pig Farms

After the successful identification of LA-MRSA from people having occupational contact with pigs, the subsequent years have been marked as the "golden period" for the prevalence of LA-MRSA and its association with human infections. In 2006, a case study performed in the Netherlands reported the pigs as a potential source of MRSA infection in humans (Huijsdens et al., 2006). According to the study, following a young lady who exhibited the symptoms of mastitis, her daughter developed pneumococcal otitis which resulted in the hospitalization. Spending 5 days at the hospital, the baby also tested positive for MRSA. To identify the potential source of MRSA infections, the family members, household animals, and companion animals were screened. Of the ten pigs tested, eight were positive for MRSA colonization. Similarly, another study from Europe reported the severe soft tissue infection, attributable to MRSA, having the origin of a pig bite (Declercq et al., 2008). A group of scientists reported the first Danish case of MRSA infection, and they speculated the occupational contact with pigs is the potential source of MRSA infection (Ruhlmann et al., 2008). A 58-year-old man who worked in a pig farm in Italy was hospitalized due to a high fever and severe pain in the buttock. The MRSA was isolated from both the patient and pig farm. Through an epidemiological investigation pertaining to the characterization of MRSA from the patient as well as the pig farm, it was revealed that the MRSA from both the sources were identical (Pan et al., 2009). Another report from Spain documented the involvement of MRSA belonging to ST1, in addition to ST 398, causing skin lesions in humans (Aspiroz et al., 2010). In this study, a 12-year-old girl living close to a pig farm was diagnosed with skin lesions and was screened for MRSA, and the isolates were molecularly characterized using multilocus sequence typing (MLST), staphylococcal cassette chromosome *mec* (SCC*mec*), staphylococcal protein A (*spa*), and accessory gene regulator (*agr*) typing techniques. Apparently, MRSA could be recovered with ST1-t127 and ST398-t011 genotypes. The study also underpinned the significant role of pig farms in Spain, in the distribution of MRSA, and its clinical manifestations in humans. On a positive note, zoonotic lineages of MRSA have been recognized as less virulent when compare to clinical strains. Conversely, another study where a group of researchers presented the transmission of MRSA resulted in severe pneumonia in new born, among the family members associated with pig farming (Hartmeyer et al., 2010). Lozano et al. (2011) studied both pig farmers and their pigs for the incidence of MRSA to demonstrate the possible transmission of this MRSA from animal to humans (Lozano et al., 2011). Through his study, he established that the MRSA isolated from both animals and humans belonged to SCC*mec* type V and *agr* type 1 and showed indistinguishable pulsed-field gel electrophoresis (PFGE) profile. Sharma and others studied the epidemiology of MRSA through a massive surveillance conducted in the UK in 2013 and 2015. They have drawn approximately 1000 samples from animals, mostly pigs, and screened for LA-MRSA. Of the

1000 sources, 12 pigs carried LA-MRSA belonging to CC398 (Sharma et al., 2016). Recently, a report from Portugal that screened 3 slaughter houses with a total of 141 pig samples revealed the presence of 28 *S. aureus*, 22 of which were methicillin resistant (MRSA). Surprisingly, all of these MRSA isolates were MLST-typed as CC398 and belonged to three different spa types: t011, t108, and t1451 (Santos et al., 2020). A study performed in Spain in 2018–2019 screened 23 *S. aureus* isolates from pigs with signs of infections (Ruiz-Ripa et al., 2021). The study documented the presence of the *mecA* gene in 21 isolates carried on SCC*mec* type V elements. CC398 and *spa* t011 were found to be more widespread types, documented by the study.

3.2 Dairy Cattle

There are a number of reports of *S. aureus* being detected in raw milk of both healthy dairy cattle and that exhibited clinical mastitis. A study with a major objective of analyzing the difference between ST1-MRSA and MSSA from cattle herds in Italy was performed by a group of researchers (Alba et al., 2015). As a result, *S. aureus* recovered from 63 animal samples were categorized as MRSA and MSSA based on their resistance status to methicillin. Both MRSA and MSSA were genotypically characterized and compared. It has been revealed that the majority of bovine isolates belonged to CC1 and had a significant level of relatedness to the human isolates. The study also underpinned the risk associated with the zoonotic capacity of CC1 MRSA and commented on the potential possibilities to be implemented at the farm level to mitigate the spillover. Another study conducted in Italy documented the occurrence and characteristics of MRSA (Basanisi et al., 2017). A total of 3760 milk samples and dairy products were collected from Southern Italy in 2008–2014 and subjected to isolation of MRSA. Of 484 isolates of *S. aureus*, 40 (8.3%) were identified as MRSA. Further, epidemiological characterization of the isolates revealed that the ST152 (t355), ST398 (t889, t108), ST1 (t127), and ST5 (t688) were the major STs and *spa* types (in brackets) prevailed in dairy cattles. All the isolates harbored SCC*mec* type V and IVa ("a" denotes the subtype). The majority of the isolates contained staphylococcal enterotoxins (SEs) such as *seg*, *sei*, *sem*, *sen*, and *seo*. The presence of Panton-Valentine leukocidin (PVL) expressing genes in 50% of the isolates tested was a major issue. PCR screening of genes detected the presence of *icaAD* complex that displayed biofilm production. Similarly, another study again from Italy reported the LA-MRSA as an agent of inflammatory infections in cattle (Magro et al., 2018). After screening a "single cattle farm" located in Eastern Poland for over 1 year, the incidence of LA-MRSA was described for the first time (Krukowski et al., 2020). The quarter-milk samples were drawn regularly and examined microbiologically. Five MRSA isolates were recovered from the same outbreak and delineated by MLST (CC398) and *spa* typing (t034). A study from Malaysia documented the pervasiveness of LA-MRSA from milk samples and nasal swabs (Aklilu & Hui Ying, 2020). The highlight of this study was the documentation of the *mecC* gene (a novel analogue of the *mecA* gene) and its co-existence with the *mecA* gene.

3.3 Other Companion Animals

Domesticating the animals such as dogs and cats in houses is a very common practice in India. They are commonly considered as members of the household rather than as animals, showing the increasing likelihood of pets coming into contact with humans. This may pose a risk of companion animals, which are carriers of pathogenic bacteria, acting as a zoonotic source when in contact with a compatible host, such as a human. The highly virulent MRSA was sporadically recovered from companion animals, convincing the experts that the animals play critical roles in the spread of MRSA to people. The first epidemic MRSA outbreak of animal origin was reported in 1988 in a geriatric caring ward (Scott et al., 1988). After screening the staff and patients of the rehabilitation center, an unusual carriage rate of MRSA was observed. Surprisingly, the source of the outbreak was traced back to a "cat" that was heavily colonized with MRSA. After removing the cat from the ward, the outbreak was drastically reduced. According to the study by Scott and his team, it is implied that in the healthy animals, MRSA can asymptomatically colonize and lead to infection under suitable conditions. Nevertheless, how the MRSA colonized in companion animals remained to be a critical question. This ambiguity was solved by a survey conducted in Germany in 2010–2012 that studied the genotypic variations of MRSA in companion animals. A very high prevalence rate of MRSA was observed in canines (62.7%), felines (46.4%), and equines (41.3%) (Vincze et al., 2014). A similar study by Loncaric et al. (2019) revealed the incidence of 90 non-duplicated MRSA isolates from companion animals in Australia (Loncaric et al., 2019). All the MRSA isolates were delineated to seven existing STs (ST398, ST8, ST225, ST22, ST152, ST1, and ST45) plus a new ST (ST5275). The grouping of isolates to "16" major *spa* types emphasized the genetic diversity of MRSA from companion animals. Similarly, in an interesting study by Harrison and others, 46 MRSA-ST22 isolates from companion animals were sequenced, and when compared with reference genomes, it was observed that the ST22 genome shared the sequence similarity with those isolates belonging to the same ST but of human origin (Harrison et al., 2014). Phylogenomics of these isolates could cluster the isolates from companion animals with human isolates from the UK. The study also documented the circulation of epidemic MRSA-15 (EMRSA-15) pandemic clade in humans as well as companion animals. From the literature, it was very clear that among the different animals, pigs and pig farms serve as the most potent source of MRSA infection/colonization.

4 Molecular Epidemiology of LA-MRSA

LA-MRSA, unlike other epidemiological types, often harbors either *mecA* or *mecC* genes as the methicillin resistance determinants which are carried on the SCC*mec* elements. This mobile genetic island was first characterized in the "pre-MRSA" N315 strain by sequence analysis of upstream and downstream regions of the *mecA* gene which is called *mec* DNA or additional DNA (Ito et al., 1999). Subsequently,

the SCC*mec* region of several other strains such as HDE288, CA05, WIS, P1, etc. was sequenced to have comprehension about this mobile genetic element (Ito et al., 2001; Oliveira et al., 2001; Ma et al., 2002; Ito et al., 2004; Takano et al., 2008). SCC*mec* is assumed to play a pivotal role in the epidemiological classification of MRSA isolates. Using the Oliveira method, MRSA can be delineated up to such six types as type I–VI (Oliveira et al., 2001). In most cases, HA-MRSA belongs to the first three types (type I–III), but CA-MRSA often carries smaller and highly mobile type IV or type V elements (Rolo et al., 2012). However, there is no such stringency for LA-MRSA to carry any particular SCC*mec* types which is attested by a study that clustered LA-MRSA to SCC*mec* types III, V, and IX (Boswihi et al., 2020). In addition to SCC*mec*, MLST and *spa* typing have also facilitated the characterization of LA-MRSA based on their sequence types and *spa* types, respectively. The first and most reported ST in LA-MRSA was ST398. The commonly found *spa* types under ST398 are t108, t011, and t034. Even now, this particular clone has been isolated from many different sources. A very recent article published in January 2021 which reported the isolation of linezolid-resistant MRSA belonging to CC398 from the pig in Spain is a typical example that shows the prevalence of this particular clone has not yet receded (Ruiz-Ripa et al., 2021). Commonly, PFGE has been considered as a "gold standard" for the bacterial delineation by which even a single nucleotide variation can be detected in bacteria. In the case of *S. aureus*, PFGE uses the *smaI* restriction enzyme to create the fingerprints which can be then compared with other outbreak-reported isolates. On the contrary, ST398 was considered as non-typeable MRSA (NT-MRSA) owing to the limitation of characterizing this clone with the help of PFGE that uses the *SmaI* restriction enzyme. This property of LA-MRSA CC398 being not typed by PFGE is attributed to its ability to synthesize C5-cytosine methyltransferase which can modify the *SmaI* recognition site (Fig. 1) (Bens et al., 2006). This dilemma was resolved by replacing the *SmaI* enzyme with a neoschizomer, namely, *Cfr*9I, that can create a nick within the *SmaI* recognition site for successfully creating the DNA fingerprint (Argudín et al., 2010).

5 Antimicrobial-Resistance Genes (ARGs) in LA-MRSA

The genetic basis of antibiotic resistance, notably in staphylococci, has been a hot topic for many years. It was always exciting to observe how the notorious staphylococci acquire resistance and adapt to the antibiotic selection pressure. Generally, horizontal or vertical gene transfer methods account for the dissemination of resistance determinants among the staphylococci. Thus, acquisition of resistome is a perpetual process, as long as the staphylococci can interact with other species carrying ARGs (Wendlandt et al., 2013). The imprudent use of antibiotics as prophylactic or growth promoters in different sectors such as animal husbandry, aquaculture settings, etc. has been considered as one of the major selection pressures for the evolution of antimicrobial resistance in bacteria. On a positive note, the current resistance profile of LA-MRSA provides sufficient treatment opportunities as

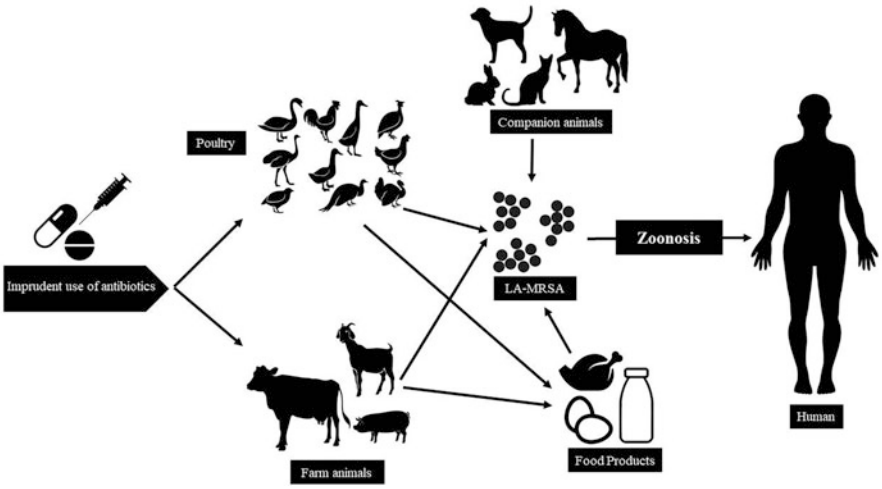


Fig. 1 A Schematic representation illustrating the potential transmission routes of livestock-associated methicillin-resistant *Staphylococcus aureus* (LA-MRSA) from animals to humans. The LA-MRSA from farm animals and poultry are transmitted to humans through occupational contact, whereas from companion animals it is through direct regular contact

they are still susceptible to a wide range of antibiotics including but not limited to the last-resort antibiotics such as vancomycin and linezolid.

Resistance to β-lactam antibiotics is majorly attributed to the ability of staphylococci to produce β-lactamase enzymes which are encoded by *blaZ* genes (Rowland & Dyke, 1989). Basically, the penicillin-binding protein which is a key protein involved in transpeptidation during bacterial cell wall synthesis is the drug target of β-lactam antibiotics. On the other hand, the bacteria that can produce β-lactamase enzyme can hydrolyze the amide bond in the β-lactam ring of the antibiotic and thereby reversing its bactericidal property (Tooke et al., 2019). Alternatively, an additional mechanism of resistance to a β-lactam antibiotic, i.e., methicillin, which is mediated by the *mecA* locus, was observed among staphylococci. The *mecA* gene is located on a highly transmissible mobile genetic element designated as SCC*mec* which has played a tremendous role in the emergence of MRSA across the globe (Ito et al., 1999). Antibiotic-sensitive staphylococci can quickly turn into resistant phenotypes through the acquisition of this genetic island.

Resistance to tetracycline, being the most commonly used antibiotic in the cattle industry, is not uncommon among the animal-sourced MRSA. Tetracycline can inhibit bacterial growth by terminating protein translation. It can interfere with the binding of amino-acyl tRNA to the A site of the 30S rRNA subunit (Chopra & Roberts, 2001). On the contrary, the bacteria have developed resistance against tetracycline by the following mechanisms: (1) pumping out the antibiotic of bacterial cells through efflux action, (2) protecting the ribosomal binding site to disable the antibiotic from binding, and (3) modification of cell membrane to block the antibiotic entry to the bacterial cell

(Speer et al., 1992). Molecular study of animal-sourced MRSA has revealed that the tetracycline resistance is predominantly controlled by the genetic expression of *tet*(K) and *tet*(L). A study by Schwarz and Cardoso (1991) documented the incidence of *tet*(K) in different staphylococcal species (majorly CoNS) isolated from animals (Schwarz & Cardoso, 1991). They also observed the co-existence of *tet*(K) with *tet*(M) gene in certain CoNS staphylococci. As far as LA-MRSA, particularly belonging to the ST398, is concerned, plasmids that carry the *tet*(L) gene seem to be predominant (Kadlec & Schwarz, 2009a).

Chloramphenicol prevents protein chain elongation owing to its interference with the peptidyl transferase activity of ribosomes (Schifano et al., 2013). Chloramphenicol resistance is mediated mainly by acetyltransferases (Fernández et al., 2012). In bovine-sourced staphylococci, the most common type of the *cat* gene, the chloramphenicol resistance determinant, is the cat_{pC221}, so-called because it is carried on the plasmid pC221 (Schwarz et al., 2004). In addition, cat_{pC223} has also been identified in bovine-sourced *S. aureus* and canine-sourced *S. haemolyticus* (Cardoso & Schwarz, 1992).

Similar to chloramphenicol, aminoglycosides also inhibit protein synthesis by targeting the bacterial ribosomes that in turn lead to the bacterial growth inhibition. There are several aminoglycosides inactivating enzymes involved in this process. Previous studies reported that the gene *aac(6′)-Ie-aph(2″)-Ia*(*aacA-aphD*) is carried on a transposon, namely, Tn*4001*, and codes for an enzyme which can exhibit acetyltransferase as well as phosphotransferase activities in addition to conferring resistance to aminoglycosides. The occurrence of this gene is very common in almost all staphylococci particularly of animal origin (Perreten et al., 2010; Gómez-Sanz et al., 2011). Additionally, another gene called *ant(4′)-Ia* (*aadD*) is assumed to be a plasmid-mediated gene found on pUB110 (McKenzie et al., 1987). Notably, studies described the integration of *aadD* gene into the type II SCC*mec* elements, which is often found to be associated with LA-MRSA, LA-MSSA, and CoNS (Kadlec & Schwarz, 2009b; Feßler et al., 2010; Hauschild et al., 2007). Similarly, the gene *aph(3′)-IIIa* (*aphA3*) that helps staphylococci to confer resistance to kanamycin, neomycin, etc. is more frequently detected in canine- and feline-sourced staphylococci (Zakour et al., 2011), whereas the gene *ant(6′)-Ia* (*aadE*) which resists streptomycin is found to be prevalent in *S. pseudintermedius* of canine and feline sources (Derbise et al., 1996). Both *aphA3* and *aadE* loci together with *sat*4, streptothricin resistance determinant, are associated with transposon Tn*5405*.

Resistance to oxazolidinones in staphylococci is assumed to be very rare but at the same time is very significant. Linezolid was truly a wonder molecule that was effective against a broad spectrum of Gram-positive bacteria including but not limited to MRSA and vancomycin-resistant enterococci (VRE) (Roger et al., 2018). The history of linezolid resistance dated back to the 1990s, and its first clinical use was recorded in 2000. Unfortunately, the first clinical case of linezolid resistance in *S. aureus* was reported in 2001 (Tsiodras et al., 2001). However, the association of the *cfr* locus that encodes the rRNA methylase, with the linezolid resistance, was elucidated only after 5 years of its first clinical case.

6 Clonal Expansion in LA-MRSA

Sequence types (STs) are assigned to LA-MRSA to distinguish them based on their molecular epidemiology. Since the beginning of LA-MRSA evolution and until now, CC398 appeared to be the most predominant clonal complex particularly in Europe and North America. There are nearly 40 STs under this clonal complex (CC398) reported in pigs; however, the major ST is ST398 (central ST), while the other STs including ST541, ST1965, ST1966, ST1967, and ST1968 are either single-locus variant (SLV) or double-locus variant (DLV) of the central ST (Porrero et al., 2012). Although not all LA-MRSA belong to ST398, MRSA belonging to ST398 are surely LA-MRSA. Notably, there is a substantial difference between ST398 isolates from human and animal sources (Stegger et al., 2010) which can be achieved by the SNP detection and the occurrence of *scn* and *tet*(M) (Stegger et al., 2013). Thus, LA-MRSA CC398 is assumed to be less virulent in humans, whereas in animals, the infection can result in mastitis.

Since the first case of LA-MRSA, the major clonal complex reported was CC398, particularly in pigs and pig farm-associated workers. However, based on the different geographical locations, an explosion in the clonality of this LA-MRSA has occurred, and that resulted in the expansion of clonal complexes. CC9 represents one such example of an expanded LA-MRSA epidemiological clone. The CC9 LA-MRSA is predominant in Asian countries (Chuang & Huang, 2015). Although its prevalence is less in other countries, Europe has witnessed its first case of CC9 MRSA in 2008 (Battisti et al., 2010). Eventually, European countries such as Germany and the UK also reported the cases of CC9 isolates from animal sources (Feßler et al., 2011; Dhup et al., 2015). Surprisingly, there is an evidence of CC9 MRSA prevalent in Europe prior to the emergence of CC398 (Espinosa-Gongora et al., 2014). However, they did not appear in the literature since they were less investigated clones than CC398. Swine has been recognized as the typical host for CC9 clones and rarely can cause infections in humans (Wan et al., 2013). Hence, MRSA CC9 has been considered as an animal pathogen.

Unlike the other two aforementioned clonal complexes, CC97 has been identified as the major reason for bovine mastitis across the globe. The incidence of CC97 strains has widely been documented in humans. It is believed that this clone first appeared in animals, and subsequently spread to people, which could have happened at least four decades ago. The first case of MRSA CC97 was reported in 2010 from Italy in a study that documented the incidence of MRSA CC97 in pigs (Battisti et al., 2010). CC1 is another major lineage of MRSA sporadically isolated from animals. Relating CC1 with animals may spark a debate because CC1 is a successful human lineage of CA-MRSA. The CC1 isolates, on the other hand, have been identified as a major lineage of MRSA circulating in nonclinical settings. Despite the fact that this chapter focuses solely on MRSA from animals, it considers CC1 because the first cases of CC1 isolates were identified in animals such as pigs and dairy cows (Alba et al., 2015). The toxigenic potential of human-sourced MRSA CC1 was attested by the presence of PVL toxins, whereas the animal-sourced MRSA CC1 lacked this toxin, but they often carried the staphylococcal enterotoxins (SEs) (Pilla et al., 2012).

7 LA-MRSA and Its Impact on Public Health

LA-MRSA is among the most common bacterial pathogens causing bovine mastitis worldwide. In addition to mastitis, LA-MRSA infections can also result in foodborne illnesses in humans. Similar to *S. aureus*, LA-MRSA also carries the same battery of toxin genes that can contribute to the onset of staphylococcal food poisoning (SFP). The people having primary and occupational contact with the animals are more prone to get infected with LA-MRSA (Cuny et al., 2015). It has been observed that LA-MRSA accounts for 15% of all isolates from human skin and soft tissue infections (Cuny et al., 2013). When people with LA-MRSA infection go to the hospital for treatment, there is a high chance for this bacterium to be passed from patients to patients through contact. A survey that reported the LA-MRSA colonization in patients accounted for 0.8% among approximately 13,855 individuals tested (Juretzek et al., 2011). Another report from North Rhine-Westphalia federal state estimated the occurrence of LA-MRSA in patients at hospital admission as an elevation from 10% to 14% (Feingold et al., 2012). Notably, the same study observed there is an increase in the LA-MRSA-attributed septicemia in this region during the same period. Here, in the first case that reported only 0.8% of occurrence, the particular area had a low population density of livestock, whereas in the second case, the population density was substantially high. These two events alone may help to explain the zoonotic potential of LA-MRSA. The possible transmission routes of MRSA infection from animals to humans are illustrated in Fig. 2.

As far as the clinical transmission of diseases is concerned, physical contact with the diseased person or the infected object is expected to play an important role. With no exception, the primary mode of LA-MRSA transmission from an infected animal to a healthy person is the physical contact. In addition, it can also be transmitted through the food chain. There are many lines of evidence demonstrating the LA-MRSA colonization/infection in humans having occupational contact with animals comprising pigs, livestock, sheep, etc. and physical contact with companion animals such as dogs, cats, etc. (Huijsdens et al., 2006; Declercq et al., 2008; Ruhlmann et al., 2008; Aspiroz et al., 2010; Basanisi et al., 2017; Magro et al., 2018; Krukowski et al., 2020). Cuny and others described the role of animal husbandry in inter-host and intra-host dissemination of LA-MRSA by stating that LA-MRSA colonization in farmers was significantly interrupted when the farmers temporarily stopped having occupational contact with the animals (Cuny et al., 2015).

8 Prevalence of LA-MRSA: Global Scenario

Ever since the first case of LA-MRSA from the Netherlands and France, Europe has witnessed several cases of LA-MRSA particularly in pig farms and associated workers (Voss et al., 2005). Thereafter, surveillance studies from neighboring European countries increased the screening of pig farms for LA-MRSA to understand their transmission dynamics. Interestingly, different parts of Europe had

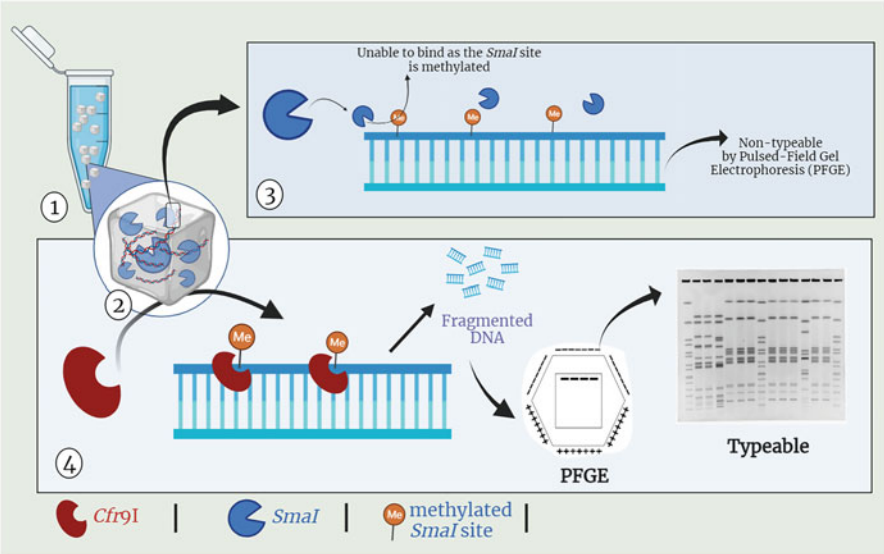


Fig. 2 Illustration of the unique property of ST398-MRSA, being non-typeable by conventional *SmaI*-pulsed-field gel electrophoresis (PFGE). (1) Restriction digestion reaction where the "plug" containing the released DNA after cell lysis is digested by restriction enzyme. (2) Enlarged view of a plug where intact DNA and restriction enzyme can be seen. (3) Because the *SmaI* site is methylated, the restriction enzyme cannot locate the site and leaves the DNA undigested; thus, no fingerprint is generated. (4) As an alternative, *SmaI* enzyme is replaced with a neoschizomer called *Cfr*9I which is insensitive to methylation and can locate the methylated *SmaI* site leading to DNA digestion

different prevalence rates of MRSA, and the actual reason for this ambiguity was undistinguishable. CC398 MRSA clone was predominant in Europe. In the UK, there is also a trend of CC398 LA-MRSA being recovered from a wide variety of animals. Similarly, in Sweden, LA-MRSA prevalence in pig farms is much higher, which contradicts the Danish government's action plans to reduce MRSA.

In the beginning, North America reported significantly fewer cases of LA-MRSA not because this lineage had no survival advantages in that environment, but because the government imposed stringent regulations that posed restrictions for the on-farm sampling (Butaye et al., 2016). The limited information from Canada and the USA reported that the LA-MRSA ST398 is a commonly identified lineage in pigs and pig farms (Molla et al., 2012). Unlike the USA and European countries where the LA-MRSA CC398 is the predominant MRSA lineage, Asian countries have often recognized CC9 as the major clone. Along with CC9, other STs including ST22 and ST221 have also been widely reported in many Asian countries (Chuang & Huang, 2015). A very recent study from China that screened pig-exposed and non-exposed farmers for the carriage of MRSA indicated that pig-exposed farmers harbor a high rate of MRSA than non-exposed farmers (Liu et al., 2021). The AMR can be contained only through a global collaborative effort, alerted by the World Health

Organizations (WHO). To accomplish this, the WHO proposed a global action plan that included the following goals: (1) to raise the awareness about antibiotic use, (2) to strengthen the AMR surveillance, and (3) to promote the sanitization and hygiene as preventive measures.

9 Prevalence of LA-MRSA: Indian Scenario

India also has evidence to substantiate the prevalence rate of LA-MRSA in animals. However, the exact picture of LA-MRSA in India is still blurred. A case study reported the occurrence of LA-MRSA in a 5-year-old cow that was admitted to Madras Veterinary College Teaching Hospital owing to reduced milk production (Chandrasekaran et al., 2014). Similar studies that evaluated the resistance profile of *S. aureus* isolated from animal samples have been reported from Uttar Pradesh (Kutar et al., 2015; Chaturvedi & Kumar, 2017), Southern Gujarat (Patel et al., 2017), and Jammu and Kashmir (Ganai et al., 2015; Ganai et al., 2016; Hamid et al., 2017). An important finding was recorded by a study that reported the presence of MRSA in mastitis samples equipped with the array ARGs (Kumar et al., 2010). The same team investigated the pathogenic potential of MRSA isolated from mastitis milk samples and discovered the presence of a number of genes associated with biofilm formation in staphylococci, including *fnbB*, *fib*, *bap*, etc. (Kumar et al., 2011). An unusual case of *mecA* carrying *S. aureus* which is "susceptible" to oxacillin and causing bovine mastitis has also been reported from India (Mistry et al., 2016). Indian studies reported the presence of MRSA from animal sources, but have not investigated the molecular epidemiology of the MRSA isolate in detail, and thus it is impossible to conclusively categorize these isolates as LA-MRSA. To contain AMR, the Indian government launched the National Action Plan to tackle AMR (NAP-AMR), with the state of Kerala being the first to implement it. The Kerala Antimicrobial Resistance Strategic Action Plan (KARSAP) was formulated in October 2018. The KARSAP takes a "One-Health" approach involving the human, nonhuman, and environmental sectors.

10 Role of Animal Husbandry in the Development of LA-MRSA

Animal husbandry is assumed to play a significant role in the rural economy particularly in India (Shanmathy et al., 2018). Livestock provides not only nutrition to millions of people but also employment for the farmers. On the other hand, this sector is also threatened by the invasive pathogenic bacteria that may cause devastating losses in this sector. However, to protect the animals from diseases, antibiotics, such as penicillin, tetracycline, etc., have been extensively used in livestock for prophylactic or non-therapeutic purposes. Considering animal husbandry as one of the fastest-growing sectors, the use of antibiotics has also been elevated rapidly. The commonly used antibiotics in animal husbandry are listed in Table 1. The antibiotic selection pressure owing to the imprudent use of antibiotics has been considered as one of the leading

Table 1 List of commonly used antibiotics in animal husbandry

Sl No	Classes of antibiotics	Animals	Uses	Reference
1	Penicillin	Beef cattle, cows, fowl, poultry, sheep, swine	Prophylaxis and nontherapeutic purposes	Dweba et al. (2018)
2	Cephalosporins	Beef cattle, cows, poultry, sheep, swine	Prophylaxis only	
3	Tetracycline	Beef cattle, dairy cows, fowl, honey bees, poultry	Prophylaxis and nontherapeutic purposes	
4	Sulfonamides	Beef cattle, poultry, swine	Prophylaxis and nontherapeutic purposes	
5	Lincosamide	Poultry, swine	Prophylaxis and nontherapeutic purposes	
6	Macrolide	Beef cattle, poultry, swine	Prophylaxis and nontherapeutic purposes	
7	Aminoglycoside	Beef cattle, goats, poultry, sheep, swine	Prophylaxis only	
8	Streptogramin	Beef cattle, poultry, swine	Prophylaxis and nontherapeutic purposes	
9	Ionophore	Beef cattle, fowl, goats, poultry, rabbits, sheep	Prophylaxis and nontherapeutic purposes	
10	Polypeptide	Fowl, poultry, swine	Prophylaxis and nontherapeutic purposes	

causes of the emergence of AMR. The farmers are using antibiotics in animal husbandry very frequently even without knowing what exactly it is, and they are least concerned about its adverse effects. The farmers believe that antibiotics are something that can improve the growth of their animals. In consequence, the antibiotics with a sublethal concentration may select the resistant bacteria. Eventually, antimicrobial-resistant bacteria thus emerged can either asymptomatically colonize or cause infections to the animals with a compromised immune response. Further, zoonosis often occurs when the colonized/infected animals come in contact with humans.

11 Conclusion

This chapter was dedicated to LA-MRSA, addressing the major aspects ranging from the history of evolution to current trends from both Indian and worldwide perspectives. At the very beginning, LA-MRSA was restricted to only a single clone which is CC398, and the later years witnessed an explosion in the clonality.

Emergence of new MRSA clones can also be expected as its prevalence has not yet receded. In many countries, though trying to mitigate, LA-MRSA remains a challenge. The future of LA-MRSA is difficult to predict based on current knowledge. Thus, the research on LA-MRSA demands more definitive characterizations. Nonetheless, it is indubitable that LA-MRSA can equally cause serious health complications in animals and humans.

References

Aklilu, E., & Hui Ying, C. (2020). First mecC and mecA positive livestock-associated Methicillin resistant *Staphylococcus aureus* (mecC MRSA/LA-MRSA) from dairy cattle in Malaysia. *Microorganisms, 8*(2), 147.

Alba, P., Feltrin, F., Cordaro, G., Porrero, M. C., Kraushaar, B., Argudín, M. A., Nykäsenoja, S., Monaco, M., Stegger, M., Aarestrup, F. M., & Butaye, P. (2015). Livestock-associated methicillin resistant and methicillin susceptible *Staphylococcus aureus* sequence type (CC) 1 in European farmed animals: High genetic relatedness of isolates from Italian cattle herds and humans. *PloS one, 10*(8), e0137143.

Argudín, M. A., Rodicio, M. R., & Guerra, B. (2010). The emerging methicillin-resistant *Staphylococcus aureus* ST398 clone can easily be typed using the Cfr9I SmaI-neoschizomer. *Letters in Applied Microbiology, 50*(1), 127–130.

Armand-Lefevre, L., Ruimy, R., & Andremont, A. (2005). Clonal comparison of *Staphylococcus aureus* isolates from healthy pig farmers, human controls, and pigs. *Emerging Infectious Diseases, 11*(5), 711.

Aspiroz, C., Lozano, C., Vindel, A., Lasarte, J. J., Zarazaga, M., & Torres, C. (2010). Skin lesion caused by ST398 and ST1 MRSA, Spain. *Emerging Infectious Diseases, 16*(1), 157.

Basanisi, M. G., La Bella, G., Nobili, G., Franconieri, I., & La Salandra, G. (2017). Genotyping of methicillin-resistant *Staphylococcus aureus* (MRSA) isolated from milk and dairy products in South Italy. *Food Microbiology, 62*, 141–146.

Battisti, A., Franco, A., Merialdi, G., Hasman, H., Iurescia, M., Lorenzetti, R., Feltrin, F., Zini, M., & Aarestrup, F. M. (2010). Heterogeneity among methicillin-resistant *Staphylococcus aureus* from Italian pig finishing holdings. *Veterinary Microbiology, 142*(3–4), 361–366.

Bens, C. C., Voss, A., & Klaassen, C. H. (2006). Presence of a novel DNA methylation enzyme in methicillin-resistant *Staphylococcus aureus* isolates associated with pig farming leads to uninterpretable results in standard pulsed-field gel electrophoresis analysis. *Journal of Clinical Microbiology, 44*(5), 1875–1876.

Boswihi, S. S., Udo, E. E., Mathew, B., Noronha, B., Verghese, T., & Tappa, S. B. (2020). Livestock-associated methicillin-resistant *Staphylococcus aureus* in patients admitted to Kuwait Hospitals in 2016–2017. *Frontiers in Microbiology, 10*, 2912.

Butaye, P., Argudín, M. A., & Smith, T. C. (2016). Livestock-associated MRSA and its current evolution. *Current Clinical Microbiology Reports, 3*(1), 19–31.

Cardoso, M., & Schwarz, S. (1992). Chloramphenicol resistance plasmids in *Staphylococcus aureus* isolated from bovine subclinical mastitis. *Veterinary Microbiology, 30*(2–3), 223–232.

Chandrasekaran, D., Venkatesan, P., Tirumurugaan, K. G., Gowri, B., Subapriya, S., & Thirunavukkarasu, S. (2014). Sub-acute mastitis associated with Methicillin Resistant *Staphylococcus aureus* in a cow: A case report. *Journal of Advanced Veterinary and Animal Research, 1*(4), 235–237.

Chaturvedi, R., & Kumar, Y. (2017). Prevalence of *Staphylococcus aureus* in bulk tank milk collected from dairies of district Allahabad India. *International Journal of Current Microbiology and Applied Science, 6*, 1297–1303.

Chen, C., & Wu, F. (2020). Livestock-associated methicillin-resistant *Staphylococcus aureus* (LA-MRSA) colonisation and infection among livestock workers and veterinarians: A systematic review and meta-analysis. *Occupational and Environmental Medicine*.

Chopra, I., & Roberts, M. (2001). Tetracycline antibiotics: Mode of action, applications, molecular biology, and epidemiology of bacterial resistance. *Microbiology and Molecular Biology Reviews, 65*(2), 232–260.

Chuang, Y. Y., & Huang, Y. C. (2015). Livestock-associated meticillin-resistant *Staphylococcus aureus* in Asia: An emerging issue? *International Journal of Antimicrobial Agents, 45*(4), 334–340.

Cuny, C., Köck, R., & Witte, W. (2013). Livestock associated MRSA (LA-MRSA) and its relevance for humans in Germany. *International Journal of Medical Microbiology, 303*(6–7), 331–337.

Cuny, C., Wieler, L. H., & Witte, W. (2015). Livestock-associated MRSA: The impact on humans. *Antibiotics, 4*(4), 521–543.

Declercq, P., Petre, D., Gordts, B., & Voss, A. (2008). Complicated community-acquired soft tissue infection by MRSA from porcine origin. *Infection, 36*(6), 590–592.

DeLeo, F. R., Otto, M., Kreiswirth, B. N., & Chambers, H. F. (2010). Community-associated meticillin-resistant *Staphylococcus aureus*. *The Lancet, 375*(9725), 1557–1568.

Derbise, A., Dyke, K. G., & El Solh, N. (1996). Characterization of a *Staphylococcus aureus* Transposon, Tn5405, located within Tn5404 and carrying the aminoglycoside resistance genes, aphA-3 and aadE. *Plasmid, 35*(3), 174–188.

Dhup, V., Kearns, A. M., Pichon, B., & Foster, H. A. (2015). First report of identification of livestock-associated MRSA ST9 in retail meat in England. *Epidemiology & Infection, 143*(14), 2989–2992.

Dweba, C. C., Zishiri, O. T., & El Zowalaty, M. E. (2018). Methicillin-resistant *Staphylococcus aureus*: Livestock-associated, antimicrobial, and heavy metal resistance. *Infection and Drug Resistance, 11*, 2497.

Espinosa-Gongora, C., Moodley, A., Lipinska, U., Broens, E. M., Hermans, K., Butaye, P., Devriese, L. A., Haesebrouck, F., & Guardabassi, L. (2014). Phenotypes and genotypes of old and contemporary porcine strains indicate a temporal change in the *S. aureus* population structure in pigs. *PLoS One, 9*(7), e101988.

Feingold, B. J., Silbergeld, E. K., Curriero, F. C., van Cleef, B. A., Heck, M. E., & Kluytmans, J. A. (2012). Livestock density as risk factor for livestock-associated methicillin-resistant Staphylococcus aureus, The Netherlands. *Emerging infectious diseases, 18*(11), 1841.

Fernández, M., Conde, S., de la Torre, J., Molina-Santiago, C., Ramos, J. L., & Duque, E. (2012). Mechanisms of resistance to chloramphenicol in Pseudomonas putida KT2440. *Antimicrobial Agents and Chemotherapy, 56*(2), 1001–1009.

Feßler, A., Scott, C., Kadlec, K., Ehricht, R., Monecke, S., & Schwarz, S. (2010). Characterization of methicillin-resistant *Staphylococcus aureus* ST398 from cases of bovine mastitis. *Journal of Antimicrobial Chemotherapy, 65*(4), 619–625.

Feßler, A. T., Kadlec, K., Hassel, M., Hauschild, T., Eidam, C., Ehricht, R., Monecke, S., & Schwarz, S. (2011). Characterization of methicillin-resistant *Staphylococcus aureus* isolates from food and food products of poultry origin in Germany. *Applied and Environmental Microbiology, 77*(20), 7151–7157.

Ganai, A. W., Kotwal, S. K., Malik, M. A., Sharma, H. K., Wani, N., & Jeelani, R. (2015). Prevalence of *Staphylococcus aureus* and Methicillin Resistant *Staphylococcus aureus* (MRSA) in clinical setting and dairy farm environment of Jammu. *Journal of Animal Research, 5*(3), 539.

Ganai, A. W., Kotwal, S. K., Wani, N., Malik, M. A., Jeelani, R. I. Z. W. A. N., Kour, S., & Zargar, R. (2016). Detection of mecA gene of methicillin resistant *Staphylococcus aureus* by PCR assay from raw milk. *Indian Journal of Animal Sciences, 86*(5), 508–511.

Gómez-Sanz, E., Torres, C., Lozano, C., Sáenz, Y., & Zarazaga, M. (2011). Detection and characterization of methicillin-resistant Staphylococcus pseudintermedius in healthy dogs in La Rioja, Spain. *Comparative Immunology, Microbiology and Infectious Diseases, 34*(5), 447–453.

Hamid, S., Bhat, M. A., Mir, I. A., Taku, A., Badroo, G. A., Nazki, S., & Malik, A. (2017). Phenotypic and genotypic characterization of methicillin-resistant *Staphylococcus aureus* from bovine mastitis. *Veterinary World, 10*(3), 363.

Harrison, E. M., Weinert, L. A., Holden, M. T., Welch, J. J., Wilson, K., Morgan, F. J., Harris, S. R., Loeffler, A., Boag, A. K., Peacock, S. J., & Paterson, G. K. (2014). A shared population of epidemic methicillin-resistant *Staphylococcus aureus* 15 circulates in humans and companion animals. *MBio, 5*(3).

Hartmeyer, G. N., Gahrn-Hansen, B., Skov, R. L., & Kolmos, H. J. (2010). Pig-associated methicillin-resistant *Staphylococcus aureus*: Family transmission and severe pneumonia in a newborn. *Scandinavian Journal of Infectious Diseases, 42*(4), 318–320.

Hauschild, T., Vuković, D., Dakić, I., Ježek, P., Djukić, S., Dimitrijević, V., Stepanović, S., & Schwarz, S. (2007). Aminoglycoside resistance in members of the Staphylococcus sciuri group. *Microbial Drug Resistance, 13*(2), 77–84.

Huijsdens, X. W., Van Dijke, B. J., Spalburg, E., van Santen-Verheuvel, M. G., Heck, M. E., Pluister, G. N., Voss, A., Wannet, W. J., & De Neeling, A. J. (2006). Community-acquired MRSA and pig-farming. *Annals of Clinical Microbiology and Antimicrobials, 5*(1), 1–4.

Ito, T., Katayama, Y., & Hiramatsu, K. (1999). Cloning and nucleotide sequence determination of the entire mec DNA of pre-methicillin-resistant *Staphylococcus aureus* N315. *Antimicrobial Agents and Chemotherapy, 43*(6), 1449–1458.

Ito, T., Katayama, Y., Asada, K., Mori, N., Tsutsumimoto, K., Tiensasitorn, C., & Hiramatsu, K. (2001). Structural comparison of three types of staphylococcal cassette chromosome mec integrated in the chromosome in methicillin-resistant *Staphylococcus aureus*. *Antimicrobial Agents and Chemotherapy, 45*(5), 1323–1336.

Ito, T., Ma, X. X., Takeuchi, F., Okuma, K., Yuzawa, H., & Hiramatsu, K. (2004). Novel type V staphylococcal cassette chromosome mec driven by a novel cassette chromosome recombinase, ccrC. *Antimicrobial Agents and Chemotherapy, 48*(7), 2637–2651.

Juretzek, T., Porta, M. A., Buehling, A., Haubold, R., Pohle, M., Schwab, F., & Bär, W. (2011). Epidemiology of methicillin-resistant Staphylococcus aureus at hospital admission in South Brandenburg, Germany. *International Journal of Medical Microbiology, 301*, 100.

Kadlec, K., & Schwarz, S. (2009a). Identification of a novel trimethoprim resistance gene, dfrK, in a methicillin-resistant *Staphylococcus aureus* ST398 strain and its physical linkage to the tetracycline resistance gene tet (L). *Antimicrobial Agents and Chemotherapy, 53*(2), 776–778.

Kadlec, K., & Schwarz, S. (2009b). Novel ABC transporter gene, vga (C), located on a multiresistance plasmid from a porcine methicillin-resistant *Staphylococcus aureus* ST398 strain. *Antimicrobial Agents and Chemotherapy, 53*(8), 3589–3591.

Khan, A. A., Ali, A., Tharmalingam, N., Mylonakis, E., & Zahra, R. (2020). First report of mecC gene in clinical methicillin resistant *S. aureus* (MRSA) from tertiary care hospital Islamabad, Pakistan. *Journal of Infection and Public Health, 13*(10), 1501–1507.

Krukowski, H., Bakuła, Z., Iskra, M., Olender, A., Bis-Wencel, H., & Jagielski, T. (2020). The first outbreak of methicillin-resistant *Staphylococcus aureus* in dairy cattle in Poland with evidence of on-farm and intrahousehold transmission. *Journal of Dairy Science, 103*(11), 10577–10584.

Kumar, R., Yadav, B. R., & Singh, R. S. (2010). Genetic determinants of antibiotic resistance in *Staphylococcus aureus* isolates from milk of mastitic crossbred cattle. *Current Microbiology, 60*(5), 379–386.

Kumar, R., Yadav, B. R., & Singh, R. S. (2011). Antibiotic resistance and pathogenicity factors in *Staphylococcus aureus* isolated from mastitic Sahiwal cattle. *Journal of Biosciences, 36*(1), 175–188.

Kutar, K., Verma, A. K., Sharma, B., Amit, K., & Yadav, S. K. (2015). Analysis of mecA gene and antibiotic resistance in *Staphylococcus aureus* isolates from bovine mastitis. *Indian Journal of Comparative Microbiology, Immunology and Infectious Diseases, 36*(1), 22–27.

Liu, Y., Li, W., Dong, Q., Liu, Y., & Ye, X. (2021). Livestock-associated and non-livestock-associated Staphylococcus aureus carriage in humans is associated with pig exposure in a dose–response manner. *Infection and Drug Resistance, 14*, 173.

Loncaric, I., Lepuschitz, S., Ruppitsch, W., Trstan, A., Andreadis, T., Bouchlis, N., Marbach, H., Schauer, B., Szostak, M. P., Feßler, A. T., & Künzel, F. (2019). Increased genetic diversity of

methicillin-resistant *Staphylococcus aureus* (MRSA) isolated from companion animals. *Veterinary Microbiology, 235*, 118–126.

Lozano, C., Aspiroz, C., Charlez, L., Gomez-Sanz, E., Toledo, M., Zarazaga, M., & Torres, C. (2011). Skin lesion by methicillin-resistant *Staphylococcus aureus* ST398-t1451 in a Spanish pig farmer: Possible transmission from animals to humans. *Vector-Borne and Zoonotic Diseases, 11*(6), 605–607.

Ma, X. X., Ito, T., Tiensasitorn, C., Jamklang, M., Chongtrakool, P., Boyle-Vavra, S., Daum, R. S., & Hiramatsu, K. (2002). Novel type of staphylococcal cassette chromosome mec identified in community-acquired methicillin-resistant *Staphylococcus aureus* strains. *Antimicrobial Agents and Chemotherapy, 46*(4), 1147–1152.

Magro, G., Rebolini, M., Beretta, D., & Piccinini, R. (2018). Methicillin-resistant *Staphylococcus aureus* CC22-MRSA-IV as an agent of dairy cow intramammary infections. *Veterinary Microbiology, 227*, 29–33.

McKenzie, T., Hoshino, T., Tanaka, T., & Sueoka, N. (1987). Correction.A revision of the nucleotide sequence and functional map of pUB110. *Plasmid, 17*(1), 83–85.

Mistry, H., Sharma, P., Mahato, S., Saravanan, R., Kumar, P. A., & Bhandari, V. (2016). Prevalence and characterization of oxacillin susceptible mecA-positive clinical isolates of *Staphylococcus aureus* causing bovine mastitis in India. *PLoS One, 11*(9), e0162256.

Molla, B., Byrne, M., Abley, M., Mathews, J., Jackson, C. R., Fedorka-Cray, P., Sreevatsan, S., Wang, P., & Gebreyes, W. A. (2012). Epidemiology and genotypic characteristics of methicillin-resistant *Staphylococcus aureus* strains of porcine origin. *Journal of Clinical Microbiology, 50*(11), 3687–3693.

Oliveira, D. C., Tomasz, A., & de Lencastre, H. (2001). The evolution of pandemic clones of methicillin-resistant *Staphylococcus aureus*: Identification of two ancestral genetic backgrounds and the associated mec elements. *Microbial Drug Resistance, 7*(4), 349–361.

Pan, A., Battisti, A., Zoncada, A., Bernieri, F., Boldini, M., Franco, A., Giorgi, M., Iurescia, M., Lorenzotti, S., Martinotti, M., & Monaci, M. (2009). Community-acquired methicillin-resistant *Staphylococcus aureus* ST398 infection, Italy. *Emerging Infectious Diseases, 15*(5), 845.

Patel, S. A., Savalia, C. V., Kumar, R., Gamit, M. A., Nair, S., Patel, R. K., & Patel, N. G. (2017). Incidence of methicillin resistant *Staphylococcus aureus* in the milk of Surti goats. *International Journal of Agricultural Science, 9*, 3637–3639.

Perreten, V., Kadlec, K., Schwarz, S., GrönlundAndersson, U., Finn, M., Greko, C., Moodley, A., Kania, S. A., Frank, L. A., Bemis, D. A., & Franco, A. (2010). Clonal spread of methicillin-resistant Staphylococcus pseudintermedius in Europe and North America: An international multicentre study. *Journal of Antimicrobial Chemotherapy, 65*(6), 1145–1154.

Pilla, R., Castiglioni, V., Gelain, M. E., Scanziani, E., Lorenzi, V., Anjum, M., & Piccinini, R. (2012). Long-term study of MRSA ST1, t127 mastitis in a dairy cow. *Veterinary Record, 170*.

Porrero, M. C., Wassenaar, T. M., Gómez-Barrero, S., García, M., Bárcena, C., Alvarez, J., Sáez-Llorente, J. L., Fernández-Garayzábal, J. F., Moreno, M. A., & Domínguez, L. (2012). Detection of methicillin-resistant *Staphylococcus aureus* in Iberian pigs. *Letters in Applied Microbiology, 54*(4), 280–285.

Price, L. B., Stegger, M., Hasman, H., Aziz, M., Larsen, J., Andersen, P. S., Pearson, T., Waters, A. E., Foster, J. T., Schupp, J., & Gillece, J. (2012). *Staphylococcus aureus* CC398: Host adaptation and emergence of methicillin resistance in livestock. *MBio, 3*(1).

Roger, C., Roberts, J. A., & Muller, L. (2018). Clinical pharmacokinetics and pharmacodynamics of oxazolidinones. *Clinical Pharmacokinetics, 57*(5), 559–575.

Rolo, J., Miragaia, M., Turlej-Rogacka, A., Empel, J., Bouchami, O., Faria, N. A., Tavares, A., Hryniewicz, W., Fluit, A. C., De Lencastre, H., & CONCORD Working Group. (2012). High genetic diversity among community-associated Staphylococcus aureus in Europe: Results from a multicenter study. *PloS One, 7*(4), e34768.

Rowland, S. J., & Dyke, K. G. (1989). Characterization of the staphylococcal beta-lactamase transposon Tn552. *The EMBO Journal, 8*(9), 2761–2773.

Ruhlmann, C. H., Kolmos, H. J., Kristiansen, J. E., & Skov, R. (2008). Pigs as an infection source for methicillin resistant *Staphylococcus aureus* infections in humans. *Ugeskrift for Laeger, 170*(43), 3436–3436.

Ruiz-Ripa, L., Bellés-Bellés, A., Fernández-Fernández, R., García, M., Vilaró, A., Zarazaga, M., & Torres, C. (2021). Linezolid-resistant MRSA-CC398 carrying the cfrgene, and MRSA-CC9 isolates from pigs with signs of infection in Spain. *Journal of Applied Microbiology, 131*, 615.

Santos, V., Gomes, A., Ruiz-Ripa, L., Mama, O. M., Sabença, C., Sousa, M., Silva, V., Sousa, T., Vieira-Pinto, M., Igrejas, G., & Torres, C. (2020). Methicillin-resistant *Staphylococcus aureus* CC398 in purulent lesions of piglets and fattening pigs in Portugal. *Microbial Drug Resistance, 26*(7), 850–856.

Schifano, J. M., Edifor, R., Sharp, J. D., Ouyang, M., Konkimalla, A., Husson, R. N., & Woychik, N. A. (2013). Mycobacterial toxin MazF-mt6 inhibits translation through cleavage of 23S rRNA at the ribosomal A site. *Proceedings of the National Academy of Sciences, 110*(21), 8501–8506.

Schwarz, S., Kehrenberg, C., Doublet, B., & Cloeckaert, A. (2004). Molecular basis of bacterial resistance to chloramphenicol and florfenicol. *FEMS Microbiology Reviews, 28*(5), 519–542.

Schwarz, S. T. E. F. A. N., & Cardoso, M. A. R. I. S. A. (1991). Molecular cloning, purification, and properties of a plasmid-encoded chloramphenicol acetyltransferase from Staphylococcus haemolyticus. *Antimicrobial Agents and Chemotherapy, 35*(7), 1277–1283.

Scott, G. M., Thomson, R., Malone-Lee, J., & Ridgway, G. L. (1988). Cross-infection between animals and man: Possible feline transmission of *Staphylococcus aureus* infection in humans? *Journal of Hospital Infection, 12*(1), 29–34.

Shanmathy, M., Gopi, M., & Beulah, P. (2018). Contribution of animal husbandry to Indian economy, its characteristics and future: A review. *Asian Journal of Agricultural Extension, Economics & Sociology, 27*, 1–7.

Sharma, M., Nunez-Garcia, J., Kearns, A. M., Doumith, M., Butaye, P. R., Argudín, M., Lahuerta-Marin, A., Pichon, B., AbuOun, M., Rogers, J., & Ellis, R. J. (2016). Livestock-associated methicillin resistant *Staphylococcus aureus* (LA-MRSA) clonal complex (CC) 398 isolated from UK animals belong to European lineages. *Frontiers in Microbiology, 7*, 1741.

Speer, B. S., Shoemaker, N. B., & Salyers, A. A. (1992). Bacterial resistance to tetracycline: Mechanisms, transfer, and clinical significance. *Clinical Microbiology Reviews, 5*(4), 387–399.

Stegger, M., Lindsay, J. A., Sørum, M., Gould, K. A., & Skov, R. (2010). Genetic diversity in CC398 methicillin-resistant *Staphylococcus aureus* isolates of different geographical origin. *Clinical Microbiology and Infection, 16*(7), 1017–1019.

Stegger, M., Liu, C. M., Larsen, J., Soldanova, K., Aziz, M., Contente-Cuomo, T., Petersen, A., Vandendriessche, S., Jimenez, J. N., Mammina, C., & Van Belkum, A. (2013). Rapid differentiation between livestock-associated and livestock-independent *Staphylococcus aureus* CC398 clades. *PloS One, 8*(11), e79645.

Takano, T., Higuchi, W., Otsuka, T., Baranovich, T., Enany, S., Saito, K., Isobe, H., Dohmae, S., Ozaki, K., Takano, M., & Iwao, Y. (2008). Novel characteristics of community-acquired methicillin-resistant *Staphylococcus aureus* strains belonging to multilocus sequence type 59 in Taiwan. *Antimicrobial Agents and Chemotherapy, 52*(3), 837–845.

Tooke, C. L., Hinchliffe, P., Bragginton, E. C., Colenso, C. K., Hirvonen, V. H., Takebayashi, Y., & Spencer, J. (2019). β-Lactamases and β-lactamase inhibitors in the 21st century. *Journal of Molecular Biology, 431*(18), 3472–3500.

Tsiodras, S., Gold, H. S., Sakoulas, G., Eliopoulos, G. M., Wennersten, C., Venkataraman, L., Moellering, R. C., Jr., & Ferraro, M. J. (2001). Linezolid resistance in a clinical isolate of *Staphylococcus aureus*. *The Lancet, 358*(9277), 207–208.

Vincze, S., Stamm, I., Kopp, P. A., Hermes, J., Adlhoch, C., Semmler, T., Wieler, L. H., Lübke-Becker, A., & Walther, B. (2014). Alarming proportions of methicillin-resistant *Staphylococcus aureus* (MRSA) in wound samples from companion animals, Germany 2010–2012. *PloS One, 9*(1), e85656.

Voss, A., Loeffen, F., Bakker, J., Klaassen, C., & Wulf, M. (2005). Methicillin-resistant *Staphylococcus aureus* in pig farming. *Emerging Infectious diseases, 11*(12), 1965.

Wan, M. T., Lauderdale, T. L., & Chou, C. C. (2013). Characteristics and virulence factors of livestock associated ST9 methicillin-resistant *Staphylococcus aureus* with a novel recombinant staphylocoagulase type. *Veterinary Microbiology, 162*(2–4), 779–784.

Wendlandt, S., Feßler, A. T., Monecke, S., Ehricht, R., Schwarz, S., & Kadlec, K. (2013). The diversity of antimicrobial resistance genes among staphylococci of animal origin. *International Journal of Medical Microbiology, 303*(6–7), 338–349.

Zakour, N. L. B., Bannoehr, J., van den Broek, A. H., Thoday, K. L., & Fitzgerald, J. R. (2011). Complete genome sequence of the canine pathogen Staphylococcus pseudintermedius. *Journal of Bacteriology, 193*(9), 2363–2364.

Prevalence of Extended Spectrum Beta Lactamase (ESBL)-Producing *E. coli*: A Systematic Overview

Ardhra Vijayan, G. K. Sivaraman, and Till Bachmann

Contents

Abstract

The introduction and widespread transmission of *Escherichia coli* that produces extended-spectrum β-lactamases (ESBLs) pose an extreme task in controlling healthcare- and community-associated infections. The transmission of genes of obduracy by means of horizontal or clonal development could possibly enhance the swiftness in incidence of ESBL *E. coli* in the environment. When the virulent pathotypes possess the ESBL genes, it results in the population with increased risk of being infected, and all the treatment strategies become ineffective. Thus, it is necessary to further investigate the ways and means of perseverance of ESBL genes among clinical as well as community settings, and its diagnosis should also be done to find unknown carriers.

A. Vijayan · G. K. Sivaraman (✉)
Microbiology, Fermentation & Biotechnology Division, ICAR- Central Institute of Fisheries Technology, Cochin, Kerala, India
e-mail: gk.sivaraman@icar.gov.in

T. Bachmann
Infection Medicine, Edinburgh Medical School: Biomedical Sciences, The University of Edinburgh, Edinburgh, UK

M. P. Mothadaka et al. (eds.), *Handbook on Antimicrobial Resistance*,
https://doi.org/10.1007/978-981-19-9279-7_24

Keywords

Escherichia coli · ESBL · Nosocomial · Pathotypes

1 Disease-Causing Characters of *E. coli* and ESBL Production

Escherichia coli is the paramount communal commensal microorganism of *Enterobacteriaceae* that occurs in both human and animal gut microflora. This also acts as symbiont in the digestive system. Presently, there are 160 serological strains and surface antigens including 171, 80, and 55 of somatic (O), capsular (K), and flagellar (H) antigens documented, respectively, in *E. coli* (Sarowska et al., 2019). The WHO reports indicate that *E. coli* is the single furthermost communal pathogen with a share of 20.1% incidence in healthcare-associated infections in mixed patient populaces (WHO, 2011). Some variants and pathotypes have been identified that are responsible for acute intestinal infections, viz., diarrhea, urinary tract infections (UTIs), bloodstream infections (BSIs), sepsis/meningitis, etc.

Pathotypes of intestinal pathogenic *Escherichia coli* (InPEC) consist of six categories, namely, pathogenic, toxigenic, hemorrhagic/Shiga toxin producing, invasive, aggregative, and diffusely adherent (Table 1) and extraintestinal pathogenic *E. coli* (ExPEC) pathotypes, uropathogenic, sepsis-related, neonatal meningitis linked, and animal pathotype, namely, avian pathogenic (Table 1).

A genetic analysis revealed that an exchange of plasmid located (typical virulent plasmid ColV) virulence gene (*iss*) is possible between human and APEC strains. This could be a reason for the genetic exchange and diversity among different

Table 1 Pathotypes of intestinal and extraintestinal *E.coli*

S. No	*E. coli* group	*Escherichia coli* categories	Abbreviated form
1	Intestinal pathogenic *Escherichia coli* (InPEC)	Enteropathogenic *Escherichia coli*	EPEC
2		Enterotoxigenic *Escherichia coli*	ETEC
3		Entero-hemorrhagic *E. coli* or Shiga toxin-producing *Escherichia coli*	EHEC/STEC 0157:H7 serotype
4		Entero-invasive *Escherichia coli*	EIEC
5		Entero-aggregative *Escherichia coli*	EAEC
6		Diffusely adherent *Escherichia coli*	DAEC
1	Extraintestinal pathogenic *Escherichia coli* (ExPEC)	Uropathogenic *Escherichia coli*	UPEC
2		Sepsis-associated *Escherichia coli*	SEPEC
3		Neonatal meningitis-associated *Escherichia coli*	NMEC
4		Animal pathotype avian pathogenic *Escherichia coli*	APEC

pathotypes (Rodriguez-Siek et al., 2005). Another virulent is AIEC, but its pathogenesis is not well defined. The heat-labile and heat-stable enterotoxins LT and ST interrupt the host cell in the majority of virulent strains of *E. coli*; *Shigella* enterotoxins, 1-shET1 and 2-shET2, and Shiga toxin-Stx by ETEC, EAEC, EIEC/ETEC, and EHEC, respectively, etc. adhesins, invasins, lipopolysaccharides, and other virulence components of ExPEC are encoded by islands of pathogenicity (PAIs), extrachromosomal elements, namely, plasmids, and supplementary mobile genetic elements (Köhler & Dobrindt, 2011). In commensal *E. coli*, mutations and/or rearrangements of genes may happen which lead to development of virulence. Among phylogenetic groups, viz., A, B1, B2, C, D, E, F, G, D, B2, and to a less significant to group A, contain makers of ESBL. The *E. coli* strains that are commensal in nature typically symbolize cluster A or B1, gastral pathotypes most frequently are placed in A, B1, or D assembly, and extraintestinal pathotypes denote B2 and D clusters (Picard et al., 1999). As per the WHO 2017 assessment, the top priority on AMR research has been assigned to ESBL-producing *Escherichia coli* and *Klebsiella pneumoniae* due to the fact that global nosocomial infection burden centered on these bacteria (WHO, 2017).

Along with these virulence mechanisms, *E. coli* is determined as a pool of antibiotic resistance determinants, and one among the shared antibiotic-unresponsive processes is owing to the existence of β-lactamase enzymes in *Enterobacteriaceae* (Pitout et al., 1998). β-Lactams are the class of most prescribed treatment regimens that usually exert their effects through the cell wall inhibition and biosynthesis through enzyme inhibition (penicillin-binding proteins (PBPs)). Generally, the β-lactam resistance may be a result of β-lactamase production (destruct the β-lactam ring), modification in PBPs (reduced β-lactam affinity), and a reduced outer membrane impermeability or active efflux. The amino acid similarity of β-lactamase enzymes was used to classify them in Ambler molecular classification, and substrate-inhibitor profile was used in a well-designed grouping of Bush-Jacoby-Medeiros (Bush & Jacoby, 2010) (Fig. 1). Disturbingly, obduracy of β-lactam in *Enterobacteriaceae* has occasioned in the advent of ESBLs, which possess hydrolyzing ability of oxyimino-β-lactams, namely, third-generation cephems besides aztreonam nonetheless with cephamycins or carbapenems, and subdued by clavulanic acid. The non-ESBL unresponsiveness associated with *Enterobacteriaceae* triggered by oversecretion impediment-impervious β-lactamases and cephalosporinase that are obdurate to inhibitors which are categorized by unyieldingness to β-lactam amalgamations due to lack of combined effect among the antimicrobials and blockers (Drieux et al., 2008).

1.1 Class A ESBLs

At present, the most frequent and predominant β-lactamase type is represented by CTX-M, and the infections caused by it are mentioned as "CTX-M pandemic." The ailments, viz., UTIs, BSIs, and intra-abdominal infections (IAIs), are mainly caused by *E. coli* that produce CTX-M enzyme (Cantón & Coque, 2006). True to its name,

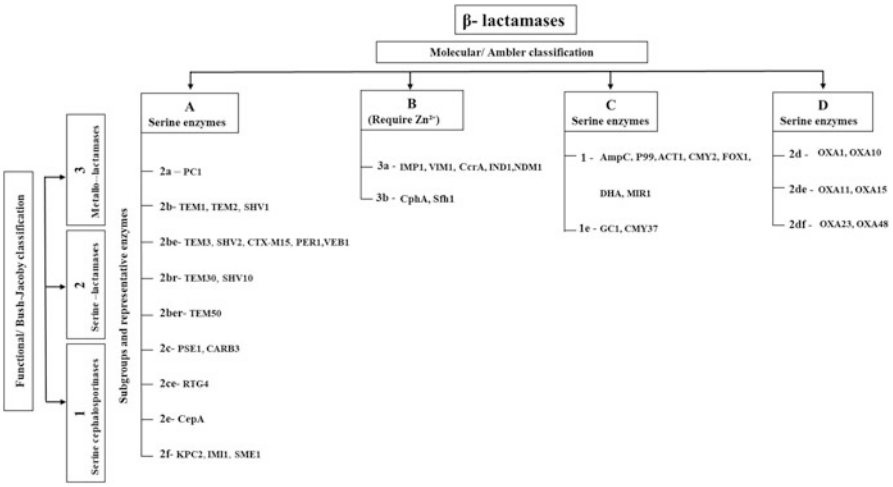


Fig. 1 Classification of β-lactamase enzyme (Bush & Jacoby, 2010)

CTX-M-type lactamases have the ability of hydrolyzing cefotaxime (Cefotaxime) but thwarted by β-lactamase deterrents such as clavulanic acid, tazobactam, avibactam, etc. (D'Andrea et al., 2013). The ESBL genes of CTX-M-type initially originated from inconsequential pathogen *Kluyvera* spp., a member of the *Enterobacteriaceae* and circulated their alleles of CTX-M to nosocomial-associated strains, viz., *E. coli* and *Klebsiella*, through transduction process (Zhao & Hu, 2013). This kind of genes belonged to the category of molecular class A or efficient group 2be and consists of six sublineages: CTX-M-1 comprising 3, 10, 12, and 15; CTX-M-2 with that of 2, 4, 5, 6, and 7, Toho-1; CTX-M-8; CTX-M-9 as well as 9, 14, 16, 18, and 19, Toho-2; CTX-M-25; and KLUC type (Cantón & Coque, 2006). Among CTX-M enzymes, 1, 14, and 15 are the frequently circulating types in *E. coli* sourced from nosocomial as well as community settings. Toho-type ESBL (named after a case from Toho University, Tokyo) is related to CTX-M-type in structure as well as cefotaxime hydrolytic activity (Ma et al., 1998).

Moreover, the association of genes of *bla*CTX-M with other drug-unresponsive genes has also been described. For example, association of CTX-M-3 to *arm*A and *rtm*B genes (16SRNA methylase), CTX-M 1 and 9 to quinolone-resistant genes like *qnr*A and *qnr*S, and CX-M-15 to *qnr*A&B and fluoroquinolone acetyltransferase (aac(6′)-Ib-cr) was reported (Poirel et al., 2006; Bogaerts et al., 2007). Likewise, specific links with certain phylogenetic groups have also been discovered: group A with CTX-M-9 and CTX-M-14 and group B2 with CTX-M-15 (Cantón & Coque, 2006).

Extended-spectrum TEM (Temoneira, a patient's named) and SHV (**s**ulf**h**ydryl **v**ariable) β-lactamases are mutual for *Escherichia coli* and *Klebsiella pneumoniae.* They are effective against penicillins, besides cephalosporins of extended-spectrum kind plus monobactams (Jacoby & Medeiros, 1991). SHV-1 β-lactamase is mostly carried in plasmid, sometimes also in chromosome (Zhao & Hu, 2013). The most

prevalent SHV variations in *Enterobacteriaceae* which have been detected all around the world are SHV-5 and SHV-12 (Yan et al., 2000), and this SHV-12 showed association with phylogroups A and D (Cantón & Coque, 2006). Like SHV-1, ampicillin-resistant enteric Gram-negative bacteria, *E. coli*, produce the most prevalent plasmid-mediated β-lactamase, TEM-1. Moreover, from the ESBL derivatives, TEM-1 and TEM-2 β-lactamase mutants show decreased clavulanate blocking, and the sulfones have emerged. TEM-3, the first ESBL phenotype of TEM variant, was reported in 1988. Nearly ten variants of TEM have been reported and named as deterrent-unresponsive TEM, namely, IRT-1, IRT-2, and IRT-3; TEM obdurate to blockers, viz., TRI-1, TRI-2, TRI-3, and TEM types 30 and 31 (Vedel et al., 1992). Complex mutant derived from TEM-1(CMT) is another ESBL phenotype which showed resistance to clavulanate and sulbactam. *E. coli*-possessing TRI variants from the clinical settings have been reported (Vedel et al., 1992). Some TEM variants were regional in nature; TEM-3 was the frequently occurring type in France (Soilleux et al., 1996). Likewise TEM-10 incidence is maximum in the USA (Wiener et al., 1999). But TEM-26 was detected worldwide (Pitout et al., 1998). There are 243 TEM variations and 228 SHV variants found now from both ESBL and non-ESBL phenotypes (NCBIa; NCBIb).

As the name indicates, KPC (*Klebsiella pneumoniae* carbapenemases)-type class A ESBL globally circulated through *K. pneumoniae*, and is usually carried by transmissible elements (Tn4401) (Naas et al., 2008). In contrast, KPC-1/2, KPC-3, and KPC-9 have been reported from *E. coli* too (Navon-Venezia et al., 2006; Pitout & Laupland, 2008). Among KPC variants, KPC-2 is the most prevalent one (Papp-Wallace et al., 2015). In general, KPC type has been demonstrated to hydrolyze oxyimino cephalosporins and carbapenems, and inhibitors (clavulanic acid and tazobactam) have only faintly inhibited it (Yigit et al., 2001).

The relationship between ESBL type and *E. coli* subgroups has been reported: CTX-M-type/subgroup D_2 (less virulent), SHV-type/subgroup $B2_3$ (highly virulent), and between TEM type/subgroup $B2_2$. Thus, the intrinsic virulence potential of *E. coli* could be predicted by ESBL type (Branger et al., 2005).

On top of β-lactamases of important type, less common class A β-lactamases (minor ESBLs) in *Enterobacteriaceae*, namely, SFO, BES, BEL, TLA, IBC GES, PER, and VEB, were categorized based on origin *Serratia fonticola*, Brazil, Belgium; in recognition of Tlahuicas Indians, integron-borne cephalosporinase Guiana, *Pseudomonas* obdurate and Vietnam, respectively, have also been reported (Paterson & Bonomo, 2005). *E. coli* strains harboring recombinant plasmids (rec) or transformant (Tc) strains in possession of these β-lactamases were well documented (Naas et al., 2008). The studies indicate (Bauernfeind et al., 1996) that *bla*PER- which encrypts class A ESBL occurred in *E. coli*. TLA-1 and VEB-1 were originally recognized in *coli* in 1993 (Mexico) and 1996 (Vietnam), respectively. VEB-1 localized on plasmid and integron, and TLA in self-transferable plasmid (Poirel et al., 1999). *E. coli* strain possesses integron-borne VEB-1 (In53) which carried additional resistance markers, including OXA10 β-lactamase aminoglycoside, rifampin ADP ribosyltransferase, chloramphenicol, and quaternary ammonium compounds also (Naas et al., 2001).

In addition, enterobacterial isolates were observed to possess PMQR (*qnrA*) (Poirel et al., 2005).

1.2 Class B ESBLs

Class B ESBLs are also called metallo-β-lactamases (MBLs) that hydrolyze the carbapenems and suppressors of β-lactamase, viz., clavulanic acid, sulbactam, and tazobactam (Meletis, 2016). Among the five different types of MBLs, namely, imipenem metallo-β-lactamase, Verona integron-encrypted metallo-β-lactamase, Sao Paulo metallo-β-lactamase, German imipenemase, and Seoul imipenemase, in short are IMP, VIM, VIM, SPM, GIM, and SIM, respectively. It is to be noted that the IMP and VIM occur frequently in *E. coli*. These MBLs' gene expressions can be mediated by plasmids or chromosomes (Smet et al., 2010). A new type of MBLs called NDM type (NDM-BL) was identified in 2009 in Sweden from *K. pneumoniae* infection, and later on, they were isolated from all over the world. The term was assigned based on the infected person location, that is, New Delhi. NDM types are highly resistant to the last resort antimicrobials like carbapenems (Yong et al., 2009).

1.3 Class C ESBLs

AmpC β-lactamases are not true ESBLs, whereas β-lactamase activity is shown by its overexpression in *E. coli* by deregulation of a chromosomal gene called derepressed mutants or promoter mutants (cAmpC). The other more frequent way is plasmid-mediated AmpC β-lactamase (pAmpC), which poses greater danger, because these genes are transferred horizontally through conjugation. AmpC β-lactamases generally give resistance against broad-spectrum cephalosporins like cephamycins, cefoxitin, moxalactam, and latamoxef. They are categorized as AmpC type (ACT) or Ambler class C (ACC), and based on source of identification, viz., R.I. (MIR-1) in Providence, Miriam Hospital, or DHA in Saudi Arabia the Dhahran hospital (Payne et al., 1992). PAmpC-β-lactamases frequently expressed resistance to aminoglycosides, amphenicols, quinolones, tetracyclines, and sulfonamides, and carried β-lactamase genes like CTX-M-3 (Chen et al., 2007), SHV, TEM-1, and VIM-1 (Miriagou et al., 2004). Incidence of non-inducible AmpC-type genes was observed in *E. coli,* and is controlled by two constituents, promoter and growth rate-dependent attenuator mechanisms (Jaurin et al., 1981). In vitro research has revealed that amdinocillin and tigecyclines are effective action against AmpC-*E. coli* (Hope et al., 2006).

1.4 Class D ESBLs

OXA (oxacillinases) kind are otherwise called class D-lactamases that hydrolyze carbapenems (CHDLs), and the genes encrypted as gene cassettes into MGE,

namely, integrons, or insertion sequences (ISs). In general, CHDLs are less suppressed by impedimentary clavulanic acid and tazobactam. In in vitro conditions, NaCl is the most effective inhibitor. Various OXA genes that have been reported from *E. coli* are OXA-2, OXA-4, OXA-7, OXA-30, and OXA-48 (Poirel et al., 2010). The frequent association between *bla*OXA-1 with genes of ESBL like *bla*CTX-M-1 or CTX-M-15 unresponsiveness to β-lactam and β-lactamase suppressor groupings is conferred. Among the OXA types, *bla*OXA-48 genes are mostly harbored in *Enterobacteriaceae* (Poirel et al., 2011).

2 ESBL Genes in *E. coli:* Dissemination and Persistence

Genes associated with ESBL were first recorded in clinical settings in 1983. But they are no longer restricted solely to the clinical setup, and have been found associated with other animals. In 1998, a dog from Spain was the first animal to be found to have clinical ESBL isolates; then, in several countries, it was discovered in other livestock (Smet et al., 2010). Horizontal gene transfer or clonal proliferations are the most common ways for ESBL genes to spread.

2.1 Gene Transfer: Horizontal Method

Plasmids are crucial transporters for HGT-related drug obduracy through the mobile genetic elements within the plasmid, as well as between the chromosome and the plasmid(s) of the same or different organisms, transposons, integrons, and insertion sequence common regions (ISCR) (Carattoli, 2013). Transmissible plasmids generally carry ESBL genes and disseminate through bacterial conjugation. After a successful entry of plasmid in a new host cell, transposable elements of plasmid origin (e.g., transposons) can further assemble antimicrobial resistance genes to the chromosome or a separate plasmid. In general, plasmids are divided into various incompatibility (Inc) groups (Novick, 1987). The exclusively reported ESBL-producing *E. coli* plasmids are IncF. But the Inc. grouping has been replaced by replicon typing because it is based on highly conserved part encoding replication initiation, control, copy number, etc., and also will give subdivisions of some Inc. groups. For example, IncF1 plasmids harbored ESBLs that comprise replicons like repFIA, FIB, and FIC. Among various Inc. types, IncF plasmids (IncFII) are mostly associated with CTX-M-15, IncN with CTX-M-32, IncHI2 with CTX-M-2 and CTX-M-9, and IncA/C connected with carbapenem unresponsiveness. It is conceivable to locate CTX-M-2 and CTX-M-9 genes on IncP, IncA/C, or IncFI plasmids. Table 2 shows examples of fully sequenced plasmids from *E. coli* bearing CTX-M and carbapenemase genes (Carattoli, 2013).

Several sequences of insertion, viz., ISEcp1 and ISCR1 (known earlier as orf 513), and the mobility of blaCTX-M genes have been concomitant to phage elements which were reported across the world (Cantón & Coque, 2006; Poirel et al.,

Table 2 Fully sequenced plasmids from *E. coli* carrying CTX-M and carbapenemase genes (Carattoli, 2013)

Plasmid family	Plasmid	Resistance gene
IncN	pHHA45	*bla*CTX-M-1
	pKC394	*bla*CTX-M-1, *bla*CTX-M-65
	pEC L46	*bla*CTX-M-15
	pKC396	*bla*CTX-M-24
IncF	pETN48, pHK01, pHK08, pHK09, pHK17a	*bla*CTX-M-14
	pC15-1a, pEC B24, pEC L8, pEK499, pEK516, pEC L46	*bla*CTX-M-15
	pXZ	*bla*CTX-M-24
	pHN7A8	*bla*CTX-M-65
IncK	pCT	*bla*CTX-M-14
IncI1	pECBactec	*bla*CTX-M-15
	pEK204	*bla*CTX-M-3
IncX4	pJIE143	*bla*CTX-M-15
IncA/C	pNDM-1 Dok01, pNDM102337, pNDM10505	*bla*NDM-1
IncL/M	pNDM-HK	
IncN2	p271A	

2008). The IS1380 family includes ISEcp1, identified in blaCTX-M gene upstream (Cantón & Coque, 2006) and showed association with *bla*CTX-M 1, 2, 25, and 9 gene clusters (Liu et al., 2007), *bla*CMY-2 gene (D'Andrea et al., 2006), and *bla*ACC 1 and 4 genes (Partridge, 2007).

The ISCR1 gene has also been reported in conjunction with blaCTX-M genes (Partridge & Hall, 2003), and ampC genes, like CMY-1, CMY-8, CMY-9, CMY-10, CMY-11, CMY-19, MOX-1, and DHA-1, were also identified from downstream of an ISCR1 which takes part in mobility of gene (Toleman et al., 2006). The third genetic element allied to blaCTX-M genes is phage-related types. This could imply that ISEcp1, ISCRI, and phage-related elements are crucial in the ESBL gene transportation among dissimilar plasmids.

In transportation of all genes of *bla*TEM, the three transposons that are important are Tn 1, 2, and 3 (Partridge & Hall, 2005). The conjugative plasmid IncI1 which is stable and large occurs mainly in *Enterobacteriaceae* in which the *bla*TEM-52 gene transports Tn3-like transposon is located (Cloeckaert et al., 2007). Mobile genetic elements for other ESBL genes like *bla*VEB-1 and *bla*GES-1 genes were recognized as a gene cassette in class I integrons (Poirel et al., 1999, 2005; Girlich et al., 2001). Moreover, *bla*GES-1 gene incidence was observed on gene cassette in class 3 integrons. As a result of these studies, ESBL genes found on integrons are classified as Ambler class D, class B. (Girlich et al., 2001), as well as class A. This could increase the likelihood of plasmid-mediated class A ESBL types propagating.

2.2 Clonal Distribution

Beyond the plasmid-driven spread of ESBL, bacterial clones ae crucial in the transmission of genes that are the root cause for not responding to antimicrobials. The clonal distribution of clinically significant phylogenetic lineages, distinguished by the sequence types (STs), is occasionally observed in ESBL *E. coli* (Ewers et al., 2012). G-ve MDR microbes, particularly enterobacters, have emerged as globally as high-hazard clones. In the case of ExPEC, the globally found highly hazardous MDR clonal ancestry is the sequence-type 131(ST131) which is closely linked to *bla*CTXM-15, and is the reason for unresponsiveness to both cephalosporins and fluoroquinolones (Nicolas-Chanoine et al., 2014). Few have contributed to the transmission of blaCTX-M-3, and *bla*KPC-2 genes too (Pitout & Laupland, 2008). General characteristics of *E. coli* ST131 are represented in Table 3 (Nicolas-Chanoine et al., 2008). The H30-Rx sublineage has grown as a result of its affiliation with cephalosporins, while the H30-R sublineage has grown as a result of its association with fluoroquinolones. Virulence mechanism and biofilm formation of the clone make them more stable and versatile than other ExPEC strains. Numerous reports have been documented and are obtainable on interfamilial transmission of ESBL-producing *ExPEC* ST131 (Ender et al., 2009). *E. coli* ST140 and ST131 are high-risk clones across the world, with carbapenem resistance genes (blaOXA-181 and blaNDM-5) carried by IncX3 and IncF plasmids (Roer et al., 2018). Another MDR *E. coli* clone ST38 possessing *bla*OXA-48 was also reported previously (Poirel et al., 2011). Other examples of MDR clones of *E. coli* are ST7, ST69, ST155, ST393, ST405, and ST648. Schaufler et al. (2016) reported the interspecies transmission and zoonotic potential of a novel viable *E. coli* ST410 clone among humans, wildlife, and the environment. STs, including ST131, 648, and 617, are shared by human beings, faunae, and the environs (Guenther et al., 2011). The incidence of ST10, 23, 48, 58, 115, 117, 131, 350, and 648 with genes like CTX-M-1

Table 3 Characteristic of high-risk clone *E. coli* ST131 (Nicolas-Chanoine et al., 2008)

Characteristic	Description
Pathogenicity	Uropathogenic
Phylogroup	B2
Plasmid	IncF types
Clonal complex	CC131
Mobile genetic element	ISEcp1
Virulence genes	*sat*, *iut*A, *mal*X, *usp*, *iha*, *hra,* and *omp*T
Serotypes	O25b:H4, O16:H5
Lineages	O25 fimH30, O16 fimH41
Resistance determinants	CTX-M-15 (H30-Rx sublineage), Flouroquinolones (H30-R sublineage)
Fitness	High metabolic potential and biofilm production
Laboratory methods for the detection	PCR, NGS, MLST, PFGE, MLVA, MALDI-TOF MS

and CTX-M-9, TEM-52, SHV-12, and/or CMY-2 was observed in chickens (Ewers et al., 2012). Companion animals have been shown to contribute to the CTX-M-1 pool in individuals by transmitting certain plasmids such as IncI1/ST3 and IncI1/ST157 (Madec et al., 2015). Thus, domestic animals are an important source of ESBL unresponsiveness transmitted by means of HGT.

2.3 Detection of ESBL *E. coli*

As potent infectious agent, *E. coli* harboring ESBL is an increasing problem worldwide. Thus, reliable and rapid screening of multiple samples is required to diagnose and govern the transmission of these virulent microbes. Some of the phenotypic (culture-dependent approach) as well as genotypic (culture-independent approach) screening and confirmatory procedures employed in identification of ESBL *E. coli* are described here. Even though phenotypic methods are regularly being used for the detection, genotypic methods are mostly preferred, owing to their precision and specificity.

The isolates of *E. coli* were verified phenotypically employing disc diffusion technique to confirm the production of ESBL based on guiding principles of CLSI USA. The process of testing is dependent on determining the exact diameter of the zones of discs of the different antibiotics. ESBL production was examined on every isolate that exhibited resistance to a minimum one of the antibiotics, namely, cefotaxime, cefpodoxime, ceftriaxone, ceftazidime, cefepime, aztreonam, etc. With regard to double-disc diffusion test, a disc containing amoxicillin/clavulanate at the rate of 20 μg and 10 μg and a cefotaxime @30 μg is used. The combined effect between cefotaxime and clavulanate was categorized as a distinctive leeway of the cefotaxime area of inhibition toward the disc of clavulanate, confirming occurrence of an ESBL (Wayne, 2009).

One of the ESBL detection procedures is the use of E-test bands of ascent applications of ceftazidime/amalgamation of ceftazidime and clavulanic acid and cefotaxime and cefotaxime in addition to clavulanic acid. The connection point of the suppression loop at the end of the test panel will be ESBL positive if cefotaxime at 0.5 and cefotaxime with clavulanic acid at 8 or ceftazidime and mixture of ceftazidime and clavulanic acid at 8 are applied to measure the MIC (Cormican et al., 1996). In another method of ESBL detection, the blend discs of cephalosporin/clavulanate are applied on Iso-Sensitest agar. A proportion of area size of cephalosporin/clavulanate to cephalosporin area 1.5 or greater confirms the incidence of ESBL. The major lacuna in this screening procedure is that it cannot detect the isolates that are involved in production of SHV-6-type ESBLs (M'Zali et al., 2000).

In the dilution method of detection, both agar and broth can be used as media, where the antibiotics are incorporated. In broth microdilution, the employment of ceftazidime, combination of ceftazidime and clavulanic acid, cefotaxime, or cefotaxime in amalgamation with clavulanic acid arrayed in μg/mL from 0.25 to 128, 0.25/4 to 128/4, 0.25 to 64, and 0.25/4 to 64/4, respectively. The MIC is

interpreted based on the growth inhibition (Queenan et al., 2004). MicroScan G-Ve Urine MIC 7 and G-Ve MIC Plus 2 panes are types of dehydrated panels used for microdilution and inferred by the scheme of Walkaway reliably indicating the presence of ESBL. The USFDA permitted the panes that hold amalgamations of ceftazidime or cefotaxime and suppressors of β-lactamase, whereas, in ESBL screening Vitek employs only cefotaxime and ceftazidime or in amalgamation with clavulanic acid at μg/mL levels of 0.5 and 4, respectively. The interpretation of positive outcomes is based on restriction of growth in the wells that are with combination of drugs in relation to the wells that had a single drug. Sensitivity and specificity greater than 90% were observed (Sanders et al., 1996).

The AmpC disc test used for identification of AmpC β-lactamase is one method which is grounded on the application of Tris EDTA to evict β-lactamases into the outer environs of bacteria making the cell wall permeable. Inactivation of cefoxitin (positive result) is obtained from either a depression or a flattening of the inhibition zone. The cefepime/cefepime in combination with clavulanic acid (PM/PML) strips also is employed in detection of Ampcβ-lactamase ESBL co-producers. In this method every strip harbors ascending levels of cefepime at one termination and at the other culmination an ascent of cefepime in combination with a persistent level of clavulanate at varying levels stretching from 0.25 to 0.50 μg/mL and 0.064 to 4 μg/mL with added + clavulanic acid at a level of 4 μg/mL, respectively. A positive outcome is recorded wherein the MIC ratio for PM/PML is $\geq$8, and the sign of a deformation or ellipse (Black et al., 2005).

The phenotypic detection methods that include automated microbiology advanced systems, viz., BD Phoenix and VITEK 2, are available for detecting the ESBLs. In the identification of ESBL producers, the system of BD Phoenix takes advantage of growth restriction characteristics of cefpodoxime, ceftazidime, ceftriaxone, and cefotaxime, in clavulanic acid occurrence or its dearth. Advantages of BD Phoenix are the ESBL identification of a variety of ESBL types, namely, SHV-2, SHV-5, SHV-12, and CTX-M-1 (Stürenburg et al., 2003). In routine screening of clinical *Enterobacter* isolates for ESBL production, the efficacy of VITEK 2 was assessed (Shanu et al., 2006) and the same seen to be useful in large-scale detection of the sample in shorter periods in clinical settings.

In the identification of unresponsiveness to β-lactam, one of the innovative methods that can be employed is matrix-assisted laser desorption/ionization time-of-flight mass spectrometry in short "MALDI-TOF MS." Centered on this, mini-sequencing process is created for quick detection of SNPs at *bla*TEM gene (Ikryannikova et al., 2008). Further development in this direction is "mass spectrometry-based assay for antibiotic susceptibility testing" also known as MAAST for rapid identification of ampicillin unresponsiveness in *E. coli* (Grundt et al., 2012). In identification of insusceptible cephalosporins of third-generation in *E. coli*, the MALDI-TOF MS β-lactamase assay was performed (Jung et al., 2014). Similarly, for recognition of ES-βL and AmpC, a panel designed through MALDI-TOF MS centered "direct-on-target microdroplet growth assay" (Correa-Martínez et al., 2019). Besides, in the identification of antibiotic insensitivity in *E. coli* and

K. pneumoniae, a partial measurable MALDI-TOF-MS-dependent technique, namely, MBT-ASTRA, is well defined (Axelsson et al., 2020).

In order to minimize time-consuming screening protocols, different kinds of selective media are available for the ESBL detection, including MacConkey agar augmented with ceftazidime, Drigalski agar added with cefotaxime, and nutrient agar by way of ceftazidime, vancomycin, and amphotericin B. Different media with chromogen also advanced to identify ESBL in hospital samples, for example, CHROMagar ESBL media and CHROMagar KPC for the fast identification of carbapenem-producing Gram-negative bacteria (CRE) (Samra et al., 2008).

Some rapid commercial test kits are available for the ESBL detection. An easy to perform swift test of ESBL NP is reliable in identification of ESBL producers which gives results within 40–45 min (Demord et al., 2021). Garcia-Fernández et al. (2015) observed that the CRE system of eazyplex®SuperBug is a reliable and effective technique in detection of carbapenemases and CTX-M-type ESBLs within 15 min. The OKN carbapenemase assay is another effective, quick, and simple to use method for confirming OXA-48, NDM, and KPC carbapenemases (Glupczynski et al., 2017). Bianco et al. (2020). The comparison studies on performance of RESIST-5 O.O.K.N.V. and NG-Test Carba 5 screening methods straight from blood cultures of *Enterobacteriaceae* isolates characterized revealed that both methods are 100% reliable in identification of KPC and OXA-48-type carbapenemases. The ESBL Nordmann/Dortet/Poirel (NDP) is another fast test in detection of ESBLs. The test exhibited 92.6% sensitivity and cent percent specificity (Nordmann et al., 2012). The ESBL producers were positively identified employing ESBL NDP rapid screening method from the specimens of blood cultures (Dortet et al., 2015). In the identification of enterobacters that produce carbapenemase and to discern diverse forms of nosocomial noteworthy carbapenemases, viz., Ambler classes A, B, and D, a biochemical screening method "NitroSpeed-Carba NP" was developed (Nordmann et al., 2020).

Among genotypic methods, polymerase chain reaction (PCR) is the foremost and trusted approach for detecting ESBL genes because of precision. For ease in detection of clone O25b-ST131 with elevated probability of generating ESBLs depending on replicate specific to allele PCR for the gene *pab*B, a PCR assay was developed (Clermont et al., 2009). For single-step result, a multiplex RT-PCR to identify the best shared β-lactamase gene of class A, namely, bla, CTX-M, SHV, TEM, and CIT-type AmpCs, was developed by Roschanski et al. (2014). Pomba et al. (2006) developed two variants of a process of multiplex PCR for detecting the β-lactam-unresponsive bla: TEM, SHV and OXA genes in UPEC animal strains. Similarly, a multiplex PCR process was designed (Kim et al., 2009) to categorize TEM, OXA, SHV, and CTX-M and pAmpC (CMY and DHA type) β-lactamases. A RT-TaqMan-M-PCR method was developed by Swayne et al. (2011) to distinguish genes encrypting five different forms of serine carbapenemases, viz., GES, IMI/NMC, KPC, etc.

One of the PCR-reliant probe-based identification methods is DNA microarray. In the identification of different CTX-M clusters and ES-βL-related single nucleotide polymorphisms in SHV and TEM distinctions in *Enterobacteriaceae*, a combination

of ligation-mediated amplification and microarray was employed by Stuart and others (2010) for detecting amplified products. One good example for advanced technology of high-throughput kind that facilitates swift recognition of all genes of TEM, SHV, and CTX-M ES-βL, including the gene of KPC-2, is Check-Points ESBL/KPC array (Naas et al., 2010). A lateral flow strip assay was recognized based on a combination of recombinase polymerase amplification (RPA) of multiplex kind using a single-stranded tag hybridization chromatographic printed-array strip (STH-PAS) for the prompt and concurrent finding of numerous *bla* genes in a solitary reaction (Kanokudom et al., 2021). It is a quick ESBL detection method that does not require any equipment and may aid clinical diagnosis. One of the future technologies for ESBL detection is the flow cytometry method. Boutal et al. (2017) established and assessed immunoassay of lateral flow type the NDM LFIA for instantaneous and trustworthy identification of enterobacters that produce carbapenemase similar to NDM. Along with NDM type, Boutal et al. (2018) performed one multiplex LFIA for identification of other carbapenemases comprising KPC, IMP, and VIM and OXA-48 types. Another original, straightforward, and quick flow cytometric assay was well documented in finding ESBLs (Faria-Ramos et al., 2013).

Thus, all these tests represent powerful diagnostic methods as they enable the screening and identification of ESBL *E. coli* that are clinically important, with less cost and time. Based on the vast diagnostic platforms, some ongoing in vitro trials for the effectiveness of new drug combinations against complicated ESBL infections have also progressed. Ceftazidime-avibactam is a combination generally active countering AmpCs belonging to A and C class and few of β-lactamases related to class D group, ceftaroline-avibactam and imipenem-relebactam countering AmpC, KPC, and OXA-48, ceftolozane-tazobactam opposing few AmpC, and plazomicin against AmpC, KPC, OXA, and VIM (Pana & Zaoutis, 2018).

The studies mentioned above showed that ESBL *E. coli* can persist in carriers for several years without the selective pressure of antibiotics, and shared within the community through food chains. In addition, due to associated resistance mechanisms, the spread of co-expressing ESBL with PMQR genes; other antimicrobials, namely, aminoglycosides; and sulfonamides could provide high risk of *E. coli* as superbugs (Wang et al., 2012). Thus, more research is needed to identify and minimize its presence, amplification, and blowout of ESBL *E. coli* from the environmental settings and new treatment strategies for their prevention.

3 Conclusion

Antimicrobial resistance, especially to β-lactam antibiotics, has been accepted as a serious health hazard due to drug ineffectiveness to severe infections. Among them, increasing concern is regarding the development of ESBL-producing *Enterobacteriaceae*, especially *E. coli*, as it causes severe hospital-originated and unrestricted infections. The evolution of various virulent pathotypes possessing ESBL genes will possibly lead to the emergence of severely hazardous *E. coli* pandemic. Thus,

continuous surveillance and monitoring on overuse or misuse of antibiotics in humans as well as veterinary practices, effective hygiene and sanitation measures, proper effluent treatment systems in healthcare settings, etc. are urgently needed for the mitigation of community spread of antibiotic resistance genes.

References

Axelsson, C., Rehnstam-Holm, A. S., & Nilson, B. (2020). Rapid detection of antibiotic resistance in positive blood cultures by MALDI-TOF MS and an automated and optimized MBT-ASTRA protocol for Escherichia coli and Klebsiella pneumoniae. *Infectious Diseases, 52*, 45–53.

Bauernfeind, A., Stemplinger, I., Jungwirth, R., Mangold, P., Amann, S., Akalin, E., Ang, O., Bal, C., & Casellas, J. M. (1996). Characterization of β--lactamase gene blaPER-2, which encodes an extended-spectrum class A β--lactamase. *Antimicrobial Agents and Chemotherapy, 40*, 616–620.

Bianco, G., Boattini, M., Van Asten, S. A., Iannaccone, M., Zanotto, E., Zaccaria, T., Bernards, A. T., Cavallo, R., & Costa, C. (2020). RESIST-5 OOKNV and NG-Test Carba 5 assays for the rapid detection of carbapenemase-producing Enterobacterales from positive blood cultures: A comparative study. *The Journal of Hospital Infection, 105*, 162–166.

Black, J. A., Moland, E. S., & Thomson, K. S. (2005). AmpC disk test for detection of plasmid-mediated AmpC β-lactamases in Enterobacteriaceae lacking chromosomal AmpC β-lactamases. *Journal of Clinical Microbiology, 43*, 3110–3113.

Bogaerts, P., Galimand, M., Bauraing, C., Deplano, A., Vanhoof, R., De Mendonca, R., Rodriguez-Villalobos, H., Struelens, M., & Glupczynski, Y. (2007). Emergence of ArmA and RmtB aminoglycoside resistance 16S rRNA methylases in Belgium. *The Journal of Antimicrobial Chemotherapy, 59*, 459–464.

Boutal, H., Naas, T., Devilliers, K., Oueslati, S., Dortet, L., Bernabeu, S., Simon, S., & Volland, H. (2017). Development and validation of a lateral flow immunoassay for rapid detection of NDM-producing Enterobacteriaceae. *Journal of Clinical Microbiology, 55*, 2018–2029.

Boutal, H., Vogel, A., Bernabeu, S., Devilliers, K., Creton, E., Cotellon, G., Plaisance, M., Oueslati, S., Dortet, L., Jousset, A., & Simon, S. (2018). A multiplex lateral flow immunoassay for the rapid identification of NDM-, KPC-, IMP-and VIM-type and OXA-48-like carbapenemase-producing Enterobacteriaceae. *The Journal of Antimicrobial Chemotherapy, 73*, 909–915.

Branger, C., Zamfir, O., Geoffroy, S., Laurans, G., Arlet, G., Thien, H. V., Gouriou, S., Picard, B., & Denamur, E. (2005). Genetic background of Escherichia coli and extended-spectrum β-lactamase type. *Emerging Infectious Diseases, 11*, 54.

Bush, K., & Jacoby, G. A. (2010). Updated functional classification of -lactamases. *Antimicrobial Agents and Chemotherapy, 54*, 969–976.

Cantón, R., & Coque, T. M. (2006). The CTX-M β-lactamase pandemic. *Current Opinion in Microbiology, 9*, 466–475.

Carattoli, A. (2013). Plasmids and the spread of resistance. *International Journal of Medical Microbiology, 303*, 298304.

Chen, Y. T., Lauderdale, T. L., Liao, T. L., Shiau, Y. R., Shu, H. Y., Wu, K. M., Yan, J. J., Su, I. J., & Tsai, S. F. (2007). Sequencing and comparative genomic analysis of pK29, a 269-kilobase conjugative plasmid encoding CMY-8 and CTX-M-3 -lactamases in *Klebsiella pneumoniae*. *Antimicrobial Agents and Chemotherapy, 51*, 3004–3007.

Clermont, O., Dhanji, H., Upton, M., Gibreel, T., Fox, A., Boyd, D., Mulvey, M. R., Nordmann, P., Ruppé, E., Sarthou, J. L., & Frank, T. (2009). Rapid detection of the O25b-ST131 clone of Escherichia coli encompassing the CTX-M-15-producing strains. *The Journal of Antimicrobial Chemotherapy, 64*, 274–277.

Cloeckaert, A., Praud, K., Doublet, B., Bertini, A., Carattoli, A., Butaye, P., Imberechts, H., Bertrand, S., Collard, J. M., Arlet, G., & Weill, F. X. (2007). Dissemination of an extended-spectrum-β-lactamase bla TEM-52 gene-carrying IncI1 plasmid in various Salmonella

enterica serovars isolated from poultry and humans in Belgium and France between 2001 and 2005. *Antimicrobial Agents and Chemotherapy, 51*, 1872–1875.

Cormican, M. G., Marshall, S. A., & Jones, R. N. (1996). Detection of extended-spectrum β--lactamase (ESBL)-producing strains by the Etest ESBL screen. *Journal of Clinical Microbiology, 34*, 1880–1884.

Correa-Martínez, C. L., Idelevich, E. A., Sparbier, K., Kostrzewa, M., & Becker, K. (2019). Rapid detection of extended-spectrum β-lactamases (ESBL) and AmpC β-lactamases in Enterobacterales: Development of a screening panel using the MALDI-TOF MS-based direct-on-target microdroplet growth assay. *Frontiers in Microbiology, 10*, 13.

D'Andrea, M. M., Arena, F., Pallecchi, L., & Rossolini, G. M. (2013). CTX-M-type β-lactamases: A successful story of antibiotic resistance. *International Journal of Medical Microbiology Supplements, 303*, 305–317.

D'Andrea, M. M., Nucleo, E., Luzzaro, F., Giani, T., Migliavacca, R., Vailati, F., Kroumova, V., Pagani, L., & Rossolini, G. M. (2006). CMY-16, a novel acquired AmpC-type β-lactamase of the CMY/LAT lineage in multifocal monophyletic isolates of Proteus mirabilis from northern Italy. *Antimicrobial Agents and Chemotherapy, 50*, 618–624.

Demord, A., Poirel, L., D'Emidio, F., Pomponio, S., & Nordmann, P. (2021). Rapid ESBL NP test for rapid detection of expanded-spectrum β-lactamase producers in Enterobacterales. *Microbial Drug Resistance, 27*, 1131–1135.

Dortet, L., Poirel, L., Nordmann, P. (2015). Rapid detection of ESBL-producing Enterobacteriaceae in blood cultures. *Emerging Infectious Diseases, 21*(3), 504–507. https://doi.org/10.3201/eid2103.141277

Drieux, L., Brossier, F., Sougakoff, W., & Jarlier, V. (2008). Phenotypic detection of extended-spectrum β-lactamase production in Enterobacteriaceae: Review and bench guide. *Clinical Microbiology and Infection, 14*, 90–103.

Ender, P. T., Gajanana, D., Johnston, B., Clabots, C., Tamarkin, F. J., & Johnson, J. R. (2009). Transmission of an extended-spectrum-β--lactamase-producing Escherichia coli (sequence type ST131) strain between a father and daughter resulting in septic shock and emphysematous pyelonephritis. *Journal of Clinical Microbiology, 47*, 3780–3782.

Ewers, C. A., Bethe, A., Semmler, T., Guenther, S., & Wieler, L. H. (2012). Extended-spectrum β-lactamase-producing and AmpC-producing Escherichia coli from livestock and companion animals, and their putative impact on public health: A global perspective. *Clinical Microbiology and Infection, 18*, 646–655.

Faria-Ramos, I., Espinar, M. J., Rocha, R., Santos-Antunes, J., Rodrigues, A. G., Cantón, R., & Pina-Vaz, C. (2013). A novel flow cytometric assay for rapid detection of extended-spectrum β--lactamases. *Clinical Microbiology and Infection, 19*, E8–15.

Garcia-Fernández, S., Morosini, M. I., Marco, F., Gijón, D., Vergara, A., Vila, J., Ruiz-Garbajosa, P., & Cantón, R. (2015). Evaluation of the eazyplex® SuperBug CRE system for rapid detection of carbapenemases and ESBLs in clinical Enterobacteriaceae isolates recovered at two Spanish hospitals. *The Journal of Antimicrobial Chemotherapy, 70*, 1047–1050.

Girlich, D., Poirel, L., Leelaporn, A., Karim, A., Tribuddharat, C., Fennewald, M., & Nordmann, P. (2001). Molecular epidemiology of the integron-located VEB-1 extended-spectrum β-lactamase in nosocomial enterobacterial isolates in Bangkok, Thailand. *Journal of Clinical Microbiology, 39*, 175–182.

Glupczynski, Y., Jousset, A., Evrard, S., Bonnin, R. A., Huang, T. D., Dortet, L., Bogaerts, P., & Naas, T. (2017). Prospective evaluation of the OKN K-SeT assay, a new multiplex immunochromatographic test for the rapid detection of OXA-48-like, KPC and NDM carbapenemases. *The Journal of Antimicrobial Chemotherapy, 72*, 1955–1960.

Grundt, A., Findeisen, P., Miethke, T., Jäger, E., Ahmad-Nejad, P., & Neumaier, M. (2012). Rapid detection of ampicillin resistance in Escherichia coli by quantitative mass spectrometry. *Journal of Clinical Microbiology, 50*, 1727–1729.

Guenther, S., Ewers, C., & Wieler, L. H. (2011). Extended-spectrum β--lactamases producing E. coli in wildlife, yet another form of environmental pollution? *Frontiers in Microbiology, 2*, 246.

Hope, R., Warner, M., Potz, N. A., Fagan, E. J., James, D., & Livermore, D. M. (2006). Activity of tigecycline against ESBL-producing and AmpC-hyperproducing Enterobacteriaceae from south-east England. *The Journal of Antimicrobial Chemotherapy, 58*, 1312–1314.

Ikryannikova, L. N., Shitikov, E. A., Zhivankova, D. G., Il'ina, E. N., Edelstein, M. V., & Govorun, V. M. (2008). A MALDI TOF MS-based minisequencing method for rapid detection of TEM-type extended-spectrum β--lactamases in clinical strains of Enterobacteriaceae. *Journal of Microbiological Methods, 75*, 385–391.

Jacoby, G. A., & Medeiros, A. A. (1991). More extended-spectrum β-lactamases. *Antimicrobial Agents Chemotherapy, 35*, 1697–1704. https://doi.org/10.1128/AAC.35.9.1697

Jaurin, B., Grundström, T., Edlund, T., & Normark, S. (1981). The *E. coli* -lactamase attenuator mediates growth rate-dependent regulation. *Nature, 290*, 221–225.

Jung, J. S., Popp, C., Sparbier, K., Lange, C., Kostrzewa, M., & Schubert, S. (2014). Evaluation of matrix-assisted laser desorption ionization–time of flight mass spectrometry for rapid detection of β-lactam resistance in Enterobacteriaceae derived from blood cultures. *Journal of Clinical Microbiology, 52*, 924–930.

Kanokudom, S., Assawakongkarat, T., Akeda, Y., Ratthawongjirakul, P., Chuanchuen, R., & Chaichanawongsaroj, N. (2021). Rapid detection of extended spectrum β-lactamase producing Escherichia coli isolated from fresh pork meat and pig cecum samples using multiplex recombinase polymerase amplification and lateral flow strip analysis. *PLoS One, 16*, e0248536.

Kim, J., Jeon, S., Rhie, H., Lee, B., Park, M., Lee, H., Lee, J., & Kim, S. (2009). Rapid detection of extended spectrum β-lactamase (ESBL) for Enterobacteriaceae by use of a multiplex PCR-based method. *Infection & Chemotherapy, 41*, 181–184.

Köhler, C. D., & Dobrindt, U. (2011). What defines extra-intestinal pathogenic *Escherichia coli*? *International Journal of Medical Microbiology, 301*, 642–647.

Liu, S. Y., Su, L. H., Yeh, Y. L., Chu, C., Lai, J. C., & Chiu, C. H. (2007). Characterisation of plasmids encoding CTX-M-3 extended-spectrum β-lactamase from Enterobacteriaceae isolated at a university hospital in Taiwan. *International Journal of Antimicrobial Agents, 29*, 440–445.

Ma, L., Ishii, Y., Ishiguro, M., Matsuzawa, H., & Yamaguchi, K. (1998). Cloning and sequencing of the gene encoding Toho-2, a class A β-lactamase preferentially inhibited by tazobactam. *Antimicrobial Agents and Chemotherapy, 42*, 1181–1186.

Madec, J. Y., Haenni, M., Métayer, V., Saras, E., & Nicolas-Chanoine, M. H. (2015). High prevalence of the animal-associated bla CTX-M-1 IncI1/ST3 plasmid in human Escherichia coli isolates. *Antimicrobial Agents and Chemotherapy, 59*, 5860–5861.

Meletis, G. (2016). Carbapenem resistance: Overview of the problem and future perspectives. *Therapeutic Advances in Infectious Disease, 3*, 15–21.

Miriagou, V., Tzouvelekis, L. S., Villa, L., Lebessi, E., Vatopoulos, A. C., Carattoli, A., & Tzelepi, E. (2004). CMY-13, a novel inducible cephalosporinase encoded by an *Escherichia coli* plasmid. *Antimicrobial Agents and Chemotherapy, 48*, 3172–3174.

M'Zali, F. H., Chanawong, A., Kerr, K. G., Birkenhead, D., & Hawkey, P. M. (2000). Detection of extended-spectrum β-lactamases in members of the family enterobacteriaceae: Comparison of the MAST DD test, the double disc and the Etest ESBL. *The Journal of Antimicrobial Chemotherapy, 45*, 881–885.

Naas, T., Mikami, Y., Imai, T., Poirel, L., & Nordmann, P. (2001). Characterization of In53, a class 1 plasmid- and composite transposon-located integron of Escherichia coli which carries an unusual array of gene cassettes. *Journal of Bacteriology, 183*, 235–249.

Naas, T., Cuzon, G., Villegas, M., Lartigue, M., Quinn, J. P., & Nordmann, P. (2008). Genetic structures at the origin of acquisition of the -lactamase blaKPC gene. *Antimicrobial Agents and Chemotherapy, 52*, 1257–1263.

Naas, T., Cuzon, G., Truong, H., Bernabeu, S., & Nordmann, P. (2010). Evaluation of a DNA microarray, the Check-Points ESBL/KPC array, for rapid detection of TEM, SHV, and CTX-M extended-spectrum β-lactamases and KPC carbapenemases. *Antimicrobial Agents and Chemotherapy, 54*, 3086–3092.

Navon-Venezia, S., Chmelnitsky, I., Leavitt, A., Schwaber, M. J., Schwartz, D., & Carmeli, Y. (2006). Plasmid-mediated imipenem-hydrolyzing enzyme KPC-2 among multiple carbapenem-resistant Escherichia coli clones in Israel. *Antimicrobial Agents and Chemotherapy, 50*, 3098–3101.

NCBIa. https://www.ncbi.nlm.nih.gov/pathogens/isolates#/refgene/SHV

NCBIb. https://www.ncbi.nlm.nih.gov/pathogens/isolates#/refgene/TEM

Nicolas-Chanoine, M. H., Blanco, J., Leflon-Guibout, V., Demarty, R., Alonso, M. P., Canica, M. M., Park, Y. J., Lavigne, J. P., Pitout, J., & Johnson, J. R. (2008). Intercontinental emergence of Escherichia coli clone O25:H4- ST131 producing CTX-M-15. *The Journal of Antimicrobial Chemotherapy, 61*, 273–281.

Nicolas-Chanoine, M. H., Bertrand, X., & Madec, J. Y. (2014). *Escherichia coli* ST131, an intriguing clonal group. *Clinical Microbiology Reviews, 27*, 543–574.

Nordmann, P., Dortet, L., & Poirel, L. (2012). Rapid detection of extended-spectrum-β-lactamase-producing Enterobacteriaceae. *Journal of Clinical Microbiology, 50*, 3016–3022.

Nordmann, P., Sadek, M., Demord, A., & Poirel, L. (2020). NitroSpeed-Carba NP test for rapid detection and differentiation between different classes of carbapenemases in Enterobacterales. *Journal of Clinical Microbiology, 58*, e00932–e00920.

Novick, R. P. (1987). Plasmid incompatibility. *Microbiological Reviews, 51*, 38195.

Pana, Z. D., & Zaoutis, T. (2018). Treatment of extended-spectrum β-lactamase-producing Enterobacteriaceae (ESBLs) infections: What have we learned until now? *F1000Res, 7*.

Papp-Wallace, K. M., Winkler, M. L., Taracila, M. A., & Bonomo, R. A. (2015). Variants of β-lactamase KPC-2 that are resistant to inhibition by avibactam. *Antimicrobial Agents and Chemotherapy, 59*, 3710–3717.

Partridge, S. R. (2007). Genetic environment of ISEcp 1 and bla ACC-1. *Antimicrobial Agents and Chemotherapy, 51*, 2658–2659.

Partridge, S. R., & Hall, R. M. (2003). In34, a complex In5 family class 1 integron containing orf513 and dfrA10. *Antimicrobial Agents and Chemotherapy, 47*, 342–349.

Partridge, S. R., & Hall, R. M. (2005). Evolution of transposons containing bla TEM genes. *Antimicrobial Agents and Chemotherapy, 49*, 1267–1268.

Paterson, D. L., & Bonomo, R. A. (2005). Extended-spectrum beta-lactamases: a clinical update. *Clinical Microbiology Reviews, 18*(4), 657–686. https://doi.org/10.1128/CMR.18.4.657-686.2005

Payne, D. J., Woodford, N., & Amyes, S. G. B. (1992). Characterization of the plasmid mediated -lactamase BIL-1. *The Journal of Antimicrobial Chemotherapy, 30*, 119–127.

Picard, B., Garcia, J. S., Gouriou, S., Duriez, P., Brahimi, N., Bingen, E., Elion, J., & Denamur, E. (1999). The link between phylogeny and virulence in Escherichia coli extra-intestinal infection. *Infection and Immunity, 67*, 546–553.

Pitout, J. D., & Laupland, K. B. (2008). Extended-spectrum β--lactamase producing Enterobacteriaceae: An emerging public-health concern. *The Lancet Infectious Diseases, 8*, 159–166.

Pitout, J. D., Thomson, K. S., Hanson, N. D., Ehrhardt, A. F., Moland, E. S., & Sanders, C. C. (1998). β-Lactamases responsible for resistance to expanded-spectrum cephalosporins in Klebsiella pneumoniae, Escherichia coli, and Proteus mirabilis isolates recovered in South Africa. *Antimicrobial Agents and Chemotherapy, 42*, 1350–1354.

Poirel, L., Naas, T., Guibert, M., Chaibi, E. B., Labia, R., & Nordmann, P. (1999). Molecular and biochemical characterization of VEB-1, a novel class A extended-spectrum b-lactamase encoded by an Escherichia coli integron gene. *Antimicrobial Agents and Chemotherapy, 43*, 573–581.

Poirel, L., Van De Loo, M., Mammeri, H., & Nordmann, P. (2005). Association of plasmid-mediated quinolone resistance with extended-spectrum b-lactamase VEB-1. *Antimicrobial Agents and Chemotherapy, 49*, 3091–3094.

Poirel, L., Leviandier, C., & Nordmann, P. (2006). Prevalence and genetic analysis of plasmid-mediated quinolone resistance determinants QnrA and QnrS in Enterobacteriaceae isolates from a French university hospital. *Antimicrobial Agents and Chemotherapy, 50*, 3992–3997.

Poirel, L., Naas, T., & Nordmann, P. (2008). Genetic support of extended-spectrum β-lactamases. *Clinical Microbiology and Infection, 14*, 75–81.

Poirel, L., Naas, T., & Nordmann, P. P. (2010). Diversity, epidemiology, and genetics of class D -lactamases. *Antimicrobial Agents and Chemotherapy, 54*, 24–38.

Poirel, L., Bernabeu, S., Fortineau, N., Podglajen, I., Lawrence, C., & Nordmann, P. (2011). Emergence of OXA-48-producing Escherichia coli clone ST38 in France. *Antimicrobial Agents and Chemotherapy, 55*, 4937–4938.

Pomba, C., Mendonça, N., Costa, M., Louro, D., Baptista, B., Ferreira, M., Correia, J. D., & Caniça, M. (2006). Improved multiplex PCR method for the rapid detection of β-lactamase genes in Escherichia coli of animal origin. *Diagnostic Microbiology and Infectious Disease, 56*, 103–106.

Queenan, A. M., Foleno, B., Gownley, C., Wira, E., & Bush, K. (2004). Effects of inoculum and β-lactamase activity in AmpC-and extended-spectrum β-lactamase (ESBL)-producing *Escherichia coli* and *Klebsiella pneumoniae* clinical isolates tested by using NCCLS ESBL methodology. *Journal of Clinical Microbiology, 42*, 269–275.

Rodriguez-Siek, K. E., Giddings, C. W., Doetkott, C., Johnson, T. J., Fakhr, M. K., & Nolan, L. K. (2005). Comparison of Escherichia coli isolates implicated in human urinary tract infection and avian colibacillosis. *Microbiology (Reading, England), 151*, 2097–2110.

Roer, L., Overballe-Petersen, S., Hansen, F., Schønning, K., Wang, M., Røder, B. L., Hansen, D. S., Justesen, U. S., Andersen, L. P., Fulgsang-Damgaard, D., & Hopkins, K. L. (2018). *Escherichia coli* sequence type 410 is causing new international high-risk clones. *Msphere., 3*, e00337-18.

Roschanski, N., Fischer, J., Guerra, B., & Roesler, U. (2014). Development of a multiplex real-time PCR for the rapid detection of the predominant β--lactamase genes CTX-M, SHV, TEM and CIT-type AmpCs in Enterobacteriaceae. *PLoS One, 9*, e100956.

Samra, Z., Bahar, J., Madar-Shapiro, L., Aziz, N., Israel, S., & Bishara, J. (2008). Evaluation of CHROMagar KPC for rapid detection of carbapenem-resistant Enterobacteriaceae. *Journal of Clinical Microbiology, 46*, 3110–3111.

Sanders, C. C., Barry, A. L., Washington, J. A., Shubert, C., Moland, E. S., Traczewski, M. M., Knapp, C., & Mulder, R. (1996). Detection of extended-spectrum-β--lactamase-producing members of the family Enterobacteriaceae with Vitek ESBL test. *Journal of Clinical Microbiology, 34*, 2997–3001.

Sarowska, J., Futoma-Koloch, B., Jama-Kmiecik, A., Frej-Madrzak, M., Ksiazczyk, M., Bugla-Ploskonska, G., & Choroszy-Krol, I. (2019). Virulence factors, prevalence and potential transmission of extraintestinal pathogenic *Escherichia coli* isolated from different sources: Recent reports. *Gut Pathogens, 11*, 10.

Schaufler, K., Semmler, T., Wieler, L. H., Wöhrmann, M., Baddam, R., Ahmed, N., Müller, K., Kola, A., Fruth, A., Ewers, C., & Guenthe, S. (2016). Clonal spread and interspecies transmission of clinically relevant ESBL-producing Escherichia coli of ST410—Another successful pandemic clone? *FEMS Microbiology Ecology, 92*, fiv155.

Smet, A., Martel, A., Persoons, D., Dewulf, J., Heyndrickx, M., Herman, L., Haesebrouck, F., & Butaye, P. (2010). Broad-spectrum β-lactamases among Enterobacteriaceae of animal origin: Molecular aspects, mobility and impact on public health. *FEMS Microbiology Reviews, 34*, 295–316.

Soilleux, M. J., Morand, A. M., Arlet, G. J., Scavizzi, M. R., & Labia, R. (1996). Survey of Klebsiella pneumoniae producing extended-spectrum β--lactamases: Prevalence of TEM-3 and first identification of TEM-26 in France. *Antimicrobial Agents and Chemotherapy, 40*, 1027–1029.

Stuart, J. C., & Leverstein-Van Hall M.A. (2010). Guideline for phenotypic screening and confirmation of carbapenemases in Enterobacteriaceae. *International Journal of Antimicrobial Agents, 36*(3), 205.

Stürenburg, E., Sobottka, I., Feucht, H. H., Mack, D., & Laufs, R. (2003). Comparison of BDPhoenix and VITEK2 automated antimicrobial susceptibility test systems for extended-

spectrum β--lactamase detection in Escherichia coli and Klebsiella species clinical isolates. *Diagnostic Microbiology and Infectious Disease, 45*, 29–34.

Swayne, R. L., Ludlam, H. A., Shet, V. G., Woodford, N., & Curran, M. D. (2011). Real-time TaqMan PCR for rapid detection of genes encoding five types of non-metallo-(class A and D) carbapenemases in Enterobacteriaceae. *International Journal of Antimicrobial Agents, 38*, 35–38.

Toleman, M. A., Bennett, P. M., & Walsh, T. R. (2006). Common regions eg orf 513 and antibiotic resistance: IS 91-like elements evolving complex class 1 integrons. *The Journal of Antimicrobial Chemotherapy, 58*, 1–6.

Vedel, G., Belaaouaj, A., Lilly, G., Labia, R., Philippon, A., Nevot, P., & Paul, G. (1992). Clinical isolates of Escherichia coli producing TRI b-lactamases: Novel TEM enzymes conferring resistance to b-lactamase inhibitors. *The Journal of Antimicrobial Chemotherapy, 30*, 449–462.

Wang, Y., He, T., Han, J., Wang, J., Foley, S. L., Yang, G. Y., et al. (2012). Prevalence of ESBLs and PMQR genes in fecal Escherichia coli isolated from the non-human primates in six zoos in China. *Veterinary Microbiology, 159*, 53–59.

Wayne, P. A. (2009). Clinical and Laboratory Standards Institute (CLSI). Performance Standards for Antimicrobial Susceptibility Testing: Nineteenth Informational Supplement. CLSI document M100-S19.

Wiener, J., Quinn, J. P., Bradford, P. A., Goering, R. V., Nathan, C., Bush, K., & Weinstein, R. A. (1999). Multiple antibiotic–resistant Klebsiella and Escherichia coli in nursing homes. *Journal of the American Medical Association, 281*, 517–523.

World Health Organization (WHO). (2011). *Report on the burden of endemic health care-associated infection worldwide*. WHO Library Cataloguing-in-Publication Data.

World Health Organization (WHO). (2017). *Global priority list of antibiotic-resistant bacteria to guide research, discovery, and development of new antibiotics*.

Yan, J. J., Wu, S. M., Tsai, S. H., Wu, J. J., & Su, I. J. (2000). Prevalence of SHV-12 among clinical isolates of Klebsiella pneumoniae producing extended-spectrum β-lactamases and identification of a novel AmpC enzyme (CMY-8) in southern Taiwan. *Antimicrobial Agents and Chemotherapy, 44*, 1438–1442.

Yigit, H., Queenan, A. M., Anderson, G. J., Domenech-Sanchez, A., Biddle, J. W., Steward, C. D., Alberti, S., Bush, K., & Tenover, F. C. (2001). Novel carbapenem-hydrolyzing β-lactamase, KPC-1, from a carbapenem-resistant strain of *Klebsiella pneumoniae*. *Antimicrobial Agents and Chemotherapy, 45*, 1151–1161.

Yong, D., Toleman, M. A., Giske, C. G., Cho, H. S., Sundman, K., Lee, K., & Walsh, T. R. (2009). Characterization of a new metallo-β-lactamase gene, blaNDM-1, and a novel erythromycin esterase gene carried on a unique genetic structure in *Klebsiella pneumoniae* sequence type 14 from India. *Antimicrobial Agents and Chemotherapy, 53*, 5046–5054.

Zhao, W. H., & Hu, Z. Q. (2013). Epidemiology and genetics of CTX-M extended-spectrum β-lactamases in Gram-negative bacteria. *Critical Reviews in Microbiology, 39*, 79–101.

Vibrio vulnificus and Its Antimicrobial Resistance

T. Muthulakshmi and Mukteswar Prasad Mothadaka

Contents

T. Muthulakshmi
MFB Division, ICAR-Central Institute of Fisheries Technology, Willingdon Island, Cochin, Kerala, India
e-mail: muthulakshmi.t@icar.gov.in

M. P. Mothadaka (✉)
Microbiology, Fermentation and Biotechnology Division, ICAR-Central Institute of Fisheries Technology (ICAR-CIFT), Cochin, Kerala, India

M. P. Mothadaka et al. (eds.), *Handbook on Antimicrobial Resistance*,
https://doi.org/10.1007/978-981-19-9279-7_25

Abstract

Vibrios are the natural inhabitants of aquatic ecosystems and cause life threatening diseases such as cholera. Halophilic, non-cholera vibrios like *Vibrio vulnificus* are autochthonous to the estuaries. The bacterial blood poisoning (septicaemia), inflammation of the lining of the intestine (gastroenteritis), and wound infections are known illnesses that are caused mainly by *V. vulnificus*. This bacterium is a pathogen of opportunity, specifically affecting immunocompromised and aged individuals when infected. In a situation, where antimicrobial resistance itself becomes a threat to the humankind, opportunistic bacteria such as *V. vulnificus* acquiring resistance to the antibiotics to which they are treated is an alarming situation. Meticulous antimicrobial profiling is necessary to reduce mortality because experimenting with different antibiotics may lead to permanent tangible and intangible losses. This chapter dealt on *V. vulnificus* characteristics as pathogen in seafood and aquaculture, development of antibiotic resistance as reported, reason for antibiotic resistance, prevention methods and alternate treatment methods.

Keywords

Vibrio vulnificus · Pathogenicity · AMR · Alternate control measures

1 Introduction

Vibrio vulnificus inhabits in brackish water environments and it is a halophilic, lactose positive bacterium, which produces green colony in TCBS agar medium due to its inability to ferment sucrose. They are commonly reported in the filter feeders like bivalves. The raw consumption of bivalves is known to cause *V. vulnificus* infection worldwide. For propagation of *V. vulnificus* free iron present in serum is very important. Iron is normally stored as hemoglobin, ferritin, and transferrin like bound forms in normal human body. When serum iron is more, it is due to disease conditions which cannot convert the iron to bound forms. In these conditions, the bacteria can grow abundantly and cause infection. It is considered as a threat to vulnerable population with predisposing disease history such as cirrhosis, alcoholism, and iron storage disorders. In the case of immunocompromised patients, the disease propagation will be very severe and it cause necrotizing fasciitis, gastroenteritis, and wound infections resulting amputation or surgical debridement.

V. vulnificus are classified as three biotypes based on the biochemical characteristics namely utilizing different sugars and amino acids. Biotype 1 previously known to infect human is the mostly encountered group found ubiquitously in the brackish water and it causes severe human disease, which can be identified by indole production and arginine utilization. Biotype 2 is indole and arginine negative bacteria previously considered as avirulent. This is observed to cause detrimental effect in fishes. Type 2 is mostly reported in water source used for Anguilla farming

eel. Now this has evolved to cause disease in humans too. As Darwin's theory of survival of the fittest suggests the acquiring of a biochemical characters, plasmid or virulence increase the survival of the organism. Israel eel fish farming has encountered with a new biotype of *V. vulnificus* with intermediate biochemical characters (Zaidenstein et al., 2008). Human infecting strains of biotype 2 has the identical plasmid profile compared to biotype 1. Roig and Amaro (2009) reported Biotype 1 has the virulence plasmid of 68–70 kilobases size. Biotype 2 of *V. vulnificus* is also called as serovar E. This primarily cause economic losses by producing disease to eels. It can occasionally affect human also. The affected eels get bacteremia in the system and high mortality results in aquaculture farms (Tison & Kelly, 1986). This is the bacteria that often associated with after wound contact of sea. *Vibrio vulnificus* is also like other vibrios susceptible to generally used prescription antibiotics (Oliver, 2006, 2013; Shaw et al., 2014; Mala et al., 2014; Miller et al., 2014; Karunasagar, 2014).

2 Biochemical Characteristics

Based on the biochemical characteristics, three biotypes reported in *V. vulnificus* which is designated as biotype1, biotype 2, and biotype 3 (Linkous & Oliver, 1999; Patrizia Serratore et al., 2017). Human pathogens mainly comes under biotype 1 and biotype 2. Human infection with vulnificus in USA are mostly with Biotype 1. These isolates are lysine, ornithine, lactose, salicin, citrate, mannitol, lactose, and indole positive. Biotype 2 is reported to cause disease in eels (Biosca et al., 1996). Biotype 3 is found to have mixed traits of both biotypes since it is a recombinant clone of the other two *V. vulnificus* populations. Biotype 1 and 2 are reported from all over the world. But biotype 3 is reported only from Israel with geographically restricted distribution (Bisharat et al., 2005). The biochemical characters are developed to suit the surrounding environment (Table 1).

Table 1 Biochemical differentiation of biotypes

S.No	Test	Biotype 1	Biotype 2	Biotype 3
1	Sucrose fermentation	–	–	–
2	Arginine dehydrolase	–	–	–
3	Lysine decarboxylase	+	+	+
4	Ornithine decarboxylase	+	–	+
5	Oxidase	+	+	+
6	Indole	+	_	+
7	Mannitol	+	_	+
8	Sorbitol	+	+	_
9	Lactose	+	+	_
10	ONPG	+	+	_
11	Citrate	+	+	_

3 Biochemical Differentiation of Biotypes

See Table 1.

4 Different Media Employed for Isolation

Based on the biochemical characteristics different media were developed to isolate *V. vulnificus*. Out of the different media, TCBS (Thiosulphate citrate bile salt sucrose agar) and CPC agar are commonly employed media for the isolation (Tables 2 and 3).

Table 2 Colony morphology of vibrios in TCBS

S. No	Bacteria	Colony morphology in TCBS
1	*Vibrio cholerae*	Yellow
2	*Vibrio parahaemolyticus*	Green
3	*Vibrio mimicus*	Green
4	*Vibrio vulnificus*	Green
5	*Vibrio harveyi*	Green
6	*Vibrio alginolyticus*	Yellow

Table 3 Different media used for *Vibrio vulnificus* other than TCBS

Media used	pH	Optimum temperature for incubation (^{0}c)	Carbohydrate source	Color of colony
Vibrio vulnificus agar	8.6	37	Salicin	Green colony with grey dark center
SDS polymyxin sucrose agar	7.6	37	Sucrose	Blue with halo
Cellobiose polymyxin colistin agar	7.6	40	Cellobiose	Yellow
Cellobiose polymyxin colistin agar	7.6	40	Cellobiose	Yellow
Modified cellobiose polymyxin B colistin agar (mCPC)	7.6	40	Cellobiose	Yellow
Vulnificus enumeration agar	8.5	37	Xgal, cellobiose, colistin	Blue green
Cellobiose colistin agar	8.5	40	Cellobiose	Yellow
Vibrio vulnificus medium	8.5	37	Cellobiose	Yellow
Vibrio vulnificus medium with colistins	8.5	37	Cellobiose	Yellow

4.1 Thiosulphate Citrate Bile Salt Sucrose Agar

This medium was constituted by Kobayashi et al. in1963. As the name implies, this contain thiosulphate, citrate, bile salt, and sucrose for the selection of vibrios. This has high concentrations of sodium thiosulphate and sodium citrate to inhibit the growth of Enterobacteriaceae. Sucrose is the main carbohydrate source for TCBS. Two types of indicators used, Thymol blue and bromothymol blue. The ideal pH is 8.6. Incubation time required is 18–24 h.

5 Colony Morphology of Vibrios in TCBS

See Table 2.

6 Different Media Employed for *Vibrio vulnificus* Other than TCBS

See Table 3.

7 *Vibrio vulnificus* Occurrence

Occurrence of *V. vulnificus* is reported from fish, shellfish, water, and sediments in numerous salinities. It is reported to be infective in the salinities between 5 and 20 ppt. and seen to be present in the salinities of 1–34 ppt (Parvathi et al., 2004). Infections of *V. vulnificus* are described worldwide in different countries, namely, Australia, Belgium, Brazil, Denmark, Germany, Holland, India, Israel, Italy, Japan, Korea, Spain, Sweden, Turkey, Taiwan, Thailand, and USA, (Dalsgaard et al., 1996; Torres et al., 2002; Oliver, 2006, 2013; Partridge et al., 2009; kim et al., 2011; Huehn et al., 2014). In Europe *V. vulnificus* was having the prevalence of 3.5–8% of the seafood samples tested. Whereas, South East Asia reported 2.4% prevalence in shrimp. In India oysters freshly harvested showed 75% prevalence for *V. vulnificus*. Whereas, 100% of the fresh oysters harvested had *V. vulnificus* in warm months at Gulf of Mexico (Jones, 2014). Mortalities of 95% resulting from consumption of seafood in USA were associated with *V. vulnificus*. Worldwide studies revealed that *V. vulnificus* is the cause of 95 cases on average with 85 require hospitalization and 35 casualties (CDC, 2013). In Chinese coastal cities, Yano et al. (2004) observed the prevalence of *V. vulnificus* in 11 species of live seafood samples (n=48).

8 Occurrence in India

Vibrios constitute up to 19–39% of the aerobic flora. The prevalence of *V. vulnificus* is about 2–13% of the total vibrios. The occurrence of 16.6% reported from marine fish collected in Cochin Coast of India in very fresh condition from vessels (Thampuran & Surendran, 1998).

9 *Vibrio vulnificus* in Seafood

The prevalence of pathogenic vibrios such as *Vibrio parahaemolyticus*, *V. mimicus*, *Vibrio vulnificus,* and *Vibrio alginolyticus* are reported in filter feeding bivalve molluscs such as oysters and clams. These can be pathogenic and are linked with disease outbreaks and gastroenteritis and septicemia associated with seafood in humans (Elliot et al., 1995). Cases of *Vibrio* infections have a specific pattern with seasonal distribution. It mainly occurs during summer and autumn, when the water temperature is to some extent on higher side with warmer currents. Salt loving vibrio distribution depends upon the salt variation in the water. Vibrios are concentrated in the filter feeding mollusk and their tissues. Raw consumption of the oysters with *V. vulnificus* can be life threatening. To counteract vibrio infections consumer needs to have awareness on the hazards of consuming live oysters and seafood. Immune compromised persons with prevailing diseases and co-morbidity are advised to avoid undercooked seafood. Shell fish and seafood needs to be cooked thoroughly to prevent microbes and other cross contamination in water (FDA, 2009). *V. vulnificus* is major concern as it accounts for 1/3 of the total seafood-related illnesses and more than 85% of the costs of direct exposure to *Vibrio* pathogen (FDA, 2009). *V. vulnificus* is the causative agent for 31% of premature mortalities in seafood-mediated infections and 18% of infections caused by the after wound direct exposure (Ralston et al., 2011).

10 Pathogenesis of *Vibrio vulnificus*

V. vulnificus is pathogenic to vulnerable populations. It is an estuarine bacterium and after consumption or exposure gastroenteritis and in extreme cases septicemia could be the consequences. The bacteria affect especially male population whose average age is above 50 years. It precisely distresses those whose serum iron is more compared to the normal persons. In healthy female, the estrogen reduces the serum iron levels which reduce the chance of infection. This causes both food and contact borne infections. Contact may be due to a sea bath after wound. It has the highest rate of fatality in vibrio infections to an extent of nearly 60%. People with chronic liver damage also get affected by *V. vulnificus*. Pathogenicity depends on the following factors such as capsule formation, pilus, filament, and siderophore such as vulnibactin.

11 Pathogenicity

The correlation between colony morphology and virulence (opaque – virulent, translucent – avirulent) was reported for *V. vulnificus* (Simpson et al., 1987). Opaqueness of colony is because of the presence of capsules in *Vibrio vulnificus* Biotype-I (Amaro et al., 1994). *V. vulnificus* infections are often reported in patients with diseases involving iron metabolism (Bogard & Oliver, 2007). In normal human serum of healthy individuals, the iron will be stored in bound form like ferritin, transferrin, and hemoglobin. When free iron is not available, *V. vulnificus* resort to iron acquisition mechanisms such as siderophore mediated that was well established in Biotype II (Amaro et al., 1994).

Kreger and Lockwood described the production of hemolysin in *V. vulnificus* against the mammalian erythrocytes in 1981. When hemocyte targeted haemolysin became a cause of concern, the new toxin called cytolysin also studied in this bacteria that causes cytopathic effect in the cell cultures, and it is further studied by injecting in to the mouse (Fan et al., 2001). *V. vulnificus* was well established as human pathogen. The studies on fish and shrimp farms by Jayashree et al. (2006) has thrown light on the diseases caused by vibrios to the cultured fish and shrimps. Vibrios *were* reported to be associated with five types of diseases, i.e., tail necrosis, shell disease, red disease, loose shell syndrome (LSS), and white gut disease (WGD) in cultured shrimps from Andhra Pradesh and among these, LSS, WGD, and red disease caused mass mortalities. The significant vibrio species studied were *V. harveyi*, *V. parahaemolyticus*, *V. alginolyticus*, *V. anguillarum*, *Vibrio vulnificus*, and *V. splendidus* (Jayasree et al., 2006; Jones, 2014).

In addition to the lysins and toxins produced, the bacteria can live in highly acidic environment such as stomach and can cross through, if necessary to cause infection. The bacteria evade stomach lining with acid neutralization capacity with the production of carboxylase and deaminase to increase the pH. In acidic pH and oxidative associated stress conditions, *V. vulnificus* can up-regulate production of lysine decarboxylase and manganese superoxide dismutase (MnSOD). Lysine decarboxylase is converted to cadeverine, putrescene, and other amines. This works as acid neutralizer and superoxide radical scavenger. *In vitro* studies on the phagocytosis of *V. vulnificus* with neutrophils of human blood revealed that if the phagocytosis is minimal, survival for longer periods is possible. The survival is inversely interrelated to the neutrophil phagocytosis. The immunocompromised individual's blood may have minimum or no neutrophil activity, which facilitates the survival and propagation of this organism (Jones & Oliver, 2009). The virulence factors, their nature, progression and the associated genes are provided in Table 4.

12 Virulence Factors

See Table 4.

Table 4 Virulence factors

Virulence factor	Nature	Progression	Gene	References
Cytolysin	Extracellular toxin	Cell lysis	*vvhA*	Bang et al. (1999), Zhang and Austin (2005)
RTX toxin	Toxin	Actin depolymerisation	*rtxA*	Kim et al. (2008), Reboucas et al. (2011)
Zinc-dependent metalloprotease	Protease	Elastase, collagenase, and caseinase activity	*vvpE*	Kothary and Kreger (1987)
Vulnibactin	Siderophore	Compete for iron	*viuB*	Ratledge and Dover (2000), Paniker et al. (2004)
Capsule	Polysaccharide	Gives protection against complement and of phagocytosis	*Wza*	Whitfield (2006), Collins and Derrick (2007)
Type IV pili	Pili	Adherence to surface epithelial cells	*pilABCD*	Gander and LARocco (1989), Paranjpye and Storm (2005)
Flagella		Initial adsorption, biofilm formation, and the subsequent invasion of the host		Lee et al. (2004)
OmpU, and IlpA membrane proteins	Outer membrane proteins	Survival in human body	*Hup A*	Jones and Oliver (2009)

13 Methods of Analysis for AMR for *Vibrio vulnificus*

Kirby and Bauer method, 1996, was the most frequently adopted method for the antimicrobial analysis of AMR in *V vulnificus*. Commonly used agar plates were Mueller–Hinton agar (Bauer et al., 1966). Since it is a halophilic vibrio, salinity commonly employed was 1–2%. The common pH used for *V. vulnificus* was 8.5 (Banerjee & Farber, 2018). In whole genome sequencing analysis, tetracycline resistance was established to be inherent in some isolates. Polymixin and colistin resistance also inherent in some isolates.

14 Antimicrobial Resistance in Aquaculture

Aquaculture industry uses antibiotics as a prophylactic measure. Some of the antibiotics used are oxytetracycline, tetracycline, quinolones, trimethoprim, and sulphonamides (Rico et al., 2012; Yano et al., 2004; Lee et al., 2004). However,

the excessive use of antibiotics not only enhances the production cost but also lead to the emergence of antibiotic resistance strains (Letchumanan et al., 2015a,b). Brazilian aquaculture also has the problem of rapidly growing antibiotic resistance. Reboucas and others observed that the shrimp hatchery waters have shown higher resistance to ampicillin (45.2%) and tetracycline (38.7%), and aquaculture vibrio isolates Florfenicol and Nitrofurantoin were effective (Reboucas et al., 2011).

With growing need for aquaculture, farmers use chemicals and antibiotics to sustain the production. Some countries permit certain antibiotics in aquaculture. Tetracycline is permitted in most of the countries (Malaysia, Philippines, and Myanmar). Oxytetracycline is permitted in some countries (USA and Europe) (Rodgers & Furones, 2009). When vibrio species from aquaculture samples were analyzed for AMR, higher variability was observed with antibiotics such as AMP, AM, TE, OTC, COT (Mariasony et al., 2021).

15 *Vibrio vulnificus* and Antibiotic Resistance in Seafood

Antibiotic resistant bacteria with MDR are a threat to fish, shellfish, farming, and also to human health (WHO, 2014). To treat an infection knowing the pathogen's resistance profile is important to lower the mortality (Elmahdi et al., 2016). Yano et al. (2004) observed that isolates of *V. vulnificus* from Chinese retail market were susceptible to ampicillin, penicillin, kanamycin, streptomycin, and erythromycin. Pan et al. (2013) has reported the molecular characteristics and antibiotic susceptibilities of *V. vulnificus* strains in shrimps from retail markets in Hangzhou, People's Republic of China. Out of 78 shrimp samples, 33 harbored *V. vulnificus*; the most-probable-number (MPN) values ranged from 3 to 1,600 g^{-1}. Isolates have shown resistance to Cefepime, Tetracycline, and Aztreonam. Vibrio species have acquired multidrug resistance due to excessive usage of antibiotics (Sudha et al., 2014; Letchumanan et al., 2015a). The antimicrobial resistance pattern in Vibrios of shrimp aquaculture, the occurrence, systems of identification, and the control measures therein are well documented (Murugadas et al., 2021).

Environment isolates of *V. vulnificus* were studied for antimicrobial resistance by Baker and Austin (2009) and they reported that the environment isolates also resistant to 3 or more group of antibiotics. The infections caused by non-cholera vibrios are treated with tetracycline, aminoglycosides, and cephalosporins. In Germany, most of the isolates were susceptible to these antibiotics (Bier et al., 2015). The German result contradicts the previous result by Baker-Austin and others (2010). These studies shows that the usage of antibiotics has the positive correlation with the antimicrobial resistance patterns. Pan et al. (2013) also have reported similar results wherein the bacteria were susceptible to ampicillin, ampicillin-sulbactam, piperacillin-tazobactam, cephalothin, ceftriaxone, cefetaxime, ceftazidime, imipenem, ciprofloxacin, levofloxacin, nalidixic acid, trimethoprim-sulfamethoxazole, chloramphenicol, and nitrofurantoin. Li et al. (1999) described the antibiotic susceptibility of 51 Vibrio strains collected from *Sparus sarba* from May 1995 to February 1997 in Hong Kong. The study observed that all strains were sensitive to ceftriaxone, streptomycin,

nalidixic acid, and rifampicin. However, four strains that were resistant to ampicillin, cefuroxime, tetracycline, trimethoprim, and aminoglycosides including gentamicin, amikacin, kanamycin, netilimicin (Li et al., 1999). In India, Sudha et al. (2014) studied *V. vulnificus* with a prevalence of 2% from Cochin that were resistant to amoxicillin, ampicillin, carbenicillin, colistin, ceftazidin, cephalothin, and streptomycin and susceptible for chloramphenicol, tetracycline, and nalidixic acid. In another study, the appropriate drugs identified to be cefotaxime and ceftriaxone, whereas cephem found to have resistance in the five out of seven isolates checked (Vaseeharan et al., 2005).

16 Mode of Antibiotic Resistance Transfer in *Vibrio vulnificus*

When vibrio database available from Latin American countries were collected and analyzed for antimicrobial resistance genes, 99.8% of the sequence harbored at least one resistance gene and MDR genes were present in 54.2% sequence types. The genes for antibiotic peptides, β lactams, and chloramphenicol were the most common to be seen. When Janecko et al. (2021) analyzed resistance genes in whole genome sequence data from vibrios viz.,*Vibrio cholerae, Vibrio parahaemolyticus,* and *Vibrio vulnificus* isolated from retail shrimps, they found 77% of the sequence type carried AMR genes. MDR genes were present in *V. vulnificus* and other vibrios.

In vibrios the antibiotic resistance determinants are present in plasmids. Plasmid is the extra chromosomal circular DNA that act as a mediator in the transfer of resistance genes. This can be transferred either vertically to next generation or horizontally to other species (Manjusha & Sarita, 2011). Generally, vibrio species contain plasmid and correlation between the presence of plasmid and antibiotic resistance also reported (Molina- Aja et al., 2002). The analysis of retail shrimps has shown the vibrio population contain 19 types of plasmids. Plasmid curing is the process of removing plasmids from the bacteria. Most of the isolates lost their one or more antibiotic resistance characters after losing plasmid. The OTC resistance is most common to lose after plasmid curing (Reboucas et al., 2011).

17 Treatment for *Vibrio vulnificus* Infections

V. vulnificus infections are treated with third generation cephalosporins and minocycline or tetracycline (Yu et al., 2017). Mortality was less when treated with cephalosporin and minocycline when compared to single therapy with cephalosporin or flouroquinolone. However, in the case of necrotizing fasciitis where the tissues are necrotized by bacteria penetration of antibiotic is difficult. In that case, an antibiotic with good tissue penetration is necessary and it is done by flouroquinolone like ciprofloxacin, moxifloxacin, levofloxacin with lower MICs compared to other antibiotics. In necrotizing fasciitis, exposure to too many antibiotics will release endotoxins, which may worsen the patient's condition. This should be taken into consideration while treating *V. vulnificus* infections (Chen et al., 2012).

Combination therapy is successful only if the antibiotics are synergistic to each other. Tigecycline which is a member of glycylcyclines was recommended for soft tissue infections and necrotizing fasciitis for good tissue penetration and soft tissue accumulations. It was found to be better than cefotoxime and minocycline single therapy (Lin et al., 2016).

Important clinical symptoms of *V. vulnificus* infection are septicaemia and wound infections. *V. vulnificus* infections in clinical level are treated with antibiotics. Correct antibiotic should be used for optimal results. In case of septicemia where the bacterial toxin induces the sepsis and wound infection where the existing wound serves as the habitat for infectious bacteria, any delay in the treatment with appropriate antibiotic may cause higher fatality rate (Rodrigues et al., 1992; Amaro et al., 1994; Moreno & Landgraf, 1998). Quinolones, cephalosporins, tetracyclines, and penicillins are the mostly used class of antibiotics against Vibrios. For vibrio treatment only antibiotic which shows correlation with reduced mortality is quinolones. As per CDC recommendation, doxycycline and ceftazidime are used for *V. vulnificus* infection in adults. Trimethoprim-sulfamethoxazole, Doxycycline and fluoroquinolone are used for treatment in children (CDC, 2013).

Cephalosporins are found to be not effective against *V. vulnificus*. Mortality was more when Cephalosporins were used in single therapy. Though fluoroquinolone single therapy found to be less effective, the mortality is less. While individual antibiotics performance is debatable, the group of antibiotics with synergistic effect are shown to give higher survival. The efficiency of cephalosporins increased with addition of doxycycline or ciprofloxacin. The ceftriaxone-doxycycline, ceftriaxone-ciprofloxacin, cefepime-doxycycline, and cefepime-ciprofloxacin groups had the highest survival rates (Trinh et al., 2017).

Other than antibiotics, debridement, and amputation also recommended as treatment for vulnificus infections in soft tissues. Antimicrobial therapy is ineffective in such cases due to thrombosis of blood vessels. Surgical debridement and amputation are necessary for the survival of the patients (Kuo et al., 2007).

18 Treatment with Antibiotics and Resistance Therein

In Italy, doxycycline which is suggested as the first line of treatment has intermediate resistance profile (Zanetti et al., 2001). *V. vulnificus* has been isolated from coastal water in Eastern Province of Saudi Arabia. The isolates were clinically significant, their plasmid profiling and the resistance towards antibiotics were assessed (Elhadi, 2012). Shaw et al. (2014) has reported intermediate resistance profile to ceftazidime. In the USA, ceftazidime which is also first line of antibiotic used for *V. vulnificus.* Sudha et al. (2014) shown resistance profile for the same antibiotic in India. Vaseeharan et al. (2005) has shown intermediate (2/7) to resistance profile (5/7) for ceftriaxone in India. Co-trimaxazole which is used for treating infection in children has resistance profile in (1/8) Italy and (8/8) in south Africa. Aminoglycosides have exhibited intermediate to resistance profile in India, Italy, Germany, China, and Hong Kong.

19 Prevention of *V. vulnificus* Infections

Prevention methods are

1. Avoiding the oyster harvested from *V. vulnificus* prone zone.
2. Avoidance of exposure of open wounds to seawater and other recreational waters.
3. Wearing protective gloves while picking and handling shellfishes.
4. Wearing protective footwear while walking in the *V. vulnificus* prone areas.
5. The products, viz., oyster and prawn, needs to be iced immediately after harvest.
6. Freezing followed by storage in high pressure is recommended for oysters in US waters.
7. In tropical waters, aquaculture farm workers need to be instructed to wash hands after coming in contact with the water, namely, feeding and other activities.

Follow up of above prevention methods helps infections related to *V. vulnificus* are prevented and automatically transmission. This in turn hinders need for employment of antibiotics and development to the antibiotics therein.

The need of the hour is pragmatic participation of all the countries in the world of WHO promulgated Global Antimicrobial Resistance Surveillance System (GLASS) to sustain global action plan on antimicrobial resistance. Despite the fact the HIG countries, namely, Denmark, Japan, Sweden, United States, and EU, pragmatized various programs in the direction of containing AMR that includes China, India, South Africa, and Thailand, in recent years as observed by Founou et al. (2016) a plan of action in association with all the countries will help to control food borne infections across the world. These pre-emptive steps restrict the transmission of microbial pathogens. In the absence of development of new antibiotics and novel therapeutics, the MDR is increasing in vibrios continuously and unhindered. The prevailing global health crisis of COVID-19 showed the importance of WASH (water, sanitation and hygiene) control of infections at health care facilities. This will help in restricting secondary infections, which in turn avoid use of antibiotics. Vaccinations too play an important role containing spread of infections. Alternative measures in prevention and control vibrio infections are provided below.

20 Alternative Control Measures

The antimicrobial resistance is a serious issue both from aquaculture and public health point of view. Accordingly, the alternate methods were developed to curtail the bacteria. Some methods involving the complete elimination of bacteria whereas some methods help in reducing the bacterial load that are necessitated to cause a disease.

Phages are viruses which feeds on bacteria. Phage therapy is a promising sector for the treatment of pathogenic bacteria such as *Vibrio vulnificus.* Most of the vulnificus affecting Phages belongs to the family of Styloviridae, Myoviridae, Siphoviridae, and Podoviridae. Some of the Phages are double-stranded DNA in

nature. Depends on the distribution of bacteria, the Phages also distributed and expressed as PFU (Plaque Forming Unit). They are present in all natural environments mainly detected from oysters where vulnificus is prevalent. Phages such as SSP002 that occur in environment were proved to be effective against *V. vulnificus* (Kalatzis et al., 2018).

Other methods that are proved to be effective are temperature-based and immunity-based approaches. Cook and Ruple (1992) described that mild heat treatment and later storage in low temperature were more acceptable from a perceptive and safety point of view. Decimal reduction time for *V. vulnificus* at 47 °C was 78 s (SO ±30 sec.) and LD50 value of 39.8 s (SO ±12.2 sec.). Heating oysters for 10 min in water at 50 °C was adequate to reduce *V. vulnificus* (Cook & Ruple,1992, Ye et al., 2012). Jones et al., 2017 reported rapid cooling after harvesting helps in reduction of *V. vulnificus*. Freezing with hydrostatic pressure, increases shelf-life (Büyükcan et al., 2009).

Vaccines exploit the adoptive immunity of human beings and other higher vertebrates. The subsequent infection's immune response is higher when the primary infection is prevailing. Bacterial vaccines are used for controlling the infection in *V. vulnificus* prone zones. Vaccine could help compared to antibiotics for controlling disease (Lafrentz & Shoemaker, 2015). The vulnivaccine against the *Vibrio vulnificus* biotype 2 helps in reducing the impact of subsequent infections. American eels immunized with outer membrane protein and immunogenic potential evaluated (Songlin et al., 2015). Passive immunization carried out for tilapia helped in controlling the disease (Lafrentz & Shoemaker, 2015). C terminal region of the pathogenic RTX induced protective immune response against *V. vulnificus* (Lee et al., 2014b). Rodents injected with recombinant RtxA1 protein with adjuvant gave long-lasting antibody response and significantly reduced bacterial load in the blood (Lee et al., 2014a). The vaccine can be delivered by (1) Oral, (2) Anal, (3) Intra peritoneal injection and prolonged immersion. Immersion and oral routes are considered to be best for fishes for the reason that they minimize the handling time. For intensive culture systems, oral vaccine is considered to be the best method. Though the vaccination improves the adoptive immunity, the inherent immune response such as lysozyme production and phagocytic responses remained same during pre and post vaccination. The virulence moiety with other vaccines like tetanus toxoid can be combined for immunotherapy. The antisera of the vibrio polysaccharide and tetanus toxoid vaccine conjugate produced high level of antibody bodies in experimental mice. The group of mice injected with vibrio polysaccharide alone did not give sufficient results. In mice intra peritoneal injection produced more antibodies compared to other methods.

Biofilm formation is the survival of bacteria under protective layers to avoid the antimicrobials and disinfectants. *V. vulnificus* is known to cause biofilms and produce the signal molecules for other bacteria. Quorum sensing is the communication system of bacteria, which helps them to form biofilm to protect against the treatment measures. The signal molecules are produced in biofilm forming bacteria. The method of disrupting the bacterial biofilm formation by stopping the signal molecule is called quorum quenching. Defoirdt et al. (2004) and (2011) studied the anti-

pathogenic compounds for quorum quenching to avoid the cell to cell communication of bacteria.

Targeting the virulence initiating gene is a new alternative method for eradication of bacteria. For that purpose, the very important gene which is essential for pathogenesis should be knocked out. During infections, the virulence genes are expressed in different manner *in vivo*. For instance, *Pyr H* gene seen initiating the disease process. *Vvps* is the gene for producing capsular polysaccharide. The deletion mutation produces mutant strains, which could not survive and replicate. By CRISPR CAS gene editing to the virulence important genes in genome will help in making avirulent population which can help in vaccine production.

21 Conclusions

In view of its potential pathogenicity, virulent nature coupled with antibiotic resistance, the *Vibrio vulnificus* needs constant surveillance in fish, fishery products, and aquatic environments so as to ensure new variants of antibiotic resistant type that evolve through different modes of transmission are in check before they spread and create chaotic conditions in wellbeing of aquatic animal and humans in terms of food safety.

References

Amaro, C., Biosca, E. G., Fouz, B., Toranzo, A. E., & Garay, E. (1994). Role of iron, capsule, and toxins in the pathogenicity of *Vibrio vulnificus* biotype 2 for mice. *Infection and Immunity, 62*(2), 759–763.

Baker-Austin, C., Stockley, L., Rangdale, R., & Martinez-Urtaza, J. (2010). Environmental occurrence and clinical impact of *Vibrio vulnificus* and *Vibrio parahaemolyticus*: A European perspective. *Environmental Microbiology Reports, 2*(1), 7–18.

Baker-Austin, C., McArthur, J.V., Lindell, A.H., Wright, M.S., Tuckfield, R.C., Gooch, J., Warner, L., Oliver, J. & Stepanauskas, R. (2009). Multisite analysis reveals widespread antibiotic resistance in the marine pathogen Vibrio vulnificus. *Microbial Ecology, 57*, 151–159.

Banerjee, S. K., & Farber, J. M. (2018). Trend and pattern of antimicrobial resistance in molluscan Vibrio species sourced to Canadian estuaries. *Antimicrobial Agents and Chemotherapy, 62*(10), e00799–e00718.

Bang, Y. B., Lee, S. E., Rhee, J. H., & Choi, S. H. (1999). Evidence that expression of the *Vibrio vulnificus* hemolysin gene is dependent on cyclic AMP and cyclic AMP receptor protein. *Journal of Bacteriology, 181*(24), 7639–7642.

Bauer, A. W., Kirby, W. M., Sherris, J. C., & Turck, M. (1966). Antibiotic susceptibility testing by a standardized single disk method. *American Journal of Clinical Pathology, 45*(4), 493–496.

Bier, N., Schwartz, K., Guerra, B., & Strauch, E. (2015). Survey on antimicrobial resistance patterns in *Vibrio vulnificus* and *Vibrio cholera* non-O1/non-O139 in Germany reveals carbapenemase-producing *Vibrio cholera* in coastal waters. *Frontiers in Microbiology, 6*, 1179.

Biosca, E.G., Oliver, J.D, & Amaro, C. (1996). Phenotypic characterization of Vibrio vulnificus biotype 2, a lipopolysaccharide-based homogeneous O serogroup within Vibrio vulnificus. *Applied and Environmental Microbiology, 62*(3), 918–927.

Bisharat, N., Cohen, D.I., Harding, R.M., Falush, D., Crook, D.W., Peto, T. & Maiden, M.C. (2005). Hybrid Vibrio vulnificus. *Emerging infectious diseases, 11*(1), 30.

Bogard, R., & Oliver, J. D. (2007). Role of iron in human serum resistance of the clinical and environmental *Vibrio vulnificus* genotypes. *Applied and Environmental Microbiology, 73*, 7501–7505.

Büyükcan, M., Bozoglu, F., & Alpas, H. (2009). Preservation and shelf-life extension of shrimps and clams by high hydrostatic pressure. *International Journal of Food Science & Technology, 44*(8), 1495–1502.

Centers for Disease Control and Prevention (CDC). (2013). *Vibrio vulnificus*. Centers for Disease Control and Prevention.

Chen, S. C., Lee, Y. T., Tsai, S. J., Chan, K. S., Chao, W. N., Wang, P. H., Lin, D. B., Chen, C. C., & Lee, M. C. (2012). Antibiotic therapy for necrotizing fasciitis caused by Vibrio vulnificus: Retrospective analysis of an 8 year period. *Journal of Antimicrobial Chemotherapy, 67*(2), 488–493.

Collins, R. F., & Derrick, J. P. (2007). Wza: A new structural paradigm for outer membrane secretory proteins? *Trends in Microbiology, 15*(3), 96–100.

Cook, D. W., & Ruple, A. D. (1992). Cold storage and mild heat treatment as processing aids to reduce the numbers of *Vibrio vulnificus* in raw oysters. *Journal of Food Protection, 55*(12), 985–989.

Dalsgaard, A., Frimodt-Møller, N., Bruun, B., Høi, L., & Larsen, J. L. (1996). Clinical manifestations and molecular epidemiology of *Vibrio vulnificus* infections in Denmark. *European Journal of Clinical Microbiology and Infectious Diseases, 15*(3), 227–232.

Defoirdt, T., Boon, N., Bossier, P., & Verstraete, W. (2004). Disruption of bacterial quorum sensing: An unexplored strategy to fight infections in aquaculture. *Aquaculture, 240*(1–4), 69–88.

Defoirdt, T., Sorgeloos, P., & Bossier, P. (2011). Alternatives to antibiotics for the control of bacterial disease in aquaculture. *Current Opinion in Microbiology, 14*(3), 251–258.

Elhadi, N. (2012). Antibiotic resistance and plasmid profiling of clinically significant *Vibrio vulnificus* isolated from Coastal Water in Eastern Province of Saudi Arabia. *British Journal of Pharmacology and Toxicology, 3*(2), 93–97.

Elliot, E. L., Kaysner, C. A., Jackson, L., & Tamplin, M. L. (1995). *Vibrio cholerae, V.parahaemolyticus, V. vulnificus* and other *Vibrio* spp. In *FDA bacteriological analytical manual* (8th ed., pp. 9.01–9.27). AOAC International.

Elmahdi, S., DaSilva, L. V., & Parveen, S. (2016). Antibiotic resistance of *Vibrio parahaemolyticus* and *Vibrio vulnificus* in various countries: A review. *Food Microbiology, 57*, 128–134.

Fan, J. J., Shao, C. P., Ho, Y. C., Yu, C. K., & Hor, L. I. (2001). Isolation and characterization of a *Vibrio vulnificus* mutant deficient in both extracellular metalloprotease and cytolysin. *Infection and Immunity, 69*(9), 5943–5948.

FDA and Food and Drug Administration (2009). *National Shellfish Sanitation program, Guide for the control of molluscan shellfish, Model ordinance*.

Founou, L. L., Founou, R. C., & Essack, S. Y. (2016). Antibiotic resistance in the food chain: A developing country-perspective. *Frontiers in Microbiology, 7*, 1881. https://doi.org/10.3389/fmicb.2016.01881. PMID: 27933044; PMCID: PMC5120092.

Gander, R. M., & LaRocco, M. T. (1989). Detection of pilus like structures on clinical and environmental isolates of *Vibrio vulnificus*. *Journal of Clinical Microbiology, 27*(5), 1015–1021.

Huehn, S., Eichhorn, C., Urmersbach, S., Breidenbach, J., Bechlars, S., Bier, N., Alter, T., Bartelt, E., Frank, C., Oberheitmann, B., & Gunzer, F. (2014). Pathogenic vibrios in environmental, seafood and clinical sources in Germany. *International Journal of Medical Microbiology, 304*(7), 843–850.

Janecko, N., Bloomfield, S. J., Palau, R., & Mather, A. E. (2021). Whole genome sequencing reveals great diversity of Vibrio spp in prawns at retail. *Microbial Genomics, 7*(9), 000647. https://doi.org/10.1099/mgen.0.000647

Jayasree, L., Janakiram, P., & Madhavi, R. (2006). Characterization of Vibrio spp. associated with diseased shrimps from culture ponds of Andhra Pradesh (India). *Journal of World Aquaculture Society, 37*, 523–532.

Jones, J. L. (2014). VIBRIO| Introduction, including *Vibrio parahaemolyticus*, *Vibrio vulnificus*, and other Vibrio species. In *Encyclopaedia of food microbiology*. Academic press (2nd edn, pp 691–698).

Jones, M. K., & Oliver, J. D. (2009). *Vibrio vulnificus*: Disease and pathogenesis. *Infection and Immunity, 77*, 1723–1733. https://doi.org/10.1128/IAI.01046-08

Jones, J. L., Lydon, K. A., Kinsey, T. P., Friedman, B., Curtis, M., Schuster, R., & Bowers, J. C. (2017). Effects of ambient exposure, refrigeration, and icing on *Vibrio vulnificus* and *Vibrio parahaemolyticus* abundances in oysters. *International Journal of Food Microbiology, 253*, 54–58.

Kalatzis, P. G., Castillo, D., Katharios, P., & Middelboe, M. (2018). Bacteriophage interactions with marine pathogenic vibrios: Implications for phage therapy. *Antibiotics, 7*(1), 15.

Karunasagar, I. (2014). Vibrio vulnificus. In Y. Motarjemi, G. Moy, & E. Tood (Eds.), *Encyclopaedia of food safety* (pp. 564–569). Elsevier.

Kim, J. R., Dool-Ri, O., Cha, M. H., Pyo, B. S., Rhee, J. H., Choy, H. E., Won Keun, O., & Kim, Y. R. (2008). Protective effect of polygoni cuspidati radix and emodin on *Vibrio vulnificus* cytotoxicity and infection. *The Journal of Microbiology, 46*(6), 737–743.

Kim, J. H., Choresca, C. H., Jr., Shin, S. P., Han, J. E., Jun, J. W., & Park, S. C. (2011). Occurrence and antibiotic resistance of *Vibrio vulnificus* in seafood and environmental waters in Korea. *Journal of Food Safety, 31*(4), 518–524.

Kothary, M. H., & Kreger, A. S. (1987). Purification and characterization of an elastolytic protease of *Vibrio vulnificus*. *Microbiology, 133*(7), 1783–1791.

Kreger, A., & Lockwood, D. (1981). Detection of extracellular toxin(s) produced by *Vibrio vulnificus*. *Infection and Immunity, 33*, 583–590.

Kuo, Y. L., Shieh, S. J., Chiu, H. Y., & Lee, J. W. (2007). Necrotizing fasciitis caused by *Vibrio vulnificus*: Epidemiology, clinical findings, treatment and prevention. *European Journal of Clinical Microbiology & Infectious Diseases, 26*(11), 785–792.

LaFrentz, B. R., & Shoemaker, C. A. (2015). Passive transfer of serum from tilapia vaccinated with a Vibrio vulnificus vaccine provides protection from specific pathogen challenge. *Aquaculture, 442*, 16–20.

Lee, J. H., Rho, J. B., Park, K. J., Kim, C. B., Han, Y. S., Choi, S. H., Lee, K. H., & Park, S. J. (2004). Role of flagellum and motility in pathogenesis of *Vibrio vulnificus*. *Infection and Immunity, 72*(8), 4905–4910.

Lee, H. S., Choi, S., Shin, H., Lee, J. H., & Choi, S. H. (2014a). *Vibrio vulnificus* bacteriophage SSP002 as a possible biocontrol agent. *Applied and Environmental Microbiology, 80*(2), 515–524.

Lee, T. H., Kim, M. H., Lee, C. S., Lee, J. H., Rhee, J. H., & Chung, K. M. (2014b). Protection against *Vibrio vulnificus* infection by active and passive immunization with the C-terminal region of the RtxA1/MARTXVv protein. *Vaccine, 32*(2), 271–276.

Letchumanan, V., Chan, K.-G., & Lee, L.-H. (2015a). An insight of traditional plasmid curing in *Vibrio* species. *Frontiers in Microbiology, 6*, 735.

Letchumanan, V., Pusparajah, P., Tan, L. T. H., Yin, W. F., Lee, L. H., & Chan, K. G. (2015b). Occurrence and antibiotic resistance of *Vibrio parahaemolyticus* from shellfish in Selangor, Malaysia. *Frontiers in Microbiology, 6*, 1417.

Li, J., Yie, J., Foo, R. W., Ling, J. M., Xu, H., & Woo, N. Y. (1999). Antibiotic resistance and plasmid profiles of Vibrio isolates from cultured silver sea bream, Sparus sarba. *Marine Pollution Bulletin, 39*(1–12), 245–249.

Lin, Y. S., Hung, M. H., Chen, C. C., Huang, K. F., Ko, W. C., & Tang, H. J. (2016). Tigecycline salvage therapy for necrotizing fasciitis caused by Vibrio vulnificus: Case report in a child. *Journal of Microbiology, Immunology and Infection, 49*(1), 138–141.

Linkous, D. A., & Oliver, J. D. (1999). Pathogenesis of *Vibrio vulnificus*. *FEMS Microbiology Letters, 174*(2), 207–214.

Mala, E., Oberoi, A., & Alexander, V. S. (2014). Vibrio isolates from cases of acute diarrhea and their antimicrobial susceptibility pattern in a tertiary care hospital. *International Journal of Basic and Applied Sciences, 3*(1), 35–37. https://doi.org/10.14419/ijbas.v3i1.1735. Accessed on: 18 Mar 2022.

Manjusha, S., & Sarita, G. B. (2011). Plasmid associated antibiotic resistance in Vibrios isolated from coastal waters of Kerala. *International Food Research Journal, 18*(3), 1171–1181.

Miller, R., Carson, J., Dalsgaard, I., Gaunt, P. S., Gieseker, C., Hawke, J., Reimschuessel, R., Smith, P., Somsiri, T., * Wu, C. (2014). Methods for broth dilution susceptibility testing of bacteria isolated from aquatic animals; approved guideline-second edition. Book Chapter, p. 56.

Molina-Aja, A., García-Gasca, A., Abreu-Grobois, A., Bolán-Mejía, C., Roque, A., & Gomez-Gil, B. (2002). Plasmid profiling and antibiotic resistance of Vibrio strains isolated from cultured penaeid shrimp. *FEMS Microbiology Letters, 213*(1), 7–12.

Moreno, M. G., & Landgraf, M. (1998). Virulence factors and pathogenicity of *Vibrio vulnificus* strains isolated from seafood. *Journal of Applied Microbiology, 84*(5), 747–751.

Murugadas, V., Pailla, S., Rao, B. M., Pillai, D., Ravishankar, C. N., & Prasad, M. M. (2021). Antimicrobial resistance in Vibrios of shrimp aquaculture: Incidence, identification schemes drivers and mitigation measures. *Aquaculture Research*. https://doi.org/10.1111/are.15142

Oliver, J. D. (2006). *Vibrio vulnificus*. In F. L. Thompson, B. Austin, & J. Swing (Eds.), *Biology of Vibrios* (pp. 349–366). American Society for Microbiol.

Oliver, J. D. (2013). *Vibrio vulnificus*: Death on the half shell. A personal journey with the pathogen and its ecology. *Microbial Ecology, 65*, 793–799. https://doi.org/10.1007/s00248-012-0140-9

Pan, J., Zhang, Y., Jin, D., Ding, G., Luo, Y., Zhang, J., Mei, L., & Zhu, M. (2013). Molecular characterization and antibiotic susceptibility of *Vibrio vulnificus* in retail shrimps in Hangzhou, People's Republic of China. *Journal of Food Protection, 76*, 2063–2206.

Panicker, G., Myers, M. L., & Bej, A. K. (2004). Rapid detection of *Vibrio vulnificus* in shellfish and Gulf of Mexico water by real-time PCR. *Applied and Environmental Microbiology, 70*(1), 498–507.

Paranjpye, R. N., & Strom, M. S. (2005). A *Vibrio vulnificus* type IV pilin contributes to biofilm formation, adherence to epithelial cells, and virulence. *Infection and Immunity, 73*(3), 1411–1422.

Partridge, D. G., Townsend, R., Larkin, S., & Parsons, H. K. (2009). *Vibrio vulnificus*: An unusual mode of acquisition and novel use of rapid susceptibility testing. *Journal of Clinical Pathology, 62*(4), 370–372.

Parvathi, A., Kumar, H. S., Karunasagar, I., & Karunasagar, I. (2004). Detection and enumeration of Vibrio vulnificus in oysters from two estuaries along the southwest coast of India, using molecular methods. *Applied and Environmental Microbiology, 70*(11), 6909–6913.

Patricia serratore (2017). In Goga, B.C., Civera, T., Bossù, T. & Novelli, E. 27th National Congress of the Italian Association of Veterinary Food Hygienists| Perugia, 13–15 Sept. *Italian Journal of Food Safety, 6*(s1).

Ralston, E. P., Kite-Powell, H., & Beet, A. (2011). An estimate of the cost of acute health effects from food- and water-borne marine pathogens and toxins in the USA. *Journal of Water and Health, 9*(4), 680–694.

Ratledge, C., & Dover, L. G. (2000). Iron metabolism in pathogenic bacteria. *Annual Reviews in Microbiology, 54*(1), 881–941.

Rebouças, R. H., de Sousa, O. V., Lima, A. S., Vasconcelos, F. R., de Carvalho, P. B., & dos Fernandes Vieira, R. H. S. (2011). Antimicrobial resistance profile of Vibrio species isolated from marine shrimp farming environments (*Litopenaeus vannamei*) at Ceará, Brazil. *Environmental Research, 111*(1), 21–24.

Rico, A., Satapornvanit, K., Haque, M. M., Min, J., Nguyen, P. T., Telfer, T. C., & Van Den Brink, P. J. (2012). Use of chemicals and biological products in Asian aquaculture and their potential environmental risks: A critical review. *Reviews in Aquaculture, 4*(2), 75–93.

Rodgers, C. J., & Furones, M. D. (2009). Antimicrobial agents in aquaculture: Practice, needs and issues. *Options Méditerranéennes, 86*, 41–59.

Rodrigues, D. P., Ribeiro, R. V., & Hofer, E. (1992). Analysis of some virulence factors of *Vibrio vulnificus* isolated from Rio de Janeiro, Brazil. *Epidemiology and Infection, 108*(3), 463–467.

Roig, F. J., & Amaro, C. (2009). Plasmid diversity in *Vibrio vulnificus* biotypes. *Microbiology, 155*(2), 489–497.

Shaw, K. S., Goldstein, R. E. R., He, X., Jacobs, J. M., Crump, B. C., & Sapkota, A. R. (2014). Antimicrobial susceptibility of *Vibrio vulnificus* and *Vibrio parahaemolyticus* recovered from recreational and commercial areas of Chesapeake Bay and Maryland Coastal Bays. *PLoS One, 9*(2), e89616.

Simpson, L. M., White, V. K., Zane, S. F., & Oliver, J. D. (1987). Correlation between virulence and colony morphology in *Vibrio vulnificus*. *Infection and Immunity, 55*, 269–272.

Songlin, G., PanPan, L., JianJun, F., JinPing, Z., Peng, L., & LiHua, D. (2015). A novel recombinant bivalent outer membrane protein of *Vibrio vulnificus* and *Aeromonas hydrophila* as a vaccine antigen of American eel (Anguilla rostrata). *Fish & Shellfish Immunology, 43*(2), 477–484.

Sony, M., Sumithra, T. G., Anusree, V. N., Amala, P. V., Reshma, K. J., Alex, S., & Sanil, N. K. (2021). Antimicrobial resistance and virulence characteristics of *Vibrio vulnificus*, *Vibrio parahaemolyticus* and *Vibrio harveyi* from natural disease outbreaks of marine/estuarine fishes. *Aquaculture, 539*, 736608.

Sudha, S., Mridula, C., Silvester, R., & Hatha, A. A. (2014). Prevalence and antibiotic resistance of pathogenic vibrios in shellfishes from Cochin market. *Indian Journal of Geo-Marine Science, 43*, 815–824.

Thampuran, N., & Surendran, P. K. (1998). Occurrence and distribution of *Vibrio vulnificus* in tropical fish and shellfish from Cochin (India). *Letters in Applied Microbiology, 26*(2), 110–112.

Tison, D. L., & Kelly, M. T. (1986). Virulence of *Vibrio vulnificus* strains from marine environments. *Applied and Environmental Microbiology, 51*, 1004–1006.

Torres, L., Escobar, S., López, A., Marco, M., & Pobo, V. (2002). Wound infection due to *Vibrio vulnificus* in Spain. *European Journal of Clinical Microbiology and Infectious Diseases, 21*(7), 537–538.

Trinh, S. A., Gavin, H. E., & Satchell, K. J. (2017). Efficacy of ceftriaxone, cefepime, doxycycline, ciprofloxacin, and combination therapy for *Vibrio vulnificus* foodborne septicaemia. *Antimicrobial Agents and Chemotherapy, 61*(12), pp.e01106–17

Vaseeharan, B., Ramasamy, P., Murugan, T., & Chen, J. C. (2005). *In vitro* susceptibility of antibiotics against *Vibrio* spp. and *Aeromonas* spp. isolated from *Penaeus monodon* hatcheries and ponds. *International Journal of Antimicrobial Agents, 26*, 285–291.

Whitfield, C. (2006). Biosynthesis and assembly of capsular polysaccharides in *Escherichia coli*. *Annual Review of Biochemistry, 75*, 39–68.

WHO. (2014). *Antimicrobial resistance global report on surveillance*. World Health Organization. Available online at: http://www.who.int/drugresistance/documents/surveillancereport/en/

Yano, Y., Yokoyama, M., Satomi, M., Oikawa, H., & Chen, S. S. (2004). Occurrence of *Vibrio vulnificus* in fish and shellfish available from markets in China. *Journal of Food Protection, 67*(8), 1617–1623.

Ye, M., Huang, Y., & Chen, H. (2012). Inactivation of *Vibrio parahaemolyticus* and *Vibrio vulnificus* in oysters by high-hydrostatic pressure and mild heat. *Food Microbiology, 32*(1), 179–184.

Yu, W., Shen, X., Pan, H., Xiao, T., Shen, P., & Xiao, Y. (2017). Clinical features and treatment of patients with *Vibrio vulnificus* infection. *International Journal of Infectious Diseases, 59*, 1–6.

Zaidenstein, R., Sadik, C., Lerner, L., Valinsky, L., Kopelowitz, J., Yishai, R., Agmon, V., Parsons, M., Bopp, C., & Weinberger, M. (2008). Clinical characteristics and molecular subtyping of *Vibrio vulnificus* illnesses, Israel. *Emerging Infectious Diseases, 14*(12), 1875.

Zanetti, S., Spanu, T., Deriu, A., Romano, L., Sechi, L. A., & Fadda, G. (2001). In vitro susceptibility of vibrio spp. isolated from the environment. *International Journal of Antimicrobial Agents, 17*(5), 407–409.

Zhang, X. H., & Austin, B. (2005). Haemolysins in vibrio species. *Journal of Applied Microbiology, 98*(5), 1011–1019.

Antimicrobial Resistance in *Cronobacter sakazakii*

Abhay Kumar, Murugadas Vaiyapuri, and Mukteswar Prasad Mothadaka

Contents

Abstract

The microbe "*Cronobacter sakazakii*" is a string test positive bacterium that inhabit different environs and thrives in adverse dry conditions. The ecosystem for Cronobacter is completely undefined. The incidence of Cronobacter is

A. Kumar (✉)
Mumbai Research Centre of CIFT, Vashi, Navi Mumbai, India
e-mail: abhay.kumar2@icar.gov.in

M. Vaiyapuri
Microbiology Fermentation and Biotechnology Division of ICAR-Central Institute of Fisheries Technology (ICAR-CIFT), Cochin, Kerala, India

M. P. Mothadaka
Microbiology, Fermentation and Biotechnology Division, ICAR-Central Institute of Fisheries Technology (ICAR-CIFT), Cochin, Kerala, India
e-mail: mm.prasad@icar.gov.in

M. P. Mothadaka et al. (eds.), *Handbook on Antimicrobial Resistance*,
https://doi.org/10.1007/978-981-19-9279-7_26

observed in different varieties of foodstuffs namely dry type, "powdered infant formula (PIF)," milk powder, tea powder, mushroom, starches, wastewater, animal meat, and fish products. Although *C. sakazakii* contaminated food has not gained health importance, mitigation measures for containment need to take into consideration the occurrence in foodstuffs, constituents, during harvest and postharvest handling, processing, product development, and deterrence, making it as a potential cradle of this contagion due to post-infection related health complications and high mortality rates. *C. sakazakii* recovered from various sources such as animal products, fish and fishery products, milk products, herbal teas, and starches exhibited resistance to different antimicrobial drugs. Despite the fact that the rate of occurrence of *C. sakazakii* is low, in view of its diversity, virulence nature, high mortality rates coupled with the emergence of obduracy to drugs, especially multiple drug resistance, and the high possibility of transfer, this candidate microbe needs complete attention in order to restrict its entry, particularly into infant foods.

Keywords

Cronobacter sakazakii · Antibiotics · Antimicrobial resistance · Meningitis

1 Introduction: History and Pathogenicity of *C. sakazakii*

Cronobacter sakazakii spreads infectious diseases in the infants. This bacterium belongs to the Enterobacteriaceae family. It is motile, string test positive, and can grow with or without oxygen but primarily prefers aerobic conditions. It is mainly affirmative for catalase, unresponsive for oxidase, and suitable for methyl α-D-glucopyranoside (MGP) and non-spore-forming organism (Drudy et al., 2006). Previously, this microbe was referred to as "yellow cloacae" (Farmer et al., 1980; Nazarowec White and Farber (1997a) and further as *Enterobacter sakazakii* in recognition of the contributions of Japanese bacteriologist Riichi Sakazakii (Iversen & Forsythe, 2003). The taxonomic approach, which includes 16S rRNA full-length sequencing and "*fluorescently labeled amplified fragment length polymorphism (f-AFLP)*" analysis with "DNA:DNA hybridization," was functional for a huge pool of these strains. The strains connote 16 distinct biotypes (Iversen et al., 2007). Centered on the available information, the organism is further classified into a novel Cronobacter gen. Nov. (Iversen et al., 2008). It causes "swelling of the liquid and membranes of the brain and spinal cord, enterocolitis and septicemia in neonatal" (Bar-oz et al., 2001), multiple cerebral infarcts, and the effect of multicyclic encephalomalacia on preterm infants with *C. sakazakii* meningitis (Gurtler et al., 2005). The epidemiology of *C. sakazakii* is relatively unknown, as infections caused by this microbe are very limited and seldom noted in the majority of global regions. The first instance of *C. sakazakii* contamination was observed in England in 1958, when two children succumbed to bacterial meningitis. Over time, however, this bacterium was defined as the "pigmented strain of *E. cloacae*." Incidences of infant *C. sakazakii* infections were documented from "Belgium,

Canada, Denmark, Germany, Greece, Israel, Netherlands, Spain, and the USA." Nine regions declared infant diseases resulting from *C. sakazakii* in the USA, and at least 76 incidents came to limelight globally during the period 1958–2003 (Iversen & Forsythe, 2003). The information available on pathogenicity and virulence factors is scant (Kothary et al., 2007). This bacterium (*C. sakazakii*) could overcome the hurdle between brain tissue and circulatory blood, further destroying endothelial microvascular brain cells (Giri et al., 2012). The natural environment of *C. sakazakii* is not yet completely comprehended; however, this microbe is extensively dispersed within the environment; food, water, soil, and vegetables are the most abundant of all living resources in existence (Iverson & Forsythe, 2004). Its most potent source is plant material, and these features contribute to the survival in unfavorable environmental conditions (Iversen & Forsythe, 2004; Mullane et al., 2006). According to the "International Commission on Microbiological Specifications for Foods," *C. sakazakii* is "a very harmful and life-alarming condition with irreversible after effects" (Iversen & Forsythe, 2003; Arroyo et al., 2011). This organism is listed with pathogens such as "*Clostridium botulinum, Listeria monocytogenes* types A and B, and *Cryptosporidium parvum*" (Iversen & Forsythe, 2003). It is highly distinguished from dairy products and cereals, vegetables, and beverages (Farmer et al., 1980; Kornacki, 1998; Cottyn et al., 2001; Kuzina et al., 2001; Soriano et al., 2001; Gassem, 2002; Leclercq et al., 2002; Cruz et al., 2004; Iversen et al., 2004; Kandhai et al., 2004). Cases of contamination of *C. sakazakii* in meat products and raw dairy products are also widely reported, but less in fish and fishery products (Farmer et al., 1985; Montgomery et al., 2002).

Studies carried out on the survival of *C. sakazakii* and different microbes belonging to Enterobacteriaceae in dry pressure PIF revealed that the organisms under scrutiny endured adverse conditions of aridity (Barron & Forsythe, 2007). The *C. sakazakii* survived in PIF for twenty-four and a half months at a temperature of 28 °C. Further, the same study revealed a tenfold decrease in counts to 3.34 log cycles within a 30-day period and to 0.58 log cycles in the first half-year period. Mild readjustment led to a reduction in counts of bacteria to 1.88 log cycles and in the subsequent 24 months, an absolute decline to 4.52 log cycles. Out of ten samples under study, 50% of the *C. sakazakii* species were seen to survive even after 2 years, indicating that some species of this bacterium may continue to be active for long-term periods in PIF. The scheme for identification of *C. sakazakii* in PIF developed by Muytjens et al. (1988), and initially the "USFDA" approved this process of detecting *C. sakazakii* in PIF as a permitted technique. Identification of *C. sakazakii* carried out employing, viz., "pre-enrichment (Buffered Peptone Water)," enrichment ("Enterobacteriaceae Enrichment, EE Broth"; "Modified Lauryl Sulfatetryptose Broth, mLSTB"), selective plating ("VRBA, EMB"), chromogenic medium ("Druggan-Forsythe-Iversen Agar"; "*E. sakazakii* Chromogenic Plating Medium"), and followed by biochemical and molecular confirmation (Simmons et al., 1989; Nazarowec White & Farber, 1997a; Restaino et al., 2006; Iversen et al., 2007, 2008; Druggan & Iversen, 2009). In determination of the level of immunity, one of the important features is age (FAO & WHO, 2008). Infants, the elderly, and pregnant women with weak immune conditions are vulnerable to foodborne diseases (WHO, 2017).

Infections due to *C. sakazakii* lead to serious mortal conditions, viz., "meningitis, septicemia and necrotizing enterocolitis in infants, and premature babies are more vulnerable than older infants" (Nazarowec White & Farber, 1997a). Despite the fact that the incidence of infections due to *C. sakazakii* is far low-slung, the mortality rate is far higher, ranging from 33% to 80%. Nearly 94% of the surviving youngsters face irreversible neurological sequelae leading to quadriplegia, growth retardation, and impairments affecting vision and hearing. These signs are due to secondary necrotic tissue in the brain (Drudy et al., 2006). Bacterial inflammation of the brain (meningitis) needs to be cured to avert the spread of disease in the patient. In this context, *C. sakazakii* with a strong unresponsiveness to antimicrobials can be more complicated to deal with, especially when young patients are under treatment. Diseases caused by *C. sakazakii* are generally managed using ampicillin aggregated with gentamicin or chloramphenicol, and the amalgamation of ampicillin and gentamicin is the preferred remedy. However, C. *sakazakii* developed obduracy to each antibiotic through the transmission of drug-unresponsive genes, in addition to β-lactam unresponsiveness through β-lactamase production. Enterobacter triggers a widespread array of penicillins and cephalosporins for β-lactamase production, and this tendency is accelerated among *C. sakazakii* at lower levels of incidence. These observations drew the attention of researchers who switched to novel third-generation carbapenems or cephalosporins blended with aminoglycoside or trimethoprim-sulfamethoxazole to ward off *C. sakazakii*. But, significant progress could not be made in treating meningitis due to *C. sakazakii* infection with different amalgamations of antimicrobials because of the furtherance of unresponsiveness of Cronobacter to these antimicrobials (Gurtler et al., 2005; Iversen & Forsythe, 2003).

The importance of the incidence of *C. sakazakii* in PIF due to its role in neonatal infections is well documented. This resulted in a diversion of attention from research to upgraded finding methods, more dependable identification processes, systems of genotyping and genomic investigations. Additional inquiry into the significance of clonal lineages and the determination of contamination routes of foodstuffs are needs of the hour (Forsythe, 2015).

1.1 Osmotic Pressure Resistance of *C. sakazakii*

C. sakazakii occurrence is usually associated with PIF and is capable of surviving in arid regions (Barone & Forsythe, 2007). Studies on *C. sakazakii* and other enterobacters for dehydrated stress conditions and survival duration in infant milk revealed that these microbes thrive in PIF at 28 °C temperatures for longer periods of near 30 months' time (Lehner et al., 2005; Barron & Forsythe, 2007). The heat resistance pattern associated with *C. sakazakii* was assessed by Nazarowec-White and Farber (1997b). In all cases of thermal process or pasteurization microbial growth restriction, the acceptable reduction is between 4.0 and 7.0 log cycles. To achieve a log reduction of 6.0–7.0 of *C. sakazakii,* a thermal process is necessitated at 60 °C for 0.25–0.29 h. This bacterium endures heat resistance for longer periods in

comparison to other Enterobacters in milk foods (Nazarowec White and Farber, 1997b).

The occurrence of "meningitis, septicemia, and necrotizing enterocolitis," especially in neonates, is attributed to *E. sakazakii*. In order to diminish the hazards of this organism isolated from Korea in baby food, the thermal properties were assessed at 52 °C, 56 °C, and 60 °C in saline solution, rehydrated PIF, and dried baby food, respectively. The D and Z values were assessed for the above categories. During the process of rehydration of PIF, the thermal inactivation was estimated by viable counts of *E. sakazakii*. The study revealed that after rehydration of PIF, no changes in count of *E. sakazakii* were observed in the first 20 min at ambient temperature, but there was a significant decrease in counts ranging from 1 to 2 CFU/g at 60 °C and 4–6 log CFU/ml at 65 °C and 70 °C for PIF and water. The PIF in general harbored 1 CUF/100 g of *E. sakazakii* in dried condition, and rehydration with enhanced temperatures of 10 °C and above than the commercial stipulated 50 °C for elimination of the risk of this organism provided a better result (Kim & Park, 2007).

1.2 Antibiotic Susceptibility of C. *sakazakii*

The antibiotic susceptibility tests are carried out employing the method of "Bauer-Kirby Disk Diffusion" (Bauer et al., 1966). The inoculum of 100 µl taken from a well-grown culture was placed on Mueller-Hinton agar with antibiotic disks under screening. Further, the plates were incubated with controls at 98.6 °F for 24 h in an aerobic condition. The results are confirmed employing the standard method of the "Clinical and Laboratory Standards Institute" (Weinstein & Lewis II, 2020).

Reports indicate that *C. sakazakii* was moderately susceptible to chloramphenicol besides ampicillin. Both antibiotics were frequently employed in managing patients with bacterial meningitis prior to culture, and susceptibility conclusions were accessible (Farmer et al., 1980). The antibiotic susceptibility of 24 *C. sakazakii* strains employing "Bauer-Kirby Disk Diffusion" showed that 100% of the isolates were completely susceptible to 4 of the 12 antibiotics tested. All the tested isolates were resistant to penicillin at 10 µg (Farmer et al., 1980).

1.3 Drug Unresponsive Nature of *C. sakazakii* Isolated from Milk and Milk Products

Production of dried formula for infant has shown an increasing trend since the turn of the century. In the present-day scenario, the infant food formulation in dried form is decidedly an advanced product and garners a significant share of the dairy trade (Knipschildt, 1986). The foodstuffs can come in contact with contaminants at diverse and multiple stages, which include: gathering, development, handling, packing, dissemination, making, marketing, stowage, reformation, and feeding

(WHO, 2017). Ingestion of contaminated food can lead to serious repercussions of food borne illnesses, especially due to opportunistic pathogens such as *C. sakazakii* (Lou et al., 2014). Antibiotic treatment is one of the most common and preferred approaches to thwart Cronobacter disease in children (Depardieu et al., 2007). Numerous studies indicate that Cronobacter species can be eliminated successfully with antibiotic drugs, but employment of antimicrobials for prolonged periods is not advisable as it will result in the advancement of strong Cronobacter resistance to drugs (Yoneyama & Katsumata, 2006; McMahon et al., 2007). The incidence of Cronobacter spp. contamination observed in PIF of different brands. All the 23 strains under study were susceptible to all 12 antibiotics tested, namely, "clavulanic acid/amoxicillin, cefotaxime, ampicillin, ceftriaxone, chloramphenicol, ciprofloxacin, gentamicin, tetracycline, nalidixic acid, co-trimoxazole, cefuroxime, and cefixime" (Terragno et al., 2009). In another study, 118 baby milk powder specimens have been collected that include various products from medical stores situated in diverse regions of Qazvin, Iran, from February to July 2018. Bacterial isolates from household and imported PMIF products exhibited higher levels unresponsiveness to antimicrobials such as "ampicillin, amoxicillin and chloramphenicol." Studies along these lines revealed a robust interface between isolates on the characteristics of the antimicrobial susceptibility that include 100% unresponsiveness to ampicillin and amoxicillin; predisposition among tetracycline and ciprofloxacin; and susceptibility to levofloxacin and amikacin (Pakbin et al., 2020).

C. sakazakii is implicated in "neonatal meningitis, septicemia, and necrotizing enterocolitis in preterm infants and newborns with mortalities ranging from 15% to 80%." "Powdered and dairy formulas (P-DF)" were identified to be major means of transmission to such an extent that the incidence of this organism in PDF led to the recall of associated products in Chile in 2017. Seven isolates were identified as C. sakazakii and were subjected to AST (Table 1). All the *C. sakazakii* isolates harbored 31 virulent genes and multiple AR genes (Parra-Flores et al., 2021), which is alarming, and the decision to recall the products justified the findings of Parra Flores et al. (2021).

2 Drug Obduracy in Clinical Isolates of *C. sakazakii*

During an investigation in two clinics from May 2007 to August 2013, 52 *Cronobacter sakazakii* were isolated from wounds, sputum, rectal swabs, tongue, throat, etc. All 52 isolates were susceptible to "amikacin, ampicillin, aztreonam, cefepime cefotaxime, ceftazidime, cefuroxime, ciprofloxacin, colistins, gentamicin, Meropenem sulbactam, sulfamethoxazole, tazobactam tigecycline and tobramycin" (Holý et al., 2019). Studies indicate Cronobacter is susceptible to most of the antibiotics. However,

Table 1 Antibiotic resistance pattern of *Cronobacter sakazakii* from different sources

				No. (%) of *C. sakazakii*																																			
				Total of 70 isolates			CH42			CH43			CH44			CH45			CH50			CH65			CH84			A total 1055 Isolates		
				R	I	S	R	I	S	R	I	S	R	I	S	R	I	S	R	I	S	R	I	S	R	I	S	R	I	S									
Antimicrobial cluster	Drug employed	Disk Code of Antibiotic drug tested	Class of antibiotic as per WHO*																																				
		Code																																					
Penicillins	Ampicillin	AMP	CI	ND*	ND*	70 (100)	+ve			+ve			+ve			+ve			+ve					+ve			+ve			3 (0.3)	1 (0.1)	1051 (99.6)								
	Ampicillin/sulbactam	SAM	CI	ND*	ND*	70 (100)																								ND*	1 (0.1)	1054 (99.9)								
	Amoxicillin/clavulanic	AMC	CI	ND*	ND*	70 (100)	+ve					+ve	+ve			+ve		+ve			+ve	+ve						+ve		Not assessed	Not assessed	Not assessed								
				ND*	ND*																																			
Cephalosporins	Cefepime	FEP	CI	ND*	ND*	70 (100)																								Not assessed	Not assessed	Not assessed								
	Ceftriaxone	GRO	CI	ND*	ND*																									Not assessed	Not assessed	Not assessed								
	Cefazolin	KZ	HI	1 (1.43)	24 (34.29)	45 (64.29)																																	
	Cephalothin	CF	HI	67 (95.71)	3 (4.29)	0 (0)	+ve				+ve		+ve			+ve			+ve			+ve			+ve														
Aminoglycosides	Gentamicin	GN	CI	ND*	ND*	70 (100)			+ve			+ve			+ve			+ve			+ve			+ve			+ve		ND*	ND*	1055 (100)								
	Tobramycin	TOB	CI	ND*	ND*	70 (100)																								Not assessed	Not assessed	Not assessed								
	Amikacin	AK	CI	ND*	2 (2.86)	68 (97.14)																								Not assessed	Not assessed	Not assessed								

(continued)

Table 1 (continued)

				No. (%) of *C. sakazakii*																									
				Total of 70 isolates			CH42			CH43			CH44			CH45			CH50			CH65			CH84			A total 1055 Isolates		
				R	I	S	R	I	S	R	I	S	R	I	S	R	I	S	R	I	S	R	I	S	R	I	S	R	I	S
Quinolones	Ciprofloxacin	CIP	CI	ND*	ND*	70 (100)			+ve			+ve			+ve			+ve			+ve			+ve			+ve	ND*	ND*	1055 (100)
Carbapenem	Imipenem	IPM	CI	ND*	ND*	70 (100)																						ND*	ND*	1055 (100)
Sulfonamides	Trimethoprim/Sulfamethoxazole	SXT	HI	ND*	ND*	70 (100)																						2 (0.2)	ND*	1053 (99.8)
Monobactams	Aztreonam	ATM	HI	ND*	ND*	70 (100)																						Not assessed	Not assessed	
Amphenicols	Chloramphenicol	C	HI	ND*	ND*	70 (100)			+ve			+ve			+ve			+ve			+ve			+ve			+ve	8 (0.8)	68 (6.5)	979 (99.8)
Tetracyclines	Tetracycline	TE	HI	ND*	ND*	70 (100)			+ve			+ve			+ve			+ve			+ve			+ve			+ve	3 (0.3)	1 (0.1)	1051 (99.6)

CI: Critically Important; HI: Highly Important. R; Resistant; I Intermediate and S; Susceptible. ND: Not detected The 70 isolates study pertains Li *et al*, (2019); The AST related to CH 42 to 45, 50, 65 and 84 related to Parra-Flores *et al* (2021); And of 1055 isolates related to Gan *et al,* (2022)

the development of drug resistance with the possibility of transfer from other isolates can be extremely hazardous to human well-being when one is cognizant of the high mortality rates of Cronobacter infections.

3 Unresponsiveness to Antimicrobial Drugs of *C. sakazakii* Isolated from Edible Mushrooms

Mushrooms are a popular food, occasionally employed as uncooked food materials due to their high nutrition, sweetness, chewiness, and other favorable sensory characteristics (Bao et al., 2013; Ye et al., 2014). Li et al. (2019) examined 668 samples of mushroom species, and 89 (13.32%) samples were contaminated with Cronobacter spp. In addition, the study showed that the isolates were sensitive to

nearly all antibiotics under screening. Yet, part of the isolates displayed high or intermediate levels of obduracy to cefazolin, cephalothin, and amikacin, which was constant and on par with previously documented studies (Lai, 2001; Molloy et al., 2009; Chon et al., 2012). The possibility of *C. sakazakii* becoming resistant at intermediate level under certain conditions cannot be ruled out (Ruiz-Bolivar et al., 2011). Genes related to "*Extended-Spectrum-β-lactamases* (*ESBLs*)" of Cronobacter spp. have already been reported (Girlich et al., 2001; Caubilla-Barron et al., 2007; Muller et al., 2014). ESBLs accord unresponsiveness to third-generation cephalosporins, monobactams, and penicillins (Vasconcellos et al., 2018).

4 Aquatic Product Sourced *C. sakazakii* and Its Obduracy to Drugs

Aquatic products have a high dietary rate and are generally used in undeveloped foods for infants. Baby food is regularly cooked at high temperatures, and all the microbes associated with these foods get eliminated without primary contact by way of a child; yet, aquatic animals and plants may be sources of environmental pollution and other food products during food preparation. Studies conducted in China on aquatic products from 2011 to 2016 focused on Cronobacter sp. (Li et al., 2020). A sum of 800 fish and fishery products was sampled that included shellfish ($n = 139$), freshwater fish ($n = 349$), and brackish water fish ($n = 312$). They were collected from local commercial outlets and malls in 44 cities across the PRC. In the same study, samples were drawn in sterile polythene containers, brought to the lab in chilled condition, and were subjected to immediate sampling sans any delay. Comprehensively, *C. sakazakii* incidence was at 3.9% (31/800), including 7, 11, and 13 samples of shellfish (5.0%), brackish water fish (3.5%), and freshwater fish (3.7%), respectively. In MPN assessment test, the harboring levels of Cronobacter sp., 96.8% (30/31) of samples had less than 10 MPN/g, and one sample had between 10 and 110 MPN/gm.

The 31 positive samples had an average contamination level of 2.05 MPN/g. In addition, 4 variants were detected in 33 isolates out of 31 samples contaminated with Cronobacter spp. In AST, all the isolates exhibited sensitivity to the antibiotics tested, viz., "ampicillin, clavulanic acid, ceftriaxone, gentamicin, tobramycin, cefepime, amikacin, ciprofloxacin, imipenem, and aztreonam." Maximum number of *Cronobacter* spp., strains showed uppermost unresponsiveness against "cephalothin (84.8%), trailed by tetracycline (6.1%), trimethoprim/sulfamethoxazole (3.0%), and chloramphenicol (3.0%)." Two of the isolates were unresponsive to three antibiotics (Table 1). The study of Li et al. (2020), which is large scale in nature, showed that 26 sequence and 33 CRISPR categories were identified, with 6 and 26 new STs and CTs, respectively, indicating enormous diversity in *Cronobacter* incidence in aquatic harvests. These findings of Li et al. (2020) are useful in opening up new vistas in the creation of operable processes for containing Cronobacter in fish in different stages of harvest, postharvest, and production.

5 *C. sakazakii* Isolated from Meat Products and Their Unresponsiveness to Drugs

In a study, the drug sensitivity pattern of 24 Cronobacter strains showed 100% susceptibility to "chloramphenicol, gentamicin, and kanamycin." Among the isolates, "97%, 92%, 87%, 67% and 13% were susceptible to nalidixic acid, streptomycin, tetracycline, carbenicillin and sulfadiazine," respectively (Farmer et al., 1980). The study of Nazarowec White and Farber (1997b) revealed two Cronobacter sp., isolated from foodstuffs, were obdurate to tetracycline and chloramphenicol. Cronobacter spp. isolates of *Anastrepha ludens* (Mexican fruit fly) origin demonstrated strong unresponsiveness to "ampicillin, cephalothin, erythromycin, novobiocin and penicillin," however, sensitive to tetracycline (Kuzina et al., 2001). Cronobacter sp. recovered from different edible products were sensitive to tetracycline and unresponsive to ampicillin (Kim et al., 2008). World over scrutiny of edible products is intense on *C. sakazakii* owing to food web globalization that is becoming a source for virulent gastrointestinal microbes (Shukla et al., 2016).

In a study, 235 specimens, including 130, 55, and 50 samples of baby milk powder, spices and herbs and, Boulogne flavored powder (BFP), were collected respectively from commercial outlets of Duhok, Iraq, during the period February–August 2019 (Tayeb et al., 2020). The products were vetted for *C. sakazakii* occurrence and suspected isolates were confirmed employing conventional biochemical characters and through PCR methods. The results indicated that "3.1% PIF, 24% BFP, and 78.2% spice and herbs were positive for *C. sakazakii* incidence," and the isolates were completely sensitive to ampicillin, tetracycline, gentamicin, and chloramphenicol, and showed resistance to other antibiotics.

The incidence of Cronobacter in general is low albeit, major aspect is the virulent nature of these microbes, which can lead to mortalities upon infection or protracted sequelae associated with the postinfection condition. In general, mainstream Cronobacter isolates are susceptible to most of the drugs, and a few studies have documented the incidence of MDR Cronobacter. Very recently, Gan et al. (2022) carried out extensive studies on PIF and powdered foods. For the purpose of the study, the authors drew 12,105 samples of PIF and other ancillary foods from 29 provinces of China during the years 2018 and 2019 (Table 1). A total of 1055 Cronobacter sp. were isolated, out of which 1048 occurred in ancillary infant foods and 7 in PIF. The susceptibility pattern of the isolates to antimicrobials drugs indicates 1% (11) were unresponsive to drugs and two of the isolates were obdurate to four antibiotics, viz., "ampicillin (AMP), tetracycline (TET), sulfamethoxazole-trimethoprim (SXT), and chloramphenicol (CHL)," demarcated as MDR. Further, they were recognized as "*Cronobacter sakazakii* sequence type 4 (ST4) (*C. sakazakii* Crono-589) and ST40 (*C. sakazakii* Crono-684)." Both MDR isolates harbored virulent genes "cusC, fkpA, flhA, hfq, hha, higB, higB1, higB2, hlyIII, nanA, nanK, nanT, ompA, ompX, vgrG1, and zpx" and 7 AMR genes, viz., "blaCSA-1, CRP, EF-Tu, emrB, GlpT, H-NS, and msbA." The genomic proportional investigations specified that food-related *C. sakazakii* could obtain AMR factors through HGT (Gan et al., 2022).

Grilled meat and meat burgers make up the bulk of the Egyptian diet due to the proliferation of fast-food restaurants and the need to stay out of the house. Buyers predict that meat products will be free from pathogen and safe to eat if managed and broiled appropriately. On the contrary, similar to any other undercooked food material, pulverized beef and its products can be contaminated at any time, from slaughter, carving, and minced meat to further processing and preservation (Sofos et al., 1999). Prepared meat products or fresh raw meals may contain antibiotic-resistant *C. sakazakii*. Many antibiotic-resistant infections spreading bacteria from fresh and prepared meat products harbored the same gene of antibiotic resistance collected from human bacteria (Aquilanti et al., 2007; Chen et al., 2012). Farmed animals provided with antibiotics through food for longer durations harbor microflora unaffected to these drugs (Swartz, 2002). Resistant infections are costly to contain and may force the farmers to employ less desirable antibiotics (Travers and Barza, 2002). The most commonly reported *C. sakazakii* infection has occurred in commercial large-scale preparations; however, bacterial infection can also occur under domestic conditions (Redmond & Griffith, 2009). *C. sakazakii* can enter the home atmosphere through deteriorated food products. *C. sakazakii* can be relocated from polluted kitchen range to baby milk powder and other prepared foods through hazardous food management protocols.

6 AMR in Domestic Kitchen Originated *C. sakazakii*

In a study by Kilonzo-Nthenge et al. (2012), a total of 78 household samples collected from home kitchens – surface swabs from different areas, gas stove, and frozen meat products – were subjected to screening for incidence of *Cronobacter sakazakii*. For estimating antibiotic obduracy, disk diffusion method was employed. The isolates of *C. sakazakii* were unresponsive frequently to "ciproxin, tetracycline, and penicillin at 57.1%, 66.6%, and 76.1%," respectively (Kilonzo-Nthenge et al., 2012). And relatively lesser percent of the isolates were unresponsive to "cefoxitin, chloramphenicol, streptomycin, ampicillin, and nalidixic acid at 9.5%, 19%, 28.6%, 33%, and 47.6%," respectively. The lowest unresponsiveness was observed with kanamycin at 4.8% level. In general, the isolates of *C. sakazakii* were unresponsive to two or more of the drugs under screening. The plausible reason for occurrence of drug unresponsive nature of *C. sakazakii* in domestic kitchenettes is contaminated meat products or other types of fresh foods. Multiple antibiotic-obdurate microbes associated with undeveloped and under-processed meat foodstuffs exhibited similarity with ARGs to that of clinical isolates (Aquilanti et al., 2007; Chen et al., 2004).

Many streaks of evidence link strong drug obdurate human infections to microbial pathogens associated with faunal-sourced foods. Types of evidence reviewed include: (1) straight studies of epidemiology, (2) chronological indications, (3) additional anecdotal signs, (4) inclinations in the unresponsive nature of Salmonellae to antimicrobial drugs, and (5) developments in drug obduracy among new pathogens such as Cronobacter. The contribution of AMR by commensal microorganisms in animals and humans cannot be ruled out among pathogenic microbes that are responsible for

infectious diseases in humans. For instance, the faunal-sourced foodborne enterococci that are unresponsive to vancomycin were associated with human gut microbiota. The period of dormancy between a new drug introduction and the advent of obduracy varies considerably, however, once this resistance reaches a certain stage in the population, the mitigation measures are extremely difficult to get pragmatized (Swartz, 2002). Controlling drug obdurate infections is not an economically viable option, and for the same reason, the majority of the users tend to depend on less expensive and low quality antimicrobials in managing infectious diseases (Travers & Barza, 2002). However, bacterial disease may also spread in the house (Redmond & Griffith, 2009). *C. sakazakii* can enter into the kitchen surroundings through food contamination. This study has shown the transmission of *C. sakazakii* into domestic kitchenettes and the development of obduracy to drugs employed in clinical settings. And also, the transfer of polluted kitchen areas to milk foods formulated for infants and other prepared foods cannot be ruled out in the absence good hygiene practices.

7 Growth Restriction of *C. sakazakii* by Botanical Products

An investigation by Stock and Wiedemann (2002) indicated thriving of C. *sakazakii* more in raw buffalo milk than in raw milk of goat and cow. Studies reveled that *C. sakazakii* was "100% unresponsive to gentamicin, ciprofloxacin, ceftriaxone, and spectinomycin, while they were resistant to rifampicin and vancomycin." *T. chebula* alcoholic extract was observed to have active antibacterial action against *C. sakazakii*. In a span of 5 min, the amalgamation of *L. fermentum*, *L. casei*, and *P. acidilactici* diminished the number of *C. sakazakii* by 50%. This study suggests that the use of raw plant extracts with antibacterial substances is advantageous over synthetic products in containing the growth of Cronobacter (Kilonzo-Nthenge et al., 2012). These plant products contain phenols, flavonoids, saponins, glycosides, oleic acid, gallic acid, linolenic acid, etc., which alter the bacterial propensity of virulence. Earlier research showed a positive outcome on herb products and bacteria of lactic acid against *C. sakazakii*. Red muscadine extracts comprise natural antioxidants, phenolic acids, and polyphenol compounds. *C. sakazakii* was challenged with red muscadine extract (Kim et al., 2009). These findings proposed employment of liquid red muscadine as a common compound in baby milk formulae to prevent the incidence of *C. sakazakii*. The extract of apple demonstrated antagonistic properties on *C. sakazakii* (Fratianni et al., 2012). The flexible release of *Cardaria draba* reported to have antimicrobial activity against *C. sakazakii* and other zoonotic diseases (Radoniae et al., 2011). A "purified pomegranate peel (POP)" extract contained polyphenolic compounds that control and kill the bacteria. The fruit product exerted detergent and disinfectant action against *C. sakazakii*. And hence, the discards from the pomegranate can be employed as natural food additives in restricting the growth of *C. sakazakii* (Gokce et al., 2019).

8 Containing *C. sakazakii*

C. sakazakii has been detected in PIF (Mullane et al., 2006). Reports indicate that the installation and maintenance of appropriate air filter systems may possibly control and impede the transmission of Cronobacter spp. Numerous reports indicate that once *C. sakazakii* enters the food chain, it will persist for prolonged periods of time in unfavorable environments in isolated modern food manufacturing surroundings, where it is supposed to acclimatize and thrive in adverse drying conditions and at high temperatures (60 °C). Thorough knowledge on molecular mechanisms associated with acclimatizing to these conditions is useful in developing mitigation measures, but much information is not available on these lines.

Many studies have analyzed the use of natural antimicrobial agents as foodstuff supplements to contain the growth of *C. sakazakii*. Al-Holy et al. (2008, 2010) assessed the consequence of employing natural biopesticides as nutritional extracts in growth containment for *C. sakazakii*. In the assessment, copper sulfate, lactic acid, and monolaurin were employed to eliminate Cronobacter. The information indicated that the application of lactic acid in combination with copper sulfate is advantageous in restricting the growth of Cronobacter in the PIF (powder infant formula) for the possible reason of synergistic effects. The liquid obtained from the botanical sources exhibited inhibitory properties against some foodborne pathogens. The potential use of the oil of the trans'-cinnamon (TC), which is primarily from the shell extract of cinnamon plant, in restricting the growth of Cronobacter was explored (Amalaradjou & Venkitanarayanan, 2011). Prebiotics appears to be useful food ingredients in recent years. Reports indicate potential nature of prebiotics in inhibiting the early progression of Cronobacter infection and, thus, in the prevention of Cronobacter-related diseases. Additionally, prebiotics have been added to the benefits of nutritional supplements, and future work will be to develop natural, noninvasive, and safe prebiotic methods to control Cronobacter infections.

In recent years, the contamination of powdered foods has shown an increased trend with *C. sakazakii* and other foodborne pathogens such as *Salmonellae* and *Bacillus* all over the world. Coupled with these contamination challenges, the absence of a proper pasteurization process is clearly felt and is asking for alternative methods. One such promising method is "intense pulsed light (IPL)" to decontaminate powdered foods in various environmental and IPL settings. The combined impact of IPL and TiO_2 photocatalysis in containing microbes was assessed by Chen et al. (2020). The study showed that the high energy force of every pulse and extraordinary crowning levels in combination with shorter periods between pulses significantly reduced microbial counts. Through TiO_2 photocatalysis, an additional $\log_{10}$ was garnered, bringing the total decline *C. sakazakii* counts to 4.71 ± 0.07 and 5.42 ± 0.10 in dry nonfat milk and wheat flour, respectively. The advantage of this process of IPL and TiO_2 photocatalysis combined treatment is that it will reduce consumption of energy while enhancing the microbial safety of powdered foods (Chen et al., 2020).

9 Conclusion

The growth of antimicrobial unresponsiveness is an extensive problem for public well-being and justifies the management of the antibiotic susceptibility of vector-borne bacterium. Studies have shown that various antibiotics symbolize foodborne pathogens and risks to customers and require a high level of hygiene, especially when cooking and keeping milk and food products for the elderly and people with compromised immune systems. The implementation of programs such as "GMP" and "HACCP" in minimizing microbiological hazards from raw material, throughout the process, to the end product (foodstuffs), so as to abate access of Cronobacter into the environs "PIF (powder infant formula)" and evade the progress of importunity of this microbe in PIF commodity, are the basic needs of the hour. Considering the virulence factors of *C. sakazakii*, multiple studies are needed for proper assessment of the risk and to introduce control strategies on AMR.

References

Al-Holy, M. A., Lin, M., Al-Qadiri, H. M., & Rasco, B. A. (2008). A comparative study between overlay method and selective-differential media for recovery of stressed *Enterobacter sakazakii* cells from infant formula. *Food Microbiology, 25*(1), 22–28. https://doi.org/10.1016/j.fm.2007.09.002

Al-Holy, M. A., Castro, L. F., & Al-Qadiri, H. M. (2010). Inactivation of *Cronobacter* spp. (*Enterobacter sakazakii*) in infant formula using lactic acid, copper sulfate and monolaurin. *Letters in Applied Microbiology, 50*, 246–251. https://doi.org/10.1111/j.1472-765X.2009.02782.x

Amalaradjou, M. A., & Venkitanarayanan, K. (2011). Proteomic analysis of the mode of antibacterial action of trans-cinnamaldehyde against *Cronobacter sakazakii* 415. *Foodborne Pathogens and Disease, 8*, 1095–1102. https://doi.org/10.1089/fpd.2011.0841

Aquilanti, L., Garofalo, C., Osimani, A., Silvestri, G., Vignaroli, C., & Clementi, F. (2007). Isolation and molecular characterization of antibiotic-resistant lactic acid bacteria from poultry and swine meat products. *Journal of Food Protection, 70*, 557–565. https://doi.org/10.4315/0362-028x-70.3.557

Arroyo, C., Cebrian, G., Pagan, R., & Condon, S. (2011). Inactivation of *Cronobacter sakazakii* by manothermosonication in buffer and milk. *International Journal of Food Microbiology, 151*(1), 21–28. https://doi.org/10.1016/j.ijfoodmicro.2011.07.035

Bao, D., Gong, M., Zheng, H., Chen, M., Zhang, L., Wang, H., et al. (2013). Sequencing and comparative analysis of the straw mushroom (*Volvariella volvacea*) genome. *PLoS One, 8*, e58294. https://doi.org/10.1371/journal.pone.0058294

Bar-Oz, B., Preminger, A., Peleg, O., Block, C., & Arad, I. (2001). *Enterobacter sakazakii* infection in newborn. *Acta Paediatrica, 90*, 356–358. https://doi.org/10.1080/080352501300067857

Barron, J. C., & Forsythe, S. J. (2007). Dry stress and survival time of Enterobacter sakazakii and other Enterobacteriaceae in dehydrated powdered infant formula. *Journal of Food Protection, 70*(9), 2111–2117.

Bauer, A. W., Kirby, W. M. M., Sherris, J. C., & Turck, M. (1966). Antibiotic susceptibility testing by a standardised single disk method. *American Journal of Clinical Pathology, 45*(4), 493–496. https://doi.org/10.1093/ajcp/45.4_ts.493

Caubilla-Barron, J., Hurrell, E., Townsend, S., Cheetham, P., Loc-Carrillo, C., Cand Fayet, O., et al. (2007). Genotypic and phenotypic analysis of *Enterobacter sakazakii* strains from an outbreak

resulting in fatalities in a neonatal intensive care unit in France. *Journal of Clinical Microbiology, 45*, 3979–3985. https://doi.org/10.1128/JCM.01075-07

Chen, S., Zhao, S., White, D., Schroeder, C. M., Lu, R., Yang, H., McDermott, P. F., Ayers, S., & Meng, J. (2004). Characterization of multiple-antimicrobial–resistant Salmonella serovars isolated from retail meats. *Applied and Environmental Microbiology, 70*, 1–7. https://doi.org/10.1128/aem.70.1.1-7.2004

Chen, Y., Noe, K. E., Thompson, S., Elems, C. A., Brown, E. A., Lampel, K. A., & Hammack, T. S. (2012). Evaluation of a revised FDA method for the detection of Cronobacter in powdered infant formula: Collaborative study. *Journal of Food Protection, 75*(6), 1144–1147. https://doi.org/10.4315/0362-028X.JFP-11-388

Chen, D., Wiertzema, J. R., Peng, P., Cheng, Y., Wang, Y., Liu, J., Ma, Y., Mosher, W., Kang, M., Min, M., Chen, P., Baumler, D. J., Chen, C., Lee, L., Vickers, Z., Feirtag, J., & Ruan, R. (2020). Catalytic intense pulse light inactivation of *Cronobacter sakazakii* and other pathogens in non-fat dry milk and wheat flour. *Food Chemistry, 332*(1), 127420. https://doi.org/10.1016/j.foodchem.2020.127420. Google Access 28th Feb, 2023.

Chon, J. W., Song, K. Y., Kim, S. Y., Hyeon, J. Y., & Seo, K. H. (2012). Isolation and characterization of *Cronobacter* from desiccated foods in Korea. *Journal of Food Science, 77*(7), M354–M358. https://doi.org/10.1111/j.1750-3841.2012.02750.x

Cottyn, B., Regalado, E., Lanoot, B., De Cleene, M., Mew, T. W., & Swings, J. (2001). Bacterial populations associated with rice seed in the tropical environment. *Phytopathology, 91*, 282–292. https://doi.org/10.1094/PHYTO.2001.91.3.282

Cruz, A. C., Fernandez, E., Salinas, E., Ramirez, P., Montiel, C., & Eslava, C. A. (2004). Characterization of *Enterobacter sakazakii* isolated from different sources. Am Soc for Microbiol 104th Gen Mtg. New Orleans, 23–27.

Depardieu, F., Podglajen, I., Leclercq, R., Collatz, E., & Courvalin, P. (2007). Modes and modulations of antibiotic resistance gene expression. *Clinical Microbiology Reviews, 20*, 79–114. https://doi.org/10.1128/CMR.00015-06

Druggan, P., & Iversen, C. (2009). Culture media for the isolation of Cronobacter spp. *International Journal of Food Microbiology, 136*(2), 169–178.

Drudy, D. O., Rourke, M., Murphy, M., Mullane, N. R., Mahony, R. O., Kelly, L., Fischer, M., Sanhaq, S., Shannon, P., & Wall, P. (2006). Characterization of a collection of *Enterobacter sakazakii* isolates from environmental and food sources. *International Journal of Food Microbiology, 110*, 127–134. https://doi.org/10.1016/j.ijfoodmicro.2006.02.008

Farmer, J. J., Asbury, M. A., Hickman, F. W., & Brenner, D. J. (1980). *Enterobacter sakazakii*-a new species of Enterobacteriacea isolated from clinical specimens. *International Journal of Systematic Bacteriology, 30*, 569–584. https://doi.org/10.1099/00207713-30-3-569

Farmer, J. J., Davis, B. R., Hickman-Brenner, F. W., Mcwhorter, A., Huntley-Carter, G. P., Asbury, M. A., Riddle, C., Wathen-Grady, H. G., Elias, C., Fanning, G. R., Steigerwalt, A. G., Ohara, C. M., Morris, G. K., Smith, P. B., & Brenner, D. J. (1985). Biochemical identification of new species and biogroups of Enterobacteriaceae isolated from clinical specimens. *Journal of Clinical Microbiology, 21*, 46–76.

Food and Agriculture Organization of the United Nations (FAO) & World Health Organization (WHO). (2008). *Enterobacter sakazakii* (*Cronobacter* spp.) in powdered follow-up formula. Meeting Report. Microbiological Risk Assessment Series No 15, Rome, 84.

Forsythe, S. J. (2015). New insights into the emergent bacterial pathogen Cronobacter, Chapter 13. In S. C. Ricke, J. R. Donaldson, & C. A. Phillips (Eds.), *Food safety* (pp. 265–308). https://doi.org/10.1016/B978-0-12-800245-2.00013-7. ISBN 9780128002452.

Fratianni, F., De Giulio, A., Sada, A., & Nazzaro, F. (2012). Biochemical characteristics and biological properties of Annurca apple cider. *Journal of Medicinal Food, 15*, 18–23. https://doi.org/10.1089/jmf.2011.0042

Gan, X., Li, M., Xu, J., Yan, S., Wang, W., & Li, F. (2022). Emerging of multidrug-resistant *Cronobacter sakazakii* isolated from infant supplementary food in China. *Microbiology Spectrum, 10*(5). https://doi.org/10.1128/spectrum.01197-22

Gassem, M. A. A. (2002). A microbiological study of sobia: A fermented beverage in the Western province of Saudi Arabia. *World Journal of Microbiology and Biotechnology, 18*, 173–177. https://doi.org/10.1023/A:1014916702466

Giri, P. A., Deshpande, J. D., Phalke, D. B., & Karle, L. B. (2012). Seroprevalence of transfusion transmissible infections among voluntary blood donors at a tertiary care teaching hospital in rural area of India. *Journal of Family Medicine and Primary Care, 1*, 48–51. https://doi.org/10.4103/2249-4863.94452

Girlich, D., Poirel, L., Leelaporn, A., Karim, A., Tribuddharat, C., Fennewald, M., et al. (2001). Molecular epidemiology of the integron-located VEB-1 extended spectrum beta-lactamase in nosocomial enterobacterial isolates in Bangkok, Thailand. *Journal of Clinical Microbiology, 39*, 175–182. https://doi.org/10.1128/JCM.39.1.175-182

Gokce, P. Y., Susan, B., & Pascal, D. (2019). Antibacterial activity of polyphenol-rich pomegranate peel extract against *Cronobacter sakazakii*. *International Journal of Food Properties, 22*(1), 985–993. https://doi.org/10.1080/10942912.2019.1622564

Gurtler, J. B., Kornacki, J. L., & Beuchata, L. R. (2005). *Enterobacter sakazakii*: A coliform of increased concern to infant health. *International Journal of Food Microbiology, 104*, 1–34. https://doi.org/10.1016/j.ijfoodmicro.2005.02.013

Holý, O., Cruz-Córdova, A., Xicohtencatl-Cortes, J., Hochel, I., Parra-Flores, J., Petrželová, J., . . . & Alsonosi, A. (2019). Occurrence of virulence factors in Cronobacter sakazakii and Cronobacter malonaticus originated from clinical samples. *Microbial Pathogenesis, 127*, 250–256.

Iversen, C., & Forsythe, S. (2003). Risk profile of *Enterobacter sakazakii* an emergent pathogen associated with infant milk formula. *Trends in Food Science and Technology, 14*, 443–454. https://doi.org/10.1016/S0924-2244(03)00155-9

Iversen, C., & Forsythe, S. (2004). Isolation of Enterobacter sakazakii and other Enterobacteriaceae from powdered infant formula milk and related products. *Food Microbiology, 21*(6), 771–777.

Iversen, C., Druggan, P., & Forsythe, S. (2004). A selective differential medium for *Enterobacter sakazakii*, a preliminary study. *International Journal of Food Microbiology, 96*, 133–139. https://doi.org/10.1016/j.ijfoodmicro.2004.01.024

Iversen, C., Lehner, A., Mullane, N., Bidas, E., Cleenwerck, I., Marugg, J., Fanning, S., Stephan, R., & Joosten, H. (2007). The taxonomy of *Enterobacter sakazakii*: Proposal of a new genus *Cronobacter* gen. nov. and descriptions of *Cronobacter sakazakii* comb. Nov., *Cronobacter sakazakii* subsp. sakazakii comb. Nov., *Cronobacter sakazakii* subsp. malonaticus comb. nov., *Cronobacter turicensis* sp. nov., *Cronobacter muytjensii* sp. nov., *Cronobacter dublinensis* sp. nov. and *Cronobacter genomo* species I. *BMC Evolutionary Biology, 7*, Article No. 64. https://doi.org/10.1186/1471-2148-7-64.

Iversen, C., Mullane, N., McCardell, B., Tal, B. D., Lehner, A., Fanning, S., Stephan, R., & Joosten, H. (2008). *Cronobacter* gen. nov., a new genus to accommodate the biogroups of *Enterobacter sakazakii*, and proposal of *Cronobacter sakazakii* gen. nov., comb. nov., *Cronobacter malonaticus* sp. nov., *Cronobacter turicensis* sp. nov., *Cronobacter muytjensii* sp. nov., *Cronobacter dublinensis* sp. nov., *Cronobacter genomo* species, and of three subspecies, *Cronobacter dublinensis* subsp. dublinensis subsp. nov., *Cronobacter dublinensis* subsp. l*ausannensis* subsp. nov. and *Cronobacter dublinensis* subsp. *lactaridi* subsp. nov. *International Journal of Systematic and Evolutionary Microbiology, 58*(6), 1442–1447. https://doi.org/10.1099/ijs.0.65577-0.

Kandhai, M. C., Reij, M. W., Gorris, L. G., Guillaume-Gentil, O., & van Schothorst, M. (2004). Occurrence of *Enterobacter sakazakii* in food production environments and households. *Lancet, 363*, 39–40. https://doi.org/10.1016/S0140-6736(03)15169-0

Kilonzo-Nthenge, A., Rotich, E., Godwin, S., Nahashon, S., & Chen, F. (2012). Prevalence and antimicrobial resistance of Cronobacter sakazakii isolated from domestic kitchens in middle Tennessee, United States. *Journal of Food Protection, 75*(8), 1512–1517.

Kim, S., & Park, J. H. (2007). Thermal resistance and inactivation of *Enterobacter sakazakii* isolates during rehydration of powdered infant formula. *Journal of Microbiology and Biotechnology, 17*(2), 364–368. Accessed Google Scholar 24th February, 2007.

Kim, K., Jang, S. S., Kim, K. K., Park, J. H., Heu, S., & Ryu, S. (2008). Prevalence and generic diversity of *Enterobacter sakazakii* in ingredients of infant foods. *International Journal of Food Microbiology, 122*, 196–203.

Kim, T. J., Silva, J. L., Weng, W. L., Chen, W. W., Corbitt, M., & Jung, Y. S. (2009). Inactivation of *Enterobacter sakazakii* by water-soluble muscadine seed extracts. *International Journal of Food Microbiology, 129*, 295–299. https://doi.org/10.1016/j.ijfoodmicro.2008.12.014

Knipschildt, M. E. (1986). Drying of milk and milk products. In R. K. Robinson (Ed.), *Modern dairy technology* (pp. 131–234). Elsevier Applied Sciences.

Kornacki, J. L. (1998). *Enterobacter sakazakii: Pursuit of a putative pathogen in a dairy powder factory (a case study)*. American Dairy Sci Assn Ann Mtg.

Kothary, M. H., McCardell, B. A., Frazar, C. D., Deer, D., & Tall, B. D. (2007). Characterization of the zinc-containing metalloprotease encoded by zpx and development of a species-specific detection method for *Enterobacter Sakazakii*. *Applied and Environmental Microbiology, 73*, 4142–4151. https://doi.org/10.1128/AEM.02729-06

Kuzina, L. V., Peloquin, J. J., Vacek, D. C., & Miller, T. A. (2001). Isolation and identification of bacteria associated with adult laboratory Mexican fruit flies, *Anastrepha ludens* (Diptera: Tephritidae). *Current Microbiology, 42*, 290–294. https://doi.org/10.1007/s002840110219

Lai, K. K. (2001). *Enterobacter sakazakii* infections among neonates, infants, children, and adults. Case reports and a review of the literature. *Medicine, 80*(2), 113–122. https://doi.org/10.1097/00005792-200103000-00004

Leclercq, A., Wanegue, C., & Baylac, P. (2002). Comparison of fecal coliform agar and violet red bile lactose agar for fecal coliform enumeration in foods. *Applied and Environmental Microbiology, 68*, 1631–1638. https://doi.org/10.1128/AEM.68.4.1631-1638.2002

Lehner, A., Riedel, K., Eberl, L., Breeuwer, P., Diep, B., & Stephan, R. (2005). Biofilm formation, extracellular polysaccharide production, and cell-to-cell signaling in various Enterobacter sakazakii strains: aspects promoting environmental persistence. *Journal of Food Protection, 68*(11), 2287–2294.

Li, C., Zeng, H., Zhang, J., He, W., Ling, N., Chen, M., Wu, S., Lei, T., Wu, H., Ye, Y., Ding, Y., Wang, J., Wei, X., Zhang, Y., & Wu, Q. (2019). Prevalence, antibiotic susceptibility, and molecular characterization of *Cronobacter* spp. isolated from edible mushrooms in China. *Frontiers in Microbiology, 10*, 283. https://doi.org/10.3389/fmicb.2019.00283

Li, C., Zeng, H., Zhang, J., Luo, D., Chen, M., Lei, T., Wu, H., Ye, Y., Ding, Y., Wang, J., Wei, X., Zhang, Y., & Wu, Q. (2020). *Cronobacter* spp. isolated from aquatic products in China: Incidence, antibiotic resistance, molecular characteristic and CRISPR diversity. *International Journal of Food Microbiology, 335*, 108857. https://doi.org/10.1016/j.ijfoodmicro.2020.108857

Lou, X., Si, G., Yu, H., Qi, J., Liu, T., et al. (2014). Possible reservoir and routes of transmission of *Cronobacter* (*Enterobacter sakazakii*) via wheat flour. *Food Control, 43*, 258–262. https://doi.org/10.1016/j.foodcont.2014.03.029

McMahon, M. A. S., Xu, J., Moore, J. E., Blair, I. S., & McDowell, D. A. (2007). Environmental stress and antibiotic resistance in food-related pathogens. *Applied and Environmental Microbiology, 73*, 211–217. https://doi.org/10.1128/AEM.00578-06

Molloy, C., Cagney, C., O'Brien, S., Iversen, C., Fanning, S., & Duffy, G. (2009). Surveillance and characterization by pulsed-field gel electrophoresis of *Cronobacter* spp. in farming and domestic environments, food production animals and retails foods. *International Journal of Food Microbiology, 136*(2), 198–238. https://doi.org/10.1016/j.ijfoodmicro.2009.07.007

Montgomery, J. M., Gillespie, D., Sastrawan, P., Fredeking, T. M., & Stewart, G. L. (2002). Aerobic salivary bacteria in wild and captive Komodo dragons. *Journal of Wildlife Diseases, 38*, 545–551. https://doi.org/10.7589/0090-3558-38.3.545

Mullane, N. R., Murray, J., Drudy, D., Prentice, N., Whyte, P., Wall, P. G., Parton, A., & Fanning, S. (2006). Detection of *Enterobacter sakazakii* in dried infant milk formula by cationic-magnetic-bead capture. *Applied and Environmental Microbiology, 72*, 6325–6330. https://doi.org/10.1128/AEM.03056-05

Muller, A., Hachler, H., Stephan, R., & Lehner, A. (2014). Presence of AmpC Beta-Lactamases, CSA-1, CSA-2, CMA-1, and CMA-2 conferring an unusual resistance phenotype in *Cronobacter sakazakii* and *Cronobacter malonaticus*. *Microbial Drug Resistance, 20*, 275–280. https://doi.org/10.1089/mdr.2013.0188

Muytjens, H. L., Roelofs-Willemse, H. A. N. N. I. E., & Jaspar, G. H. (1988). Quality of powdered substitutes for breast milk with regard to members of the family Enterobacteriaceae. *Journal of Clinical Microbiology, 26*(4), 743–746.

Nazarowec White, M., & Farber, J. M. (1997a). Incidence, survival and growth of *Enterobacter sakazakii* in infant formula. *Journal of Food Protection, 60*(3), 226–230. https://doi.org/10.4315/0362-028X-60.3.226. Accessed Google Scholar 27 Feb 2023.

Nazarowec White, M., & Farber, J. M. (1997b). Thermal resistance of *Enterobacter sakazakii* in reconstituted dried-infant formula. *Letters in Applied Microbiology, 24*(1), 9–13. https://doi.org/10.1046/j.1472-765x.1997.00328.x. Accessed Google Scholar 27 Feb 2023.

Pakbin, B., Mahmoudi, R., Mousavi, S., Allahyari, S., Amani, Z., Peymani, A., Qajarbeygi, P., & Hoseinabadi, Z. (2020). Genotypic and antimicrobial resistance characterizations of Cronobacter sakazakii isolated from powdered milk infant formula: A comparison between domestic and imported products. *Food Science & Nutrition, 8*(12), 6708–6717. https://doi.org/10.1002/fsn3.1965

Parra-Flores, J., Holý, O., Riffo, F., Lepuschitz, S., Maury-Sintjago, E., Rodríguez-Fernández, A., Ariadnna Cruz-Córdova, A., Xicohtencatl-Cortes, J., Mancilla-Rojano, J., Troncoso, M., Figueroa, G., Ruppitsch, W., & Stephen Forsythe, S. (2021). Profiling the virulence and antibiotic resistance genes of *Cronobacter sakazakii* strains isolated from powdered and dairy formulas by whole-genome sequencing. *Frontiers in Microbiology, 12*, 10.3389/fmicb.2021.694922. Accessed Google Scholar on 1st March 2023.

Radoniae, A., Blaževiæ, I., Masteliæ, J., Zekiæ, M., Skoèibušiæ, M., & Maraviæ, A. (2011). Phytochemical analysis and antimicrobial activity of Cardariadraba (L.) Desv. volatiles. *Chemistry & Biodiversity, 8*, 1170–1181. https://doi.org/10.1002/cbdv.201000370

Redmond, E. C., & Griffith, C. J. (2009). The importance of hygiene in the domestic kitchen: Implications for preparation and storage of food and infant formula. *Perspectives in Public Health, 129*, 69.

Restaino, L., Frampton, E. W., Lionberg, W. C., & Becker, R. J. (2006). A chromogenic plating medium for the isolation and identification of Enterobacter sakazakii from foods, food ingredients, and environmental sources. *Journal of Food Protection, 69*(2), 315–322.

Ruiz-Bolivar, Z., Neuque-Rico, M. C., Poutou-Pinales, R. A., Carrascal-Camacho, A. K., & Mattar, S. (2011). Antimicrobial susceptibility of Listeria monocytogenes food isolates from different cities in Colombia. *Foodborne Pathogens and Disease, 8*, 913–919. https://doi.org/10.1089/fpd.2010.0813

Shukla, S., Lee, G., Song, X., Park, S., & Kim, M. (2016). Immunoliposome-based immunomagnetic concentration and separation assay for rapid detection of Cronobacter sakazakii. *Biosensors and Bioelectronics, 77*, 986–994.

Simmons, B. P., Gelfand, M. S., Haas, M., Metts, L., & Ferguson, J. (1989). Enterobacter sakazakii infections in neonates associated with intrinsic contamination of a powdered infant formula. *Infection Control & Hospital Epidemiology, 10*(9), 398–401.

Sofos, J. N., Kochevar, S. L., Bellinger, G. R., Buege, D. R., Hancock, D. D., Ingham, S. C., Morgan, J. B., Reagan, J. O., & SMITH AC. (1999). Sources and extent of microbiological contamination of beef carcasses in seven United States slaughtering plants. *Journal of Food Protection, 62*(2), 140–145. https://doi.org/10.4315/0362-028x-62.2.140

Soriano, J. M., Rico, H., Molto, J. C., & Manes, J. (2001). Incidence of microbial flora in lettuce meat and Spanish potato omelette from restaurants. *Food Microbiology, 18*, 159–163. https://doi.org/10.4315/0362-028x-63.9.1273

Stock, I., & Wiedemann, B. (2002). Natural antibiotic susceptibility of *Enterobacter amnigenus*, *Enterobacter cancerogenus*, *Enterobacter gergoviae* and *Enterobacter sakazakii* strains.

Clinical Microbiology and Infection, 8, 564–578. https://doi.org/10.1046/j.1469-0691.2002.00413.x

Swartz, M. N. (2002). Human diseases caused by foodborne pathogens of animal origin. *Clinical Infectious Diseases, 34*, S111–S122. https://doi.org/10.1086/340248

Tayeb, B. A., Mohamed Sharif, Y. H., & Ameen, A. M. (2020). Incidence rate and antibiotic resistance profile of *Cronobacter sakazakii* isolated from various food products. *Food Research, 4*(6), 2217–2223. https://doi.org/10.26656/fr.2017.4(6).304

Terragno, R., Salve, A., Pichel, M., Epszteyn, S., Brengi, S., & Binsztein, N. (2009). Characterization and subtyping of *Cronobacter* spp. from imported powdered infant formulae in Argentina. *International Journal of Food Microbiology, 136*, 193–197. https://doi.org/10.1016/j.ijfoodmicro.2009.10.013

Travers, K., & Barza, M. (2002). Morbidity of infections caused by antimicrobial-resistant bacteria. *Clinical Infectious Diseases, 3*, S131–S134. https://doi.org/10.1086/340251

Vasconcellos, L., Carvalho, C. T., Tavares, R. O., de Mello Medeiros, V., de Oliveira Rosas, C., Silva, J. N., et al. (2018). Isolation, molecular and phenotypic characterization of *Cronobacter* spp. in ready-to-eat salads and foods from Japanese cuisine commercialized in Brazil. *Food Research International, 107*, 353–359. https://doi.org/10.1016/j.foodres.2018.02.048

Weinstein, M. P., & Lewis, J. S., II. (2020). The clinical and laboratory standards institute subcommittee on antimicrobial susceptibility testing: Background, organization, functions, and processes. *Journal of Clinical Microbiology, 58*(3), e01864–e01819. https://doi.org/10.1128/JCM.01864-19

World Health Organization. (2017). The burden of foodborne diseases in the WHO European region, Publications, WHO Regional Office for Europe UN City, Marmorvej 51 DK-2100 Copenhagen Ø, Denmark.

Ye, Y., Li, H., Wu, Q., Chen, M., Lu, Y., & Yan, C. (2014). Isolation and phenotypic characterization of Cronobacter from dried edible macrofungi samples. *Journal of Food Science, 79*, M1382–M1386. https://doi.org/10.1111/1750-3841.12513

Yoneyama, H., & Katsumata, R. (2006). Antibiotic resistance in bacteria and its future for novel antibiotic development. *Bioscience, Biotechnology, and Biochemistry, 70*, 1060–1075. https://doi.org/10.1271/bbb.70.1060

Vibrio mimicus and Its Antimicrobial Resistance in Fish and Aquatic Environments

Ezhil S. Nilavan and Mukteswar Prasad Mothadaka

Contents

E. S. Nilavan (✉)
Microbiology, Fermentation and Biotechnology, ICAR-Central Institute of Fisheries Technology (ICAR-CIFT), Cochin, Kerala, India

Kerala University of Fisheries and Ocean Studies, Cochin, Kerala, India
e-mail: theezhilnilavan@gmail.com

M. P. Mothadaka
Microbiology, Fermentation and Biotechnology Division, ICAR-Central Institute of Fisheries Technology (ICAR-CIFT), Cochin, Kerala, India

M. P. Mothadaka et al. (eds.), *Handbook on Antimicrobial Resistance*,
https://doi.org/10.1007/978-981-19-9279-7_19

Abstract

Vibrio mimicus is an important bacterial pathogen occurring in brackish water and seawater habitats, and it is now emerging as an important cause of seafood-linked bacterial infections. *V. mimicus* infections have been documented in the United States as well as many tropical nations, including India, China, Bangladesh, Thailand, and Africa. There is an increasing concern that *V. mimicus* may delineate an important and increasing clinical issue in India in the future. Also, many factors underlie the requisite for a broader understanding of this pathogen within the Indian context. First, *V. mimicus* incidences are on the rise, and they tend to follow regional climatic patterns, with outbreaks peaking during the summer months. These reports are particularly alarming given current predictions of the warming of marine waters due to climate change. Besides, a variety of epidemiological causes, namely, rise in global seafood consumption and trade as well as an increase in the number of vulnerable populations eating seafood items, have been identified. Finally, comprehensive surveillance data is scarce on *V. mimicus* occurrence in fish and the marine ecosystem, which probably conceals the real therapeutic burden of certain diseases in humans and aquatic animals. This chapter deals with the highly publicized data on the antibiotic-resistant strains of *V. mimicus* that have been detected in food and the aquatic environment, as well as prospective regulatory strategies to reduce this antimicrobial resistance.

Keywords

V. mimicus · Seafood · PCR · AMR

1 Introduction

Food security is a serious public health issue across the world. Foodborne infections have a detrimental effect on human well-being and survival. Serious foodborne illnesses have resulted in severe economic losses. Every year, contaminated food triggers 600 million cases of seafood-borne illness globally, affecting approximately one-tenth of the population on planet Earth, and mortalities of nearly 420,000 people (WHO, 2015).

Pathogenic microorganisms are microbiological agents that can cause foodborne disease outbreaks. There are numerous emerging bacterial foodborne pathogens such as *Listeria monocytogenes*, *Salmonella typhi*, *Aeromonas hydrophila, Clostridium botulinum*, *Cronobacter sakazakii*, Vibrio spp., *Escherichia coli* O 157, and *Plesiomonas shigelloides*, which are reported from tropical and subtropical countries of the world (WHO, 2015). These bacterial pathogens can cause life-threatening diseases in infants, the aged, lactating females, and immune compromised people, and liable for a wide range of diseases with serious consequences on human health and the economy especially in developing and underdeveloped countries.

Gram-negative bacteria belonging to the Vibrionaceae family, including the genus Vibrio, are frequently encountered in aquatic habitats such as marine, estuarine, and aquaculture environments. As a result, they have become a common element of aquatic life, particularly seafood species. Over 100 different bacterial species have been found, but only 12 of them, notably *Vibrio cholerae*, *Vibrio vulnificus*, *Vibrio parahaemolyticus*, and *Vibrio mimicus*, are the main causes of food-borne vibriosis in individuals all over the world.

Among vibrios, *V. mimicus* is an emerging foodborne pathogen causing gastroenteritis and cholera-like diarrhea reported in many countries. The causative agent is a Gram negative, non-halophilic, catalase positive, oxidase positive, motile, facultative anaerobe that occurs naturally in freshwater, brackish water, and marine environment. *V. mimicus* is reported to cause huge economic loss to aquaculture industry. Rare case of wound infection due to *V. mimicus* also reported in few occasions. This bacterium has been isolated from seawater, turtle eggs, bivalve mollusk, finfish, shellfish, aquatic weeds, and aquatic birds in Australia, Nigeria, Bangladesh, China, Thailand, India, and the United States (Wong et al., 1995; Adebayo-Tayo et al., 2011; Chowdhury et al., 1989; Geng et al., 2014; Chitov et al., 2009; Nair et al., 1991; Kay et al., 2012; CDC, 2019; Nilavan et al., 2021).

V. mimicus has been isolated from coasts, estuaries, rivers, lakes, and lagoons of fresh and salt water in temperate or warm zones. The populations of this microorganism do not remain constant throughout the year because environmental factors such as pH, salinity, and temperature affect its development. It has been reported that in summer, the concentration of these microbes increases because heat promotes their proliferation; in contrast, populations decrease in winter (when the temperature of the water column decreases) (Chowdhury et al., 1989; Vieira et al., 2001). A plethora of seafoods, including fish, crab, mussels, lobster, catfish, freshwater prawns, shrimp, mullet, clams, and turtle eggs, are linked to several outbreaks of *V. mimicus* infection. It is worth noting that consumption of raw or undercooked seafood is the most common way for the *V. mimicus* infection to spread to humans.

V. mimicus was first reported as an atypical *V. cholerae* in 1981. *V. mimicus* infection was first reported in 1981 in Bangladesh, where it was involved in two cases of otitis, one in a person of 39 years and the other in a child, both caused by exposure to seawater. In another report, 17 cases of gastroenteritis occurred in adults who had consumed raw oysters, as well as infections in wounds exposed to seawater (Davis et al., 1981; Shandera et al., 1983). With the passage of time, more cases have been reported in other parts of the world, including countries like India, China, Mexico, Nigeria, Malaysia, the United States, Costa Rica, Bangladesh, China, and Japan. In India, first diarrheal cases of *V. mimicus* were reported in the year 1993 in Kolkata (Mitra et al., 1993). In the United States, approximately 12 cases of vibriosis are attributed to *V. mimicus* annually (Newton et al., 2012). The incidence of *V. mimicus* in different nations across the world is depicted in Table 1.

Table 1 Countries of occurrence of *V. mimicus*

Place	Source	Reference
Asia		
Japan	Seafood	Chowdhury et al., 1989; Alam et al., 1997; Miyoshi et al., 1997
Taiwan	Seafood	Wong et al., 1993
China	Human, environment	Chowdhury et al., 1989
India	Human, environment	Davis et al., 1981; Chowdhury et al., 1989; Ramamurthy et al., 1994
Oceania		
New Zealand	Prawn	Davis et al., 1981
Philippines	Human	Davis et al., 1981
Australia	Crayfish	Wong et al., 1995; Payne et al., 2004
Africa		
Egypt	Human	Davis et al., 1981
Senegal	Seafood	Schandevyl et al,, 1984
Europe		
France	Mussels, coastal water	Hervio-Heath et al., 2002
Italy	Seawater	Baffone et al., 2001
Romania	Human	Janda et al., 1988
America		
Brazil	Environment	Vieira et al., 2001
Ecuador	Shrimp	Vandenberghe et al., 1999
Costa Rica	Tortoise eggs	Campos et al., 1996
Mexico	Seafood	Davis et al., 1981
The United States	Seafood	Davis et al., 1981
Canada	Human ear	Davis et al., 1981

2 Habitat and Distribution

V. mimicus thrives in aquatic habitat and has been isolated from fresh and salt water coasts, estuaries, rivers, lakes, and lagoons in temperate or warm zones. The populations of this microorganism do not remain constant throughout the year because environmental factors such as pH, salinity, and temperature affect its development. It has been reported that in the summer season, the concentration of these microbes will increase because heat promotes their proliferation; in contrast, the *V. mimicus* population decreases in winter (when the temperature of the water column decreases) (Vieira et al., 2001). It has been observed that under unfavorable conditions these bacteria go into a state of decreased respiratory activity called the "viable non-culturable" state and therefore cannot be detected by conventional detection methods (Okada et al., 2005).

2.1 Epidemiology

V. mimicus is recognized as an emerging human pathogen and is also important economically because it is also pathogenic for crustaceans and some fish of economic importance (Guardiola-Avila et al., 2015a).

2.2 *Vibrio mimicus* as a Fish Pathogen

One of the main illnesses that impede the growth of aquaculture and cause a significant economic catastrophe each year is vibriosis. Historically, *V. mimicus* was referred to as a biochemically abnormal *V. cholera* (Davis et al., 1981). It is a different bacterium of significant medical consequence that can infect people and cause occasional instances of diarrhea and dysentery (Chitov et al., 2009). *V. mimicus* is the only non-halophilic Vibrio other than *V. cholerae*, which was reported to cause infection in cultured red swamp crawfish (*Procambarus clarkii*) in the United States and red claw crawfish (*Cherax quadricarinatus*) in Australia. This pathogen was able to cause huge mortality and economic loss in Chinese aquaculture systems. In China, *V. mimicus* infection in freshwater yellow catfish caused 100% mortality (Geng et al., 2014). The infection was characterized by an ulcer, petechial hemorrhages, and a loss of muscular integrity. Additionally, *V. mimicus* was recently found in ornamental fish from several nations, including Singapore, China, Thailand, etc (Zhang et al., 2014).

3 Pathogenesis

V. mimicus is capable of causing several clinical conditions, such as gastroenteritis, otitis, wound infection, cholera-type diarrhea, dysentery, and, very rarely, septicemia. The infective dose is still unknown, but it has been considered that it may be similar to that of *Vibrio cholerae* (10^4 to 10^9 CFU), due to the close relationship between the two (Reidl & Klose, 2002). The incubation period can vary from person to person, from a few hours after ingestion of food to 3–4 days, depending on the inoculum with which the contact was made and the immune status of the person. Many virulence factors have been described, such as hemolysins, hemagglutinin, proteases, siderophores, and enterotoxins (Guardiola-Avila et al., 2015b).

3.1 Virulence Factors

As of now, the system of pathogenicity of *V. mimicus* is obscure; be that as it may, some virulence factors have been reported, like hemolysins, hemagglutinin, proteases, siderophores, and enterotoxins (Shinoda et al., 2004). The genes responsible for virulence in *V. mimicus* that were reported in various studies are shown in Table 2.

Table 2 List of virulent genes reported across the world

Genes	Oligonucleotide sequence 5′-3’	Amplicon size (bp)	Annealing temperature	References
cp	F:GAAGAATTTRTAAAAGAAGAACA R: GAAAGGACCTTCTTTCACGTTG	451	55 °C	Shinoda et al., 2004
tox R	F: ATGTTCGATTAGGACAC R: TACTCACACACTTTGATGGC	779	60 °C	Mantri et al., 2006
omp U	F: ACGCTGACGGAATCAACCAAAG R: GCGGAAGTTTGGCTTGAAGTAG	869	62 °C	Singh et al., 2002
zot	F: TCGCTTAACGATGGCGCGTTT R: AACCCCGTTTCACTTCTACCCA	947	62 °C	Singh et al., 2002
VPI	F: GCAATTTAGGGGCGCGACGT R: CCGCTCTTTCTTGATCTGGTAG	618	52 °C	Xie et al., 2005
ctx	F:CTCAGACGGGATTTGTTAGGCACG R: TCTATCTCTGTAGCCCCTATTACG	301	55 °C	Bi et al., 2001
dnaj	F: CAGGTTTGTGCACGGCGAAGA R: CTTGAAGAAGCGGTTCGTGCA	177	52 °C	Nhung et al., 2007
vmh	F: GGTAGCCATCAGTCTTATCACG R: ATCGTGTCCCAATACTTCACCG	389	55 °C	Bi et al., 2000
sodB	F: CATTCGGTTCTTTCGCTGAT R: GAAGTGTTAGTGATTGCTAGAT	121	57 °C	Raissy et al., 2015

3.2 Hemolysins

This microorganism’s most studied virulence factor is a hemolysin known as VMH (*Vibrio mimicus* hemolysin). This hemolysin is a thermolabile protein of about 66 kDa that shares 81.60% of its activated state with the HlyA of *V. cholerae*. The VMH is a species-specific gene that can be found in environmental strains as well as those recovered from clinical studies. The VMH is capable of forming pores of 2.8–3.5 nm in diameter on the surface of host cells. It also includes the production of AMP in the enterocytes and activates an ion channel of CI-CFTR, which causes an imbalance of electrolytes and causes diarrhea (Shinoda et al., 2004).

Another hemolysin, one that is thermostable, named Vm-TDH, has been discovered in *V. mimicus*. The second thermolysin is a duplex with 21 KDa subunits and is similar to *V. parahaemolyticus* TDH. The ability to cause dysentery-like diarrhea is conferred by Vm-TDH. Because the TDH gene is on a transposon, *V. mimicus* obtains it through lateral DNA transfer (Terai et al., 1991).

3.3 Proteases

V. mimicus has been found to contain several different types of proteases. One of these, with hemagglutinin activity called *Vibrio mimicus* protease (VMP), is capable of altering blood vessel permeability, causing edema and fluid accumulation in the rabbit-linked loop assay. Its activity is dependent on calcium ions, and it has been postulated that this may play a key role in pathogenicity (Chowdhury et al., 1986).

3.4 Factors that Affect Adherence

In order to survive in a variety of habitats, pathogenic microorganisms are thought to need the ability to cling and colonize abiotic or biotic substrates. The capacity to attach to the intestinal epithelium is the first step in colonization for several enteropathogenic bacteria, and it is a need for causing diarrhea. Adhesins are a group of macromolecules that regulate these interactions. The lipopolysaccharide (Vm-LPSHA), a thermostable outer membrane protein (Vm-OMPHA) of 39 KDa, and a protease (Vm-HA/Protease) have also been identified in *V. mimicus* and are all involved in adherence to the intestinal epithelium. These compounds have a good interaction with the glycoprotein on enterocyte surfaces (Alam et al., 1997).

3.5 Iron Acquisition Mechanisms

Several biological functions of bacterial development and pathogenicity depend heavily on iron. It is anticipated that the capacity of human pathogenic microorganisms to use iron will be crucial for both the creation of infections in their host and the survival of those strains in the host's environment. Bacteria primarily absorb iron through the secretion of siderophores, which are low-molecular-weight iron-binding peptides. Vibrios, like the majority of other species, need iron. It has been reported that iron is a key determinant in virulence for several Vibrio species. As a result, microbes require effective iron uptake systems. Siderophores are small molecules with a strong affinity for iron, which they can remove from mineral complexes or transmembrane proteins like lactoferrin and ferritin. So-called catecholate siderophores, such as vibriobactin, are synthesized in the Vibrio genus. Nevertheless, the siderophores aerobactin has been identified as the primary siderophores in *V. mimicus* that is more closely linked to Enterobacteriaceae (Moon et al., 2004).

Bacterial cells in a community can communicate among themselves through a process called quorum sensing (QS), which enables organisms to coordinate the regulation of gene expression and subsequent phenotype changes. The importance of quorum sensing in the pathogenic *V. mimicus* has been well documented. (Abdel-Sattar et al., 2016) reported that the toxin produced by the *V. mimicus* strain was under the regulation of the master regulatory protein Lux R.

3.6 Enterotoxins

V. mimicus can produce a heat-stable enterotoxin (Vm-ST) and a heat-labile enterotoxin (Vm-LT), both of which are linked to gastroenteritis, as well as cholera toxin (CT), which is similar in structure to that produced by *Vibrio cholerae* and is responsible for clinical presentation.

3.7 Mobile Genetic Elements

V. mimicus produced a physicochemical toxin that was biologically, functionally, and immunologically indistinguishable from the CT synthesized by *V. cholerae*, and hence, it was suspected that it was probably the same toxin (Spira and Fedorka cray, 1984). Years later, (Acuña et al., 1999) reported the presence of strains of *V. mimicus* with toxigenic capacity and involved in cases of cholera-type diarrhea, finding cholera toxin genes in their genome. Almost at the same time, it was shown that the toxigenic strains of *V. mimicus* (as well as those of *V. cholerae*) require two genetic elements to be toxigenic: a filamentous bacteriophage called CTX and the pathogenicity island of Vibrio 1 (VPI1) (Faruque et al., 1999; Boyd et al., 2000a). In recent years, strains of *V. mimicus* have been characterized that have IPV 1 and the CTX phage integrated into their genome (Faruque et al., 2005). Strains that have the CTX element integrated into their genome and are able to express it but do not contain the VPI element have also been described. This indicates that there are other mechanisms to acquire the CTX as well as for its regulation (Lazar & Waldor, 1998).

3.8 Pathogenicity Island of Vibrio 1 (VPI-1)

VPI1 has a size of approximately 40 kb, a G-C distinct from that of *V. cholerae* and is inserted near the ssrA gene (which is very similar to an RNAt gene) by means of *att* sequences (Schmidt & Hensel, 2004). It has many open reading frames (ORFs) with similarity to bacteriophages. For this reason, Karaolis in 1999 proposed that the island is actually a bacteriophage (which he called VPI), although this topic is still controversial because the way within this element there is a group of genes necessary for the synthesis and regulation of TCP (the pilus co-regulated with the toxin), a type IV pilus that functions as a colonization factor and as a receptor of the CTX phage (Karaolis et al., 1999). These pili are a polymer of the TcpA protein, which could be part of the capsid of the phage VPI. It is known that the TCP favors the colonization of the intestine through bacterium-bacterium interactions during the formation of the microcolonies (Reidl & Klose, 2002). The island also has other loci that are co-regulated by proteins encoded on the island, as well as some others encoded on the chromosome of the bacterium (toxT and po). For example, it is a transmembrane regulator that is directly responsible for activating the transcription of the pili genes or the ctxAB genes of the CTX phage, as well as genes that are related to the colonization of the intestine (Karaolis et al., 1999; Schmidt & Hensel, 2004).

3.9 Bacteriophages

CTX: It has been demonstrated that the generation of toxigenic strains of *V. cholerae* O1 and O139 from non-toxigenic strains is due to the presence of a filamentous bacteriophage that contains in its genome the genes of the cholera toxin (Waldor & Mekalanos, 1996; Faruque & Mekalanos, 2003). The CTX phage carries in its

genome the ctxAB operon, which corresponds to the cholera toxin responsible for severe diarrhea.

In 2000, it was described that the CTX phage can infect both *V. cholerae* and *V. mimicus* (Boyd et al., 2000a). The phage has a size of approximately 6.9 kb and is organized into two functional domains: (a) the region of the nucleus, where the genes necessary for morphogenesis are found, such as some capsid proteins (Psh, Cep, OrfU, and Ace) and proteins that assemble the virus (Zot). Some of these proteins, besides being fundamental for the virus, have been given a role as enterotoxins, as is the case with the toxin accessory to cholera and the toxin of the zonula occludens (encoded in the genes ace and zot, respectively). (b) The RS2 region, which contains genes for replication (rstA), integration (rstB), and regulation (rstR) of the virus and two intergenic regions called ig-1 and ig-2 (Boyd et al., 2000a).

Organization of the bacteriophage CTX (Source: Boyd et al., 2000b): The ctxAB genes have a G-C content different from the rest of the phage, which indicates that they were acquired recently during the evolution of the phage. This explains, in part, the presence of strains of *V. cholerae* and *V. mimicus* that contain the zot gene but are negative for ctxAB (Boyd et al., 2000b; Faruque et al., 2001). This virus is usually integrated into the genome of its host, through attRS sites although the replicative form has also been detected, which produces more toxin than when the phage is integrated into the chromosome (Boyd et al., 2000b).

The ctxAB genes have G-C content different from the rest of the phage, which indicates that they were acquired recently during the evolution of the phage. This explains, in part, the presence of strains of *V. cholerae* and *V. mimicus* that contain the zot gene but are negative for ctxAB (Boyd et al., 2000b; Faruque et al., 2001). This virus is usually integrated into the genome of its host through attRS sites, although the replicative form has also been detected, which produces more toxin than when the phage is integrated into the chromosome (Boyd et al., 2000b).

3.10 ToxR

In response to changes in the environment, microorganisms respond by expressing or repressing genes depending on the stimulus (temperature, pH, or osmolality). In *V. mimicus* and *V. cholerae*, it has been described that most of the genes associated with virulence are regulated by the ToxR protein; the regulation of this protein comprises the approximate expression of 20 genes (Skorupski & Taylor, 1997). ToxR is a transcriptional activator of the two-component system type: the protein is divided into three domains, one transmembrane to anchor to the cell membrane, another that serves as a sensor of periplasmic space conditions and one that is cytoplasmic and that has the ability to bind to DNA. It is known that for this protein to function, it must be a dimer, being able to dimerize with itself or with other transcriptional activators such as ToxS, ToxT, or TcpP (Osorio & Klose 2000). An interesting aspect of this protein is that it controls the expression of genes present in mobile elements, both of the VPI-1 (formation of the TCP pili) and of the phage CTX (it can be linked directly to the promoter of ctxAB). It is also likely to regulate

the expression of *vhm* A hemolysin and some outer membrane proteins that are related to resistance to bile, the modulation of adherence, and the regulation of the mobility of the flagellum. Therefore, it is considered the master regulator of virulence (Provenzano et al., 2000).

4 Methods of Identification of *V. mimicus*

Traditionally, the classification of vibrios is based on their biochemical identification. Thiosulfate-citrate-bile salts-sucrose agar (TCBS) is the conventionally used selective medium for the isolation of *V. mimicus* from clinical and environmental samples. The colony morphology of *V. mimicus* in this medium is distinguishable from *V. cholerae* due to its non-sucrose fermenting green color colonies after direct streaking of clinical samples or after enrichment in alkaline peptone water (pH 8.6). After preliminary screening in the TCBS, a set of biochemical tests are essential for the biochemical confirmation of *V. mimicus*. Those include Gram's reaction, salt utilization tests, motility, catalase test, the oxidase test, and the amino acid decarboxylase test. Vibrio species cannot be reliably distinguished with existing biochemical testing techniques, particularly for strains isolated from food or the aquatic environment. While using the biochemical tests to identify Vibrio species, the type of selective media, salt percentage, and incubation temperature of the medium can influence the observed reaction.

More than 25 new bacteria species have been discovered between 2008 and 2012, further complicating the situation since new Vibrio types are indeed being reported at a fast pace. The majority of the newly identified species lack complete biochemical profiles, which makes it difficult to accurately create a taxonomy key for such species based solely on phenotypic tests.

Biochemical identification for Vibrio spp. is no longer thought to be as reliable as genetic approaches. Many unique vibrios can be identified using molecular techniques like PCR, and the majority of these tests are superior to the traditional detection methods. Molecular methods targeting species-specific genes have more advantages than traditional methods. Many molecular methods are available for the detection of *V. mimicus* in seafood and aquatic environments. PCR, real-time PCR, colony hybridization, and loop-mediated isothermal amplification (LAMP) are a few of them. The advantage of these molecular biology techniques is that, in addition to their sensitivity, they may be used to detect the vibrios in foods, the environment, and other substances without isolating a colony.

4.1 Antimicrobial Resistance Profile of *V. mimicus*

A growing global hazard to public health is posed by the development of antimicrobial resistance (AMR) in vibrios and a decline in the potency of routinely used antibiotics. The antimicrobial resistance profile of *V. mimicus* in the US and other nations such as India, China, Mexico, Africa, Bangladesh, and Nigeria is reviewed

in this chapter. Most antimicrobials used in veterinary and human medicine are generally effective against *V. mimicus*. However, investigations revealed that *V. mimicus* has developed various antibiotic resistances as a result of antibiotic abuse in agriculture and aquaculture. Antimicrobial resistance patterns were also similar in both environmental and clinical strains. Across all nations, higher resistance was reported towards ampicillin, ampicillin, amoxyclav, and tetracycline. Multidrug-resistant bacteria inhabiting aquatic environments are a serious issue in aquaculture as well as public health. The response of *V. mimicus* to different antibiotics in different parts of the world is common for some antibiotics and varied for others. The global scenario of resistance pattern of *V. mimicus* is presented in Table 3.

The mode of action of antibiotics, the mechanism of antibiotic resistance, the genetic basis of antibiotic resistance, the factors responsible for diffusion and dissemination of antibiotic resistance, and the possible advancement of *V. mimicus* infection from seafood and aquatic sources (Figs. 1, 2, 3, and 4) is shown below.

To measure antimicrobial resistance in pathogenic Vibrio species like *V. mimicus*, a variety of standardized techniques have been developed. A few of the techniques include the broth microdilution method, disk diffusion method, and gradient-based disk diffusion methods.

4.2 Region-Wise Antimicrobial Resistance Profiles of *Vibrio mimicus*

4.2.1 India

Nilavan et al. (2021) examined the antibiotic resistance patterns of 41 *V. mimicus* strains isolated from fish and shellfish in and around Cochin, Kerala, India. The agar well diffusion assay was used to test the resistance to 12 antibiotics. Results of the study revealed that 100% of the strains were resistant to ampicillin, 85% of the strains were resistant to amoxyclav, and all were sensitive to other antibiotics, including tetracycline, sulfamethoxazole, kanamycin, and fluoroquinolone.

In a similar investigation by Sudha et al. (2014), six potentially pathogenic Vibrio strains, including *V. mimicus*, were recovered from four retail market places in Kerala, India. AMR profile: this study reports 100% resistance to ampicillin, amoxyclav, and cephalothin (100%). Further isolates were susceptible to chloramphenicol and nalidixic acid.

Another study from Vaseeharan et al. (2005) recorded the resistance of *V. mimicus* in aquaculture settings. Fifty percent of the isolates were resistant to ampicillin, ceftriaxone, ciprofloxacin, and kanamycin.

4.2.2 Australia

Akinbowale et al. (2006) studied the antimicrobial susceptibility of 100 *V. mimicus* isolates from Australian aquaculture farms. The agar well diffusion method was used

Table 3 Global scenario of *V. mimicus* response to different antibiotics

Sl. No	Susceptible	Intermediate	Resistant	Country	Reference
1	Sulfamethoxazole Imipenem Aztreonam Gentamicin Tetracycline Nitrofurantoin Nalidixic acid Oxytetracycline	Cefoxitin	Ampicillin	Brazil	Reboucas et al., 2011
2	Streptomycin Kanamycin Tetracycline Trimethoprim-sulfamethoxazole	Chloramphenicol Gentamicin	Ampicillin	Bangladesh	Chowdhury et al., 1986
3	–	–	Novobiocin Kanamycin	The United States	Voll et al., 1986
4	Sulfamethoxazole Imipenem Aztreonam Gentamicin Tetracycline Nitrofurantoin Nalidixic acid Oxytetracycline	–	Ampicillin Amoxicillin Cephalothin	India	Sudha et al., 2014 Nilavan et al., 2021
5	–	–	Ampicillin Furazolidone Nalidixic acid Streptomycin Trimethoprim + sulfamethoxazole	Iran	Raissy et al., 2015

6	Amikacin Trimethoprim + sulfamethoxazole Ampicillin Ciprofloxacin Chloramphenicol Cefotaxime Nalidixic acid Ceftazidime Imipenem Aztreonam Streptomycin Nitrofuran Tetracycline Cefoxitin Gentamicin Sulfamethoxazole	–	–	Spain	Garcia-Aljaro et al., 2014

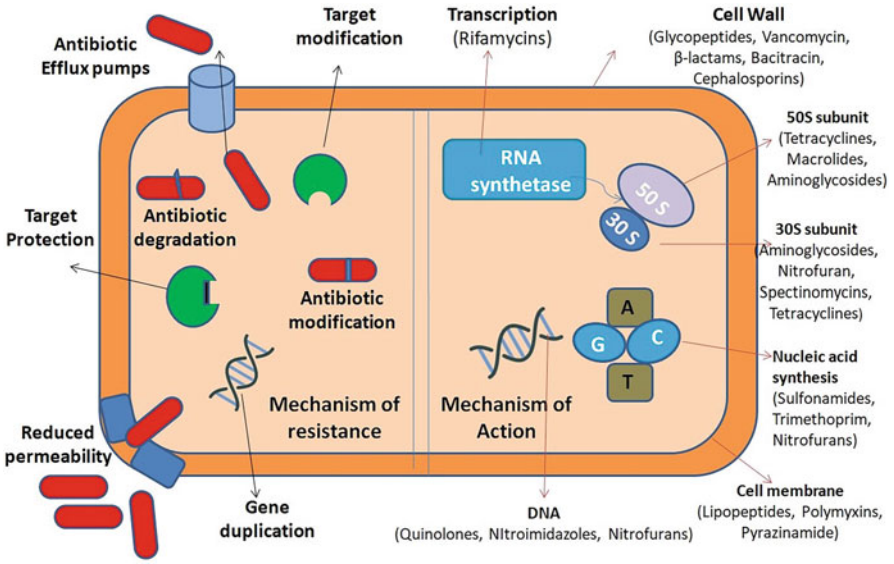
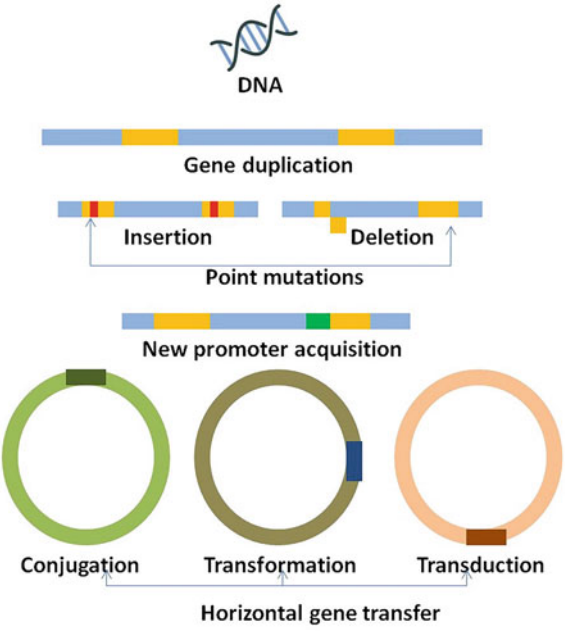


Fig. 1 Schematic overview of antibiotic modes of action and subsequent mechanisms of antibiotic resistance

Fig. 2 Genetics of antibiotic resistance



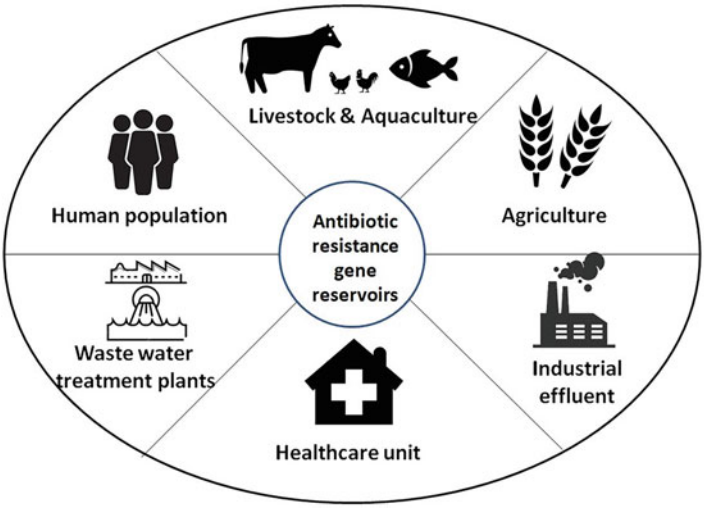


Fig. 3 Factors responsible for transmission and dissemination of antibiotic resistance

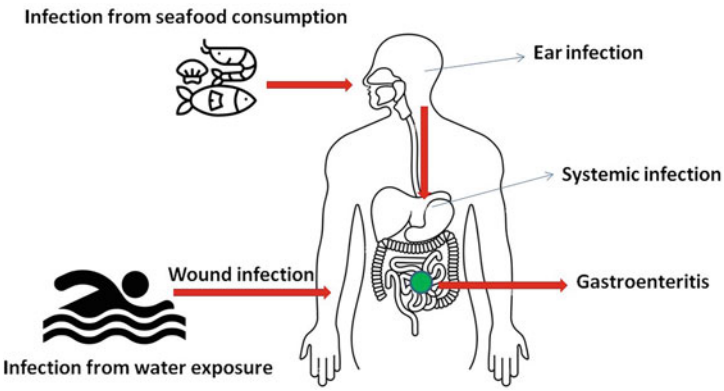


Fig. 4 Schematic overview of *Vibrio mimicus* infection caused by seafood and water exposure

to assess the sensitivity of 100 isolates. Resistance to ampicillin and erythromycin was prevalent; ciprofloxacin was effective against all isolates.

4.2.3 Nigeria

Beshiru et al. (2020) have carried out a study to evaluate the AMR patterns of vibrios recovered from seafood. The findings revealed that all the strains were sensitive to colistin and gentamicin but had different degrees of resistance to the other antibiotics

tested. Resistance to trimethoprim, penicillin, amoxicillin, chloramphenicol, meropenem, and streptomycin was found in the majority of *V. mimicus* strains.

4.2.4 Bangladesh

Chowdhury et al. (1986) examined the antibiotic susceptibility profiles of 25 environmental and 19 clinical *V. mimicus* strains. Environmental bacteria were resistant to streptomycin, kanamycin, and trimethoprim + sulfamethoxazole, but clinical strains were sensitive. Environmental bacteria were resistant to ampicillin in certain cases (44%), while clinical strains were not. Resistance to chloramphenicol and gentamicin was observed in all the tested isolates.

5 Conclusion

Due to ocean warming and an increase in sea surface temperature, numerous vibrios are emerging as pathogenic to humans, and now this bacterium has been considered one of the major disease-causing organisms. *V. mimicus* also caused serious economic losses in the Chinese aquaculture industry. In Bangladesh, (Begum & Khan, 2001) studied the prevalence and distribution of both *V. cholerae* and *V. mimicus* in different elements of the aquatic environment, and 10.80% of *V. mimicus* strains were isolated from aquatic plants, surface water, snails, oysters, and soil sediment. The abundance of *V. mimicus* was highest in snails and lowest in soil sediment.

V. mimicus infection is often self-limited. No chemotherapy is required in mild cases, but patients need to drink plenty of liquids to replace fluids lost due to diarrhea. In cases of severe infections, a combination of third-generation cephalosporins (ceftazidime, cefotaxime, and ceftriaxone) is the therapy of choice. *V. mimicus* is reported to survive at refrigeration temperatures, and it can be killed by heating and disinfectants. At present, no vaccine is available, so the infection can be prevented by thoroughly cooking the seafood before eating, using clean and potable water in the kitchen, adequate refrigeration of foods, and preventing cross-contamination of processed and raw foods. Furthermore, fish consumers need to be aware of the hazards of consuming untreated or inadequately cooked seafood as well as kitchen hygiene.

Many *V. mimicus* strains were antibiotic-resistant in fishery settings, posing a significant threat to human and fish health. Some researchers have also discovered R-plasmid-mediated resistance. Antimicrobial resistance control and drug use monitoring in the fishery sector must be promoted to enhance antibiotic management of public health and food safety-related activities. To detect new disease strains of *V. mimicus*, improved worldwide public health surveillance is needed. More research into the etiology, co-morbidities, and molecular pathogenesis is required.

Aquaculture is expanding to fulfill consumers' needs, especially in Asia, and the world's fish production is still growing exponentially. Producers may continue to use antimicrobials as a preventative measure and a treatment for bacterial infections in aquatic animals due to a lack of knowledge about their use and potential risks to

humans. The effectiveness of medicines used in healthcare situations has decreased, which impedes and delays hospitalization plans.

This chapter provides a global overview of *V. mimicus* characteristics, isolation, identification, and methods of detection of antimicrobial resistance. This information would be beneficial in coming up with a workable alternative to the growing antimicrobial resistance problem. The possible effects of antibiotics used in the aquaculture industry on public health vary and may be regionally specific. If suitable control measures are not recommended, antibiotic residues will actively move into aquatic habitats. To stop the detrimental effects of resistant microorganisms on human and animal health, society, legislators, governmental organizations, and the fishery sectors should cooperate and fight together. Since protecting public health must come first, producers require motivation to employ nonantibiotic methods such as phages and probiotics. Together, we can successfully manage the environment's increasing antimicrobial resistance among Vibrio species.

References

Abdel-Sattar, E.-S., Miyoshi, S.-I., & Elgaml, A. (2016). Regulation of *Vibrio mimicus* metalloprotease (VMP) production by the quorum-sensing master regulatory protein, lux R. *Journal of Basic Microbiology, 56*. https://doi.org/10.1002/jobm.201600002. Accessed 26 Dec 2022.

Acuña, M. T., Díaz, G., Bolaños, H., Barquero, C., Sánchez, O., Sánchez, L. M., … & Campos, E. (1999). Sources of *Vibrio mimicus* contamination of turtle eggs. *Applied and Environmental Microbiology*, *65*(1), 336–338. https://doi.org/10.1128/AEM.65.1.336-338.1999. Accessed 26 Dec 2022.

Adebayo-Tayo, B. C., Okonko, I. O., Esen, C. U., Odu, N. N., Onoh, C. C., & Igwiloh, N. J. P. (2011). Occurrence of potentially pathogenic Vibrio species in seafoods obtained from Oron creek. *World Applied Sciences Journal, 15*, 985–991.

Akinbowale, O. L., Peng, H., & Barto, M. D. (2006). Antimicrobial resistance in bacteria isolated from aquaculture sources in Australia. *Journal of Applied Microbiology, 100*(5), 1103–1113. https://doi.org/10.1111/j.1365-2672.2006.02812.x. Accessed 26 Dec 2022.

Alam, M., Miyoshi, S. I., Tomochika, K. I., & Shinoda, S. (1997). *Vibrio mimicus* attaches to the intestinal mucosa by outer membrane hemagglutinin specific to polypeptide moieties of glycoproteins. *Infection and Immunity, 65*(9), 3662–3665.

Baffone, W., Citterio, B., Vittoria, E., Casaroli, A., Pianetti, A., Campana, R., & Bruscolini, F. (2001). Determination of several potential virulence factors in *Vibrio*spp. isolated from sea water. *Food Microbiology, 18*(5), 479–488.

Begum, A., & Khan, S. I. (2001). Abundance of *Vibrio cholerae* non-O1 and *Vibrio mimicus* in various aquatic components of the pond ecosystem in Matlab Bangladesh. *Microbes and Environments, 16*(1), 19–23.

Beshiru, A., Okareh, O. T., Okoh, A. I., & Igbinosa, E. O. (2020). Detection of antibiotic resistance and virulence genes of Vibrio strains isolated from ready-to-eat shrimps in Delta and Edo states Nigeria. *Journal of Applied Microbiology, 129*, 17–36. https://doi.org/10.1111/jam.14590. Accessed 26 Dec 2022.

Bi, K., Shi, L., Maehara, Y., Miyoshi, S. I., Tomochika, K. I., & Shinoda, S. (2000). Analysis of *Vibrio mimicus* clinical strains by arbitrarily primed polymerase chain reaction. *Microbiology and Immunology, 44*(2), 149–153.

Bi, K., Miyoshi, S. I., Tomochika, K. I., & Shinoda, S. (2001). Detection of virulence associated genes in clinical strains of *Vibrio mimicus*. *Microbiology and Immunology, 45*(8), 613–616.

Boyd, E. F., Heilpern, A. J., & Waldor, M. K. (2000a). Molecular analyses of a putative CTXφ precursor and evidence for independent acquisition of distinct CTXφs by toxigenic Vibrio cholerae. *Journal of Bacteriology, 182*(19), 5530–5538. Accessed 26 Dec 2022.

Boyd, E. F., Moyer, K. E., Shi, L., & Waldor, M. K. (2000b). Infectious CTXΦ and the vibrio pathogenicity Island prophage in *Vibrio mimicus*: Evidence for recent horizontal transfer between *V. mimicus* and *V. cholerae*. *Infection and Immunity, 68*(3), 1507–1513. Accessed 26 Dec 2022.

Campos, E., Bolaños, H., Acuña, M. T., Díaz, G., Matamoros, M. C., Raventos, H., Sánchez, L. M., Sánchez, O., & Barquero, C. (1996). *Vibrio mimicus* diarrhea following ingestion of raw turtle eggs. *Applied and Environmental Microbiology, 62*(4), 1141–1144.

Centers for Disease Control and Prevention (CDC). (2019). Vibrio species causing Vibriosis. https://www.cdc.gov/vibrio/faq.html. Accessed 26 Dec 2022.

Chitov, T., Kirikaew, P., Yungyune, P., Ruengprapan, N., & Sontikun, K. (2009). An incidence of large foodborne outbreak associated with *Vibrio mimicus*. *European Journal of Clinical Microbiology & Infectious Diseases, 28*, 421–424. https://doi.org/10.1007/s10096-008-0639-7

Chowdhury, M. A., Aziz, K. M., Rahim, Z., & Kay, B. A. (1986). Antibiotic resistance patterns of *Vibrio mimicus* isolated from human and environmental sources in Bangladesh. *AntimicrobialAgents and Chemotherapy, 30*(4), 622–623.

Chowdhury, M. A., Yamanaka, H., Miyoshi, S., Aziz, K. M., & Shinoda, S. (1989). Ecology of *Vibrio mimicus* in aquatic environments. *Applied and Environmental Microbiology, 55*(8), 2073–2078.

Davis, B. R., Fanning, G. R., Madden, J. M., Steigerwalt, A. G., Bradford, H. B., Smith, H. L., & Brenner, D. J. (1981). Characterization of biochemically atypical *Vibrio cholerae* strains and designation of a new pathogenic species *Vibrio mimicus*. *Journal of ClinicalMicrobiology, 14*(6), 631–639.

Faruque, S. M., & Mekalanos, J. J. (2003). Pathogenicity islands and phages in *Vibrio cholerae* evolution. *Trends in Microbiology, 11*(11), 505–10. https://doi.org/10.1016/j.tim.2003.09.003. PMID: 14607067.

Faruque, S. M., Rahman, M. M., Asadulghani Islam, K. N., & Mekalanos, J. J. (1999). Lysogenic conversion of environmental *Vibrio mimicus* strains by CTXΦ. *Infection and Immunity, 67*(11), 5723–5729. https://doi.org/10.1128/IAI.67.11.5723-5729.1999. Accessed 26 Dec 2022.

Faruque, S. M., Rahman, M. M., Hasan, A. M., Nair, G. B., Mekalanos, J. J., & Sack, D. A. (2001). Diminished diarrheal response to *Vibrio cholerae* strains carrying the replicative form of the CTXΦ genome instead of CTXΦ lysogens in adult rabbits. *Infection and Immunity, 69*(10), 6084–6090. Accessed 26 Dec 2022.

Faruque, S. M., Naser, I. B., Islam, M. J., Faruque, A. S. G., Ghosh, A. N., Nair, G. B., … & Mekalanos, J. J. (2005). Seasonal epidemics of cholera inversely correlate with the prevalence of environmental cholera phages. *Proceedings of the National Academy of Sciences, 102*(5), 1702–1707. https://doi.org/10.1073/pnas.040899210. Accessed 26 Dec 2022.

Garcia-Aljaro, C., Riera-Heredia, J., & Blanch, A. R. (2014). Antimicrobial resistance and presence of the SXT mobile element in Vibrio spp. isolated from aquaculture facilities. *The New Microbiologica, 37*(3), 339–346.

Geng, Y., Liu, D., Han, S., Zhou, Y., Wang, K. Y., Huang, X. L., Chen, D. F., Peng, X., & Lai, W. M. (2014). Outbreaks of vibriosis associated with *Vibrio mimicus* in freshwater catfish in China. *Aquaculture, 433*, 82–84.

Guardiola-Avila, I., Noriega-Orozco, L., Acedo-Félix, E., Lara, A. E., & Tapia-Olea, M. M. (2015a). Presence of the hemolysin gene of *Vibrio mimicus* in fish and seafood products in Sonora Méxic. *Journal of Food Research, 4*(1), 66.

Guardiola-Avila, I., Noriega-Orozco, L., Gómez-Gil, B., & Acedo-Félix, E. (2015b). Factores de virulencia de *Vibrio mimicus*. *Revista de Ciencias Biológicas y de la Salud, 17*(2), 38–49.

Hervio-Heath, D., Colwell, R. R., Derrien, A., Robert-Pillot, A., Fournier, J. M., & Pommepuy, M. (2002). Occurrence of pathogenic vibrios in coastal areas of France. *Journal of Applied Microbiology, 92*(6), 1123–1135.

Janda, J. M., Powers, C., Bryant, R. G., & Abbott, S. L. (1988). Current perspectives on the epidemiology and pathogenesis of clinically significant *Vibrio* spp. *Clinical Microbiology Reviews, 1*(3), 245–267.

Karaolis, D. K., Somara, S., Maneval, D. R., Johnson, J. A., & Kaper, J. B. (1999). A bacteriophage encoding a pathogenicity Island, a type-IV pilus and a phage receptor in cholera bacteria. *Nature, 399*(6734), 375–379. https://doi.org/10.1038/20715. Accessed 26 Dec 2022.

Kay, M. K., Cartwright, E. J., Maceachern, D., Mccullough, J. O. E. L., Barzilay, E. Z. R. A., Mintz, E. R. I. C., Duchin, J. S., Macdonald, K., Turnsek, M., Tarr, C., Talkington, D., Newton, A. N. N. A., & Marfin, A. A. (2012). *Vibrio mimicus* infection associated with crayfish consumption , Spokane, Washington, 2010. *Journal of Food Protection, 75*, 762–764. https://doi.org/10.4315/0362-028X.JFP-11-410

Lazar, S., & Waldor, M. K. (1998). Tox R-independent expression of cholera toxin from the replicative form of CTXΦ. *Infection and Immunity, 66*(1), 394–397. https://doi.org/10.1128/IAI.66.1.394-397.1998. Accessed 26 Dec 2022.

Mantri, C. K., Mohapatra, S. S., Ramamurthy, T., Ghosh, R., Colwell, R. R., & Singh, D. V. (2006). Septaplex PCR assay for rapid identification of Vibrio cholerae including detection of virulence and int SXT genes. *FEMS Microbiology Letters, 265*(2), 208–214.

Mitra, U., De, S. P., Bhattacharya, M. K., Dutta, P., Dutta, D., Rasaily, R., Banerjee, D., Nag, A., Chowdhury, A. S., & Bhattacharya, S. K. (1993). Acute diarrhoea caused by *Vibrio mimicus* in Calcutta. *The Journal of the Association of Physicians of India, 41*(8), 487–488. PMID: 8294349. Accessed 26 Dec 2022.

Miyoshi, S. I., Sasahara, K., Akamatsu, S., Rahman, M. M., Katsu, T., Tomochika, K. I., & Shinoda, S. (1997). Purification and characterization of a hemolysin produced by *Vibrio mimicus*. *Infection and Immunity, 65*(5), 1830–1835.

Moon, Y. H., Tanabe, T., Funahashi, T., Shiuchi, K. I., Nakao, H., & Yamamoto, S. (2004). Identification and characterization of two contiguous operons required for aerobactin transport and biosynthesis in *Vibrio mimicus*. *Microbiology and Immunology, 48*(5), 389–398. https://doi.org/10.1111/j.1348-0421.2004.tb03528.x. Accessed 26 Dec 2022.

Nair, G. B., Bhadra, R. K., Ramamurthy, T., Ramesh, A., & Pal, S. C. (1991).*Vibrio cholerae* and other Vibrios associated with paddy field cultured prawns. *Food Microbiology, 8*, 203–208. https://doi.org/10.1016/0740-0020(91)90051-3

Newton, A., Kendall, M., Vugia, D. J., Henao, O. L., & Mahon, B. E. (2012). Increasing rates of vibriosis in the United States, 1996–2010: Review of surveillance data from 2 systems. *Clinical Infectious Diseases, 54*(suppl_5), S391–S395.

Nhung, P. H., Ohkusu, K., Miyasaka, J., Sun, X. S., & Ezaki, T. (2007). Rapid and specific identification of 5 human pathogenic vibrio species by multiplex polymerase chain reaction targeted to dnaJ gene. *Diagnostic Microbiology and Infectious Disease, 59*(3), 271–275.

Nilavan, E., Vaiyapuri, M., Sadanandan Sheela, G., Nadella, R. K., Thandapani, M., Kumar, A., & Mothadaka, M. P. (2021). Prevalence of *Vibrio mimicus* in fish, fishery products, and environment of south west coast of Kerala India. *Journal of AOAC International, 104*(3), 790–794.

Okada, K., Iida, T., Kita-Tsukamoto, K., & Honda, T. (2005). Vibrios commonly possess two chromosomes. *Journal of Bacteriology, 187*(2), 752–757.

Osorio, C. R., & Klose, K. E. (2000). A Region of the Transmembrane Regulatory Protein ToxR That Tethers the Transcriptional Activation Domain to the Cytoplasmic Membrane Displays Wide Divergence among Vibrio Species ABSTRACT. *Journal of Bacteriology, 182*(2), 526–528 https://doi.org/10.1128/JB.182.2.526-528.2000

Payne, M., Oakey, J., & Owens, L. (2004). The ability of two different *Vibrio*spp. bacteriophages to infect *Vibrio harveyi*, *Vibrio cholerae* and *Vibrio mimicus*. *Journal of Applied Microbiology, 97*(4), 663–672.

Provenzano, D., Schuhmacher, D. A., Barker, J. L., & Klose, K. E. (2000). The virulence regulatory protein ToxR mediates enhanced bile resistance in Vibrio cholerae and other pathogenic Vibrio species. *Infection and Immunity, 68*(3), 1491–1497. https://doi.org/10.1128/IAI.68.3.1491-1497.2000. Accessed 26 Dec 2022.

Raissy, M., Rahimi, E., Azargun, R., Moumeni, M., Rashedi, M., & Sohrabi, H. R. (2015). Molecular detection of Vibrio spp. in fish and shrimp from the Persian Gulf. *Journal of Food Biosciences and Technology, 5*(2), 49–52.

Ramamurthy, T., Albert, M. J., Huq, A., Colwell, R. R., Takeda, Y., Takeda, T., Shimada, T., Mandal, B. K., & Nair, G. B. (1994). *Vibrio mimicus* with multiple toxin types isolated from human and environmental sources. *Journal of Medical Microbiology, 40*(3), 194–196.

Reboucas, R. H., de Sousa, O. V., Lima, A. S., Vasconcelos, F. R., de Carvalho, P. B., & dos Fernandes Vieira, R. H. S. (2011). Antimicrobial resistance profile of Vibrio species isolated from marine shrimp farming environments (Litopenaeusvannamei) at Ceará Brazil. *Environmental Research, 111*(1), 21–24.

Reidl, J., & Klose, K. E. (2002). *Vibrio cholerae* and cholera: Out of the water and into the host. *FEMS Microbiology Reviews, 26*(2), 125–139. https://doi.org/10.1111/j.1574-6976.2002.tb00605.x. Accessed 26 Dec 2022.

Schandevyl, P. E. T. E. R., Van Dyck, E. D. D. Y., & Piot, P. E. T. E. R. (1984). Halophilic *Vibrio* species from seafish in Senegal. *Applied andEnvironmental Microbiology, 48*(1), 236–238.

Schmidt, H., & Hensel, M. (2004). Pathogenicity islands in bacterial pathogenesis. *Clinical Microbiology Reviews, 17*(1), 14–56. https://doi.org/10.1128/CMR.17.1.14-56.2004. Accessed 26 Dec 2022.

Shandera, W. X., Johnston, J. M., Davis, B. R., & Blake, P. A. (1983). Disease from infection with *Vibrio mimicus*, a newly recognized *Vibrio* species: Clinical characteristics and epidemiology. *Annals of Internal Medicine, 99*(2), 169–171.

Shinoda, S., Nakagawa, T., Shi, L., Bi, K., Kanoh, Y., Tomochika, K.-I., Miyoshi, S.-I., & Shimada, T. (2004). Distribution of Virulence-Associated Genes in Vibrio mimicus Isolates from Clinical and Environmental Origins. *Microbiology and Immunology, 48*(7), 547–551. https://doi.org/10.1111/j.1348-0421.2004.tb03551.x

Singh, D. V., Isac, S. R., & Colwell, R. (2002). Development of a hexaplex PCR assay for rapid detection of virulence and regulatory genes in *Vibrio cholerae* and *Vibrio mimicus*. *Journal of Clinical Microbiology, 40*(11), 4321–4324.

Skorupski, K., & Taylor, R. K. (1997). Control of the ToxR virulence regulon in Vibrio cholerae by environmental stimuli. *Molecular Microbiology, 25*(6), 1003–1009. Accessed 26 Dec 2022.

Spira, W. M., & Fedorka-Cray, P. J. (1984). Purification of enterotoxins from *Vibrio mimicus* that appear to be identical to cholera toxin. *Infection and Immunity, 45*(3), 679–684. https://doi.org/10.1128/iai.45.3.679-684.1984. Accessed 26 Dec 2022.

Sudha, S., Mridula, C., Silvester, R., & Hatha, A. A. M. (2014). Prevalence and antibiotic resistance of pathogenic Vibrios in shellfishes from Cochin market. *Indian Journal of Geo-Marine Sciences, 43*(5), 815–824.

Terai, A., Baba, K., Shirai, H., Yoshida, O., Takeda, Y., & Nishibuchi, M. (1991). Evidence for insertion sequence-mediated spread of the thermostable direct hemolysin gene among Vibrio species. *Journal of Bacteriology, 173*(16), 5036–5046. https://doi.org/10.1128/jb.173.16.5036-5046.1991. Accessed 26 Dec 2022.

Vandenberghe, J., Verdonck, L., Robles-Arozarena, R., Rivera, G., Bolland, A., Balladares, M., Gomez-Gil, B., Calderon, J., Sorgeloos, P., & Swings, J. (1999). Vibrios associated with *Litopenaeusvannamei* larvae, post-larvae, broodstock, and hatchery probionts. *Applied and Environmental Microbiology, 65*(6), 2592–2597.

Vaseeharan, B., Ramasamy, P., Murugan, T., & Chen, J. C. (2005). In vitro susceptibility of antibiotics against Vibrio spp. and Aeromonas spp. isolated from Penaeus monodon hatcheries and ponds. *International Journal of Antimicrobial Agents, 26*(4), 285–291. https://doi.org/10.1016/j.ijantimicag.2005.07.005. Accessed 26 Dec 2022.

Vieira, V. V., Teixeira, L. F. M., Vicente, A. C. P., Momen, H., & Salles, C. A. (2001). Differentiation of environmental and clinical isolates of *Vibrio mimicus* from *Vibrio cholerae* by multilocus enzyme electrophoresis. *Applied and Environmental Microbiology, 67*(5), 2360–2364.

Voll, M. J., Colwell, R. R., & Maneval, D. (1986). Characterisation of non-01 *Vibrio cholerae* and *V. mimicus* isolated from fresh water in Bangladesh. *Journal of Diarrheal Diseases Research*, 216–221.

Waldor, M. K., & Mekalanos, J. J. (1996). Lysogenic conversion by a filamentous phage encoding cholera toxin. *Science, 272*(5270), 1910–1914. https://doi.org/10.1126/science.272.5270.1910. Accessed 26 Dec 2022.

WHO. (2015). Estimates of the global burden of foodborne diseases, *World Health Organisation*, https://apps.who.int/iris/bitstream/handle/10665/199350/9789241565165_eng.pdf?sequence=1.

Wong, H. C., Shieh, W. R., & Lee, Y. S. (1993). Toxigenic characterization of vibrios isolated from foods available in Taiwan. *Journal of Food Protection, 56*(11), 980–982.

Wong, F. Y. K., Fowler, K., & Desmarchelier, P. M. (1995). Vibriosis due to *Vibrio mimicus* in Australian freshwater crayfish. *Journal of Aquatic Animal Health, 7*(4), 284–291.

Xie, Z. Y., Hu, C. Q., Chen, C., Zhang, L. P., & Ren, C. H. (2005). Investigation of seven Vibrio virulence genes among Vibrio alginolyticus and Vibrio parahaemolyticus strains from the coastal mariculture systems in Guangdong China. *Letters in Applied Microbiology, 41*(2), 202–207.

Zhang, X., Li, Y. W., Mo, Z. Q., Luo, X. C., Sun, H. Y., Liu, P., Li, A. X., Zhou, S. M., & Dan, X. M. (2014). Outbreak of a novel disease associated with *Vibrio mimicus* infection in fresh water cultured yellow catfish, *Pelteobagrus fulvidraco*. *Aquaculture, 432*, 119–124.

Part IV

Drivers of AMR and Recent Trends in Detection and Characterization of AMR Pathogens

Drivers of Antimicrobial Resistance

Aravind Reghukumar

Contents

Abstract

Individuals and healthcare systems all around the globe face a burden related to antimicrobial resistance (AMR) both in terms of their physical health and their financial well-being. The evolution of unresponsiveness to antibiotics in microorganisms presents a danger to the effectiveness of a number of different antimicrobials. AMR is as complex as climate change, and tackling it effectively requires coordination and cooperation between different sectors, namely, human health, animal health, fisheries and aquaculture, environment, food production, agriculture sectors, etc. Knowledge of the causes and processes behind AMR is essential to plan and execute strategies to mitigate the danger to biosecurity and

A. Reghukumar (✉)
Department of Infectious Diseases, Govt. Medical College, Thiruvananthapuram, India

M. P. Mothadaka et al. (eds.), *Handbook on Antimicrobial Resistance*,
https://doi.org/10.1007/978-981-19-9279-7_27

human well-being. Even though the development of AMR in microorganisms occurs as a part of natural phenomenon, selection burden on microbes isaugmented by indiscriminate use of antimicrobials in humans, animal husbandry, agriculture, fisheries and environment. "*Antibiotic-resistant bacteria (ARB)*" and "*antimicrobial-resistant genes (ARGs)*" increase is dependent on infection-regulatory standards, hygiene, availability of potable water, access to quality antimicrobials, travel, and immigration. AMR poses both health and economic burden for patients and healthcare systems across the world. The upsurge of ARB poses a risk to the effectiveness of a number of different antibiotics. Multiple interconnected variables, such as a high infectious disease burden, inadequate public health infrastructure, lack of suitable diagnostic assistance, inadequate infection control methods, propensity of practitioners to continue using empirical treatment approaches, sale of antibiotics sans prescription, use of antibiotics for metaphylaxis and as growth promoters in farming, fisheries and aquaculture, and effluents with antibiotic residues from hospitals and pharmaceutical industry, have amplified the crisis of AMR.

Keywords

Antimicrobial resistance · Antimicrobial stewardship · One Health approach · Antibiotic residues · Antibiotic-resistant genes

1 Introduction

Antimicrobial resistance (AMR) is a critical issue that affects public health all over the world. In addition to the antibiotic misuse that occurs in people, the riskiest trends contributing to rising AMR include the employment of antibiotics in animals to enhance growth and avoid illness, aquaculture, and fisheries. Even though there are many factors that influence AMR, the most pertinent ones causing an increase in AMR include the unscrupulous use and handling of antimicrobial drugs in various sectors which include animal manure application to soil and the inadequate handling of effluents from pharmaceutical industry, healthcare institutions, and farms. According to estimates, more than a million people die each year throughout the world as a result of infectious diseases that are resistant to antimicrobials. If remedial measures are not implemented to control the scourge of AMR, it is projected that by the year 2050 the human mortalities world over due to infections caused by extremely drug resistant microbes will be 10 million per year. This number is predicted to be the single largest cause of death worldwide and will be more than the number of deaths due to cancer and road traffic accidents. Global economy also will be set back to the tune of 100 trillion US dollars if steps are not initiated to control AMR (Tackling drug resistant infections globally, 2016).

According to the findings of the first comprehensive study on the worldwide effect of antimicrobial resistance (AMR), which was published in *The Lancet*, AMR was responsible for 1.27 million deaths in 2019, while infectious diseases resistant to

antimicrobials were a factor in 4.95 million fatalities. Estimates for 204 nations and territories show that antimicrobial resistance is a worldwide health problem, with the most severe effects occurring in countries with low and moderate incomes (LMICs). These newly estimated numbers serve as a timely reminder that COVID-19 is just one among the pandemics of public health hazard confronted by the planet. AMR is the invisible pandemic that nobody talks about. AMR is a significant factor contributing to the mortality rate associated with a wide variety of infections, including but not limited to those that are generally considered to be serious, such as pneumonia and sepsis, but also to those that are straightforward and perceived as easily treatable, such as infections of the urinary tract or infected wounds.

In November 2015, mcr-1 (mobilized colistin resistance-1) gene (conferring plasmid-mediated obduracy to colistin) was detected in *E. coli* from swines in china and later subsequently in humans from almost all countries in the world (Liu et al., 2016). This mutation renders polymixins the last-ditch antibiotic against Gram-negative bacteria ineffective. Even more alarming is the fact that *mcr-1* is the first recognized horizontal gene transfer pathway for polymixins obduracy (Cong Shen et al., 2020). Growing concern about an imminent post-antibiotic era and economic repercussions of such a grim scenario forced the UN assembly to hold a special session (fourth time in its history to discuss a health related issue) to discuss the growing threat of antimicrobial resistance on September 21, 2016. In the meeting, sustainable, multisectoral approaches to combat antimicrobial resistance under One Health umbrella were planned after carefully assessing the seriousness and scope of AMR to derail global health systems (Laxminarayan et al., 2016).

Microbes acquire AMR as a part of natural selection in response to evolutionary pressures. The selection pressure on microbes due to the unscrupulous employment of antibiotics for human well-being, faunal farming aquaculture, agriculture, and due to drug deposits in untreated effluents from hospitals and pharmaceutical industry can accelerate the acquirement of AMR. Use, misuse, and overuse of antimicrobials in all these sectors can accelerate AMR. Emergence of drug-resistant microbes right from the period when penicillin and sulfonamides were introduced as antibiotics gave early clues to the clinical dimensions and impact of AMR. In contrast, the environmental dimensions in the acquirement and dissemination of AMR were understood much later. The exact contribution of environment in the propagation of AMR is yet to be fully appreciated. It is now widely known how ARB and ARGs may enter the environment via the air, food, soil, and water. The factors responsible for it, viz., water and air quality, sanitation practices, urbanization, etc., are being extensively studied. Antibiotic residues are present both in rural and urban environments. Concentration of antibiotic residues in the rural environment is driven by veterinary use of antibiotics, whereas that in the urban environment depends on effluents from hospitals and pharmaceutical industry. Antibiotic consumption by individuals can also result in dissemination of ARBs and ARGs to environment. Important drivers of AMR that vary between economically underprivileged, privileged, and advanced nations include socioeconomic risk factors like poverty, extent of corruption, standards of sanitation, access

to clean water, air, food, and good quality antibiotics. Consumption, disposal, and fate of antibiotics within local environment vary widely between LMICs and HICs (Mendelson et al., 2016).

Environment is a reservoir and source of AMR, and it is crucial in the evolution, selection, and broadcast of strong unresponsiveness in microbes. ARGs are capable of being passed from one microorganism to another by both vertical and horizontal gene transfer. A major impeding factor to stop the spread of AMR is ARGs in environmental bacteria. These "resistomes" from different environs have the ability to impart obduracy to diseases via a process known as horizontal gene transfer (Forsberg et al., 2014). Practice of using animal manure in agriculture leads to increase in the concentration of ARGs in soil. The formation of environmental resistomes is significantly influenced by changes in population density, sanitary infrastructure, and disposal methods for solid waste (Aminov, 2009). There is a possibility that the spread of resistance might be influenced by high population densities in cities located inside LMICs that are plagued by inadequate sanitation and disposal of solid waste. Untreated effluents from hospital and drug manufacturing units, wastewater and sewage treatment plans, farm waste, and farm environment are also responsible for driving AMR (Martinez, 2009a). Regulatory and research-based interventions to reduce release of antibiotic residues in environment are a critical measure required to slow AMR.

AMR is as complex as climate change and requires a "One Health" approach with intersectoral coordination between human, animal, fisheries, aquaculture, environment, food production, agriculture sectors, etc.

2 Principal Drivers of AMR

1. Use, misuse, and overuse of drugs for the well-being of humans.
2. Extensive use of antibiotics in animal husbandry, agriculture, fisheries, and aquaculture. Animal husbandry accounts for 70% of antibiotic consumption in the world.
3. Net present value and profitability index of antibiotics are very low, and hence pharmaceutical corporations are disinclined to finance more for novel antimicrobial development as returns on the investments made are not assured.
4. Regulatory roadblocks: Gaining regulatory clearance is sometimes a challenge even for those pharmaceutical firms that have a positive outlook on their ability to find and develop novel antibiotics.
5. The overlapping social and economic elements, such as inadequate arrangements for public well-being, poverty, migration, cheap and unregulated sales of antibiotics, lack of access to clean water, air, and food, high disease burden, especially in LMICS, and poor sanitation.
6. Antibiotic residues and ARGs in the environment due to effluents from hospitals, pharmaceutical industry, farms, etc.
7. Poor governance and corruption have also been implicated as a driver of AMR.

3 Improper Use of Antimicrobials in the Human Sector

The rapid progress that modern medicine saw in the twentieth century with regard to complex surgeries, organ transplant programs, cancer chemotherapy, use of immunosuppressants for autoimmune diseases, advances in critical care, etc., was made possible by the discovery of antimicrobials that saved millions of lives. The umbrella of effective antimicrobials is essential for the advancement of modern medicine. However, the swift advent of obduracy to drugs and concomitant dwindling of antimicrobial pipeline has become fully fledged to be a public health hazard. In the span of less than a century with the dawn of these wonder drugs, we have reached a situation where the healthcare industry is plagued by organisms that are multi-, xeno-, and pan drug-resistant (MDR, XDR, and PDR). The abuse and misuse of antimicrobial drugs, in addition to the paucity of new drug research by the pharmaceutical industry as a result of decreased economic incentives and rigorous regulatory requirements, have been cited as one of the significant causes of the AMR issue (Bell et al., 2014). The "*Centre for Disease Control and Prevention (CDC)*" has labeled a number of bacteria as presenting urgent, severe, and worrying risks to the healthcare system. This will result in significant clinical and financial burden being placed on patients and their families (CDC, 2019). Coordinated efforts to implement antibiotic stewardship policies, renewing research efforts to come up with newer antibiotics, and strict hospital infection control policies are the need of the hour in supporting the healthcare industry to thwart the advancement and total blowout of AMR.

Use, misuse, and overuse of antimicrobials are considered to be one of the major drivers of antimicrobial resistance. Studies have clearly shown that more than 50% of antibiotic prescription in outpatient, inpatient, and critical care settings are unnecessary. Using antibiotics for treating viral infections, continuing course of antibiotics for more duration than is necessary to treat the diagnosed infection syndrome, using broad-spectrum antibiotics in situations where narrow-spectrum antibiotics would have been enough, not practicing de-escalation of antibiotics based on culture results, continuing prophylactic antibiotics initiated prior to surgery for extended periods than is recommended, and buying drugs sans prescription from the point of sale are some of the modifiable drivers of antimicrobial resistance. All these factors need to be addressed through antimicrobial stewardship, which is administering the proper medication to the factual patient at the proper time, in the appropriate dosage, for the suitable length of time. Antimicrobial stewardship is a coordinated program that aims at responsible use of antimicrobials with the aim of optimizing patient outcomes, minimizing collateral damage, and thereby preventing the occurrence and evolution of AMR organisms (Barlam et al., 2016). Collateral damage is defined as the selection of drug-resistant microbes in human gut microbiota by the unscrupulous use of antibiotics. Even in nations with relatively low prescription rates, there is still the problem of unnecessary antibiotic prescriptions. Appropriate antibiotic stewardship policies and effective information, education, and communication [IEC] strategies targeting both the prescribers and the general public are essential to address this issue.

The Access, Watch, and Reserve (AWaRe) categorization of antibiotics was included in the Grade of Indispensable Drugs as per the Classification of WHO in 2017. This was done with the intention of preventing the future development and dissemination of antimicrobial resistance. This categorization serves as the foundation for antibiotic stewardship programs at the local, national, and global levels, with the end goal of mitigating the effects of antimicrobial resistance (AMR). In October 2019, WHO AWaRe classification database comprising of details of 180 antibiotics was created. This database is meant to be an interactive tool for countries for optimizing and monitoring antimicrobial use. Use of unscientific fixed–dose (FDC) combinations can also fuel antimicrobial resistance. The AWaRe database also lists antibiotics whose use is not recommended by the WHO like FDCs of multiple-broad-spectrum antibiotics. The WHO Essential Medicines List Antimicrobial Working Group has bracketed antibiotics into three stewardship groups: Access, Watch, and Reserve (AWaRe), with the goal of drawing attention to the significance of the possibility of antimicrobial resistance and the need for the right use of antibiotics (WHO, 2019).

3.1 Access Cluster Antibiotics

This cluster consists of medications with lesser possibility for developing resistance and have effectiveness against a wide variety of susceptible pathogenic microbes that are often found in the environment. There are a total of 48 antibiotics in the Access group, 19 of which are listed at the top of the Grade of Indispensable Drugs as per WHO Classification to be used as either the first- or second-choice empiric therapy for certain infectious diseases (WHO, 2019).

3.2 Watch Group Antimicrobials

This group of antimicrobial drugs exhibit a greater potential for developing resistance, and it also represents majority of the "*critically important antimicrobials (CIA)*" employed for the well-being of humans. Antibiotics that are part of the Watch group have to be given top priority as essential targets for stewardship programs and monitoring. There are 110 antibiotics in the Watch Group, 11 of which are individually listed at the top of the Grade of Indispensable Drugs as per WHO Classification (WHO, 2019).

3.3 Reserve Group Antibiotics

Antibiotics that belong to this category are the kind that ought to be saved for the treatment of diseases brought on by MDR organisms. The antibiotics in the reserve group are regarded to be a "last resort" choice, and their usage should be limited to very particular individuals and circumstances only after all other options have been

tried and shown to be ineffective or inappropriate. Antibiotics that are classified as reserve group are safeguarded and given higher priority in stewardship efforts. This category encompasses twenty-two different antibiotics. In the Grade of Indispensable Drugs WHO Classification, seven different antimicrobial drugs from Reserve category are mentioned as separate entries. Antibiotic utilization metrics with regard to Reserve antibiotics is crucial in planning and executing stewardship strategies (WHO, 2019).

The WHO, under the 13th General Program of Work, has set an indicator based on AWaRe classification "to monitor access to essential medicines and thereby progress towards Universal Health Coverage." The WHO has specified that by 2023 all countries should calculate antibiotic utilization metrics and 60% of antimicrobial consumption in a country should be from the Access group.

The cognizable aspect of the advent of AMR in humans upon continuous usage of drugs does not follow a linear path. Confounding factors such as interfaces of drug–pathogen, microbial connections with hosts, rate of genetic transformations in bacteria, development clones that are obdurate to drugs, transmission dynamics of ARGs between people, faunae, and the environs, resistance gene heterogeneity in microbes, and also attainment of transformation in various loci impacting different drugs as well as pleiotropic drug obduracy make the association difficult to understand. At the community level, other factors such as vaccination coverage rates, sanitation and hygeine standards, exodus, and travel can also influence the development of antimicrobial resistance.

4 Antimicrobial Use for Food Production

Worldwide nearly three-fourth of antimicrobial utilization is in faunal farming for treatment, food production, and disease prevention. Antibiotics are used in veterinary medicine not only for treating microbial infections but also for prophylaxis and metaphylaxis in livestock, poultry, and pet animals. Antimicrobial growth promoters [AGPs] as subtherapeutic quantities of antibiotics are employed as growth stimulants (GPS) in animal feed (Andersson & Hughes, 2014). In the evolution of AMR, the critical part is microbes coming in contact with AGPs (Andersson & Hughes, 2014). The use of AGPs as feed additives has been found to change the gut flora of treated animals and boost drug obduracy transfer intra-animal and with the environmental microbiome. AGPs are administered to animals for longer periods of time in mixed with water for consumption or food to accelerate the rate of growth (You & Silbergeld, 2014). AGPs are being procured and used in many places across the world without the need for a veterinarian opinion or prescription. The application of drugs for prophylactic purpose in faunae is defined as the employment of antimicrobial drugs to vulnerable faunas that are otherwise healthy in order to avoid the development of infectious illnesses in such animals. The practice of giving therapeutic amounts of an antimicrobial medication to all of the animals in a group that contains some animals that are infected is technically termed "metaphylaxis." The use of metaphylaxis may serve both as a therapy for animals that have

previously contracted the disease and as a preventative approach for healthy animals that bear the risk of illness (Landers et al., 2012).

High population density in livestock and poultry farms without adequate biosecurity measures may result in the transmission of antimicrobial resistant microbes, genes and antibiotic residues to environment. Rapid dissemination of microbes can occur in such farms necessitating forceful contagion control approaches that include the employment of drugs. A magnitude of application of drugs in animal husbandry, horticulture, fisheries, and aquaculture varies among different nations. Many studies have shown that selection pressure on microbes due to antimicrobial application outside of human medicine, viz., in animal husbandry, fish production, and agriculture, has been a major driver of antimicrobial resistance. For this reason, "*One Health Approach*" is essential for containing AMR. Antimicrobials that are used to treat infectious illnesses in animals may be the same as or very comparable to antimicrobials that are used to treat infectious diseases in people. It is possible for resistant germs to transfer from one host to another, whether they originate in people, animals, or the environment. Borders between humans and animals or different geographic regions are meaningless to AMR. In view of the reason that more people are anticipated to eat animal protein, mitigation strategies for antimicrobial resistance containment in animal sector are critical. Country-level regulatory agencies to emphasize responsible use of antibiotics have "tolerance limits" that are set for veterinary medicine residues in animal diets, including antibiotics, viz., milk, meat, eggs, etc. An example of the misuse of antibiotics in veterinary medicine is with regard to the treatment of mastitis in dairy animals. After intra-mammary injection of antibiotics, milking should be done only after a specified waiting period to avoid antibiotic residues in milk. Most often the farmers do not follow waiting period due to a multitude of reasons. Unqualified medical professionals and availability of antibiotics without prescription of a veterinarian add to the problem. The presence of antibiotic residues in milk, seafood, and meat is a significant public health challenge that should be addressed at the earliest.

All antimicrobials used in veterinary medicine are not currently used for treatment in humans. But antibiotic classes, viz., tetracyclines, penicillins, sulfonamides, and colistin, are also used to treat human infections. The WHO has established standards for cataloging of antimicrobials employed in the animal husbandry as "critically important," "highly important," and "important" depending on their significance in treatment of human diseases. Quinolones, cephalosporins of the third generation and higher, glycopeptides, polymyxins, macrolides, and ketolides are the "Highest Priority Critically Important Antimicrobials" according to the most recent version of the list of critically important antibiotics (CIA) (6th Revision, 2018) published by the World Health Organization (WHO). The CIA list is updated periodically by the tripartite of WHO, FAO, and OIE (World Organization for Animal Health). The tripartite itself clearly demonstrates that the problem of AMR can be addressed by a One Health approach (Highest priority critically important antimicrobials, 2019). In 2022, with the addition of UNEP [United Nations Environment Programme] the tripartite was expanded to a quadripartite.

Due to factors such as rapid population expansion, changing urbanization patterns, and increased food production, Southeast Asia is sometimes referred to be the "hot bed" of antimicrobial resistance (Yam et al., 2019). It has been hypothesized that the rapid rate of population expansion in Southeast Asia, in conjunction with the region's intensive food and agricultural production, might result in an increased likelihood of drug-resistant diseases in people (State of Food and Agriculture in Asia and the Pacific Region, 2020). The "*Food and Agriculture Organization (FAO)*" has emphasized the need of educating farmers on the hazards of using medically vital drugs in food-producing animals as AGPs, and also to focus on enforcement of regulatory standards in production of food.

In the treatment of carbapenem-unresponsive Gram-negative infections in humans, one of the the last resort drugs is colistin which is being extensively used for many decades in animals, especially in swine, to control enteric infections. The rise of *mcr-1* is associated with extensive employment of colistin in Chinese farms, and in 2016, China put an end to the practice of colistin administration in food-producing animals. Despite the fact that employment of colistin as a growth promoter has been legally prohibited in the majority of countries, the drug is nevertheless used for therapeutic purposes in animals. The "*World Health Organization (WHO)*" issued a statement in November 2017 recommending limitations on the application of medically essential drugs such as colistin in food-producing animals, which includes ban on using these antibiotics as AGPs. Colistin's application in promoting development and preventing sickness in poultry and livestock will lead to the development of unresponsiveness to colistins in microbes of farms, contaminate air in farm surroundings, and is likely to spread to humans through meat. This is a very dangerous scenario as colistin is the lifeline for more than 50% of critically ill patients in ICUs with bloodstream infections due to carbapenem-resistant Enterobacterales. With very few new antibiotics against Gram-negative infections in the pipeline, all efforts should be made to prevent the emergence of colistin resistance in humans, poultry, and livestock. NDM-1 has been isolated from milk samples in India (Ghatak et al., 2013).

5 Role of Aquatic Environment as Driver of AMR

Aquatic environments can act as a reservoir for acquisition and distribution of ARGs and ARB (Karkman et al., 2018). It is possible for water bodies to get contaminated with antibiotic residues, ARB, and ARGs owing to the effluents that are discharged by hospitals, residences, municipal sewage treatment facilities, animal farms, land application of animal wastes, and slaughterhouses. Effluents from pharmaceutical manufacturing plants can directly contaminate the aquatic environment. The majority of drugs employed for human and animal health will ultimately sink into environment (Martinez, 2009b).

"*Wastewater treatment plants (WWTPs)*" receive significant quantities of chemicals and microbial loads from various sources like hospitals, farms, pharmaceutical industry, etc. WWTPs are ideal settings for exchange of genetic material between microbes, thereby fostering AMR. Within WWTPs, horizontal

gene transfer (also known as HGT) may take place between bacteria because of the pressure applied just to only certain groups by antibiotics, metals, and other chemical compounds (Karkman et al., 2018). Many studies have confirmed that it is very difficult to achieve zero effluent discharge from WWTPs. The effluent released from WWTPs might contain antibiotic residues, ARB, and ARGs. Hence, the effluent discharge from WWTPs to marine habitats, groundwater, and surface water and soil can result in selection pressure on microbes from these sources due to interactions with ARB and ARGs, leading to expansion of obduracy to antimicrobials. ARB and ARGs may be introduced to a new environment when recovered wastewater is used for agricultural irrigation and recreational activities, which creates a perfect setting for the development of unresponsiveness to drugs (Rodriguez-Mozaz et al., 2015). Anthropological contact possibly with ARB and ARGs can occur while washing, during water sports activities, irrigating crops, and by ingestion of foods that has been grown using domestic waste water. However, contaminated water consumption is the most common way that humans are exposed to ARB and ARGs. The techniques used to clean drinking water and wastewater are not totally efficient in screening out the genes responsible for antibiotic resistance (ARGs) (Rodriguez-Mozaz et al., 2015).

As far as marine ecosystems are concerned, despite significant dilution of contaminated waters, fecal-coliform such as *E. coli* continue to be sustainable reservoirs ARG and is of serious concern (Fig. 1).

6 Environmental Sanitation and AMR

Environment also plays a pivotal role in driving AMR. Use of metals like copper in agriculture as a bactericide or fungicide can lead to antimicrobial resistance. Nitrogen-containing fertilizers can affect the soil content of ARGs and can cause alterations in microbial composition of soil. The role of poor sanitation in blowout of antibiotic residues, ARB, and ARGs is very significant. Nearly 50% of the world's population in many of the underdeveloped nations do not have access to proper sanitation facilities, which prevents them from properly discarding human waste (Martinez, 2009b). Besides, a significant amount of sewerages is drained directly into aquatic bodies sans being treated at initial stages, which leads to the extreme pollution of such water bodies with antibiotic residues, microbes that are obdurate to drugs, and ARGs (Marathe et al., 2017). More than 70% of the urban sewage that is produced does not go through treatment facilities in the majority of the LMICs. The practice of disposing unused antimicrobials in landfills and water bodies can also lead to antimicrobial resistance. A drug take-back program as part of extended producer responsibility is necessary to prevent environmental contamination with unused drugs. The detection of microbes with antimicrobial-resistant genes in wildlife can be used as a surrogate tool to assess the environmental dimensions of unresponsiveness to antimicrobial drugs.

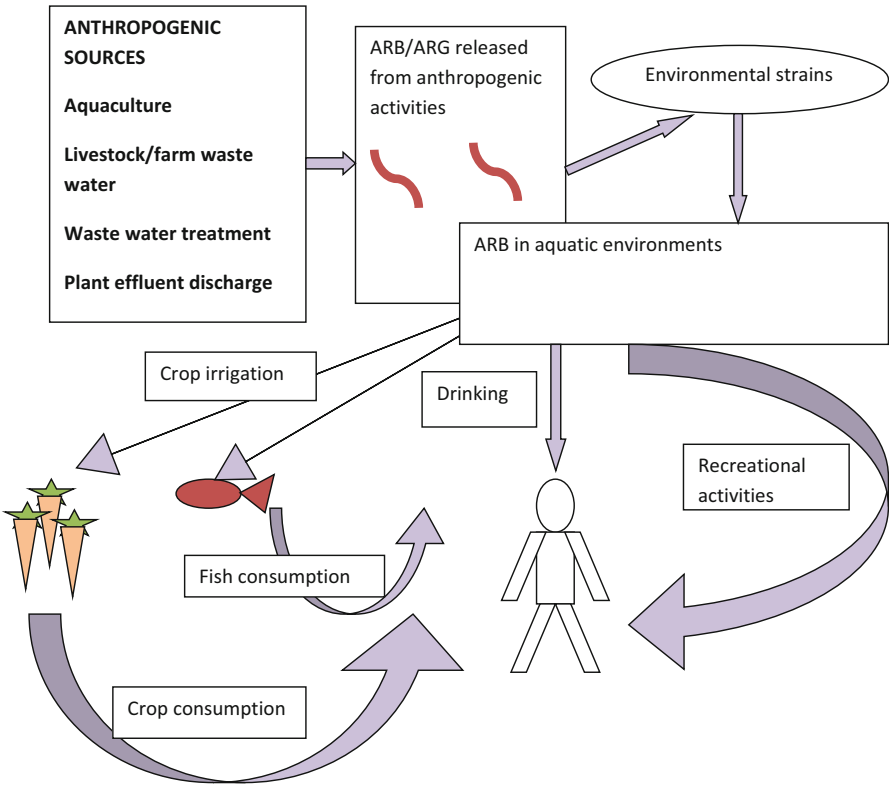


Fig. 1 Schematic representation of environmental dimensions of AMR

7 "*Infection Prevention and Control [IPC]*" Methods and AMR Transmission

One of the major reasons for healthcare-associated infections (HAIs) is cross-infection through the hands of healthcare workers or through instruments used. Adhering to good IPC practices, five moments of hand hygiene, ventilator-associated pneumonia bundle, catheter related urinary tract infection bundle, central line-related bloodstream infection bundle, surgical-site infection bundle, etc., are important in preventing HCAI due to MDR, XDR, and PDR microbes and prevent further spread within the unit and hospital. Breach in IPC measures can result in HCAI due to drug-resistant microbes. The resultant ARGs can get into hospital sewage and thereby into environment, resulting in genes getting transferred horizontally (HGT) with environmental microbiome.

The transmission dynamics of microbes unresponsive to antimicrobials have been modeled, and the insights acquired have contributed to a better understanding of how

human-to-human transmission is pivotal in the progress of obduracy to antimicrobials. Substandard sanitary conditions in many LMICs lead to feco-oral transmission of resistant enterobacterales and ARGs. Whole-genome sequencing has helped in delineating transmission points with regard to HAI outbreaks, for instance, in methicillin-resistant *Staphylococcus aureu*s contact transmission due to breach in IPC have been identified as the single most factor responsible for ongoing transmission. Hence, good IPC measures are important not only to prevent the emergence of antimicrobial resistance but also to prevent further spread.

8 Rapid Diagnostic Tools and AMR

In order to practice effective antimicrobial stewardship, correct diagnosis has to be made at the earliest using rapid diagnostic tools, namely, automated culture systems or molecular methods for pathogen and resistance mechanism identification. Point-of-care rapid diagnostic tools are an integral aspect of diagnostic stewardship. In clinical settings, it may be difficult to tell the difference between upper and lower respiratory illnesses caused by bacteria and those caused by viruses. Molecular methods like nucleic acid amplification tests (NAAT), real-time PCR, multiplex PCR, etc., will help in identifying viral pathogens within hours. This will help in preventing unnecessary application of antimicrobial drug in the handling of viral origin diseases. Speed of diagnosis is crucial for effective antimicrobial stewardship. Rapid diagnostic tools employing "*Mass spectrometry, namely, matrix-assisted laser desorption/ionization–time of flight (MALD-TOF)*", cytogenetic methods like "*peptide nucleic acid fluorescence in situ hybridization [PNA-FISH]*" and whole-genome sequencing with turnaround time of hours will play a crucial role in diagnostic and antimicrobial stewardship (Rapid diagnostic testing in antimicrobial stewardship, 2017).

9 Social Factors and AMR

In the use, overuse, and misuse of antimicrobial drugs, social factors of varied kinds play a crucial role, especially in economically underprivileged nations. Easy access to antibiotics due to unrestricted over-the-counter (OTC) sale in certain countries has largely influenced the antibiotic consumption behavior of the public. Self-medication, easy access to antibiotics without prescription, seeking healthcare from pharmacies and informal healthcare providers, lack of knowledge on appropriate use of antibiotics, etc., are some of the important social factors responsible for antimicrobial resistance (Collignon & Beggs, 2019).

10 Cultural Activities and AMR

Some cultural activities can act as drivers of AMR. One of the most significant cultural practices that have been linked to the possible attainment and transmission of spread of ARB and ARGs is the practice of participating in religious mass

meetings that include bathing in rivers. A comparison was made between the levels of fecal coliform and blaNDM-1 that were found in the water and sediments in the Upper Ganges before and during the season when pilgrims visit the area. During the time of the pilgrimage, the levels of blaNDM-1 in the river were twenty times higher than at other times of the year (Ahammad et al., 2014). This observation indicates that pilgrimage sites might serve as harbingers for wider dissemination of *blaNDM-1* and other ARGs. This research shows the impact that cultural activities have on the development of antimicrobial resistance as well as the need of improving waste disposal during pilgrimages in order to meet the recommendations of the study.

11 Poor Governance, Corruption, and AMR

The extent of AMR is directly related to the quality of governance and prevalence of corruption, which is an important socioeconomic determinant of AMR. In a study done by Peter Collignon et al. (2015) comparing the extent of AMR with corruption in European countries, the impact of corruption on the prevalence of AMR was statistically significant and is associated with increase in antimicrobial resistance. Poor governance and corruption correlate better than antibiotic consumption volumes with resistance rates (Frost et al., 2019). When the quality of governance is poor, regulatory control on antibiotic use in all sectors will be less effective, which can lead to AMR. Measures to curb the spread of AMR also will be less effective in the absence of good governance. Poor governance will lead to less supervision and laxity in enforcement of laws, which in turn can worsen AMR crisis. Food and water safety, sanitation standards, poverty, etc., which are major drivers of AMR, are intrinsically linked to the quality of governance and extent of corruption prevailing. Dysfunctional public institutions were responsible for variation in antibiotic utilization metrics between different regions. For this reason, better controls on corruption are essential to effectively address the challenge of AMR.

12 Travel, Migration, and Spread of AMR

Travel, trade, and mass migration play an important role in the dissemination of AMR to different geographical locations. Global travel is one of the major modes of transmission in the spread of ARB and ARGs. This issue has to be addressed at multiple levels. Visitors to regions with high AMR burden should be vaccinated (typhoid, cholera vaccine etc) and should practise good infection control and safe sex practices. Economically underdeveloped and developing nations have to enhance the investments in environmental hygiene and sanitation, as well as access to clean water and food. It is necessary to conduct effective surveillance in order to get an understanding of the role mobility of people, animals, and food items plays in the transmission of resistance (Frost et al., 2019). The migratory wild birds too have been implicated as potential reservoirs and spreaders of antimicrobial-resistant organisms and genes (Elsohaby et al., 2021).

13 Conclusion

AMR is a global problem and has been recognized by the WHO as one of the principal public well-being challenges. The advent and rapid dissemination of antibiotic impervious bacteria have seriously hampered the efficacy of the currently available drugs. Improper use of antibiotics in agriculture, aquaculture, healthcare settings of fauna, fisheries, humans, along with ecological pollution with antibiotic residues, ARB, and ARG, are the major drivers for the emergence and spread of AMR. The absence of access to potable water, exposed sewage structures, suboptimal contagion containment in clinical practices, farms without good biosecurity, suboptimal regulation of antimicrobial use in farms, high flock densities, etc., can lead to antimicrobial resistance. Socioeconomic factors, viz., poverty, poor governance, and corruption, play a very important role in the expansion and transmission of AMR. Lacunae on the availability for better antimicrobial drugs, rapid diagnostic tools, inadequate AMR surveillance, inadequate or nonexistent programs for contagion deterrence and restriction, weak laboratory capacity, and practice of over-the-counter sale of antibiotics sans prescription are also major concerns that should be addressed. AMR has become a core political, social, and economic problem. AMR like climate change should be tackled by a multi-pronged approach with multi-disciplinary intersectoral co-ordination and collaboration under principles of One Health.

References

Ahammad, Z. S., Sreekrishnan, T. R., Hands, C. L., Knapp, C. W., & Graham, D. W. (2014). Increased waterborne blaNDM-1 resistant gene abundances associated with seasonal human pilgrimages to the upper Ganges river. *Environmental Science & Technology, 48*, 3014–3020.

Aminov, R. I. (2009). The role of antibiotics and antibiotic resistance innature. *Environmental Microbiology, 11*, 2970–2988.

Andersson, D. I., & Hughes, D. (2014). Microbiological effects of sublethal levels of antibiotics. *Nature Reviews Microbiology, 12*, 465–478.

Barlam, T. F., Cosgrove, S. E., Abbo, L. M., MacDougall, C., Schuetz, A. N., Septimus, E. J., Srinivasan, A., Dellit, T. H., Falck-Ytter, Y. T., Fishman, N. O., Hamilton, C. W., Jenkins, T. C., Lipsett, P. A., Malani, P. N., May, L. S., Moran, G. J., Neuhauser, M. M., Newland, J. G., Ohl, C. A., Samore, M. H., Seo, S. K., & Trivedi, K. K. (2016). Implementing an antibiotic stewardship program: Guidelines by the Infectious Diseases Society of America and the Society for Healthcare Epidemiology of America. *Clinical Infectious Diseases, 62*(10), e51–e77. https://doi.org/10.1093/cid/ciw118

Bell, B. G., Schellevis, F., Stobberingh, E., Goossens, H., & Pringle, M. (2014). A systematic review and meta-analysis of the effects of antibiotic consumption on antibiotic resistance. *BMC Infectious Diseases, 14*, 13.

CDC. (2019). *Antibiotic resistance threats in the United States, 2019*. U.S. Department of Health and Human Services, CDC. www.cdc.gov/DrugResistance/Biggest-Threats.html. https://doi.org/10.15620/cdc:82532.

Collignon, P., & Beggs, J. J. (2019). Socioeconomic enablers for contagion: Factors impelling the antimicrobial resistance epidemic. *Antibiotics, 8*(3), 86. https://doi.org/10.3390/antibiotics8030086

Cong Shen, Lan-Lan Zhong, Yongqiang Yang, Yohei Doi, Paterson, D. L., Stoesser, N., et al. (2020, May 1). Dynamics of mcr-1 prevalence and mcr-1-positive Escherichia coli after the cessation of colistin use as a feed additive for animals in China: A prospective cross-sectional and whole genome sequencing-based molecular epidemiological study. *Lancet Microbe, 1*(1), E34–E43.

Elsohaby, I., Samy, A., Elmoslemany, A., Alorabi, M., Alkafafy, M., Aldoweriej, A., Al-Marri, T., Elbehiry, A., & Fayez, M. (2021). Migratory wild birds as a potential disseminator of antimicrobial-resistant bacteria around Al-Asfar Lake, Eastern Saudi Arabia. *Antibiotics (Basel), 10*(3), 260. https://doi.org/10.3390/antibiotics10030260

Forsberg, K. J., Patel, S., Gibson, M. K., et al. (2014). Bacterial phylogenystructures soil resistomes across habitats. *Nature, 509*, 612–616.

Frost, I., Van Boeckel, T. P., Pires, J., Craig, J., & Laxminarayan, R. (2019). Global geographic trends in antimicrobial resistance: The role of international travel. *Journal of Travel Medicine, 26*(8), taz036. https://doi.org/10.1093/jtm/taz036

Ghatak, S., Singha, A., Sen, A., Guha, C., Ahuja, A., Bhattacharjee, U., Das, S., Pradhan, N. R., Puro, K., Jana, C., Dey, T. K., Prashantkumar, K. L., Das, A., Shakuntala, I., Biswas, U., & Jana, P. S. (2013). Detection of New Delhi metallo-beta-lactamase and extended-spectrum beta-lactamase genes in Escherichia coli isolated from mastitic milk samples. *Transboundary and Emerging Diseases, 60*(5), 385–389. https://doi.org/10.1111/tbed.12119. Epub 2013 Jul 19.

Highest priority critically important antimicrobials. WHO Food Safety. (2019, May) WHO CIA list 6th rev.: https://www.who.int/foodsafety/publications/antimicrobials-sixth/en/

Karkman, A., Do, T. T., Walsh, F., & Virta, M. P. J. (2018). Antibiotic-resistance genes in waste water. *Trends in Microbiology, 26*(3), 220–228. https://doi.org/10.1016/j.tim.2017.09.005. Epub 2017 Oct 13.

Landers, T. F., Cohen, B., Wittum, T. E., & Larson, E. L. (2012). A review of antibiotic use in food animals: Perspective, policy, and potential. *Public Health Reports, 127*, 4–22.

Laxminarayan, R., Matsoso, P., Pant, S., Brower, C., Røttingen, J. A., Klugman, K., & Davies, S. (2016). Access to effective antimicrobials: A worldwide challenge. *Lancet, 387*(10014), 168–175. https://doi.org/10.1016/S0140-6736(15)00474-2. Epub 2015 Nov 18.

Liu, Y. Y., Wang, Y., Walsh, T. R., Yi, L. X., Zhang, R., Spencer, J., Doi, Y., Tian, G., Dong, B., Huang, X., Yu, L. F., Gu, D., Ren, H., Chen, X., Lv, L., He, D., Zhou, H., Liang, Z., Liu, J. H., & Shen, J. (2016). Emergence of plasmid-mediated colistin resistance mechanism MCR-1 in animals and human beings in China: A microbiological and molecular biological study. *The Lancet Infectious Diseases, 16*(2), 161–168. https://doi.org/10.1016/S1473-3099(15)00424-7. Epub 2015 Nov 19.

Marathe, N. P., Pal, C., Gaikwad, S. S., Jonsson, V., Kristiansson, E., & Larsson, D. G. J. (2017). Untreated urban waste contaminates Indian river sediments with resistance genes to last resort antibiotics. *Water Research, 124*, 388–397. https://doi.org/10.1016/j.watres.2017.07.060. Epub 2017 Jul 25.

Martinez, J. L. (2009a). The role of natural environments in the evolution of resistance traits in pathogenic bacteria. *Proceedings of the Royal Society B: Biological Sciences, 276*, 2521–2530.

Martinez, J. L. (2009b). Environmental pollution by antibiotics and by antibiotic resistance determinants. *Environmental Pollution, 157*, 2893–2902.

Mendelson, M., Røttingen, J. A., Gopinathan, U., Hamer, D. H., Wertheim, H., Basnyat, B., Butler, C., Tomson, G., & Balasegaram, M. (2016). Maximising access to achieve appropriate human antimicrobial use in low-income and middle-income countries. *Lancet, 387*(10014), 188–198. https://doi.org/10.1016/S0140-6736(15)00547-4. Epub 2015 Nov 18.

Rapid diagnostic testing in antimicrobial stewardship. CIDRAP Antimicrobial Stewardship Project. Policy Update October 2017.

Rodriguez-Mozaz, S., Chamorro, S., Marti, E., Huerta, B., Gros, M., Sànchez-Melsió, A., Borrego, C. M., Barceló, D., & Balcázar, J. L. (2015). Occurrence of antibiotics and antibiotic resistance genes in hospital and urban wastewaters and their impact on the receiving river. *Water Research, 69*, 234–242. https://doi.org/10.1016/j.watres.2014.11.021. Epub 2014 Nov 24.

State of Food and Agriculture in Asia and the Pacific Region, including Future Prospects and Emerging Issues FAO Regional Conference for Asia and the Pacific Thirty-fifth Session Thimphu, Bhutan, 17–20 February 2020.

Tackling drug resistant infections globally: Final report and recommendations: The review on AMR Chaired by Jim O. Neill (2016, May).

WHO. (2019). *The 2019 WHO AWaRe classification of antibiotics for evaluation and monitoring of use*.

Yam, E., Hsu, L., Yap, E. H., et al. (2019). Antimicrobial resistance in the Asia Pacific region: A meeting report. *Antimicrobial Resistance and Infection Control, 8*, 202. https://doi.org/10.1186/s13756-019-0654-8

You, Y., & Silbergeld, E. K. (2014, June 10). Learning from agriculture: Understanding low dose antimicrobials as drivers of resistome expansion. *Frontiers in Microbiology*. https://doi.org/10.3389/fmicb.2014.00284

Molecular Mechanisms of Antimicrobial Resistance

Murugadas Vaiyapuri, Ahamed Basha Kusunur, and Madhusudana Rao Badireddy

Contents

M. Vaiyapuri
Microbiology Fermentation and Biotechnology Division of ICAR-Central Institute of Fisheries Technology (ICAR-CIFT), Cochin, Kerala, India
e-mail: murugadas.v@icar.gov.in

A. B. Kusunur
Visakhapatnam Research Centre of ICAR-Central Institute of Fisheries Technology (ICAR-CIFT), Visakhapatnam, Andhra Pradesh, India
e-mail: kusunur.ahamedbasha@icar.gov.in

M. R. Badireddy (✉)
Visakhapatnam Research Centre of ICAR-CIFT, ICAR-Central Institute of Fisheries Technology (ICAR-CIFT), Visakhapatnam, Andhra Pradesh, India
e-mail: bm.rao@icar.gov.in

M. P. Mothadaka et al. (eds.), *Handbook on Antimicrobial Resistance*,
https://doi.org/10.1007/978-981-19-9279-7_28

Abstract

Antibiotics were the biggest discovery in the twentieth century and have positively impacted infection treatment in human and animal health. After several decades of use and misuse of antibiotics, the world is now racing toward pre-antibiotic era with the development of resistance in bacteria to the maximum number of antibiotics. The development and spread of resistance are mediated through several mechanisms evolved due to the immense genetic plasticity of bacteria. Understanding the underlying molecular basis of antimicrobial resistance (AMR) is pertinent to develop new drugs or design appropriate strategies to prevent the emergence of resistance. The present chapter is a ready reckoner for the mode of action of antibiotics, mechanism of AMR, and transfer of its resistance.

Keywords

Antimicrobial resistance · Antimicrobial resistance genes · Molecular mechanism · Biofilm · AMR · ARG

1 Introduction

Antibiotics though originally defined as compounds produced by bacteria that either kills or inhibits the growth of other related or unrelated bacteria but now includes any class of organic molecule that specifically interacts with bacterial targets and destroys / inhibits them (Davies & Davies, 2010; Zimdahl, 2015). The term was described in 1942 by Selman Waksman. Antibiotics have been used in human and veterinary medicine to combat bacterial infections and prevent post-surgery sepsis since their discovery. Antibiotics elicit their detrimental effect on the growth and survival of bacteria by several mechanisms, namely, inhibition of bacterial cell wall synthesis (penicillin, ampicillin, cefotaxime, ceftazidime, cefazolin, meropenem, imipenem, vancomycin), disruption of bacterial cell membrane (daptomycin, polymixin, clistin), inhibition of protein synthesis (gentamicin, kanamycin, amikacin, tobramycin, tetracycline, doxycyline, linezolid, chloramphenicol, azithromycin, clarithromycin, fusidic acid), or inhibition nucleic acid synthesis (ciprofloxacin, ofloxacin, norfloxacin, sulfamthoxazole, trimethoprim and rifampin) (Coates et al., 2011; Kapoor et al., 2017; Gray & Wenzel, 2020).

Antibiotics have been inappropriately used both in human health care and in animal agriculture. Antimicrobial resistance (AMR), *i.e.,* non-responsiveness of bacteria to previously susceptible antibiotics, has serious and adverse consequences for human and animal health care and is recognized as a major public health threat of the twenty-first century (Prestinaci et al., 2015). The emergence of AMR is generally attributed to the genetic plasticity of bacteria that enables them to survive in antibiotic laden environment (Munita & Arias, 2016). Although the AMR is a natural process but anthropogenic practices hasten the processes that play a role in the selection of antimicrobial-resistant bacteria. An estimated 20,000 antibiotic resistance genes (ARG

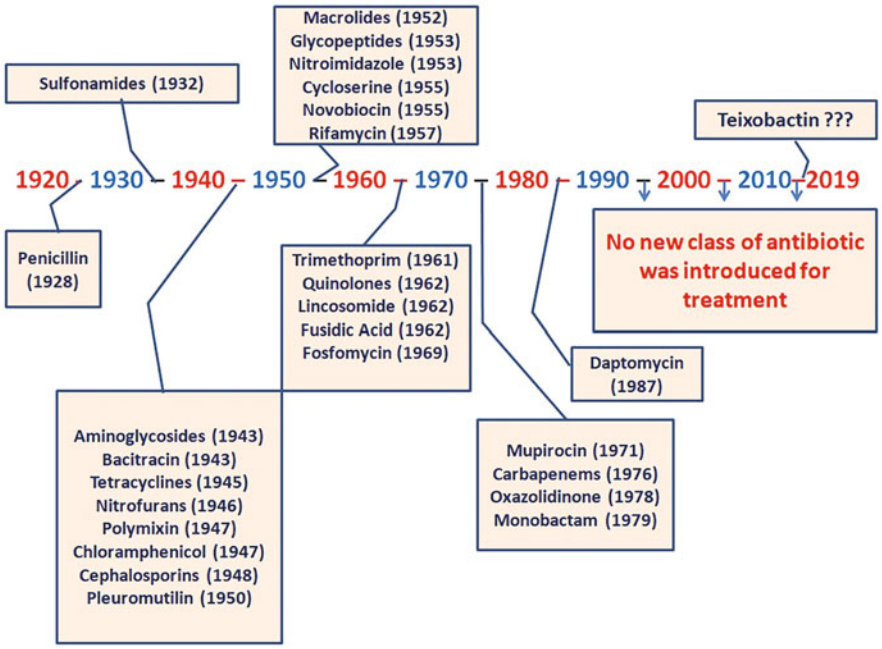


Fig. 1 Time line of discovery of antibiotics

genes) are detected in the bacterial genomes that empower them to resist virtually all antibiotics that are currently available (Aslam et al., 2018). The situation reached an alarming proposition as virtually no new class of antibiotic introduced in the last three decades for health care use (Fig. 1). Health care professionals warn of an imminent return to pre-antibiotic era jeopardizing human life as antibiotic resistant bacteria make people succumb to common infections, small wounds and routine surgeries (Davies & Davies, 2010; World Health Organization, 2020).

In a broader sense, the increase in the AMR are due to the following reasons; increased consumption of antimicrobials in medical and veterinary fields for therapy and prophylaxis; inadequate stewardship in prescribing of antimicrobials for therapy; and ignorance of the patients.

Understanding the molecular mechanism of AMR is pertinent to develop novel drugs or design innovative strategies to prevent the emergence of resistance. Bacteria can be either inherently resistant to certain classes of antibiotics groups or they can acquire resistance during their lifetime.

Before the understanding of the mechanism of AMR, it is very imperative to understand the mechanism of action of antibiotics based on the variation in the cellular structure of Gram positive and negative bacteria (Table 1). Antibiotics were naturally produced by the several bacterial and fungal organisms as tools of self-defense (Table 2) and exert their activity on different cellular target of susceptible bacteria (Fig. 2).

Table 1 Distinction between Gram positive and Gram-negative bacteria for variation in AMR

Properties	Gram positive	Gram negative
Outer membrane	Outer membrane consists of single but relatively thick peptidoglycan layer	Outer membrane consists of multiple layers (usually three) with phospholipids and peptidoglycans.
Resistance	Sensitive to majority of the antibiotics	Resistant to most of the antibiotics due to the alteration of outer membrane proteins
Uptake of substances	Peptidoglycans helps in uptake of most of the disinfectants which diffuse through the cells and kill the cells	Hydrophobic nature of the outer membrane helps in escaping from the harmful disinfectants

Table 2 List of antibiotics produced by microorganisms

Antibiotic	Naturally produced from microbes	Target of action
Penicillin	*Penicillium notatum*	D-Ala metabolizing enzyme
Cephalosporin C	*Acremonium chrysogenum*	
Carbapenems - thienamycin	*Streptomyces cattleya*	
Clavulanic acid	*Streptomyces clavuligerus.*	β-lactamase inhibitor
Erythromycin	*Streptomyces erythreus*	50S ribosome peptidyltransferase
Tylosin	*Streptomyces fradiae*	
Vancomycin	*Streptomyces (Amycolatopsis orientalis)*	Transglycosylase in murein synthesis
Gentamicin (kanamycin, Neomycin, tobramycin)	*Micromonospora echinospora Streptomyces kanamyceticus*	30S ribosome
Spectinomycin	*Streptomyces spectabilis*	
Streptomycin	*Streptomyces griscus*	
Kasugamysin	*Streptomyces kasugaensis*	
Tetracycline	*Streptomyces aureofaciens and Streptomyces rimosus*	
Rifamycin	*Micromonospora rifamycinica and Salinispora arenicola*	RNA polymerase
Chloramphenicol	*Streptomyces venezuelae*	Peptidyl transferase
Lincomycin	*Streptomyces lincolnensis*	
Puromycin	*Streptomyces alboniger*	A-/P site, 50S ribosome

2 Intrinsic Resistance

The natural occurrence of resistance determinants in the bacterial chromosome that primarily arise due to chromosomal mutations is termed as intrinsic resistance (Cox & Wright, 2013). Usually, all species share this form of resistance within the same genus or species; for example, most intrinsic resistant to penicillin G is noticed in gram-negative bacteria. Antibiotic producing bacteria such as actinomycetes possess mechanisms to subsist in the presence of antibacterial compounds by them as means

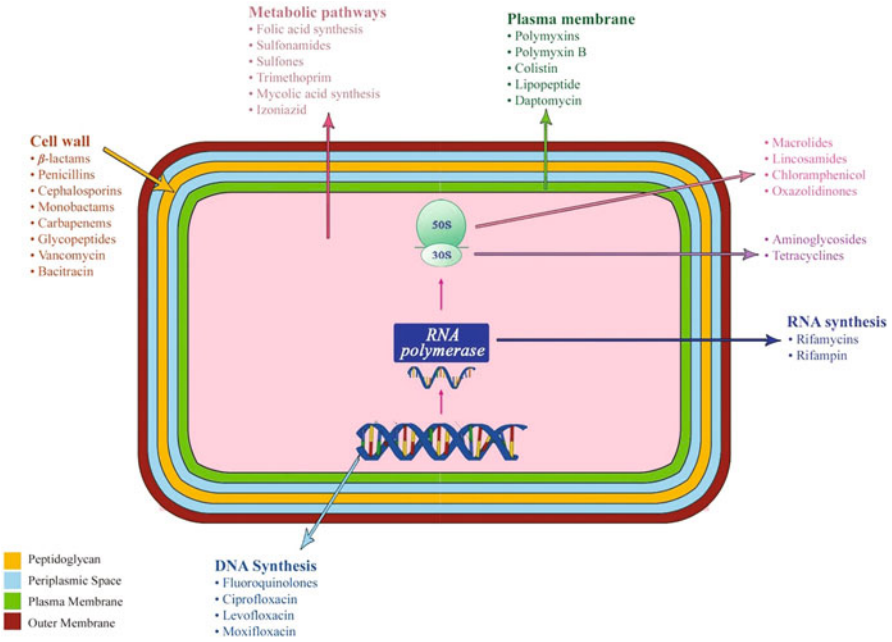


Fig. 2 Site of action of different antibiotics

to avoid self-destruction. Similarly, *Streptomyces rimosus* possesses an efflux system for tetracycline. Moreover, soil bacteria have developed the capacity to utilize antibiotics as carbon and nitrogen sources and are considered to be reservoirs of ARGs. The sum total of all the 'r' genes in nature is termed as the environmental resistome (Arzanlou et al., 2017; Kostyanev & Can, 2017).

3 Acquired Resistance

Bacteria "acquire" resistance to an antibiotic to which they were originally susceptible. This type of resistance results either from mutations in the chromosomal gene that can be transmitted vertically across bacterial generations or due to horizontal gene transfer (HGT) acquisition of resistance genes from different bacteria through mobile genetic elements (MGE). Chromosomal mutations, albeit occur at a relatively lower frequency (10^{-7} to 10^{-9}) but are extremely relevant in terms of emergence of AMR in bacteria that is genetically passed on to the next generation. However, the most relevant mode of resistance emergence is through the HGT that occurs through bacterial conjugation (the transfer of resistance genes from a donor bacterium to a receipient bacterium via physical contact and sex pili); or through transduction (the bacteriophage mediated transfer of genetic material transfer between bacteria) or by transformation (the uptake of

free DNA in the environment by bacteria). The gene transfer is common within the bacteria of the same genus but gene exchange has been observed between different genera including evolutionary different genera (van Hoek et al., 2011; Arzanlou et al., 2017).

4 Mechanisms of AMR

Antibiotic resistance mechanisms are broadly categorized into four strategies, viz., modification of the antibiotic, prevent the antibiotic from reaching its target site, changes and/or bypass of target sites, and biofilm based antibiotic resistance (Benveniste & Davies, 1973; Alekshun & Levy, 2007; Beceiro et al., 2013; Wright, 2016). Few mechanisms are unique to gram-negative or gram-positive bacteria, while majority of the mechanisms are exhibited by both gram-positive and -negative bacteria (Figs. 3 and 4).

4.1 Mechanism of AMR Through Modification of Antibiotic Molecule (Attacking Strategy)

Bacteria adopt an attacking strategy to directly encounter and destroy the antibiotic molecule present in its immediate vicinity. This is performed in two ways, viz., by producing enzymes that modify the antibiotic or by producing enzymes that completely destroy the antibiotic.

4.1.1 Enzymes that Alter the Antibiotic Molecule

Bacteria produce certain enzymes that induce chemical changes in the antibiotic molecule and modify it leading to the loss of antimicrobial property. This mechanism is evidenced in gram positive and gram-negative bacteria. Aminoglycosides (amikacin, gentamicin, kanamycin, streptomycin) inhibit protein synthesis in

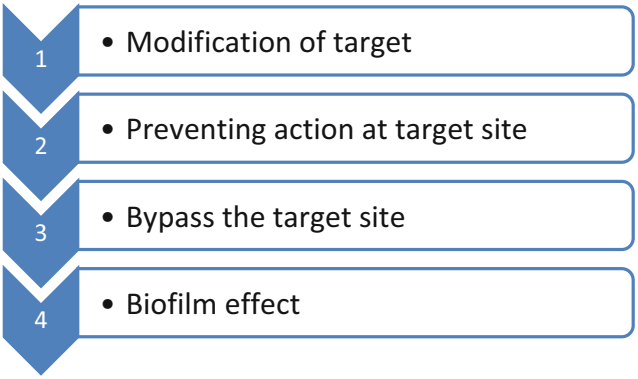


Fig. 3 Major mechanisms of AMR

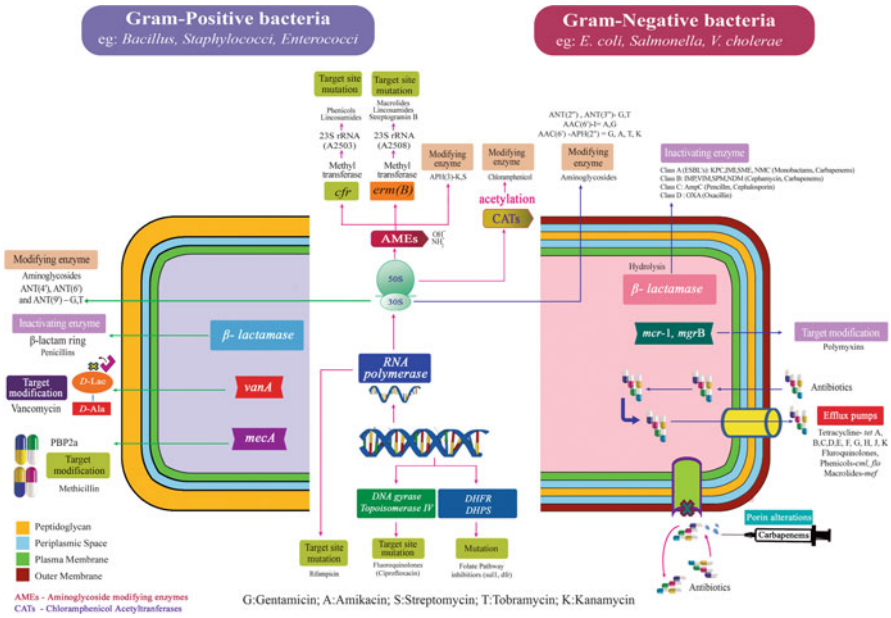


Fig. 4 Molecular mechanisms of Antimicrobial Resistance

bacteria at 30S ribosome level but are most vulnerable to this mode of resistance. Altertaion in the aminoglycoside molecule is brought out by aminoglycoside modifying enzymes (AMEs) that are enzymes that can covalently change the hydroxyl or amino groups in aminoglycosides (Lin et al., 2018).

Biochemical modifications that are frequently performed by the aminoglycoside modifying enzymes (AMEs) include acetylation (acetyltransferase; AAC), adenylation (adenyltransferase; ANT), and phosphorylation (phosphotransferase; APH). The AAC (6′)-I is an acetytransferase enzyme that is commonly seen Gram-negative bacteria such as Acinetobacter, Enterobacteriacea, and Pseudomonas and prevents the action of amikacin and gentamicin. APH (3) enzyme is a phosphotransferase enzyme that is extensively distributed in both, gram-positive and gram-negative bacteria and modifies the activity of kanamycin and streptomycin, however, the APH (3) enzyme does not change gentamicin and tobramycin. Acetyltransferase, Adenyltransferases, alter the activity of gentamicin and tobramycin. Gram-positive bacteria usually harbor ANT (4′), ANT (6′), and ANT (9′) genes whereas Gram-negative bacteria usually harbor the genes encoding for ANT (2″) and ANT (3″). AAC (6′)-APH (2″) enzyme is a bifunctional AME that performs both, acetylation and phosphorylation, and confers resistance to almost of the aminoglycosides except streptomycin. AAC (6′)-APH (2′) is seen in Gram-positive coocci, *viz*., Enterococci and Staphylococci (Ramirez et al., 2013; Garneau-Tsodikova & Labby, 2016). Many bacteria produce chloramphenicol acetyltransferases (CATs) that modify chloramphenicol. The *cat* genes are present in gram-positive and -negative

bacteria. The *Cat* genes are mostly found in MGEs like plasmids and transposons; however, there are a few that have been found on chromosomes (Doi et al., 2016).

4.1.2 Enzymes that Destroy the Antibiotic Molecule

Bacteria produce certain enzymes that completely annihilate the antibiotic molecule making it incapable of executing its antibacterial activity. β-lactam group of antibiotics are destroyed by β-lactamases enzyme that are usually encoded by *bla* genes located on the chromosome or localized in MGEs (Ambler, 1980; Hall & Barlow, 2005). More than thousand different β-lactamases have been reported in bacteria and are classified either based on amino acid sequence identity into 4 groups (A, B, C, and D) as per Ambler classification or based on substrate specificity into 4 categories as per Bush-Jacoby classification. Extended Spectrum β-Lactamase (ESBL) hydrolyzes penicillins, monobactams, and third generation cephalosporins but harbor limited activity against cephamycins and carbapenems (Kim et al., 2009; Bush & Jacoby, 2010).

Class A β-lactamases posess the amino acid serine residue in their catalytic site and clavulanic acid inhibits their activity. Monobactams, but not cephamycins, are among their active spectrum. Class A β-lactamases are either chromosomally encoded (e.g., imipenem hydrolyzing enzyme [IMI], Serratia marcescens enzyme [SME], non-metallo-enzyme carbapenemase [NMC]) or plasmid mediated (e.g., Klebsiella pneumoniae carbapenemase [KPC], GES carbapenemases.

Class B β-lactamases require a metal ion (usually Zinc) as co-factor for the nucleophilic attack on β-lactam ring of a many β-lactams, including carbapenems. Class B β-lactamases are effective on cephamycins but has poor activity against aztreonam. Unlike Class A, Class B β-lactamases are not inhibited by clavulanic acid and tazobactam but are inhibited by ion-chelating agents such as EDTA. Class B β-lactamases are actegorized into 4 families, viz., IMP, VIM, SPM, and NDM. IMP type β-lactamases were first reported in *Serratia marcescens* but were later observed in *Acinetobacter*, *Enterobacteriaceae*, and *Pseudomonas*. VIMtype β-lactamases (Verona integron-encoded metallo β-lactamase) were first reported from Verona, Italy, in *P. aeruginosa*. NDM type enzymes (New Delhi Metallo β-lactamase) were first reported in *K. pneumoniae* in Sweden on a person who was previous history of hospitalization in New Delhi, India. Class C β-lactamases hydrolyze all penicillins and cephalosporins but do not reliably hydrolyze aztreonam. Smilar to Class B, Class C β-lactamases are inhibited neither by clavulanic acid nor tazobactam. AmpC is clinically the important β-lactamase. Class D β-lactamases hydrolyze oxacillin. They are weakly inhibited by clavulanic acid. Class D β-lactamases are prevalent in *Acinetobacter baumanii* and have also been reported in several other Gram-negative bacteria such as *K. pneumoniae, E. coli*, *P. aeruginosa*, and *Enterobacter*. OXA-11 from *P. aeruginosa* hydrolyzes third and OXA-23 from *A. baumannii* hydrolyzes carbapenems (Pfeifer et al., 2010; Nordmann et al., 2012).

Understanding the resistance mechanism has led to drug development to circumventing the activity of β-Lactamases. Irreversible “suicide inhibitors” such as clavulanic acid was discovered to counter the action of β-lactamases and are being extensively used in human health care. Clavulanic acid isolated from *Streptomyces*

clavuligerus showed little antimicrobial activity alone, but when it used in combination with combined with amoxicillin, clavulanic acid significantly lowered the MICs of amoxicillin against *E. coli*, *K. pneumoniae*, *Proteus mirabilis*, and *S. aureus*. Other combinations include ticarcillin-clavulanate, ampicillin-sulbactam, and piperacillin-tazobactam. Metallo β-lactamase inhibitors such as thiol derivatives (thiomandelic acid), pyridine dicarboxylates, succinate derivatives, tricyclic natural products, trifluoromethyl ketones have been reported to inhibit the activity of β-lactamase enzyme (Drawz & Bonomo, 2010).

4.2 Mechanism of AMR Through Prevention of Antibiotic Penetration and Efflux (Defensive Strategy)

This is a defensive strategy adopted by bacteria to escape from the invading antibiotic molecule. This is done in two ways, viz., prevent the entry of antibiotics into the bacterial cell by decreasing cell permeability to or extrude the antibiotics that had entered the bacterial cell by employing efflux pumps.

4.2.1 Prevention of Entry of Antibiotics

Most of the antibiotics target the intracellular components of bacterial cell. The target of some antibiotics is located in the cytoplasmic membrane. Bacteria have devised mechanisms to limit the antibiotic influx from the external environment. To exert their action on Gram-negative bacteria, antibiotic molecules must penetrate their outer membrane (OM). OM acts as the first barrier that prevents the influx of antibiotics such as vancomycin. OM permeability also affects β-lactams, tetracyclines, and some fluoroquinolones. Porins are water-filled channels present in the OM that are used by antibiotics to cross this protective barrier. Alterations of porins could be adversely affects the influx of antibiotics. Bacteria achieve alterations in porin function through a shift in the type of porins and level of porin expression.

4.2.2 Efflux Pumps to Flush Out Antibiotics from the Bacterial Cell

Gram-positive and -negative bacteria deploy efflux to directly transport antibiotics from cytosol or periplasmic space of the bacterial cell to the external environment. It is believed that 5 to 10% of bacterial genes are involved in cellular transport, with efflux pumps accounting for a major fraction of these genes. Efflux pump capable of pumping out tertracycline from cytoplasm was initially reported in *E. coli*. Efflux pumps are categorized based on their structure, energy source, and transporter substrate into five major families. Energy source wise, ABC (ATP binding cassette family) family utilizes ATP hydrolysis while other four families, viz., 1. resistance nodulation cell division family – RND; 2. major facilitator superfamily- MFS; 3. small multidrug resistance family – SMR; and multidrug and toxic compound extrusion family MATE that are utilizing the proton motive force. Distribution wise, RND family is reported mainly in gram-negative bacteria but SMR, ABC, MFS, and MATE families are reported in both gram-positive and gram-negative bacteria (Webber & Piddock, 2003; Schindler & Kaatz, 2016).

Efflux pumps are structurally either single component transporter system or multiple component transporter systems. The multi-component efflux pump is a tripartite complex comprising of a periplasmic adaptor protein (*AcrA* and *MexA*) linked to the inner membrane by a fatty acid, an integral inner membrane transporter (*AcrB* and *MexB*) and an outer membrane channel (*TolC* in *E. coli*, OprM in *P. aeruginosa*). RND family efflux pumps have tripartite composition and have been reported in clinically significant AMR gram-negative bacteria, viz., *Escherichia coli* (*AcrB*), *Pseudomonas aeruginosa* (*MexB*), and *Salmonella typhimurium* (*AcrB*). MFS family efflux pumps are distributed in clinically significant Gram-positive bacteria, viz., *Staphylococcus aureus* (*NorA*) and *Streptococcus pneumoniae* (*PmrA*) (Poole, 2005; Lin et al., 2015).

Several compounds that act as efflux pump inhibitors have been reported. PAβN (phenyl arginine beta naphthylamide) is known to inhibit RND pumps involved in fluoroquinolone efflux and strongly decrease the MICs of a several antibiotics. Quinoline derivatives increase the intracellular concentration of radiolabelled norfloxacin and chloramphenicol. MBX2391 (pyranopyridine derivative) is a strong inhibitor of *AcrB* pump in *Enterobacteriaceae* and D13–9001 (pyridopyrimidine compound) inhibits efflux pumps in *P. aeruginosa* (Li & Nikaido, 2009; Sun et al., 2014).

4.3 Mechanism of AMR Through Changes in the Antibiotic Target Sites in Bacteria

Bacteria evade the detrimental action of antibiotics by modifying the target site of antibiotics in the bacterial cell and achieve it either by protecting the target site or modifying the target site.

4.3.1 Target Protection

Bacteria achieve resistance toward antibiotics such as tetracycline (*TetM*, *TetO*), fluoroquinolones (*Qnr*), and fusidic acid (*FusB* and *FusC*) through target protection mechanism. Tetracycline attaches to the bacterial ribosomes that result in antibacterial activity. *TetO* and *TetM* dislodges the tetracycline from its binding site by altering the bacterial ribosomal conformation, prevents rebinding of tetracycline, and allow resumption of protein synthesis by the bacterium. Bacteria evade quinolone resistance (*Qnr*) by competing with DNA gyrase and topoisomerase IV for the binding sites in bacterial DNA (Roberts, 1996; Roberts, 2005; Grossman, 2016).

4.3.2 Target Modification

Bacteria decrease the affinity of the antibiotic for the target site by modifying the target site (Wright, 2005). Enzymatic alterations of the antibiotic binding site result in resistance to macrolides, lincosamides, and phenicols. Erythromycin ribosomal methylation (*erm*) genes encode for an enzyme that catalyzes mono- and dimethylation of the 50S ribosomal subunit of bacterial ribosomes and confer resistance to macrolides, lincosamides, and streptogramin B. Similarly, *cfr* gene

encodes for enzyme of the S-adenosyl-L-methionine family that confers resistance to phenicols and lincosamides. Point mutation in the genes encoding the target site confers resistance to fluoroquinolones, rifampin, and oxazolidinone. Mutaions in the gyrase (gyrA-gyrB) and topoisomerse IV (parC-parE) confer fluoroquinolone resistance. Bacteria are capable of replacing the original target and evolve innovative targets that function biochemically similar to the original target but at the same time are uninhibited by the antibiotics. *S. aureus* acquires the *mecA* gene, which encodes the penicillin binding protein (PBP2a), which has a low affinity for all β-lactam antibiotics (penicillins, cephalosporins, and carbapenems). Bacteria bypass the antibiotic target by increasing the synthesis of antibiotic targets with an aim to overpower the antibiotic by increasing the amounts of targets available. Bacteria achieve resistance to trimethoprim-sulphamethoxazole through over production of dihydrofolate reductase (Markley & Wencewicz, 2018).

4.4 Biofilm-Based Antibiotic Resistance

Bacteria sheathed in biofilm resist aggressive antibiotic treatment. Subinhibitory concentrations of antibiotics such as aminoglycosides trigger biofilm formation in *E. coli* and *P. aeruginosa*. *S. aureus* encased in biofilm are protected from oxacillin, cefotaxime, and vancomycin due to the inability of these antibiotics to diffuse through biofilms. Exopolysaccharide, Psl, produced by *P. aeruginosa* during the early stages of biofilm development plays a role in resistance to ciprofloxacin, colistin, polymixin B, and tobramyscin. Extracellular DNA present in the bacterial biofilm matrix increases biofilm resistance to antimicrobial agents. Antibiotic destroying enzymes such as β-lactamases present in the biofilm matrix prevents β-lactam antibiotics from reaching the cellular targets (Hall & Mah, 2017; Cepas et al., 2019) (Table 3).

4.5 DNA Damaging Antibiotics and Induced Resistance

Induced resistance is a mechanism wherein the antibiotic is directly or indirectly involved in developing resistance. DNA damaging antibiotics are the key elements in fragmenting DNA and letting it add to the gene pool from where the other bacteria can acquire resistance determinants. SOS response is the response of bacteria at times of DNA damage. In this response, when the DNA is damaged, the microbial cell arrests its cell cycle and starts to repair the cell. The DNA damaging antibiotics such as quinolones will induce drug resistance with SOS response and also independent of SOS response (López et al., 2007). Quinolones and Beta lactams induce SOS response by activating Rec A-mediated response. In normal cells, the SOS-mediated response is repressed by LexA. In DNA affected cells, the single-stranded or affected DNA induce the RecA protein, and it inactivates the repressor Lex A. SOS response use DNA polymerases to repair the fragmented DNA. Since the DNA polymerases of bacteria are error prone which cause mutations that lead to antibiotic resistance. This process is

Table 3 Mechanisms of AMR: Antibiotic group - wise and genes involved in the resistance

Antibiotic group	Antibiotic Resistance mechanism and Genes responsible for the resistance	Reference
Aminoglycosides	acetyl-, phosphoryl- and nucleotidyl- transferases (enzymes that alter the antibiotic molecule) encoded by *aacA-aphD*, *ant3*, *aadA* genes 16S rRNA methylases (modification of target site)	Strommenger et al. (2003)
Phenicols	Chloramphenicol acetyltransferase encoded by *cat* genes, inactivate chloramphenicol (Enzymes that alter the antibiotic molecule). Ribosomal methylase encoded by *cfr* genes that modifies the ribosome so that florfenicol cannot bind, resulting in resistance (modification of target site) *cml* and *flo* genes encode for specific efflux pumps (Efflux Pumps to flush out antibiotics) *catA and cml* genes	Gerzova et al. (2014)
β-Lactams	β-lactamases, Extended-spectrum β-lactamases (ESBLs) enzymes (enzymes that destroy the antibiotic molecule). *AmpC*, *MIR-1, P99*, *CMY-2*, *FOX-1 ACT-1*, genes which cause hydrolysis of cephalosporins than the benzyl penicillin and cephamycins. *GC1*, *CMY-37* genes, which cause hydrolysis of cephalosporin, viz., ceftazidime. *PC1* gene, which causes hydrolysis of Benzylpenicillin (Penicillin) and not the Cephalosporins. *SHV-1, TEM-1*, *TEM-2* genes, which cause hydrolysis of benzylpenicillin and early cephalosporins. *SHV-2*, *TEM-3*, *PER-1*, *CTX-M-15*, *VEB-1* genes, which cause hydrolysis of oxyimino *β-lactams,* i.e., extended spectrum *cephalosporins, monobactams* (*cefotaxime, ceftazidime*, ceftriaxone, cefepime, aztreonam). *TEM-30*, *SHV-10* genes cause resistance to clavulanic acid, sulbactam, and tazobactam. *TEM-50* gene causes increased hydrolysis of oxyimino β-lactams combined with resistance to clavulanic acid, sulbactam, and tazobactam, i.e., Extended-spectrum cephalosporins monobactams. *PSE-1*, *CARB-3* genes causes increased hydrolysis of carbenicillin. *RTG-4* gene causes hydrolysis of carbenicillin, cefepime, and cefpirome *OXA-1*, *OXA-10* genes cause hydrolysis of cloxacillin or oxacillin. *OXA-11*, *OXA-15* genes hydrolyze cloxacillin or oxacillin and oxyimino β-lactams (ES cephalosporin) *OXA-23*, *OXA-48* genes hydrolyze cloxacillin or oxacillin and carbapenems. *CepA* gene hydrolyzes cephalosporins but this activity is inhibited by the addition of clavulanic acid but not aztreonam. *KPC-2*, *IMI-1*, *SME-1* genes cause hydrolysis of carbapenems, oxyimino b-lactams, cephamycins. *IMP-1*, *VIM-1*, *CcrA*, *IND-1* genes cause broad-spectrum	Ellington et al. (2007) Dallenne et al. (2010) Poirel et al. (2011)

(continued)

Table 3 (continued)

Antibiotic group	Antibiotic Resistance mechanism and Genes responsible for the resistance	Reference
	hydrolysis including carbapenems but not monobactams. *L1*, *FEZ-1, GOB- 1*, *CphA*, *Sfh-1, CAU-1*, genes cause preferential hydrolysis of carbapenems. *mecA* gene carried by a mobile genetic element SCC mec causes extremely low binding affinity to βlactam antibiotics Novel *mecA* homologue called mecLGA251 or mecC element	
Glycopeptides	*van* genes (modification of target site) *VanA*, *VanB*, *VanC*, *VanD*, *VanE*, *VanG*, *VanL*, *VanM and VanN*.	Bhatt et al. (2015)
Macrolides	Over 40 *erm* genes were reported so far. Four major classes, viz., erm(A), erm(B), erm(C), and erm(F). *mph* genes in Gram-negative; *vat* genes in Gram-positive bacteria (Enzymes that destroy the antibiotic molecule). *mef* genes (Efflux Pumps to flush out antibiotics) Methylase encoded by *erm* genes (modification of target site) *erm*, *ere* and *msr* genes	Strommenger et al. (2003) Volokhov et al. (2003)
Quinolones	Aminoglycoside-resistance enzyme, AAC (6′)-lb-cr (enzymes that alter the antibiotic molecule) *qnr* genes (Protection of target site) Mutations in *gyr* and/or *par* genes (modification of target site) *gyrA*, *gyrB*, *parC,* and *parE* Reserpine-sensitive efflux and an unspecific efflux (Efflux pumps to flush out antibiotics) qepAgene	Yang et al. (2013) Ciesielczuk et al. (2013) Goudarzi and Fazeli (2017)
Tetracyclines	*tet A*, *tet B*, *tet C*, *tet D*, *tet E*, *tet G*, *tet H*, *tet J*, *tet K* genes (Efflux Pumps to flush out antibiotics) The genes involved in the ribosomal protection are *tetM*, *tetO*, *tetS*, *tetT*, *tetW*, *tetW(P)*, *tet32*, *tet36*, *otrA* genes. The genes responsible for the inactivation of tetracyclines are *tetX*, *tet47* to *tet56* genes	Ng et al. (2001) Preena et al. (2020)
Co-trimoxazole	Over-expressing DHPS (Protection of target site) *dhfr* genes, *Sul* genes	Schmidt et al. (2001) Frank et al. (2007) Seyfried et al. (2010)

well studied in *E. coli* using quinolones and betalactams. In SOS independent response, which happens with ciprofloxacin like type II topoisomerase inhibitors, when DNA is affected, it activates RecBCD and RecFor pathways independent of SOS. When single-stranded DNA is annealed and mismatch repaired, it paves way to form mutS mutant (DNA repairing enzyme mutant). Trimethoprim also reduces the speed of replication of DNA and induce SOS response.

Possible precautions are

1. Avoid the use of DNA damaging antibiotics.
2. Use underclose supervision with appropriate dosage.
3. Avoid prophylactic usage of these drugs.

4.6 Oxidative Stress: Reason for Antibiotic Induced Resistance Flow

Oxidative stress is the main mechanism of destruction of bacterial cells by quinolones, beta lactams and aminoglycosides. These antibiotics create peroxides and other oxidative components for the killing of cells. When sub-optimal levels of antibiotics are used, it generates reactive oxygen species, which cause mutations in the DNA. Prolonged sub-optimal usage of these drugs as a prophylactory measure creates mutated population. Later, the natural selection selectively allows the resistant population to transfer their genes for survival. The reactive oxygen species (ROS) is harmful chemicals. They prevent oxygen-requiring organisms from respiration normally. Antibiotics at sub-lethal levels that generate ROS will enhance the MIC for a wide spectrum of antibiotics, regardless of the drug target. This kind of mutation is prevented by ROS scavengers such as thiourea. MDR efflux pumps resulted by *AcrAB* mutations are mediated through ROS. Super oxide responsive systems can directly damage DNA and cause accumulation of mutations. Oxidative damage to DNA activates SOS repair, which also enables then to have resistance to wide variety of antibiotics. This implies that treatment with an aminoglycoside can give resistance not only to aminoglycosides but also to other antibiotics.

4.7 Eliminating Commensals: Antibiotic-Induced Resistance Flow

Antibiotic treatment is assumed to eliminate the commensal organisms in the gut. Commensals are organisms dwelling in the gut atmosphere and help to digest certain nutrients in the food. The commensal bacteria also secrete some proteins such as bacteriocins that restrict survival and growth of other bacteria. The elimination of commensals opens intestinal niches to resistant flora. Vancomycin resistant Enterococci (VRE) survive and proliferate in the absence of commensals. Commensal microbes increase the mucosal innate immune responses thereby inhibit the VRE indirectly. VRE present in the gut colonize the small intestine, caecum, and colon when combination of metronidazole, neomycin, and vancomycin is administered.

4.8 Antibiotic Resistance Through Antibiotic Preparation

Antibiotic producing bacteria have antibiotic resistant genes to escape from the product. During the preparation process, the remains of bacterial resistance genes

are isolated and resultant of that retrieved from antibiotic preparations. It was demonstrated that during the antibiotic preparations such as macrolides (erythromycin), aminoglycosides (streptomycin, tobramycin, tylosin, oxytetracycline, vancomycin, and cefoxitin were observed to contain remnants of the bacterial DNA. The genes such as oxytetracycline resistant gene and streptomycin-3-phosphotransferase gene were detected in the preparations of streptomycin and tetracycline. These findings suggest that while taking antibiotics for treatment we also inadvertently take the resistant genes, and this may result in the resistance for the particular antibiotic during subsequent infection control therapy (Webb & Davies, 1993).

4.9 Cell Wall Deficient Bacteria and Antibiotic Resistance

Cell wall affecting antibiotics such as beta lactams and glycopeptides kill the bacteria through inhibiting the murine synthesis. These cells wall less bacteria get exposed to the external environment and eventually die. However, there are two important possibilities that might arise. The cell wall deficient bacteria, for their survival, can uptake the DNA from the external environment through transformation. The second possibility is that the cell wall deficient bacteria may leak their contents in to protoplasmic pool that provides other commensals the power to resist other antibiotics. The transfer of plasmids encoding antibiotic resistance was from Staphylococcus to *Bacillus subtilis* was demonstrated with short antibiotic treatment. *E. coli* were also known to absorb foreign DNA with antibiotic treatment. In intestinal tract, it is common for cell wall deficient forms to obtain and release DNA to the gene pool.

Novel resistance genes causing AMR are constantly being discovered around the world due to pathogenic bacteria's genetic plasticity. These new genes can be categorized as part of any of the mechanisms of action mentioned above for mediating resistance (Blair et al., 2015). Researchers all over the world are exploiting this newly discovered molecular pathway to develop creative tactics for future drug development in the fight against AMR (Ali et al., 2018). Recently, a strong tool known as the CRISPR-Cas editing tool was discovered, and it has a promising future in research for treating AMR bacteria by targeting the removal of resistance genes (Gomaa et al., 2014). Re-sensitizing bacteria to antibiotics is also being investigated as a potential alternate approach of managing AMR infections (Goh et al., 2015).

5 Conclusion

The extreme genetic plasticity of bacteria and the stress of survival in antibiotic-laden environment made bacteria devise several resistance mechanisms to evade the detrimental effects of antibiotics. The emergence of superbugs (multi drug resistant – (MDR); extremely drug resistant – (XDR); and pan drug resistant – (PDR)) resistant to currently available antibiotics is seriously limiting the therapeutic options in human and animal health care. The multiplicity of mechanisms involved in the resistance development in various classes of antibiotics indicates the complexity in determination

of the resistance phenotypes. However, the genetic methods especially PCR, qPCR, and microarray remain indispensable tools for detecting the genes responsible for antibiotic resistances. The characterization of the antibiotic resistance genes in different bacteria including pathogens will provide an insight to the molecular mechanisms involved in the phenotypic expression of antibiotic resistance. Understanding the existing antibiotic resistance mechanisms in bacteria provides opportunities to explore ways for novel drug discovery and mitigate drug resistance.

6 Cross-References

► Molecular Tools for Characterizing AMR Pathogens

References

Alekshun, M. N., & Levy, S. B. (2007). Molecular mechanisms of antibacterial multidrug resistance. *Cell, 128*(6), 1037–1050.

Ali, J., Rafiq, Q. A., & Ratcliffe, E. (2018). Antimicrobial resistance mechanisms and potential synthetic treatments. *Future Science OA, 4*(4), FSO290.

Ambler, R. P. (1980). The structure of β-lactamases. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences, 289*, 321.

Arzanlou, M., Chai, W. C., & Venter, H. (2017). Intrinsic, adaptive and acquired antimicrobial resistance in Gram-negative bacteria. *Essays in Biochemistry, 61*(1), 49–59.

Aslam, B., Wang, W., Arshad, M. I., Khurshid, M., Muzammil, S., Rasool, M. H., Nisar, M. A., Alvi, R. F., Aslam, M. A., Qamar, M. U., & Salamat, M. K. F. (2018). Antibiotic resistance: A rundown of a global crisis. *Infection and Drug Resistance, 11*, 1645.

Beceiro, A., Tomas, M., & Bou, G. (2013). Antimicrobial resistance and virulence: A successful or deleterious association in the bacterial world? *Clinical Microbiology Reviews, 26*, 185–230.

Benveniste, P., & Davies, J. (1973). Mechanisms of antibiotic resistance in bacteria. *Annual Review of Biochemistry, 42*, 471–506.

Bhatt, P., Sahni, A. K., Praharaj, A. K., Grover, N., Kumar, M., Chaudhari, C. N., & Khajuria, A. (2015). Detection of glycopeptide resistance genes in enterococci by multiplex PCR. *Medical Journal Armed Forces India, 71*(1), 43–47.

Blair, J., Webber, M. A., Baylay, A. J., Ogbolu, D. O., & Piddock, L. J. (2015). Molecular mechanisms of antibiotic resistance. *Nature Reviews Microbiology, 13*(1), 42–51.

Bush, K., & Jacoby, G. A. (2010). Updated functional classification of β-lactamases. *Antimicrobial Agents and Chemotherapy, 54*, 969–976. https://doi.org/10.1128/AAC.01009-09

Cepas, V., López, Y., Muñoz, E., Rolo, D., Ardanuy, C., Martí, S., & Soto, S. M. (2019). Relationship between biofilm formation and antimicrobial resistance in gram-negative bacteria. *Microbial Drug Resistance, 25*(1), 72–79.

Ciesielczuk, H., Hornsey, M., Choi, V., Woodford, N., & Wareham, D. W. (2013). Development and evaluation of a multiplex PCR for eight plasmid-mediated quinolone-resistance determinants. *Journal of Medical Microbiology, 62*(12), 1823–1827.

Coates, A. R., Halls, G., & Hu, Y. (2011). Novel classes of antibiotics or more of the same? *British Journal of Pharmacology, 163*(1), 184–194. https://doi.org/10.1111/j.1476-5381.2011.01250.x

Cox, G., & Wright, G. D. (2013). Intrinsic antibiotic resistance: mechanisms, origins, challenges and solutions. *International Journal of Medical Microbiology, 303*(6–7), 287–292.

Dallenne, C., Da Costa, A., Decre, D., Favier, C., & Arlet, G. (2010). Development of a set of multiplex PCR assays for the detection of genes encoding important beta-lactamases in Enterobacteriaceae. *The Journal of Antimicrobial Chemotherapy, 65*, 490–495.

Davies, J., & Davies, D. (2010). Origins and evolution of antibiotic resistance. *Microbiology and Molecular Biology Reviews, 74*, 417–433. https://doi.org/10.1128/MMBR.00016-10

Doi, Y., Wachino, J. I., & Arakawa, Y. (2016). Aminoglycoside resistance: The emergence of acquired 16S ribosomal RNA methyltransferases. *Infectious Disease Clinics, 30*(2), 523–537.

Drawz, S. M., & Bonomo, R. A. (2010). Three decades of β-lactamase inhibitors. *Clinical Microbiology Reviews, 23*(1), 160–201.

Ellington, M. J., Kistler, J., Livermore, D. M., & Woodford, N. (2007). Multiplex PCR for rapid detection of genes encoding acquired metallo-β-lactamases. *Journal of Antimicrobial Chemotherapy, 59*(2), 321–322.

Frank, T., Gautier, V., Talarmin, A., Bercion, R., & Arlet, G. (2007). Characterization of sulphonamide resistance genes and class 1 integron gene cassettes in Enterobacteriaceae, Central African Republic (CAR). *Journal of Antimicrobial Chemotherapy, 59*(4), 742–745.

Garneau-Tsodikova, S., & Labby, K. J. (2016). Mechanisms of resistance to aminoglycoside antibiotics: Overview and perspectives. *Medchemcomm, 7*(1), 11–27.

Gerzova, L., Videnska, P., Faldynova, M., Sedlar, K., Provaznik, I., Cizek, A., & Rychlik, I. (2014). Characterization of microbiota composition and presence of selected antibiotic resistance genes in carriage water of ornamental fish. *PLoS One, 9*(8), e103865.

Goh, S., Loeffler, A., Lloyd, D. H., Nair, S. P., & Good, L. (2015). Oxacillin sensitization of methicillin-resistant Staphylococcus aureus and methicillin-resistant Staphylococcus pseudintermedius by antisense peptide nucleic acids in vitro. *BMC Microbiology, 15*(1), 1–10.

Gomaa, A. A., Klumpe, H. E., Luo, M. L., Selle, K., Barrangou, R., & Beisel, C. L. (2014). Programmable removal of bacterial strains by use of genome-targeting CRISPR-Cas systems. *MBio, 5*(1), e00928–e00913.

Goudarzi, M., & Fazeli, M. (2017). Quinolone resistance determinants qnr, qep, and aac (6′)-Ib-cr in extended-spectrum B-lactamase producing Escherichia coli isolated from urinary tract infections in Tehran, Iran. *Medical Journal, 18*, 1–7.

Gray, D. A., & Wenzel, M. (2020). Multitarget approaches against multi-resistant superbugs. *ACS Infectious Diseases, 6*(6), 1346–1365.

Grossman, T. H. (2016). Tetracycline antibiotics and resistance. *Cold Spring Harbor Perspectives in Medicine, 6*(4), a025387.

Hall, B. G., & Barlow, M. (2005). Revised Ambler classification of β-lactamases. *Journal of Antimicrobial Chemotherapy, 55*(6), 1050–1051.

Hall, C. W., & Mah, T. F. (2017). Molecular mechanisms of biofilm-based antibiotic resistance and tolerance in pathogenic bacteria. *FEMS Microbiology Reviews, 41*(3), 276–301.

Kapoor, G., Saigal, S., & Elongavan, A. (2017). Action and resistance mechanisms of antibiotics: A guide for clinicians. *Journal of Anaesthesiology, Clinical Pharmacology, 33*(3), 300–305. https://doi.org/10.4103/joacp.JOACP_349_15

Kim, J., Jeon, S., Rhie, H., Lee, B., Park, M., Lee, H., & Kim, S. (2009). Rapid detection of extended spectrum β-lactamase (ESBL) for Enterobacteriaceae by use of a multiplex PCR-based method. *Infection and Chemotherapy, 41*(3), 181–184.

Kostyanev, T., & Can, F. (2017). The global crisis of antimicrobial resistance. In *Antimicrobial Stewardship* (pp. 3–12). Academic Press.

Li, X. Z., & Nikaido, H. (2009). Efflux-mediated drug resistance in bacteria. *Drugs, 69*(12), 1555–1623.

Lin, J., Nishino, K., Roberts, M. C., Tolmasky, M., Aminov, R., & Zhang, L. (2015). Mechanisms of antibiotic resistance. *Frontiers in Microbiology, 6*, 34.

Lin, J., Zhou, D., Steitz, T. A., Polikanov, Y. S., & Gagnon, M. G. (2018). Ribosome-targeting antibiotics: modes of action, mechanisms of resistance, and implications for drug design. *Annual Review of Biochemistry, 87*, 451–478.

López, E., Elez, M., Matic, I., & Blázquez, J. (2007). Antibiotic-mediated recombination: Ciprofloxacin stimulates SOS-independent recombination of divergent sequences in Escherichia coli. *Molecular Microbiology, 64*(1), 83–93.

Markley, J. L., & Wencewicz, T. A. (2018). Tetracycline-inactivating enzymes. *Frontiers in Microbiology, 9*, 1058.

Munita, J. M., & Arias, C. A. (2016). Mechanisms of antibiotic resistance. *Microbiology spectrum, 4*(2), 4–2.

Ng, L. K., Martin, I., Alfa, M., & Mulvey, M. (2001). Multiplex PCR for the detection of tetracycline resistant genes. *Molecular and Cellular Probes, 15*, 209–215.

Nordmann, P., Dortet, L., & Poirel, L. (2012). Carbapenem resistance in Enterobacteriaceae: Here is the storm! *Trends in Molecular Medicine, 18*(5), 263–272.

Pfeifer, Y., Cullik, A., & Witte, W. (2010). Resistance to cephalosporins and carbapenems in Gram-negative bacterial pathogens. *International Journal of Medical Microbiology, 300*(6), 371–379.

Poirel, L., Walsh, T. R., Cuvillier, V., & Nordmann, P. (2011). Multiplex PCR for detection of acquired carbapenemase genes. *Diagnostic Microbiology and Infectious Disease, 70*(1), 119–123.

Poole, K. (2005). Efflux-mediated antimicrobial resistance. *Journal of Antimicrobial Chemotherapy, 56*(1), 20–51.

Preena, P. G., Swaminathan, T. R., Rejish Kumar, V. J., & Bright Singh, I. S. (2020). Unravelling the menace: detection of antimicrobial resistance in aquaculture. *Letters in Applied Microbiology, 71*(1), 26–38.

Prestinaci, F., Pezzotti, P., & Pantosti, A. (2015). Antimicrobial resistance: A global multifaceted phenomenon. *Pathogens and Global Health, 109*(7), 309–318. https://doi.org/10.1179/2047773215Y.0000000030

Ramirez, M. S., Nikolaidis, N., & Tolmasky, M. (2013). Rise and dissemination of aminoglycoside resistance: The aac (6′)-Ib paradigm. *Frontiers in Microbiology, 4*, 121.

Roberts, M. C. (1996). Tetracycline resistance determinants: Mechanisms of action, regulation of expression, genetic mobility, and distribution. *FEMS Microbiology Reviews, 19*(1), 1–24.

Roberts, M. C. (2005). Update on acquired tetracycline resistance genes. *FEMS Microbiology Letters, 245*(2), 195–203.

Schindler, B. D., & Kaatz, G. W. (2016). Multidrug efflux pumps of Gram-positive bacteria. *Drug Resistance Updates, 27*, 1–13.

Schmidt, A. S., Bruun, M. S., Dalsgaard, I., & Larsen, J. L. (2001). Characterization of class 1 integrons associated with R-plasmids in clinical Aeromonas salmonicida isolates from various geographical areas. *The Journal of Antimicrobial Chemotherapy, 47*, 735–743.

Seyfried, E. E., Newton, R. J., Rubert, K. F., Pedersen, J. A., & McMahon, K. D. (2010). Occurrence of tetracycline resistance genes in aquaculture facilities with varying use of oxytetracycline. *Microbial Ecology, 59*, 799–807.

Strommenger, B., Kettlitz, C., Werner, G., & Witte, W. (2003). Multiplex PCR assay for simultaneous detection of nine clinically relevant antibiotic resistance genes in Staphylococcus aureus. *Journal of Clinical Microbiology, 41*(9), 4089–4094.

Sun, J., Deng, Z., & Yan, A. (2014). Bacterial multidrug efflux pumps: Mechanisms, physiology and pharmacological exploitations. *Biochemical and Biophysical Research Communications, 453*, 254–267.

van Hoek, A. H., Mevius, D., Guerra, B., Mullany, P., Roberts, A. P., & Aarts, H. J. (2011). Acquired antibiotic resistance genes: An overview. *Frontiers in Microbiology, 2*, 203. https://doi.org/10.3389/fmicb.2011.00203

Volokhov, D., Chizhikov, V., Chumakov, K., & Rasooly, A. (2003). Microarray analysis of erythromycin resistance determinants. *Journal of Applied Microbiology, 95*(4), 787–798.

Webb, V., & Davies, J. (1993). Antibiotic preparations contain DNA: A source of drug resistance genes? *Antimicrobial Agents and Chemotherapy, 37*, 2379–2384.

Webber, M. A., & Piddock, L. J. V. (2003). The importance of efflux pumps in bacterial antibiotic resistance. *Journal of Antimicrobial Chemotherapy, 51*, 9–11.

World Health Organization. (2020). Lack of new antibiotics threatens global efforts to contain drug-resistant infections. *New release Geneva*.

Wright, G. D. (2005). Bacterial resistance to antibiotics: Enzymatic degradation and modification. *Advanced Drug Delivery Reviews, 57*(10), 1451–1470.

Wright, G. D. (2016). Antibiotic adjuvants: Rescuing antibiotics from resistance. *Trends in Microbiology, 24*(11), 862–871.

Yang, H., Duan, G., Zhu, J., Zhang, W., Xi, Y., & Fan, Q. (2013). Prevalence and characterisation of plasmid mediated quinolone resistance and mutations in the gyrase and topoisomerase IV genes among Shigella isolates from Henan, China, between 2001 and 2008. *International Journal of Antimicrobial Agents, 42*, 173–177.

Zimdahl, R. L. (2015). *Six chemicals that changed agriculture*. Academic Press.

Avenues in the Determination of AMR in Human Health

Dhiviya Prabaa Muthuirulandi Sethuvel,
Yamuna Devi Bakthavatchalam, and Balaji Veeraraghavan

Contents

Abstract

Resistance to antibiotics is a silent pandemic threatening the success of modern medicine. Multiple resistance mechanisms exist in bacteria that confer resistance to various antibiotics. It is often characterized by acquiring antimicrobial-resistant

D. P. Muthuirulandi Sethuvel · Y. D. Bakthavatchalam · B. Veeraraghavan (✉)
Department of Clinical Microbiology, Christian Medical College, Vellore, Tamil Nadu, India
e-mail: vbalaji@cmcvellore.ac.in

M. P. Mothadaka et al. (eds.), *Handbook on Antimicrobial Resistance*,
https://doi.org/10.1007/978-981-19-9279-7_29

genes (ARG) by means of horizontal gene transfer (HGT), tailed by the expansion of clones and genetic elements involved in its maintenance. Overuse or inappropriate use of antibiotics increases antibiotic resistance, as well as disease severity, hospital stay length, adverse effects, death risk, healthcare costs, and hospital readmission. The wide application of antimicrobial drugs in managing poultry and animal infections is well known. Antimicrobials used in food production promote selective pressure, leading to AMR in humans. In the absence of suitable scrutiny, it is difficult to evaluate the extent and distribution of this disease. Furthermore, the current national measures for the regulation of antimicrobial use have not dealt with this issue. Existing efforts do not appear adequate to deal with the issue as it arises. In this chapter, clinically significant pathogens were examined for their resistance mechanisms and their health and economic implications. The primary risks of AMR and the control measures in place are discussed.

Keywords

Antimicrobial resistance · Antibiotic consumption · WHO priority organisms · Newer agents · One Health approach · Genomics

1 Introduction

In the present-day scenario, unresponsiveness of microbes to drugs, that is, antimicrobial resistance (AMR), is the leading global health crisis causing 1.27 million mortalities per annum, out of which 15.75% were attributed to drug-resistant *Escherichia coli* (Baekkeskov et al., 2020; Murray et al., 2022). By the year 2050, AMR is predicted to result in ten million deaths (O'Neill, 2016). There are three major factors contributing to this crisis: (1) overemployment of drugs resulting in the development and selection of drug-obdurate pathogens; (2) human mobility facilitates global bacterial spread; and (3) excessive use of antimicrobials can contribute to strong selective pressure resulting in microbial evolution (Michael et al., 2014). Multidrug-resistant bacteria (MDR bacteria) are found in all niches of animals, humans, and the environment, and the pathogens are interconnected within this triad (Aslam et al., 2018). In order to improve surveillance based on facts, the WHO introduced "*Global Antimicrobial Resistance Surveillance System (GLASS)*" in 2015 (Gandra et al., 2020). Antimicrobial resistance surveillance provides the basis for detecting emerging trends, monitoring interventions, and developing new drugs (Kostyanev & Can, 2017). An understanding of how antimicrobial resistance develops, evolves, and propagates is critical for the development of effective ways of tracking resistance spread and optimizing treatment. A recent advancement in genome sequencing technologies has revolutionized diagnosis and antimicrobial resistance surveillance (Hendriksen et al., 2019). By combining this technology with bioinformatics tools and online databases, it is possible to provide rich genome information about infectious pathogens, which can be used to combat antimicrobial resistance (Hendriksen et al., 2019).

The impact of AMR on public health and risk factors associated with AMR will be discussed in this chapter. The current regulatory measures and potential control strategies relating to this issue with a particular emphasis on novel antibiotics will be covered.

2 Antimicrobial Resistance: A Global Crisis

Antibiotic-resistant diseases afflict more than two million people annually, killing at least 23,000 individuals, according to the "*Centers for Disease Control (CDC)*" of the United States (CDC, 2013). According to estimates, MDR microorganisms lead to 25,000 fatalities per year. The key pathogens commonly known as "ESKAPE" include *Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa,* and *Enterobacter* spp. (Prestinaci et al., 2015). Resistance to antibiotics can either arise spontaneously or through selective pressure in response to antibiotic exposure and other chemicals that kill or inhibit bacteria (Halpern, 2009; Baekkeskov et al., 2020). AMR is spread by a variety of variables, including social, economic, ecological influences, and behavioral patterns of humans (Littmann & Viens, 2015). Antibiotic resistance leads to prolonged hospital stays and an increase in healthcare costs.

Despite the fact that the current system has significant shortcomings, the WHO Report on AMR investigations of surveillance networks at regional and at global levels in 2014 indicated the possibility of the issue in many parts of the world. Over the last decade, global antibiotic consumption has risen steadily to 65% (expressed in specified doses per day) in 76 countries (Klein et al., 2018). Additionally, epidemiological studies have linked antibiotic consumption directly to bacterial resistance (Ventola, 2015). Studies have demonstrated that bacteria exposed to subtherapeutic antimicrobial doses become resistant through genetic alterations, such as altered expressions of gene, HGT, and mutagenesis, making treatment more difficult (Viswanathan, 2014; Wistrand-Yuen et al., 2018).

There are four major areas where antibiotic resistance can develop: human healthcare, animal husbandry, animal production, and the environment. Everyone agrees that the primary cause of AMR is human and farm animal intake of antibiotics (Baekkeskov et al., 2020). The annual antibiotic consumption in the animal industry is at least 60,000 tons (Laxminarayan & Chaudhury, 2016). This will significantly rise in the near future as consumer demand for meat and other animal products rises. This hastens the advent of obduracy to drugs by raising the evolutionary selection burden on different microbes (Wu, 2017). Information on the employment of antibiotics in the animal sector is hard to come by because of insufficient surveillance systems (Wu, 2017).

India, the largest antibiotic-consuming nation in the world, is observed to show a drop in the employment of ampicillin and co-trimoxazole and at the same time show enhanced levels of quinolone and carbapenem consumption (Van Boeckel et al., 2014). India sells more over-the-counter carbapenem than any other country in the world, which increases carbapenem resistance (Laxminarayan & Chaudhury, 2016).

Over-prescription is a result of diagnostic ambiguity, particularly when the clinical profile of a viral or bacterial etiology is identical. Cost-effective diagnostics can give clinicians instant input to help them make policy decisions.

The Development of AMR in Gram-Positive Bacteria of WHO Priority: Three pathogens were identified as high-priority pathogens: "methicillin-resistant *Staphylococcus aureus* (MRSA)," *Staphylococcus aureus,* which is susceptible at the intermediate level to or resistant to vancomycin, and vancomycin-resistant *Enterococcus faecium*. *Streptococcus pneumoniae*, a pathogen that is resistant to penicillin, was classified as medium priority.

2.1 *Staphylococcus aureus*

S. aureus infections are common in nosocomial and community settings. The probability of developing infection with *S. aureus* colonization is high. A substantial proportion of humans have been colonized with *S. aureus*, responsible for bacteremia, infections related to skin and soft tissue (SSTIs), pneumonia, and infective endocarditis (Hamdan-Partida et al., 2010). Recently, treating *S. aureus* infections has become progressively challenging due to the enhanced capability of bacteria to grow obduracy to antimicrobials. Since the early 1940s, when penicillin resistance was first described, subsequent resistance to beta-lactams, aminoglycosides, tetracyclines, fluoroquinolones, vancomycin, linezolid, and daptomycin has been reported (Table 1).

2.2 Penicillin-Resistant *S. aureus* (PRSA)

Penicillin G was introduced in the early 1940s and was found to be effective against all staphylococcal infections (Lowy, 2003). The *bla*Z gene is responsible for imparting penicillin obduracy to *S. aureus*, which encodes beta-lactamase that hydrolyzes beta-lactam rings. Since the 1960s, PRSA has also been reported in community settings (Chambers & Deleo, 2009). There has been an increase in the prevalence of PRSA, and almost 90% of both community and hospital strains were penicillin-resistant (Gardam, 2000).

2.3 Methicillin or Oxacillin-Resistant *Staphylococcus aureus* (MRSA)

Methicillin was first used in clinical settings to treat PRSA infections in the 1960s. The modified form of penicillin-binding protein PBP2a was produced due to mecA gene in MRSA cells that hinders adherence of beta-lactam antibiotics. In general, the MRSA strains are unresponsive to entire beta-lactams, beta-lactam combinations, carbapenems, and monobactams, but not cephalosporins with anti-MRSA activity. The Staphylococcal Cassette Chromosome (SCCmec) carries mecA, the mobile genetic component

Table 1 Antimicrobial resistance mechanism in *Staphylococcus aureus*

Antibiotic	Mechanism of resistance	Gene responsible
Penicillin	Hydrolyze beta-lactam ring	Blaz gene on plasmid
Beta-lactams (cephalosporin, carbapenem, monobactams)	Low affinity to PBP2	*mec*A encoded PBP2a
Aminoglycosides	Enzymes that alter aminoglycoside	*(aph (3′)-IIIa, aac (6′)-Ie-aph (2″),* and *ant (6)-Ia*
Macrolides and clindamycin	Methylation of binding sites in ribosomes and efflux pumps	*ermA, ermB, ermC, ermF, msr*A
Tetracycline	Modification of binding sites and efflux pumps	*tet*K, *tet*L, *tet*M, *tet*O
Fluoroquinolones	Topoisomerase IV and DNA gyrase mutations	*par*C, *gyr*A
Trimethoprim-sulfamethoxazole	Mutation in dihydrofolate reductase (DHFR) gene	*dfrA* (*dfrS1*), *dfrG,* and *dfrK*
Rifampicin	RNA polymerase gene mutations	*rpo*B
Glycopeptides (vancomycin, teicoplanin)	Cell wall solidification, VISA	Unclear
	Altered cell wall antecedent (VRSA)	*Van* A
Lipopeptides (daptomycin)	Altered charge of the cell membrane	*mpr*F gene
Oxazolidinones (linezolid)	Modification of binding sites	Point mutation in 23S rRNA or ribosomal proteins L3/L4
	23S rRNA subunit methylation	*Cfr* gene – Plasmid-mediated

(Jensen & Lyon, 2009). There are 11 different types of SCC*mec* types (Baig et al., 2020). Consequently, multidrug-resistant *S. aureus* is characterized by this SCC*mec* element's ability to acquire and accumulate resistance genes in chromosomes. Typically, MRSA exhibits resistance to aminoglycosides, tetracyclines, and erythromycin. There has been a report of resistance to oxacillin mediated by a *mec*A homolog known as *mec*C (Ford, 2017). A 70% similarity was found between mecC and mecA. The gene mecC encoded PBP2a exhibited 63% identity to mecA encoded PBP2a. In a study by Cartwright and colleagues, 89% of mecC-bearing MRSA were susceptible to oxacillin, suggesting that induction by cefoxitin is an effective adjunct in classifying all strains as resistant to the drug (Cartwright et al., 2013).

Borderline oxacillin-resistant *S. aureus* (BORSA) is poorly characterized; oxacillin MICs are often found to be 1–8 g/ml, and unlike MRSA, they do not have a modified form of the PBP2a, which is expressed by the mecA/C genes (Hryniewicz & Garbacz, 2017). Occasionally, point mutations in PBP genes or hyperproduction of beta-lactamases contribute to oxacillin resistance. It is neither true that BORSA strains are resistant to methicillin nor that they are truly susceptible to it. However, they are commonly misinterpreted, posing a clear epidemiological and therapeutic risk.

2.4 Vancomycin Heteroresistance

In view of the enhanced application of glycopeptides in clinical settings in recent years, the vancomycin susceptibility of MRSA has declined significantly. Studies on "heteroresistant vancomycin-intermediate *S. aureus*" (hVISA) and "*vancomycin-intermediate S. aureus*" (VISA) were well documented way back in 1997. The presence of MRSA isolates within the hVISA phenotype is more common than in VISA. With reference to routine antimicrobial susceptibility test, hVISA is susceptible to vancomycin; however, it comprises a subset of cells with MICs intra the VISA range. These bacteria are present in subpopulations with a frequency of 1×10^6 and exhibit varying degrees of vancomycin resistance (Liu & Chambers, 2003). For detecting hVISA strains, however, no precise definition, breakpoint, or optimal method exists. A comprehensive review and meta-analysis of global data on hVISA and VISA revealed that the pooled prevalence for hVISA is 6% and for VISA it is 3% (Zhang et al., 2015). "The standard method employed for proper documentation of hVISA subpopulation is the "population analysis profile (PAP)" (Wootton et al., 2001). The area under the curve (AUC) is computed using this method and compared to the hVISA reference strain Mu3 (ATCC 700698). This quantitative technique allowed for the definition of hVISA and VSSA with PAP/AUC ratios of 0.9 and 0.9, respectively.

The determinants of hVISA and VISA at the molecular level are still not well understood. Recent advancements in DNA sequencing technology have revealed some of the mechanisms behind hVISA/VISA. Chromosome mutations altered cellular biogenesis. The marks include modified cell wall metabolism, resulting in enhanced production of D-Ala-D-Ala and cell wall turnover, thickening of cell walls, and diminished peptidoglycan cross-linking (Hiramatsu et al., 2014). Several mutations were linked to hVISA/VISA phenotypes. Vancomycin resistance-associated two-component system (vraSR-TCS), glycopeptide resistance detection (graSR) TCS, walKR TCS, teicoplanin resistance-associated operon (tcaRAB), and RNA polymerase encoding rpoB gene were the most representative systems implicated in the mechanism of hVISA/VISA (Bakthavatchalam et al., 2019; Howden et al., 2014). RpoB or one of these regulatory gene mutations may have a role in the development of hVISA (Howden et al., 2014).

2.5 Vancomycin-Resistant *S. aureus*

The acquisition of *van*A gene results in vancomycin resistance in *S. aureus* (Limbago et al., 2014). As shown in Table 2, cases of VRSA have been reported.

3 Reduced Daptomycin Susceptibility

Daptomycin nonsusceptibility is strongly correlated with hVISA/VISA (Cui et al., 2006). A study found that 15% of hVISAs and 38% of VISAs were nonsusceptible to daptomycin (Kelley et al., 2011). hVISA and VISA exhibit cross-resistance

Table 2 Isolates of VRSA reported across the world

Year of isolation	Country	Source
2002	Michigan, USA	Ulcers and catheter
2002	Pennsylvania, USA	Plantar ulcer
2004	New York, USA	Urine
2005	Michigan, USA	Toe wound
2005	Michigan, USA	Surgical-site wound
2005	Michigan, USA	Plantar ulcer
2006	Michigan, USA	Triceps wound
2007	Michigan, USA	Toe wound
2007	Michigan, USA	Wound infection
2009	Michigan, USA	Plantar foot wound
2010	Delaware, USA	Wound drainage
2010	Delaware, USA	Vaginal swab
2012	Delaware, USA	Foot wound
2005	Kolkata, India	Pus
2011	Mashhad, Iran	Tracheal wash
2012	Tehran, Iran	Foot ulcer
2007	Tehran, Iran	Soft-tissue wound
2007	Tehran, Iran	Post-cardiac surgery wound

through multiple pathways. In hVISA/VISA strains, subsequent mutations in *wal*KR TCS and *mpr*F result in reduced daptomycin susceptibility.

3.1 Antimicrobial Resistance in *Enterococcus* Sp.

Enterococcus faecalis and *Enterococcus faecium* are more commonly involved in causing infections. Vancomycin-resistant *E. faecium* (VREfm) has significantly increased during the past few decades (Hollenbeck & Rice, 2012). A limited number of treatment options and poor outcomes are associated with invasive VRE infections. Resistance mechanisms of enterococci are listed in Table 3 (Kakoullis et al., 2021).

3.2 Resistance to Ampicillin and Cephalosporins

E. faecalis is now far less likely to develop beta-lactam resistance than *E. faecium* does, especially resistance to ampicillin (Guzman Prieto et al., 2016). Mutations in PBP5 confer ampicillin resistance in *E. faecium* and *E. faecalis* (Miller et al., 2014). Intrinsic aminoglycoside resistance in *E. faecalis* and *E. faecium* necessitates susceptibility testing to high-level aminoglycosides, which helps in understanding the synergistic action of the ampicillin amalgamations with elevated levels of aminoglycosides.

Table 3 Antimicrobial resistance mechanism in *Enterococcus* sp.

Antibiotic	Mechanism of resistance	Gene responsible
Penicillin/ampicillin	Decreased affinity for PBPs	Mutation in pbp5 Blaz
Cephalosporins and carbapenems	Decreased affinity for PBPs	–
Aminoglycosides (gentamicin, streptomycin)	Aminoglycoside-modifying enzyme (AMEs)	AAC, ANT, APH
Macrolides (erythromycin)	Target modification, inhibiting ribosomal translation	*erm*A, *erm*B, *vat*D (satA), *vat*H, *vgb*A, *vat*E (*sat*G)
Tetracyclines (tetracycline, minocycline)	Ribosomal methylation	*tet*K, *te*tL, *te*tM, *tet*O, and *tet*S
Quinolones (levofloxacin)	Targeted mutation at drug-binding sites	*gyr*A and B, *par*C
Ansamycins (rifampicin)	Target modification of DNA-dependent RNA polymerase	*rpo*B
Glycopeptides (vancomycin/ teicoplanin)	D-alanine-D-alanine moiety replaced with D-alanine-D-lactose	VanA, VanB, VanC, VanD, VanE, VanG, VanL, VanM, VanN
Lipopeptides (daptomycin)	Altered cell membrane	LiaFSR, YycFG Cls, GdpD
Oxazolidinones (linezolid)	Changes at the binding location	23S rRNA gene mutation, cfr, optrA, poxtA

3.3 Vancomycin Resistance

Enterococci develop vancomycin resistance when the alteration in amino acids takes place from D-Ala-D-Ala to D-Ala-D-lactate or D-Ala-D-serine. Multiple variants of the van operon confer vancomycin resistance (Table 4) (Gorrie et al., 2019). Among them, *van*A and *van*B dominate exist in the transposons Tn1546 and Tn1549, respectively (Guzman Prieto et al., 2016). VanB solely provides resistance to vancomycin, as opposed to vanA, which also imparts resistance to teicoplanin. Other conjugative plasmids, viz., Inc18-, pRUM-, pMG1-, and pHT-like, are also in charge of their extensive dispersion in addition to the vanA-carrying transposon Tn1546 (Wardal et al., 2017).

3.4 Linezolid Resistance

Linezolid unresponsiveness is conferred by the mutation G2576T in domain V of 23S rRNA or by attaining genes, viz., cfr, *optr*A, and *poxt*A (Bakthavatchalam et al., 2021). In the 23S rRNA, methyltransferase (designated cfr, for chloramphenicol-florfenicol resistance) methylates the adenine at position 2503 (A2503) (Kehrenberg et al., 2005). The cfr dissimilarities in enterococci such as A, B, C, D, and E were

Table 4 Geno and phenotypic characterization of vancomycin resistance operon in *Enterococcus* sp.

Van operon	Level of resistance	
	Vancomycin	Teicoplanin
VanA	High	High
VanB	High (variable)	Susceptible
VanC	Low	Susceptible
VanD	Variable	Variable
VanE	Low to moderate	Susceptible
VanG	Low	Susceptible
VanL	Low	Susceptible
Vann	High	High
Vann	Low	Susceptible

well documented. On the contrary, the unresponsiveness of enterococci to linezolid is uncommon worldwide with an occurrence of 1% or less (ATLAS database; https://atlas-surveillance.com). The current rise in linezolid-resistant enterococci can be attributed to the gene optrA, which has been widely identified as one of the transferable linezolid resistance determinants. OptrA encodes a ribosome protection protein that gives resistance to tedizolid in addition to phenicols and oxazolidinones.

3.5 AMR Mechanisms in WHO Priority Gram-Negative Organisms

Due to their near-universal resistance to antibiotics currently on the market, Gram-negative pathogens are especially worrisome. Enterobacterales, *Pseudomonas aeruginosa,* and *Acinetobacter baumannii* cause most Gram-negative infections in healthcare settings. One of the important developments is that enhanced levels of MDR Gram-negative infections are becoming a common occurrence in the community (Ventola, 2015). The main resistance mechanism in Gram-negative pathogens includes enzymes that are involved inactivating antibiotic and acquiring mobile genetic elements (MGEs) with genes of drug obduracy genes, efflux, and porin loss (Breijyeh et al., 2020).

3.6 Enterobacterales

Extended spectrum beta-lactamase (ESBLs)-producing Enterobacterales cause 26,000 hospital-acquired infections (HAIs) and 1700 deaths per year (Ventola, 2015). The TEM and SHV variants genes that encrypt class A beta-lactamase impart obduracy to cephalosporins, monobactams, and penicillins. While CTX-M variants (CTX-Munich) hydrolyze cefotaxime more effectively than ceftazidime. ampC beta-lactamase hydrolysis and cephalosporins are not inhibited by tazobactam, clavulanate, and sulbactam (Breijyeh et al., 2020).

Carbapenem-resistant Enterobacteriaceae (CRE) are unresponsive to the majority of the antibiotics (Ventola, 2015; Breijyeh et al., 2020). The choice of treatments that we are left with are polymyxins, tigecycline, fosfomycin, and aminoglycosides. About 600 people die every year from the infections caused by carbapenem-resistant *E. coli* and *Klebsiella* species (CDC, 2013; Ventola, 2015). There are two types of CREs: carbapenemase-producing CREs, which carry carbapenemase-encoding genes on the mobile elements, and non-carbapenemase-producing CREs, mainly mediated by the chromosomes (van Duin, 2017; Breijyeh et al., 2020). In the family of Enterobacteriaceae, the five important carbapenemases are imipenemase, *K. pneumoniae* carbapenemase, New Delhi metallo-beta lactamases, oxacillinase, and Verona integron-encoded metallo-β-lactamase; in short, IMP, KPC, NDM, OXA-48, and VIM, respectively. Some species of Enterobacteriaceae such as *Morganella morganii*, *Providencia* spp., and *Proteus* spp. have intrinsic imipenem resistance (Breijyeh et al., 2020).

3.7 *Acinetobacter baumannii*

Many antibiotics, including carbapenems, are no longer effective against *Acinetobacter* species (Ventola, 2015). The extended resistome and virulome, formation of biofilm, and resistance to host immune effectors have led to the classification of *A. baumannii* as one of the most dangerous ESKAPE pathogens (Vrancianu et al., 2020). *A. baumannii* is frequently associated with hospital-acquired infections and develops resistance through multiple resistance mechanisms such as production of beta-lactamases, efflux, and porin. Acinetobacter-derived cephalosporinase are ampC variants to which penicillins and cephalosporins are ineffective but not cefepime or carbapenems. Presence of class D beta-lactamase OXA-23 and/or NDM confers resistance to almost all antibiotics.

3.8 *Pseudomonas aeruginosa*

Difficult-to-treat *P. aeruginosa* strains are proven to lack activity against cephalosporins, aminoglycosides, fluoroquinolones, and carbapenems (CDC, 2013). As a result, treating *P. aeruginosa* infections has become very challenging. *P. aeruginosa* is a well-known opportunistic pathogen in people with cystic fibrosis and immunocompromised people. In addition to chromosome-mediated overexpression of efflux pumps and outer membrane impermeability, resistance genes may also be acquired (Pang et al., 2019). *P. aeruginosa*'s adaptive resistance involves the development of biofilms in infected lungs.

Moreover, *P. aeruginosa* has an inducible ampC gene that inactivates beta-lactam antibiotics. Several studies have shown that class C beta-lactamases inhibit anti-pseudomonal cephalosporins. Six different metallo beta-lactamases have been reported in *P. aeruginosa*: VIM, NDM, IMP, DIM (Dutch imipenemase), FIM (Florence imipenemase), GIM (Germany imipenemase), and SPM (Sao Paulo

Table 5 Prevailing antimicrobial resistance mechanisms, associated mobile genetic elements, and clonal types from India

Organisms	Acquired AMR genes	Mobile genetic elements	Clonal types
E. coli	$bla_{\text{TEM-1B}}$, $bla_{\text{CTX-M15}}$, $bla_{\text{CMY-42}}$, $bla_{\text{NDM-5}}$, $bla_{\text{OXA-181}}$	IncFII, IncX3 plasmid IS6	ST410, ST405, ST167, ST131
K. pneumoniae	$bla_{\text{TEM-1}}$, $bla_{\text{CTX-M-15}}$, bla_{NDM}, $bla_{\text{OXA48-like}}$	IncF, ColKP3 plasmid ISEcp1, ISSsu9 Tn*4401*,	ST231, ST11, ST14, ST15, ST147, ST2096
A. baumannii	bla_{PER} $bla_{\text{OXA-23}}$, $bla_{\text{NDM-1}}$	*repAci6* plasmid ISAba1, ISAba125 Tn*2006* AbaR4 resistance island	International clone 2 (ST208, ST451, ST195)
P. aeruginosa	bla_{VIM}, $bla_{\text{NDM-5}}$	Class 1 integron	ST357, ST235, ST111

IS, insertion sequence; *Tn*, transposon

metallo-β-lactamase) (Hong et al., 2015). These assimilated genes are distributed primarily through integrons (Pang et al., 2019). The multifaceted antibiotic resistance strategy of *P. aeruginosa* makes conventional antibiotics ineffective and continues to be challenging to treat patients. It is becoming increasingly important to develop alternative therapeutic strategies. As a result, combination therapies are likely to be the most effective treatment. Table 5 summarizes the prevailing resistance mechanisms seen in *P. aeruginosa* isolates.

4 Need for Newer Antimicrobial Agents

Gram-negative bacteria possess multilayered resistance mechanisms, making treatment challenging, and there is a severe dearth of novel treatment options. There is a severe dearth of antibiotics with a comprehensive spectrum of coverage (WHO, 2017). The novel beta-lactam/beta-lactamase inhibitors, namely, ceftazidime–avibactam, cefiderocol meropenem–imipenem–relebactam, and vaborbactam, are game changers in managing CRE infections. However, many low- and middle-income group countries remain burdened by the accessibility and affordability of these new antibiotics. Obduracy to ceftazidime-avibactam is described in clinical isolates (Aitken et al., 2016; Humphries et al., 2015). The challenge of treating metallo beta-lactamases-producing Gram-negative pathogens remains unresolved. Antimicrobial resistance might be aggravated by the current COVID-19 pandemic.

In the past, only one out of five infectious disease drugs made it through the initial phase of human testing and received FDA approval. Only a limited percentage of patients are eligible to participate in conventional clinical studies, making it especially difficult to develop effective antibiotics against highly drug-obdurate bacteria. According to the most recent assessment of the pipeline, 43 novel antibiotics are

currently under development and are likely to treat numerous unresponsive bacteria. Despite this, it is obvious that not enough antibiotics are being developed to meet the demands of both current and future patients, especially given the inevitable rise of antibiotic resistance.

5 Rapid Diagnostic Tests

By offering quick and precise diagnosis, rapid diagnostic tests (RDTs) have transformed the management of infectious diseases. Several advanced methods are available for the detection of pathogens. One of the most often used tools for classifying the bacterial genus and species is "matrix-assisted laser desorption/ionization-time of flight (MALDI-TOF) mass spectrometer." Susceptibility testing combined with rapid phenotypic identification has recently become available for detecting pathogens and reporting antimicrobial resistance (Table 6). Among them, accelerate phenosystem provides rapid and reliable antimicrobial susceptibility testing within 7 h.

In recent years, newer technologies have made it possible to identify pathogens and genotypic markers of resistance directly from blood culture samples or whole blood samples (Table 7). Clinical laboratories use molecular assays to find genes that code for carbapenem resistance (IMP, KPC, NDM, OXA-48-type, and VIM) or phenotypic tests to detect carbapenemase production (CarbaNP, mCIM/eCIM). Several commercial molecular diagnostic techniques are also offered detection of carbapenem resistance with rapid turnaround time (Table 8).

6 One Health Approach

Studies have reported antibiotic resistance genes in environmental, hospital, as well as community microbes. Within this ecosystem, AMR genes are horizontally transferred (HGT) between different genera and species (Baquero & Nombela, 2012). The origin of the CTX-M genes in species of *Kluyvera* is one of the well-known examples (Canton & Coque, 2006). The genetic determinants of environmental AMR are a rich source of potentially transferable resistance to pathogens. When bacteria are exposed to stress, such as antibiotics, their gene transfer rate increases. In India, antibiotics are overused and used irrationally. There is currently no way to trace the pattern of AMR spread or identify the source of resistance. To get insight into the distinctions between healthy and unhealthy bacteria, metagenomics can be utilized to examine the microbiota of healthy volunteers and patients. The discovery of AMR genetic determinants in the environment will also significantly improve the knowledge of the dynamics of AMR transmission.

ARG are not discrete entities, and microbiota microorganisms are challenging to culture. When MGEs such as integrons, plasmids, and transposons are acquired by bacterial cells, they can spread and move around in the environment (Baquero, 2004). In addition to direct contact, the use of animal waste as fertilizer in agriculture

Table 6 Automated system that provides identification with antimicrobial susceptibility testing

Technology	Company	Turnaround time	ID and AST	AST	Provides MIC	Level of commercialization
MBT-ASTRA	Bruker Daltonik	2–4 h	–	–	✓	Commercial
Direct-on-target microbial growth assay	Instrument providers	4 h	–	–	✓	Experimental
FISH (fluorescent probes, microscope)	XpressFISH	2–4 h	✓	–	–	Commercial
FISH microscopy	Accelerate diagnostics	6.5 h	✓	✓	✓	Commercial
Nanoparticle-based detection	Light diagnostics	4 h	✓	✓	✓	Commercial, under testing
Photon fluorescence microscopy	ArcDia	2–4 h	✓	✓	✓	Commercial, under testing

Table 7 Molecular-based methods that are approved and in development for the detection of pathogens and antimicrobial-resistant genes

iCubate, Inc.	iCubate System	Microarray	*S. aureus, S. epidermidis, E. faecium*, *S. pneumoniae* resistance genes (*mec*A, *van*A/B) *E. coli, K. pneumoniae, K. oxytoca, Enterobacter cloacae complex, Proteus* spp., and *S. marcescens*, *P. aeruginosa,* and *A. baumannii complex,* pipeline: Mycobacterium, gastrointestinal, and respiratory tests
Curetis GmbH	Unyvero™ system	Multiplex PCR, and array hybridization	Blood culture panel: 36 bacterial targets and 16 resistant genes, including *mec*A/C, *vanA/B,* CTX-M, KPC, IMP, VIM, NDM, OXA-48, OXA-23, OXA-24/40 andOXA-58 Respiratory panel: 19 targets includes bacteria and fungi as well as resistance markers listed in blood culture panel; 88 pathogens and 15 resistance markers make up the UTI panel
Bruker-Hain Diagnostics	GeneType® assays and Fluoro type® system	PCR with fluorescence	Gram-negative and gram-positive pathogens: *S. aureus*, *E. faecium*, and *S. pneumoniae, E. coli, K. pneumonia, E. aerogenes, E. cloacae*, and *E. sakazakii*, *P. aeruginosa,* and *A. baumannii* Other kits*: Clostridium difficile, helicobacter pylori,* and assays for MTB complex
GenMark diagnostics	ePlex® system	Microchip-based detection	Gram-positive panel: *Staphylococcus aureus* and *enterococcus faecium,* as well as resistance genes *mecA/C, vanA/BB*. Gram-negative panel: *Klebsiella pneumoniae, Enterobacter* sp., *H. influenza, P. aeruginosa,* and *A. baumannii* and resistant genes, ($bla_{CTX\text{-}M}$, bla_{KPC}, bla_{VIM}, bla_{NDM}, bla_{IMP}, and bla_{OXA}) Blood culture for fungal infection
ID and resistance from whole blood			
SeeGene	Seeplex™, Allplex™ Anyplex™ Magicplex™	Real-time PCR	MagicPlex™: Identification of 27 bacterial pathogens, *S. aureus, S. pneumoniae, E. faecium, E. coli*, *K. pneumoniae, P. aeruginosa, A. baumannii,* and *S. Typhi* Seeplex™, Anyplex™, and Allplex assays; detects resistant genes

(continued)

Table 7 (continued)

Immunoassays and other methods for the detection of antibacterial resistance			
NG biotech	Flow immunoassays	Monoclonal antibody-based detections	Detection/confirmation of resistance genes from culture: bla_{NDM}, bla_{KPC}, bla_{NDM}, bla_{VIM}, and bla_{OXA-48}, bla_{CTX-M}, bla_{mcr-1}
Coris BioConcept	RESIST assays	LFIA	bla_{KPC}, bla_{NDM}, bla_{VIM}, bla_{OXA-48} like *H. Pylori, Escherichia coli*, and *C. difficile* tests for bacterial pathogen identification in stool samples
Identification of pathogens and/or detection of resistance			
SpinDiag	LabDisk	Nested PCR; microfluidics with disk-based test cartridge	25 bacterial targets nasal swabs, RTIs, and STIs

also contributes to the spread of disease-causing microorganisms from animals to people (van den Bogaard & Stobberingh, 2000). In 2016, the first report on colonoscopic fecal microbiota transplantation for ulcerative colitis was released by Indian researchers (Seth et al., 2016).

The human gut microbiome is an important source of AMR (Salyers et al., 2004). The frequently employed drugs change the dynamics of the gut's microbial ecology by killing infections as well as commensal and beneficial bacteria. The antibiotic ciprofloxacin affects about one-third of the bacteria, diminishing diversity and balance (Dethlefsen et al., 2008). Antibiotic use affects the gut microbiome negatively in the elderly, leaving them more susceptible to pathogenic illness (Robinson et al., 2010). The gut microbiome of tribal people revealed the presence of *Faecalibacterium*, *Blautia*, *Eubacterium*, *Clostridium*, *Ruminococcus*, and *Roseburia* (Dehingia et al., 2015). Further, the gut microbiome has been observed to transport ARGs between continents, including the Indian peninsula and Central Africa (Bengtsson-Palme et al., 2015). Despite not taking any antibiotics, there was a copious incidence of ARG, particularly those encoding unresponsiveness to beta-lactams, trimethoprim, and sulfonamides by 2.6-, 7.7-, and 6.7-fold enhancement, respectively. The gut microbiota profile varies greatly between individuals within or between communities, but the metabolic processes required by the gut are conserved.

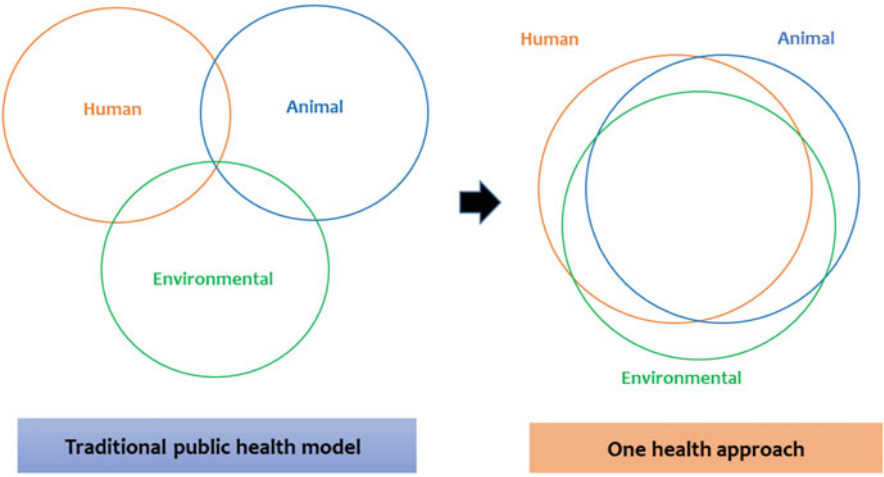
The mode of action of antibiotic obduracy distribution requires further investigations to lengthen the clinical lives of antibiotics. Despite selective pressure, ARGs' incidence recorded in the environment (and clinical antibiotic resistance) is unlikely to vanish anytime soon. Antibiotics are widely used in India both for human and animal sectors. Animal manure and wastewater sludge are extensively treated with anaerobic digestion (AD) due to their cost-effectiveness and ability to yield bioenergy. A significant hazard can occur in human and animal health from the

Table 8 Characteristics of select commercial molecular tests for carbapenemase detection approved for the detection of carbapenemases in Enterobacterales isolates

Assay	Time of results	Source	Detection of carbapenemases gene	Sensitivity (%)	Specificity (%)
Xpert Carba-R	2 hrs	Isolate	KPC, IMP, VIM, NDM, OXA-48 like mecA	100	100
BioFire film Array	1–2 h	Blood culture	KPC	NA	NA
Nanosphere Verigene BC-GN	2 h	Blood culture	KPC, NDM, VIM, IMP, OXA	NA	NA
EntericBio CPE assay	2 h	Isolate, swabs	KPC, IMP, VIM, NDM, OXA-48 like, GES-5, IMI, OXA-23	100	100
Check- direct CPE assay	3.5 h	Rectal swab/ isolate	KPC, OXA-48, including OXA-181, VIM, and NDM	100	94
AID line probe assay	5 h	Various clinical specimens	KPC, IMP, VIM, NDM, OXA-48, SIM, SPM, AIM, BIC, DIM, GIM, IMI, NMC-A	97.7	NA
Hyplex MBL ID system	5 h	Various clinical specimens	VIM and IMP	98	98.6
BB MAX™ CRE assay	2 h	Rectal swab/ isolate	KPC, NDM, oxa-48	93.1	97.3
Check-MDR 103 XL	6.5 h	Isolate	KPC, OXA-48, VIM, NDM, GES, GIM, SPM, OXA-23 like, Oxa-24 like	100	100
Eazyplex® SuperBug CRE	15 min	Blood culture	KPC, NDM, VIM	100	100

discards of ARBs and ARGs to the environment because the residual of AD treatment is frequently utilized in agriculture as fertilizer or soil enhancement.

It is crucial to deepen our understanding of how AMR transmission is impacted by present management systems. Identifying alternatives to destroy pathogens and reduce antimicrobial-resistant gene (ARG) transmission seems to be a major challenge. Therefore, it is crucial to comprehend ARGs' effects on the ecosystem more thoroughly. The burden and status of AMR in the community and environment, as well as possible sources of resistance, will be revealed through a metagenomic approach. Utilizing suitable wastewater treatment strategies and cutting-edge disinfection techniques, intervention tactics include the elimination of antibiotic residues and resistant bacteria.



7 Genomics to Track Antimicrobial Resistance Dynamics

Human and environmental microbiome research has revealed the presence and similarity of AMR genes. Vancomycin, tetracycline, and beta-lactam antibiotic resistance genes and efflux pumps are the most prevalent forms of resistance mechanisms discovered in environmental metagenomes. These drugs are commonly utilized in veterinary and human medicine. In all metagenomes, hits for membrane fusion proteins and efflux pumps were found in 30% and 21% of AMR genetic determinants, respectively (Nesme et al., 2014). Various vancomycin resistance genes and tetracycline ribosomal protective protein genes were also discovered. Beta-lactamases were less frequent, whereas the PBP accounted for 5.4% of annotated reads.

MGEs are crucial for the development and spread of antibiotic resistance. For carbapenem-resistant *E. coli*, IncF plasmids are more prevalent (93%) than Col (43%) or IncL plasmids (40%). IncF plasmids are more common (93%) in

carbapenem-resistant *E. coli* than Col (43%) or IncL plasmids (40%). In *P. aeruginosa* and *A. baumannii*, VIM, NDM, IMP, and OXA-23/24-like are spread by integrons of class I, respectively. Additionally, *A. baumannii* strongly associates the blaOXA-23/24 gene with the ISAba1 insertional element. Because veterinary medicine may employ these antibiotics in a way that contributes to human resistance, fluoroquinolone resistance is of special concern (Durso et al., 2011). The genes for beta-lactamases of class D, macrolide resistance efflux pumps, 23S rRNA methyltransferases, tetX inactivation enzymes, and trimethoprim-resistant dihydrofolate reductases were found in samples of agricultural vegetables, whereas resistance mechanisms with broad substrate specificity were found in vegetables grown organically.

8 National Action Plan for AMR in India

The burden of drug-resistant strains is highest in Asian countries (Porter & Grills, 2016). There is a dearth of an antimicrobial policy, accepted treatment standards, an action plan to stop AMR, and studies on the public health effects of AMR in India. To tackle the threat that AMR poses to human health, the World Health Assembly (WHA) developed a Global AMR Action Plan in 2015. The National Action Plan for Containing AMR was released by the Indian Ministry of Health and Family Welfare in April 2017 (Ranjalkar & Chandy, 2019; Dixit et al., 2019; Sidjabat et al., 2011).

There are several initiatives underway in India to address this issue. An antibiotic policy is being developed to be incorporated into hospital guidelines. Hospitals are urged to apply for certification with the National Accreditation Board for Hospitals as government's initiatives will result in prudent application of drugs (WHO, 2011). In agriculture, fisheries, and in veterinary growth products, antimicrobials must be avoided. The required actions must be made to guarantee access to important pharmaceuticals and stop the over-the-counter sale. Periodically, communities as well as various types of healthcare providers should participate in educational and awareness activities.

A key project initiated by the ICMR is the Antimicrobial Resistance Surveillance and Research Network, which analyzes and publishes data on drug resistance across the country. This network compiles data on six priority pathogens identified by the World Health Organization and updates the antibiotic policy. Government of India legislation was introduced in January 2020 to limit harmful antibiotic residues released by pharmaceutical plants. To reduce antimicrobial resistance and infections linked to healthcare, a program called antimicrobial stewardship (AMS) has also been created (Walia et al., 2015). The following are a few possible strategies to control AMR in the country (Mittal et al., 2020).

Possible Strategies to Combat AMR

1. Diagnosing infectious diseases rapidly and accurately.
2. Identifying and understanding the key factors promoting antibiotic resistance.
3. Implementing a nationwide surveillance program for antibiograms.

4. Rationalizing antibiotic use.
5. Developing new antibiotics capable of combating prevailing resistance mechanisms.
6. Developing combination antibiotic therapy is necessary in cases of drug-resistant bacteria causing severe bacterial infections.

9 Conclusion

In view of ease in accessibility to drugs sans prescription and dearth of infection diagnostics, Asiatic regions top the rates of antibiotic resistance. To mitigate the risk of AMR, a coordinated effort is necessary to establish surveillance and appropriate guidelines. Developing effective treatment strategies requires rapid diagnosis of infection and antimicrobial resistance surveillance.

References

Aitken, S. L., Tarrand, J. J., Deshpande, L. M., Tverdek, F. P., Jones, A. L., Shelburne, S. A., Prince, R. A., Bhatti, M. M., Rolston, K., Jones, R. N., Castanheira, M., & Chemaly, R. F. (2016). High rates of nonsusceptibility to Ceftazidime-avibactam and identification of New Delhi metallo-β-lactamase production in enterobacteriaceae bloodstream infections at a major cancer center. *Clinical Infectious Diseases: An Official Publication of the Infectious Diseases Society of America, 63*(7), 954–958.

Aslam, B., Wang, W., Arshad, M. I., Khurshid, M., Muzammil, S., Rasool, M. H., Nisar, M. A., Alvi, R. F., Aslam, M. A., Qamar, M. U., & Salamat, M. K. F. (2018). Antibiotic resistance: A rundown of a global crisis. *Infection and Drug Resistance, 11*, 1645.

Baekkeskov, E., Rubin, O., Munkholm, L., and Zaman, W. (2020). "Antimicrobial Resistance as a Global Health Crisis," in Oxford Research Encyclopedia of Politics. Editor E. Stern (Oxford University Press), 1–24. https://doi.org/10.1093/acrefore/9780190228637.013.1626

Baig, S., Rhod Larsen, A., Martins Simões, P., Laurent, F., Johannesen, T. B., Lilje, B., Tristan, A., Schaumburg, F., Egyir, B., Cirkovic, I., Nimmo, G. R., Spiliopoulou, I., Blanc, D. S., Mernelius, S., Moen, A., David, M. Z., Andersen, P. S., & Stegger, M. (2020). Evolution and population dynamics of clonal complex 152 community-associated methicillin-resistant Staphylococcus aureus. *mSphere, 5*(4), e00226-20.

Bakthavatchalam, Y. D., Babu, P., Munusamy, E., Dwarakanathan, H. T., Rupali, P., Zervos, M., John Victor, P., & Veeraraghavan, B. (2019). Genomic insights on heterogeneous resistance to vancomycin and teicoplanin in methicillin-resistant Staphylococcus aureus: A first report from South India. *PLoS One, 14*(12), e0227009.

Bakthavatchalam, Y. D., Vasudevan, K., Babu, P., Neeravi, A. R., Narasimhan, V., & Veeraraghavan, B. (2021). Genomic insights of optrA-carrying linezolid-resistant enterococcus faecium using hybrid assembly: First report from India. *Journal of Global Antimicrobial Resistance, 25*, 331–336.

Baquero, F. (2004). From pieces to patterns: Evolutionary engineering in bacterial pathogens. *Nature Reviews Microbiology, 2*(6), 510–518.

Baquero, F., & Nombela, C. (2012). The microbiome as a human organ. *Clinical Microbiology and Infection, 18*, 2–4.

Bengtsson-Palme, J., Angelin, M., Huss, M., Kjellqvist, S., Kristiansson, E., Palmgren, H., Larsson, D. J., & Johansson, A. (2015). The human gut microbiome as a transporter of antibiotic

resistance genes between continents. *Antimicrobial Agents and Chemotherapy, 59*(10), 6551–6560.

Breijyeh, Z., Jubeh, B., & Karaman, R. (2020). Resistance of gram-negative bacteria to current antibacterial agents and approaches to resolve it. *Molecules, 25*(6), 1340.

Cantσn, R., & Coque, T. M. (2006). The CTX-M β-lactamase pandemic. *Current Opinion in Microbiology, 9*, 466–475.

Cartwright, E. J., Paterson, G. K., Raven, K. E., Harrison, E. M., Gouliouris, T., Kearns, A., Pichon, B., Edwards, G., Skov, R. L., Larsen, A. R., Holmes, M. A., Parkhill, J., Peacock, S. J., & Török, M. E. (2013). Use of Vitek 2 antimicrobial susceptibility profile to identify mecC in methicillin-resistant Staphylococcus aureus. *Journal of Clinical Microbiology, 51*(8), 2732–2734.

Centers for Disease Control and Prevention, Office of Infectious Disease. (2013, April). *Antibiotic resistance threats in the United States, 2013*. Available at: http://www.cdc.gov/drugresistance/threat-report-2013. Accessed 28 Jan 2015.

Centres for Disease Control and Prevention, US Department of Health and Human Services. (2013). *Antibiotic resistance threats in the United States*. Atlanta: CDC. Available from: http://www.cdc.gov/drugresistance/pdf/ar-threats-2013-508.pdf.

Chambers, H. F., & Deleo, F. R. (2009). Waves of resistance: Staphylococcus aureus in the antibiotic era. Nature reviews. *Microbiology, 7*(9), 629–641.

Cui, L., Tominaga, E., Neoh, H. M., & Hiramatsu, K. (2006). Correlation between reduced daptomycin susceptibility and vancomycin resistance in vancomycin-intermediate Staphylococcus aureus. *Antimicrobial Agents and Chemotherapy, 50*(3), 1079–1082.

Dehingia, M., Talukdar, N. C., Talukdar, R., Reddy, N., Mande, S. S., Deka, M., & Khan, M. R. (2015). Gut bacterial diversity of the tribes of India and comparison with the worldwide data. *Scientific Reports, 5*(1), 1–12.

Dethlefsen, L., Huse, S., Sogin, M. L., & Relman, D. A. (2008). The pervasive effects of an antibiotic on the human gut microbiota, as revealed by deep 16S rRNA sequencing. *PLoS Biology, 6*(11), e280.

Dixit, A., Kumar, N., Kumar, S., & Trigun, V. (2019). Antimicrobial resistance: Progress in the decade since emergence of New Delhi Metallo-β-lactamase in India. *Indian journal of community medicine: Official publication of Indian Association of Preventive & social medicine, 44*(1), 4.

Durso, L. M., Harhay, G. P., Bono, J. L., & Smith, T. P. (2011). Virulence-associated and antibiotic resistance genes of microbial populations in cattle feces analyzed using a metagenomic approach. *Journal of Microbiological Methods, 84*(2), 278–282.

Ford, B. A. (2017). mecC-harboring methicillin-resistant Staphylococcus aureus: Hiding in plain sight. *Journal of Clinical Microbiology, 56*(1), e01549–e01517.

Gandra, S., Alvarez-Uria, G., Turner, P., Joshi, J., Limmathurotsakul, D., & van Doorn, H. R. (2020). Antimicrobial resistance surveillance in low-and middle-income countries: Progress and challenges in eight south Asian and southeast Asian countries. *Clinical Microbiology Reviews, 33*(3), e00048–e00019.

Gardam, M. A. (2000). Is methicillin-resistant Staphylococcus aureus an emerging community pathogen? A review of the literature. *Canadian Journal of Infectious Diseases, 11*(4), 202–211.

Gorrie, C., Higgs, C., Carter, G., Stinear, T. P., & Howden, B. (2019). Genomics of vancomycin-resistant enterococcus faecium. *Microbial Genomics, 5*(7), e000283.

Guzman Prieto, A. M., van Schaik, W., Rogers, M. R., Coque, T. M., Baquero, F., Corander, J., & Willems, R. J. (2016). Global emergence and dissemination of enterococci as nosocomial pathogens: Attack of the clones? *Frontiers in Microbiology, 7*, 788.

Halpern, N. E. (2009). Antibiotics in food animals: The convergence of animal and public health, science, policy, politics and the law. *The Drake Journal of Agricultural Law, 14*, 401.

Hamdan-Partida, A., Sainz-Espuñes, T., & Bustos-Martínez, J. (2010). Characterization and persistence of Staphylococcus aureus strains isolated from the anterior nares and throats of healthy carriers in a Mexican community. *Journal of Clinical Microbiology, 48*(5), 1701–1705.

Hendriksen, R. S., Bortolaia, V., Tate, H., Tyson, G. H., Aarestrup, F. M., & McDermott, P. F. (2019). Using genomics to track global antimicrobial resistance. *Frontiers in Public Health, 7*, 242.

Hiramatsu, K., Katayama, Y., Matsuo, M., Aiba, Y., Saito, M., Hishinuma, T., & Iwamoto, A. (2014). Vancomycin-intermediate resistance in Staphylococcus aureus. *Journal of Global Antimicrobial Resistance, 2*(4), 213–224.

Hollenbeck, B. L., & Rice, L. B. (2012). Intrinsic and acquired resistance mechanisms in enterococcus. *Virulence, 3*(5), 421–433.

Hong, D. J., Bae, I. K., Jang, I. H., Jeong, S. H., Kang, H. K., & Lee, K. (2015). Epidemiology and characteristics of metallo-β-lactamase-producing Pseudomonas aeruginosa. *Infection & chemotherapy, 47*(2), 81–97.

Howden, B. P., Peleg, A. Y., & Stinear, T. P. (2014). The evolution of vancomycin-intermediate Staphylococcus aureus (VISA) and heterogenous-VISA. *Infection, Genetics and Evolution: Journal of Molecular Epidemiology and Evolutionary Genetics in Infectious Diseases, 21*, 575–582.

Hryniewicz, M. M., & Garbacz, K. (2017). Borderline oxacillin-resistant Staphylococcus aureus (BORSA) – A more common problem than expected? *Journal of Medical Microbiolgy, 66*(10), 1367–1373.

Humphries, R. M., Yang, S., Hemarajata, P., Ward, K. W., Hindler, J. A., Miller, S. A., & Gregson, A. (2015). First report of ceftazidime-avibactam resistance in a KPC-3-expressing Klebsiella pneumoniae isolate. *Antimicrobial Agents and Chemotherapy, 59*(10), 6605–6607.

Jensen, S. O., & Lyon, B. R. (2009). Genetics of antimicrobial resistance in Staphylococcus aureus. *Future Microbiology, 4*(5), 565–582.

Kakoullis, L., Papachristodoulou, E., Chra, P., & Panos, G. (2021). Mechanisms of antibiotic resistance in important gram-positive and gram-negative pathogens and novel antibiotic solutions. *Antibiotics (Basel, Switzerland), 10*(4), 415.

Kehrenberg, C., Schwarz, S., Jacobsen, L., Hansen, L. H., & Vester, B. (2005). A new mechanism for chloramphenicol, florfenicol and clindamycin resistance: Methylation of 23S ribosomal RNA at A2503. *Molecular Microbiology, 57*(4), 1064–1073.

Kelley, P. G., Gao, W., Ward, P. B., & Howden, B. P. (2011). Daptomycin non-susceptibility in vancomycin-intermediate Staphylococcus aureus (VISA) and heterogeneous-VISA (hVISA): Implications for therapy after vancomycin treatment failure. *The Journal of Antimicrobial Chemotherapy, 66*(5), 1057–1060.

Klein, E. Y., Van Boeckel, T. P., Martinez, E. M., Pant, S., Gandra, S., Levin, S. A., Goossens, H., & Laxminarayan, R. (2018). Global increase and geographic convergence in antibiotic consumption between 2000 and 2015. *Proceedings of the National Academy of Sciences, 115*(15), E3463–E3470.

Kostyanev, T., & Can, F. (2017). The global crisis of antimicrobial resistance. In *Antimicrobial stewardship* (pp. 3–12). Academic Press.

Laxminarayan, R., & Chaudhury, R. R. (2016). Antibiotic resistance in India: Drivers and opportunities for action. *PLoS Medicine, 13*(3), e1001974.

Limbago, B. M., Kallen, A. J., Zhu, W., Eggers, P., & McDougal, L. K. (2014). Albrecht VS. Report of the 13th vancomycin-resistant Staphylococcus aureus isolate from the United States. *Journal of Clinical Microbiology, 52*(3), 998–1002.

Littmann, J., & Viens, A. M. (2015). The ethical significance of antimicrobial resistance. *Public health ethics, 8*(3), 209–224.

Liu, C., & Chambers, H. F. (2003). Staphylococcus aureus with heterogeneous resistance to vancomycin: Epidemiology, clinical significance, and critical assessment of diagnostic methods. *Antimicrobial Agents and Chemotherapy, 47*(10), 3040–3045.

Lowy, F. D. (2003). Antimicrobial resistance: The example of Staphylococcus aureus. *The Journal of Clinical Investigation, 111*(9), 1265–1273.

Michael, C. A., Dominey-Howes, D., & Labbate, M. (2014). The antimicrobial resistance crisis: Causes, consequences, and management. *Frontiers in Public Health, 2*, 145.

Miller, W. R., Munita, J. M., & Arias, C. A. (2014). Mechanisms of antibiotic resistance in enterococci. *Expert Review of Anti-Infective Therapy, 12*(10), 1221–1236.

Mittal, A. K., Bhardwaj, R., Mishra, P., & Rajput, S. K. (2020). Antimicrobials misuse/overuse: Adverse effect, mechanism, challenges and strategies to combat resistance. *The Open Biotechnology Journal, 14*(1), 107.

Murray, C. L. J., et al. (2022). Antimicrobial Resistance Collaborators. Global burden of bacterial antimicrobial resistance in 2019: a systematic analysis. *Lancet, 399*(10325), 629–655. 10.1016/S0140-6736(21)02724-0. Epub 2022 Jan 19. Erratum in: Lancet. 2022 Oct 1;400(10358): 1102. PMID: 35065702; PMCID: PMC8841637.

Nesme, J., Cécillon, S., Delmont, T. O., Monier, J. M., Vogel, T. M., & Simonet, P. (2014). Large-scale metagenomic-based study of antibiotic resistance in the environment. *Current Biology, 24*(10), 1096–1100.

O'Neill, J. (2016). *Tackling drug-resistant infections globally: Final report and recommendations*. Wellcome Trust.

Pang, Z., Raudonis, R., Glick, B. R., Lin, T. J., & Cheng, Z. (2019). Antibiotic resistance in Pseudomonas aeruginosa: Mechanisms and alternative therapeutic strategies. *Biotechnology Advances, 37*(1), 177–192.

Porter, G., & Grills, N. (2016). Medication misuse in India: A major public health issue in India. *Journal of Public Health, 38*(2), e150.

Prestinaci, F., Pezzotti, P., & Pantosti, A. (2015). Antimicrobial resistance: A global multifaceted phenomenon. *Pathogens and global health, 109*(7), 309–318.

Ranjalkar, J., & Chandy, S. J. (2019). India's National Action Plan for antimicrobial resistance – An overview of the context, status, and way ahead. *Journal of Family Medicine and Primary Care, 8*(6), 1828.

Robinson, C. J., Bohannan, B. J., & Young, V. B. (2010). From structure to function: The ecology of host-associated microbial communities. *Microbiology and Molecular Biology Reviews, 74*(3), 453–476.

Salyers, A. A., Gupta, A., & Wang, Y. (2004). Human intestinal bacteria as reservoirs for antibiotic resistance genes. *Trends in Microbiology, 12*(9), 412–416.

Seth, A. K., Rawal, P., Bagga, R., & Jain, P. (2016). Successful colonoscopic fecal microbiota transplantation for active ulcerative colitis: First report from India. *Indian Journal of Gastroenterology, 35*(5), 393–395.

Sidjabat, H., Nimmo, G. R., Walsh, T. R., Binotto, E., Htin, A., Hayashi, Y., Li, J., Nation, R. L., George, N., & Paterson, D. L. (2011). Carbapenem resistance in Klebsiella pneumoniae due to the New Delhi Metallo-β-lactamase. *Clinical Infectious Diseases : An Official Publication of the Infectious Diseases Society of America, 52*(4), 481.

Van Boeckel, T. P., Gandra, S., Ashok, A., Caudron, Q., Grenfell, B. T., Levin, S. A., & Laxminarayan, R. (2014). Global antibiotic consumption 2000 to 2010: An analysis of national pharmaceutical sales data. *The Lancet Infectious Diseases, 14*(8), 742–750.

van den Bogaard, A. E., & Stobberingh, E. E. (2000). Epidemiology of resistance to antibiotics: Links between animals and humans. *International Journal of Antimicrobial Agents, 14*(4), 327–335.

van Duin, D. (2017). Carbapenem-resistant Enterobacteriaceae: What we know and what we need to know. *Virulence, 8*, 379–382.

Ventola, C. L. (2015). The antibiotic resistance crisis: Part 1: Causes and threats. *Pharmacy and Therapeutics, 40*(4), 277.

Viswanathan, V. K. (2014). Off-label abuse of antibiotics by bacteria. *Gut Microbes, 5*(1), 3–4.

Vrancianu, C. O., Gheorghe, I., Czobor, I. B., & Chifiriuc, M. C. (2020). Antibiotic resistance profiles, molecular mechanisms and innovative treatment strategies of Acinetobacter baumannii. *Microorganisms, 8*(6), 935.

Walia, K., Ohri, V. C., & Mathai, D. (2015). Antimicrobial stewardship programme (AMSP) practices in India. *The Indian Journal of Medical Research, 142*(2), 130.

Wardal, E., Kuch, A., Gawryszewska, I., Żabicka, D., Hryniewicz, W., & Sadowy, E. (2017). Diversity of plasmids and Tn1546-type transposons among VanA enterococcus faecium in Poland. *European Journal of Clinical Microbiology & Infectious Diseases: Official Publication of the European Society of Clinical Microbiology, 36*(2), 313–328.

Wistrand-Yuen, E., Knopp, M., Hjort, K., Koskiniemi, S., Berg, O. G., & Andersson, D. I. (2018). Evolution of high-level resistance during low-level antibiotic exposure. *Nature Communications, 9*(1), 1–12.

Wootton, M., Howe, R. A., Hillman, R., Walsh, T. R., Bennett, P. M., & MacGowan, A. P. (2001). A modified population analysis profile (PAP) method to detect hetero-resistance to vancomycin in Staphylococcus aureus in a UK hospital. *The Journal of Antimicrobial Chemotherapy, 47*(4), 399–403.

World Health Organization. (2017, February 27). *WHO publishes list of bacteria for which new antibiotics are urgently needed*. News release. https://www.who.int/news-room/detail/27-02-2017-who-publishes-list-of-bacteria-for-which-new-antibiotics-are-urgently-needed.

World Health Organization. *WHO global strategy for containment of antimicrobial resistance*. Available from: http://www.who.int/emc. Last accessed 16 Apr 2011.

Wu, Z. (2017). *Balancing food security and AMR: A review of economic literature on antimicrobial use in food animal production*. China Agricultural Economic Review (Vol. 9, p. 14).

Zhang, S., Sun, X., Chang, W., Dai, Y., & Ma, X. (2015). Systematic review and meta-analysis of the epidemiology of vancomycin-intermediate and heterogeneous vancomycin-intermediate Staphylococcus aureus isolates. *PLoS One, 10*(8), e0136082.

Detection of Antimicrobial Resistance in Veterinary Bacterial Pathogens

M. Mini and R. Ambily

Contents

Abstract

For decades, antimicrobial resistance (AMR) in bacteria is detected using standardized phenotypic methods like disc diffusion and broth dilution. World Health Organization categorized molecular tests for AMR into four classes, viz., sequence-based, hybridization-based, amplification-based, and immunoassays. Sequence-based assays include whole genome sequencing and nanopore in which genome sequences are evaluated to find out the resistant genes. Hybridization-based tests involve the use of hybridized nucleic acid probes that

M. Mini (✉) · R. Ambily
Department of Veterinary Microbiology, College of Veterinary and Animal Sciences, Mannuthy, Thrissur, Kerala, India
e-mail: mini@kvasu.ac.in; ambily.r@kvasu.ac.in

M. P. Mothadaka et al. (eds.), *Handbook on Antimicrobial Resistance*,
https://doi.org/10.1007/978-981-19-9279-7_30

target gene sequences for their specific detection. In amplification-based tests like polymerase chain reaction (PCR) and loop-mediated isothermal amplification (LAMP), target gene sequence is amplified into multiple copies permitting detection. The basis for immunoassays such as lateral flow immunoassay and nucleic acid-based lateral flow assay is the capacity of the antibody to bind to the target genes as well as their products to allow detection. However, novel resistance mechanisms cannot be detected absolutely by these molecular methods, since a clear understanding of the responsible DNA sequences is essential. To examine and anticipate the resistance of bacterial isolates from sequence data, various high-throughput bioinformatics tools are available. Recent studies are centered on the development of novel tools that recognize genes associated with AMR and the single nucleotide polymorphisms (SNPs) directly from short reads and produce comprehensive and customizable output. As far as the detection of degree of resistance in a particular setting and the mechanisms of resistance are concerned, molecular and phenotypic AMR diagnostics complement each other.

Keywords

Antimicrobial resistance · Hybridization · Amplification · Immunoassays · Molecular diagnostic tests

1 Introduction

The ability of bacteria, viruses, and parasites to resist an antimicrobial agent (antibiotics, antivirals, and antimalarials) from acting against it is called antimicrobial resistance. This will lead to ineffectiveness of standard treatments and hence, infections may persist and spread to others (WHO, 2018). This is rapidly becoming a major public health risk that hinders decades of advances in treating diseases. The upsurge of bacteria harboring AMR is a universal problem, because there is a scarcity of antibiotics to treat multidrug-resistant (MDR) bacterial infections in human beings and animals. India's National Health Policy 2017 recognizes AMR as a problem and insists effective action to address it. In India's "National Action Plan – Antimicrobial Resistance (NAP-AMR)," six strategic priorities were identified, which include creating awareness and understanding of antimicrobial resistance, strengthening laboratories thereby enhancing knowledge through surveillance and research, minimizing the infection, optimizing antimicrobial usage in health, animal, and food sectors, developing an economic case for sustainable investment that takes account of the needs of all countries and increasing investment in new medicines, diagnostic tools, vaccines, and other interventions, and strengthening India's leadership on AMR through international collaborations. Strategic Priority 2 aims to reinforce knowledge and evidence by surveillance of AMR which consists of two focus areas, viz., strengthening labs in human, animal, food, as well as environment sectors and surveillance of antimicrobial resistance in these areas. The widely accepted methods for surveillance include conventional phenotypic

methods such as disc diffusion, broth dilution, and agar dilution. These methods assess the ability of the bacteria to multiply in the presence of a particular antimicrobial agent. However, molecular methods are widely employed for the detection of antimicrobial resistance nowadays. These tests are not only useful in confirming phenotypic tests but also serve as practical tools which throw light into the mechanisms of AMR in certain pathogens. The gene amplification methods, sequence-based methods, immunological assays, as well as the hybridization assays are included in this category. Various point of care test devices employing smartphone technology are also developed for antimicrobial susceptibility testing and reported to have sufficient accuracy. This chapter reviews the methods that can be employed for the detection of antimicrobial resistance in bacteria of veterinary importance.

2 Diagnostic Methods

The formation of national as well as regional reference laboratories is vital for the synchronization of antimicrobial susceptibility testing (AST), analysis, and implementation of suitable techniques to ensure accuracy and reproducibility. Microbiological laboratories should establish and maintain an authorized quality management program and must obtain a third-party accreditation that consolidates methodologies for AST to be employed within the range of that accreditation. It is essential that they must meet the criteria of International Laboratory Accreditation Cooperation [ILAC] standards. The requirement for participation in proficiency testing programs is mandatory. In order to assess quality control in laboratories, for quality assurance and proficiency testing, specific bacterial reference strains are necessary.

3 Conventional Methods

Conventionally, detection of AMR in bacteria is usually carried out using standardized phenotypic methods of AST like disc diffusion, e-test, and broth dilution (CLSI, 2019). The test demands harmonization of AST test parameters such as media, inoculum, incubation time, quality controls, choice of antimicrobial and interpreting criteria, etc. Quantitative susceptibility data comprise calculation of "Minimum Inhibitory Concentration" (MIC).

4 Selection of Antimicrobials

The choice of appropriate antimicrobials for AST is a hard task due to the vast numbers of agents available. In case of veterinary pathogens, the availability of the patent preparation for the particular species of animal is also important. The FAO/OIE/WHO conducted an expert workshop on "Non-human antimicrobial usage and antimicrobial resistance" that promotes generating a directory of vital antimicrobials used in veterinary field for AST and reporting. The most suitable antimicrobial agent is

selected by each OIE member after discussion with the suitable bodies. Antimicrobial agents of the same class usually have similar in vitro activities to the selected bacteria. Hence, selection of antimicrobial agent that predicts susceptibility to members of the same class is essential. Some bacteria are primarily resistant to some antimicrobial classes; hence, it is confusing to test certain agents for activity in vitro. The nature of intrinsic resistance against these organisms has to be determined either from the available publications or through laboratory testing. The number of antimicrobial agents to be tested should be in accordance with the guideline used (CLSI/EUCAST/ISO). It should contain representatives of each class to make sure the significance and practicality of AST (WHO, 2017). To monitor the emergence of unexpected resistance, regular review of microorganisms that are presently susceptible to distinct antimicrobials is recommended. Poor response to treatment is also an indication of emerging resistance.

For the identification of the pure culture of bacteria that is proposed to be subjected to AST, standard protocols need to be followed in order to ensure consistent and accurate identification up to genus and/or species level. For future analysis, the isolates should be stored by lyophilization or by cryogenic preservation at −70 °C to −80 °C.

After isolating bacteria in "pure culture," concentration of the inoculum is calculated using a nephelometer or spectrophotometer to ensure a defined number of colony forming units to obtain accurate and repeatable susceptibility results. The organisms used for susceptibility testing should be from a "24-hour culture." The composition of the media (cations, thymidine or thymine, supplements) and the preparation should be in accordance with standard guidelines (CLSI/EUCAST/ISO). The selection of methodology for AST depends upon reproducibility, reliability, and accuracy of the test as well as cost-effectiveness, simplicity of performance, and adaptability to automated or semiautomated systems. The antimicrobial agent of relevance in that specific OIE member, microorganisms tested, and the availability of suitable validation data also influence the methodology to be selected.

5 Antimicrobial Susceptibility Testing Methodologies

Disc diffusion, broth dilution, and agar dilution are the three methods that constantly provide reproducible and repeatable results when done correctly (CLSI, 2018).

The advantage of disc diffusion method is that it is cost-effective and can be modified by changing antimicrobial discs when required. Large numbers of isolates can be screened, and identification of isolates for additional testing by new methods like determination of MICs is possible. The protocol is by including appropriate control organisms for which a target zone size range is kept for each of the important antimicrobial agents being tested in the disc diffusion test. This is the common method for the testing of rapidly growing bacterial pathogens.

The least concentration of the antimicrobial agent that prevents the visible growth of bacterium in either broth or on agar is determined using broth and agar dilution method, respectively. In broth dilution method, bacterial suspension with known

optimal concentration is checked for sensitivity using different concentrations of each antimicrobial agent (serially diluted twofold) in liquid medium. In agar dilution method, different concentrations of antibacterial agent are added to an agar medium in twofold dilutions serially and then, a quantified amount of bacterial culture is inoculated to the agar surface. This is the most consistent method for calculation of MIC for some antimicrobials like fosfomycin and mecillinam and for certain bacteria where broth dilution methods are not well established. In agar dilution methods, multiple bacteria can be tested, although not possible with some bacteria that forms swarming type of colonies like *Proteus* spp. The MIC endpoints can be identified effectively and the antibiotic concentration range can be extended. Semiautomatic inoculum replicators are also available commercially which are capable of transferring even 32–60 distinct bacteria to each agar plate. Unless automated, they demand substantial economic and technical resources and are very cumbersome too. The plates should not be kept beyond 1–3 weeks of preparation. The endpoints are not easily interpreted all the time. Agar dilution is the standard method in case of fastidious organisms like anaerobes and *Helicobacter* species (CLSI, 2018).

Broth microdilution method employing cation-adjusted Mueller-Hinton broth (CAMHB) is commonly used in case of fastidious organisms. The medium can be supplemented with lysed horse blood at the rate of 2.5% to 5% v/v. Depending on the pathogen, there are specified media like Brucella broth or *Haemophilus* Test Medium (HTM). In its document M07.2, CLSI has described the standard procedures including methods of preparation of medium and inoculum, dilutions of drugs used, conditions of incubation, as well as criteria for analyzing the MIC endpoints. In several clinical laboratories, modified agar disk diffusion method is also used for testing some fastidious species of bacteria like *Campylobacter jejuni*, *Campylobacter coli*, *Moraxella catarrhalis*, and *Pasteurella* spp. Sufficient studies have to be carried out to standardize this technique for many fastidious bacteria, like *Helicobacter pylori* for which only the broth microdilution or agar dilution method is currently performed.

Although the basic technique is the same, there are several modifications for antimicrobial sensitivity testing of veterinary pathogens. In case of *Listeria monocytogenes*, which is a foodborne pathogen, only MIC testing (i.e., dilution testing or MIC stripes) is suggested and disc testing is not recommended. It requires inoculation by direct colony suspension in media with supplements and incubation for 20–24 h at 35 °C in the presence of ambient air for sufficient growth. The organism is reported to be susceptible to penicillin, ampicillin ($\leq$2 ug/mL), and sulfamethoxazole ($\leq$0.5/9.5 ug/mL), and it shows inherent resistance to cephalosporins. The zone diameter cutoff for benzylpenicillin (concentration 1 U $\geq$ 13 mm sensitive), ampicillin (2 ug $\geq$ 16 mm sensitive), trimethoprim-sulfamethoxazole (1.25/23.75 ug $\geq$ 29 mm sensitive), erythromycin (15 u $\geq$ 25 mm sensitive), and meropenem (10 ug $\geq$ 26 mm sensitive) are given as per the EUCAST standards.

In case of *Haemophilus influenzae*, MH broth supplemented with 5% horse blood (lysed) along with 20 mg/L β-nicotinamide adenine dinucleotide (NAD) is used, and organisms are inoculated at $5x10^5$ CFU/mL of the broth and incubated at 35 $\pm$ 1 °C in the presence of 5% CO_2 for 18 $\pm$ 2 h. The lowest concentration of the

antimicrobial agent that inhibits visible growth of the bacteria completely is considered as the MIC. In case of agar disc diffusion method, MH agar supplemented with 5% defibrinated horse blood and 20 mg/L β-NAD is utilized, and incubation should be done at 35 ± 1 °C in the presence of 5% CO_2 for 18 ± 2 h, as in the case of broth microdilution method. The results should be read after removing the lid of the Petri plate and observing from the front portion of the plate in reflected light.

In case of potentially hazardous zoonotic bacteria, especially those involved in bioterrorism, it is better if the laboratory cultural procedures are avoided and the antimicrobial sensitivity is determined directly from the environmental samples. *Yersinia pestis*, *Bacillus anthracis*, and *Francisella tularensis* are some bacteria that are included in this category. Although conventional methods like agar disk diffusion and broth dilution techniques can be employed, the development of simple, rapid, and novel techniques with high specificity and sensitivity was reported to be highly beneficial. Micro-agar PCR test is one such antibiotic sensitivity test method that eliminates the cumbersome hazardous steps of bacterial isolation, quantification, and enrichment. By this technique, correct therapeutic MIC values can be identified. The advantage of this method is that antimicrobial susceptibility of both fast-growing and slow-growing bacteria can be analyzed accurately, within a short period of time directly from the clinical or environmental samples, without involving isolation. Thus, the therapy can be initiated at an early stage in the susceptible individuals exposed to the infective agent, even before exhibition of clinical signs by them. The media used is MHA. Autoclaved molten agar is added to tubes. Antibiotics are serially diluted and added to molten agar in tubes. These are dispensed in 96-well microtiter plates. To this, environmental samples are added (in duplicates). These plates with different concentrations of the tested antibiotics were incubated at the different temperatures that are optimum for each bacterium. In case of *B. anthracis* and *F. tularensis*, it is 37 °C, whereas for *Y. pestis*, it is 28 °C. Quantification of bacteria is done by quantitative PCR.

In case of leptospirosis, which is caused by highly fastidious organisms belonging to the genus Leptospira, antimicrobial susceptibility testing was reported to be conducted by broth dilution method (Hospenthal & Murray, 2003). Each 96-well round-bottom plate was added with serial twofold dilutions of antibiotic solutions, negative controls (medium only) and positive controls (bacteria without antimicrobial agent), all in Ellinghausen–McCullough–Johnson–Harris (EMJH) medium. The inoculum was prepared from 7-day-old cultures of Leptospira grown in EMJH medium at 30 °C. The amount of organisms in the inoculum can be determined by use of dark field microscopy.

6 Other Specific Bacterial Resistance Tests

Many of the veterinary pathogens like *E. coli* are visible on culture plates only if the colonies contain approximately 5×10^6 bacteria. As *E. coli* is a common veterinary pathogen that is associated with a variety of disease conditions in animals including mastitis in cow, navel ill in calves, and colibacillosis in poultry, rapid detection of the

antimicrobial susceptibility is essential for prompt initiation of therapy. Microscopic techniques facilitate visualization of microcolonies containing even 120 cells (London et al., 2010). Rapid Micro Biosystems Inc. developed the Growth Direct System which utilizes digital imaging for detecting microcolonies by blue light illumination followed by focusing the cellular autofluorescence straight onto a CCD chip without any magnification. In visual plate counting technique, the average time for *E. coli* detection is reported to be 8.5 h, whereas this autofluorescence method provides result in 3.1 h, as per the published reports.

The automated microscopy systems give real-time growth curves and bacterial counts. In order to facilitate rapid online antimicrobial susceptibility testing, Accelerated Diagnostics (USA) has marketed "multiplexed automated digital microscopy (MADM)" that applies the principle of fluorescent in situ hybridization (FISH) (Metzger et al., 2014).

Charnot-Katsikas et al. (2017) has evaluated Accelerate Pheno system in clinical studies involving urinary tract and bloodstream infections. Although developed for human patients, this technique can be applied in veterinary diagnostics. It involves removal of impurities from clinical samples like urine and blood by electrophoresis in which the impurities are run into a gel. Following this procedure, the polarity of the electric field is reversed, and this causes repulsion of the microbes back to the fluid. In this technique, fluorescent signal is measured in the bacterial cultures that are grown in MH media. Accelerate Pheno system is a growth-based diagnostic antimicrobial susceptibility system that is approved by FDA.

Gradient strips that diffuse a predetermined concentration of antimicrobials are available commercially which helps to calculate antimicrobial MICs. But these are not suggested for AST of antimicrobial agent, colistin, because of the large size of this molecule and hence, poor diffusivity in agar (Matuschek et al., 2018). The AST method adopted depends on the cultural characters of the microorganism and the purpose of testing. The detection of resistance phenotypes can be done by various novel tests. Chromogenic cephalosporin-based tests (e.g., nitrocefin) which give reliable and rapid results for β-lactamase detection is one such example (CLSI, 2018).

The detection of "extended-spectrum β-lactamase" (ESBL) (CLSI, 2018) activity in certain bacteria is possible by standard disc diffusion AST methods involving specific cephalosporins (cefotaxime and ceftazidime) individually and also along with a β-lactamase inhibitor such as clavulanic acid, and the zones of inhibition formed can be measured. A latex agglutination test was evaluated for the detection of penicillin-binding protein 2a (PBP 2a) in methicillin-resistant staphylococci (Stepanovic et al., 2006). In order to ensure accurate results, it is important to test known positive and negative control strains along with clinical isolates.

7 Modern Methods in Detecting AMR

In addition to phenotypic methods, molecular techniques are also frequently employed to detect the underlying genetic mechanisms responsible for AMR. Phenotypic methods evaluate the capacity of the organism to multiply in the

presence of a specific antibiotic. By employing molecular diagnostic tests, mutations can be detected in the genes responsible for resistance to a particular group of antimicrobial agent. Genome-based diagnostics are useful tools in confirming phenotypic tests, through which the mechanisms of certain AMR can be confirmed. The isolates of *E. coli* or *Klebsiella pneumoniae* that are phenotypically resistant to third-generation cephalosporins can be tested for different ESBLs and the gene that codes for the resistance can be characterized. However, by molecular tests, unknown resistance genes cannot be detected. Only previously identified resistance genes or mutations can be detected, and this necessitates testing for phenotypic resistance in surveillance to ensure accurate identification and classification of the isolates. Lack of correlation between the molecular and phenotypic test results are often noticed. Particularly, DNA amplification-based tests lead to false-negative results since the gene concerned with the resistance phenotype is not analyzed. Similarly, DNA contamination can lead to false-positives. Moreover, these nucleic acid amplification technologies cannot give MICs or it cannot give a direct indication of which antibiotic should be used. However, many commercial diagnostic panels are commercialized by Qiagen, Bosch, or BioMerieux for the identification of specific resistance genes. This is not applicable in case of certain pathogens in which antibiotic resistance has been reported less frequently, *viz. Mycoplasma, Legionella, Bordetella* and *Chlamydia*. In case of these organisms, a detailed antibiogram is not needed and hence, the results are clinically relevant.

Laboratories are classified into three types based on the use of molecular tools and AMR surveillance (WHO, 2019). They are Type 1 laboratories, which have no previous experience in surveillance of AMR or molecular methods; Type 2 laboratories, which have previous experience in antimicrobial susceptibility testing but no previous experience in molecular diagnostics (a new National Research Laboratory); and Type 3 laboratories, having experience in both antimicrobial susceptibility testing and use of molecular methods in surveillance of AMR (a fully established National Research Laboratory).

According to WHO, the most validated molecular tests for detection of AMR are divided into four categories:

1. Based on sequence – In sequence-based tests such as whole genome sequencing and nanopore, resistance genes are detected based on analysis of genome sequences.
2. Based on hybridization – In hybridization-based tests such as arrays and fluorescent in situ hybridization (FISH), gene sequences are targeted using hybridized nucleic acid probes.
3. Amplification methods – In amplification-based tests like PCR as well as loop-mediated isothermal amplification (LAMP), target gene sequences are amplified to allow detection.
4. Immunoassays – Immunoassays such as lateral flow immunoassay and nucleic acid lateral flow assay are based on binding of antibody to target genes or their products allowing detection.

7.1 Polymerase Chain Reaction

Polymerase chain reaction has been employed as a quick and reliable diagnostic technique since its invention in 1983 and is considered as an inevitable technique in all molecular biology laboratories. Over the years, the methodology has undergone various modifications. In Type 1 laboratories, AMR is detected using automated, integrated devices with single-use amplification test cartridges. Here, freeze-dried reagents are used for cartridges that can be stored at room temperature. There are devices having rechargeable batteries, anticipating power failures. In order to detect several resistance markers in a single step, multiplex PCR can be used. For instance, carbapenemase and ESBL genes can be detected at the same time, facilitating identification of MDR organisms.

In LAMP-based diagnostics also, fully automated, integrated devices with single-use test cartridges are used. They are quicker and more powerful than PCR. The positive reactions can be interpreted visually based on increased turbidity in the reaction container. They are reported to be having 10–100 times more sensitive compared to conventional PCR (Khan et al., 2018). Another advantage of LAMP is that expensive thermal cyclers or electrophoresis systems are not required.

7.2 DNA Microarray

Markers for all the major pathogens and resistance markers are incorporated into an array and thus, several molecular resistance and species markers can be identified simultaneously. In order to generate a labelled probe, a PCR step is required. Other machines are also required to read and interpret the signal (e.g., laser and optical detector). If multiple genes are tested at the same time, statistical correction is required. The expense depends on the number of markers tested.

A comparatively simple and rapid test that detects several resistance markers at the same time is the line probe assay. For example, penicillinases and metallo-β-lactamases can be detected simultaneously by line probe test. However, for sample preprocessing, several equipment and reagents and steps to avoid contamination are required.

7.3 Hybridization Techniques

Fluorescence in situ hybridization (FISH) is used for detecting resistance markers directly from bacterial cells. It involves a fluorescence laser microscope or mercury vapor bulb or light-emitting diodes as cost-effective substitutes. The care of the lens should be assured by purified water. Fluorescence laser microscope necessitates frequent servicing by trained personnel. The PNA-FISH technology utilizing peptide nucleic acid (PNA) probes facilitates quick and specific binding than DNA probes as per Cerqueira et al. (2011). This principle is used in recently developed QuickFish technology aiming 16S rRNA (Enroth et al., 2019). A novel XpressFish technology

when used in combination with the QuickFish-based method is proved to be effective in diagnosis of methicillin resistance in Staphylococcus by detecting *mecA* gene in a period of 2 h after the blood culture is positive (Salimnia et al., 2014).

Antibiotic sensitivity testing system based on biosensors been developed which involves the quantification of 16srRNA molecules. In rapidly dividing bacterial cells, the concentration of RNA is abundant. Their presence indicates active metabolism of bacteria and thus it indirectly provides a measurement of bacterial concentration (Halford et al., 2013). This is the principle involved in biosensor-based antibiotic susceptibility system.

7.4 Nucleic Acid Lateral Flow Immunoassays

Immunodiagnostic tests can be used for the detection of bacterial pathogens. Several simple lateral flow assays are now available. For the detection of antimicrobial susceptibility, these can be incorporated into biosensors or into nucleic acid-based tests. This is now available for the diagnosis of influenza viruses. The antibodies are immobilized onto immunochromatographic strips, biosensors, or nanoparticles, which facilitates its binding specifically to targets. In this technique, the detection antibody is labeled with enzymes or fluorescent dyes which emit a quantitative signal. Kitao et al. (2010) has reported a lateral flow test that detects resistance to chloramphenicol in *Pseudomonas aeruginosa* in clinical samples. The same principle can be applied in detecting methicillin-resistant *Staphylococcus aureus*. This was reported by Yamada et al. (2013). They developed a lateral flow test based on penicillin-binding protein 2a-specific (PBP2a) chicken IgY antibody. The promptness of PBP2a *Staphylococcus aureus* culture colony test in identifying MRSA (detection possible within 6 min) was also observed by Delport et al. (2016). Carbapenem group of drugs, which are the last resort in antibiotic therapy, is reported to be gaining resistance, especially in bacteria belonging to the family *Enterobacteriaceae*. This is due to the presence of carbapenemases. In order to identify carbapenemases, especially, KPC, NDM type, or OXA-48-like, commercial kits (Coris Bioconcept, Belgium) are launched and their efficacy has been evaluated (Glupczynski et al., 2017). Lateral flow devices for the identification of carbapenemases like KPC, VIM, NDM, OXA-48-like, TX-M15, and IMP were developed and found to be effective in isolates from clinical cases (Boutal et al., 2017). Lateral flow tests can be considered to be quick, cheap, and easy-to-use techniques which can detect several resistance markers in a single step. The test can be carried out without electricity. It necessitates reagents and equipment for preprocessing of samples and training to avoid contamination.

7.5 Whole Genome Sequencing

The utility of sequencing the whole genome is employed in personalized medicine. It is a significant tool to combat AMR. Whole genome sequencing provides fast

pathogen identification and aids in epidemiological typing, as well as detection of genes associated with antimicrobial resistance. This technique gives immense quantity of data in fragmented form and hence, sophisticated software is essential to interpret the results. European Committee on Antimicrobial Susceptibility Testing reviewed the development status of WGS for antimicrobial susceptibility testing. Various databases are available that captures gene sequences associated with antimicrobial resistance. The database, ResFinder, analyzes AMR genes from data sets of whole genomes employing basic local alignment search tool (BLAST). In 2017, this database was updated so as to facilitate detection of mutations at chromosomal level by means of PointFinder. The data regarding chromosomal mutations that confer antimicrobial resistance to certain pathogens such as *Campylobacter*, *Escherichia coli*, *Mycobacterium tuberculosis*, and *Salmonella* are available with this. Currently, several bioinformatic resources are freely available for the detection of determinants of AMR based on amino acid or DNA sequence. These include ARG-ANNOT, Genefinder, CARD, MEGARes, AMRFinder, KmerResistance, SRST2, ARIBA, and ResFinder (Hendriksen et al., 2019).

Whole genome sequencing as a tool for antimicrobial susceptibility testing is, however, either weak or nonexistent for most bacteria and thus inadequate for a decision-making in clinical settings. This can be resolved by developing a single database, including all known resistance genes/mutations, thus facilitating comparison between these. In veterinary diagnostics, WGS is evaluated in many clinical cases. In a study conducted in non-aureus staphylococci, 405 isolates (348 from nonclinical mastitis and 57 clinical mastitis isolates) were sequenced using a MiSeq platform. A total of randomly selected 20 multidrug-resistant isolates were submitted to NCBI. Four databases were utilized for evaluation of antibiotic resistance genes and other resistance determinants, viz., ARG-ANNOT v3 (Antibiotic Resistance Gene-ANNOTation), ResFinder (Center for Genomic Epidemiology), Comprehensive Antibiotic Resistance Database (CARD), and MegaRES v1.0.1, as reported by the authors. These four databases were combined to a separate database (Nobrega et al., 2018). There are certain databases which are species specific. "Tuberculosis Drug Resistance Database" for *Mycobacterium tuberculosis* is one such database. These are helpful in recognizing mechanisms of resistance in these pathogens where the AMR is primarily from chromosomal mutations.

7.6 Matrix-Assisted Laser Desorption Ionization–Time of Flight Mass Spectrometry (MALDI-TOF MS)

Matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS) is employed for primary identification of bacteria because of its simplicity and accuracy in clinical settings (Oviano & Bou, 2018).

New commercial technique includes the GeneXpert system (Cepheid, France), which is capable of detecting genes coding for a variety of carbapenemases (Burillo et al., 2016) and "methicillin-resistant *Staphylococcus aureus*" (MRSA) can be detected by the Xpert MRSA test (Yarbrough et al., 2017). Another biochemical

method for prompt detection of antimicrobial resistance is the Carba-NP test. The change in color of the buffer in the presence of a pH indicator is measured. Polymerase chain reaction and whole genome sequencing are routinely used globally for decreasing the risk posed by AMR worldwide. The increased access to data pertaining to genome sequence aids in better understanding of the mechanisms of AMR as well as the diversity.

The smell-print characters of specific bacteria and their metabolic profile can be utilized for the detection of antibiotic degradation products and growth-related molecules employing devices that detect volatile compounds. These are called electronic noses. These are applied in diagnostics for pathogen identification (Persaud & Dodd, 1982). Later, Lai et al. (2002) developed the Cyranose system that differentiate controls and samples positive for *Haemophilus influenzae*, *S. aureus*, *P. aeruginosa*, and *S. pneumoniae* in upper respiratory tract infections. The technique was modified and evaluated by Lewis et al. (2017) and found that gas chromatography coupled with ion mobility spectrometry, called as GC-IMS E-nose, could differentiate bacterial respiratory tract infections from those caused by viruses. An ion mobility spectrometry sensor that discriminates methicillin resistant and sensitive staphylococci were evaluated by Saviauk et al. (2018) and reported to be 83% sensitive and 100% specific. This was utilized for the identification for anaerobic pathogens like *Clostridium perfringens* with moderate accuracy.

Point of care test devices employing smartphone technology have been applied in personalized human medication. Smartphone-based technologies were evaluated for antimicrobial susceptibility testing of urinary pathogens (Kadlec et al., 2014). They were used in combination with a microphotometric system in which micro-well plates were coated with antimicrobial agent and a tetrazolium salt, followed by addition of the bacterial culture. The metabolic activity caused change in color of the indicator from yellow to orange. Similarly, automated technologies with three-dimensional printing attachments were also evaluated and reported to be 99.23% accurate in testing susceptibility of *Klebsiella pneumoniae* to different classes of antibiotics (Feng et al., 2016). Barnes et al. (2018) also evaluated smartphone-based technology in colorimetric analysis of urine strips. Hernández-Neuta et al. (2019) analyzed the use of smartphones in conventional growth-based antimicrobial susceptibility testing. These tests can be employed in veterinary field as well for the detection of antimicrobial susceptibility of animal pathogens.

8 Limitations and Challenges for Molecular AMR Diagnostics

Only known resistance genes or mutations can be detected by molecular tests. The cost-efficacy of molecular tests in routine clinical and laboratory settings is not satisfactory. Molecular tests are used mainly in public health surveillance systems rather than in clinical settings. The ability of various tests to detect resistance mechanisms is dubious. Continuous funding is required for molecular AMR testing. Many a times, the correlation of results of molecular tests with that of phenotypic tests are imperfect and their clinical interpretation is also difficult. The results vary

with bacterial species and antimicrobial class. Inadequate awareness of mechanisms of resistance often leads to poor sensitivity of the test. Awareness on molecular mechanisms of antimicrobial resistance must be improved. Proof-of-principle studies may be supplemented to evaluate molecular AMR diagnostics for surveillance purpose.

9 Conclusion

Despite the developments in molecular diagnostics, conventional growth-based tests are widely used in clinical settings for AST. With existing molecular AMR diagnostics, novel mechanisms caused by changes in known resistance genes are detectable. However, complete understanding of the mechanisms of resistance is not possible, as their design depends on the previous knowledge of the responsible DNA sequences. There are several bioinformatics tools that aid in analyzing and predicting the resistance of a bacterial isolate based on sequencing data. New tools that identify genes associated with AMR and single nucleotide polymorphisms and generate exhaustive output are being developed. Molecular and phenotypic tests for AMR diagnosis of AMR complement one another in analyzing the extent of resistance in a given setting. They also aid in understanding the underlying mechanisms responsible for resistance.

References

Barnes, L., Heithoff, D. M., Mahan, S. P., Fox, G. N., Zambrano, A., & Choe, J. (2018). Smartphone-based pathogen diagnosis in urinary sepsis patients. *eBioMedicine, 36*, 73–82. https://doi.org/10.1016/j.ebiom.2018.09.001

Boutal, H., Naas, T., Devilliers, K., Oueslati, S., Dortet, L., & Bernabeu, S. (2017). Development and validation of a lateral flow immunoassay for rapid detection of NDM-producing enterobacteriaceae. *Journal of Clinical Microbiology, 55*, 2018–2029. https://doi.org/10.1128/JCM.00248-17

Burillo, A., Marín, M., Cercenado, E., Ruiz-Carrascoso, G., Pérez-Granda, M. J., Oteo, J., & Bouza, E. (2016). Evaluation of the Xpert Carba-R (Cepheid) assay using contrived bronchial specimens from patients with suspicion of ventilator-associated pneumonia for the detection of prevalent carbapenemases. *PLoS One, 11*, e0168473. https://doi.org/10.1371/journal.pone.0168473

Cerqueira, L., Fernandes, R. M., Ferreira, R. M., Carneiro, F., Dinis-Ribeiro, M., Figueiredo, C., et al. (2011). PNA-FISH as a new diagnostic method for the determination of clarithromycin resistance of helicobacter pylori. *BMC Microbiology., 11*, 101. https://doi.org/10.1186/1471-2180-11-101

Charnot-Katsikas, A., Tesic, V., Love, N., Hill, B., Bethel, C., & Boonlayangoor, S. (2017). Use of the accelerate pheno system for identification and antimicrobial susceptibility testing of pathogens in positive blood cultures and impact on time to results and workflow. *Journal of Clinical Microbiology, 56*, e01166–e01117. https://doi.org/10.1128/JCM.01166-17

Clinical and Laboratory Standards Institute. (2018). *Performance standards for antimicrobial susceptibility testing; 28th international supplement M100-S21* (25p). CLSI.

Clinical and Laboratory Standards Institute. (2019). *Performance standards for antimicrobial susceptibility testing; 29th international supplement M100-S21* (25p). CLSI.

Delport, J. A., Mohorovic, I., Burn, S., McCormick, J. K., Schaus, D., & Lannigan, R. (2016). Rapid detection of meticillin-resistant staphylococcus aureus bacteraemia using combined three-hour short-incubation matrixassisted laser desorption/ionization time-of-flight MS identification and alere culture colony PBP2a detection test. *Journal of Medical Microbiology, 65*, 626–631. https://doi.org/10.1099/jmm.0.000285

Enroth, H., Retz, K., Andersson, S., Andersson, C., Svensson, K., & Ljungström, L. (2019). Infectious diseases evaluation of QuickFISH and maldi sepsityper for identification of bacteria in bloodstream infection. *Infectious Diseases, 51*, 249–258. https://doi.org/10.1080/23744235.2018.1554258

Feng, W., Zhou, D., Wang, Q., Luo, W., Zhang, D., Sun, Q., & Xia, P. (2016). Dissemination of IMP-4-encoding pIMP-HZ1-related plasmids among Klebsiella pneumoniae and Pseudomonas aeruginosa in a Chinese teaching hospital. *Scientific Reports, 6*, 1–7.

Glupczynski, Y., Jousset, A., Evrard, S., Bonnin, R. A., Huang, T.-D., & Dortet, L. (2017). Prospective evaluation of the OKN K-SeT assay, a new multiplex immunochromatographic test for the rapid detection of OXA-48 like, KPC and NDM carbapenemases. *Journal of Antimicrobials and Chemotherapy, 72*, 1955–1960. https://doi.org/10.1093/jac/dkx089

Halford, C., Gonzalez, R., Campuzano, S., Hu, B., Babbitt, J. T., & Liu, J. (2013). Rapid antimicrobial susceptibility testing by sensitive detection of precursor rRNA using a novel electrochemical biosensing platform. *Antimicrobial Agents and Chemotherapy, 57*, 936–943. https://doi.org/10.1128/AAC.00615-12

Hendriksen, R. S., Bortolaia, V., Tate, H., Tyson, G. H., Aarestrup, F. M., & McDermott, P. F. (2019). Using genomics to track global antimicrobial resistance. *Frontiers of Public Health, 7*, 242. https://doi.org/10.3389/fpubh.2019.00242

Hernández-Neuta, I., Neumann, F., Brightmeyer, J., Ba Tis, T., Madaboosi, N., & Wei, Q. (2019). Smartphone-based clinical diagnostics: Towards democratization of evidence-based health care. *Journal of Internal Medicine, 285*, 19–39. https://doi.org/10.1111/joim.12820

Hospenthal, D. R., & Murray, C. K. (2003). In vitro susceptibilities of seven Leptospira species to traditional and newer antibiotics. *Antimicrobial Agents and Chemotherapy, 47*, 2646–2648.

Kadlec, M. W., You, D., Liao, J. C., & Wong, P. K. (2014). A cell phone–based microphotometric system for rapid antimicrobial susceptibility testing. *Journal of Laboratory Automation, 19*, 258–266.

Khan, M., Wang, R., Li, B., Liu, P., Weng, Q., & Chen, Q. (2018). Comparative evaluation of the LAMP assay and PCR-based assays for the rapid detection of *Alternaria solani*. *Frontiers of Microbiology, 9*, 2089. https://doi.org/10.3389/fmicb.2018.02089

Kitao, T., Miyoshi-Akiyama, T., Shimada, K., Tanaka, M., Narahara, K., & Saito, N. (2010). Development of an immunochromatographic assay for the rapid detection of AAC(6′)-Iae-producing multidrug-resistant *Pseudomonas aeruginosa*. *Journal of Antimicrobials and Chemotherapy, 65*, 1382–1386. https://doi.org/10.1093/jac/dkq148

Lai, S. Y., Deffenderfer, O. F., Hanson, W., Phillips, M. P., & Thaler, E. R. (2002). Identification of upperrespiratory bacterial pathogens with the electronic nose. *Laryngoscope, 112*, 975–979. https://doi.org/10.1097/00005537-200206000-00007

Lewis, J. M., Savage, R. S., Beeching, N. J., Beadsworth, M. B. J., Feasey, N., & Covington, J. A. (2017). Identifying volatile metabolite signatures for the diagnosis of bacterial respiratory tract infection using electronic nose technology: A pilot study. *PLoS One, 12*, e0188879. https://doi.org/10.1371/journal.pone.0188879

London, R., Schwedock, J., Sage, A., Valley, H., Meadows, J., Waddington M., & Straus, D. (2010). An automated system for rapid non-destructive enumeration of growing microbes. *PLoS ONE, 5*, e8609. https://doi.org/10.1371/journal.pone.0008609

Matuschek, E., Åhman, J., Webster, C., & Kahlmeter, G. (2018). Antimicrobial susceptibility testing of colistin - evaluation of seven commercial mic products against standard broth microdilution for *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Acinetobacter* spp. *Clinical Microbiology and Infection, 24*(8), 865–870.

Metzger, S., Frobel, R. A., & Dunne, W. M. (2014). Rapid simultaneous identification and quantitation of *Staphylococcus aureus* and *Pseudomonas aeruginosa* directly from bronchoalveolar lavage specimens using automated microscopy. *Diagnostic Microbiology and Infectious Disease, 79*, 160–165. https://doi.org/10.1016/j.diagmicrobio.2013.11.029

Nobrega, D. B., Naushad, S., Naqvi, S. A., Condas, L., Saini, V., Kastelic, J. P., Luby, C., De Buck, J., & Barkema, H. W. (2018). Prevalence and genetic basis of antimicrobial resistance in non-*aureus* staphylococci isolated from Canadian dairy herds. *Frontiers in Microbiology, 9*, 256. https://doi.org/10.3389/fmicb.2018.00256

Oviano, M., & Bou, G. (2018). Matrix-assisted laser desorption ionization-time of flight mass spectrometry for the rapid detection of antimicrobial resistance mechanisms and beyond. *Clinical Microbiology Review, 32*(1), e00037-18.

Persaud, K., & Dodd, G. (1982). Analysis of discrimination mechanisms in the mammalian olfactory system using a model nose. *Nature, 299*, 352–355. https://doi.org/10.1038/299352a0

Salimnia, H., Fairfax, M. R., Lephart, P., Morgan, M., Gilbreath, J. J., & Butler-Wu, S. M. (2014). An international, prospective, multicenter evaluation of the combination of advanDx staphylococcus QuickFISH BC with mecA XpressFISH for detection of methicillinresistant staphylococcus aureus isolates from positive blood cultures. *Journal of Clinical Microbiology, 52*, 3928–3932. https://doi.org/10.1128/JCM.01811-14

Saviauk, T., Kiiski, J. P., Nieminen, M. K., Tamminen, N. N., Roine, A. N., & Kumpulainen, P. S. (2018). Electronic nose in the detection of wound infection bacteria from bacterial cultures: A proof-of-principle study. *European Surgical Research, 59*, 1–11. https://doi.org/10.1159/000485461

Stepanovic, S., Hauschild, T., Dakic, I., Al-Doori, Z., Švabic-Vlahovic, M., Ranin, L., & Morrison, D. (2006). Evaluation of phenotypic and molecular methods for detection of oxacillin resistance in members of the *Staphylococcus sciuri* group. *Journal of Clinical Microbiology, 44*, 934–937.

World Health Organisation. (2017). *Global antimicrobial resistance surveillance system (GLASS) report: Early implementation 2016–2017* (164p). World Health Organisation.

World Health Organisation. (2018). *Tackling antimicrobial resistance (AMR) together* (Working paper 1.0: Multisectoral coordination). World Health Organisation, 32p.

World Health Organisation. (2019). *Molecular methods for antimicrobial resistance (AMR) diagnostics to enhance the Global Antimicrobial Resistance Surveillance System* (55p). World Health Organisation.

Yamada, K., Wanchun, J., Ohkura, T., Murai, A., Hayakawa, R., & Kinoshita, K. (2013). Detection of methicillin-resistant staphylococcus aureus using a specific anti-PBP2a chicken IgY antibody. *Japanese Journal of Infectious Diseases, 66*, 103–108. https://doi.org/10.7883/yoken.66.103

Yarbrough, M. L., Warren, D. K., Allen, K., Burkholder, D., Daum, R., Donskey, C., Knaack, D., LaMarca, A., May, L., Miller, L. G., Parenti, D. M., Peterson, L., Tan, T. Y., Widen, R., Hernandez, D. R., Wolk, D. M., & Burnham, C. A. (2017). Multicenter evaluation of the Xpert MRSA NxG assay for detection of methicillin-resistant *Staphylococcus aureus* in nasal swabs. *Journal of Clinical Microbiology, 56*, e01381–e01317. https://doi.org/10.1128/JCM.01381-1

Trends in the Determination of Antimicrobial Resistance in Aquaculture and Fisheries

G. K. Sivaraman, Visnuvinayagam Sivam, and Ardhra Vijayan

Contents

Abstract

Antimicrobial resistance (AMR) and its transfer is considered a new pollutant in the aquatic system as it acts as root cause of shifting drug-obdurate genes to human consumers. During aquaculture production, microorganisms acquire antimicrobial resistance genes (ARGs) via cross-contamination with essential inputs such as inlet water, feed, manure, etc., or intentionally added prophylactic agent for maintaining the stock's survivability away from any kind of disease outbreak. And hence, monitoring of antimicrobial contamination from various sources is the need of the hour to establish criterions for monitoring by the expert/consultant policy makers. For this purpose, operable programs of scrutiny on drug unresponsiveness of microbes require swift and accurate methods to assess the occurrence, spread, and control of diseases is mandatory.

G. K. Sivaraman (✉) · A. Vijayan
Microbiology, Fermentation & Biotechnology Division, ICAR- Central Institute of Fisheries Technology, Cochin, Kerala, India
e-mail: gk.sivaraman@icar.gov.in

V. Sivam
Microbiology, Fermentation and Biotechnology Division, ICAR-Central Institute of Fisheries Technology (ICAR-CIFT), Kochi, India

M. P. Mothadaka et al. (eds.), *Handbook on Antimicrobial Resistance*,
https://doi.org/10.1007/978-981-19-9279-7_31

Keywords

Antimicrobial resistance · Antibiotic residues · Aquaculture and fisheries · AMR diagnostics · Microbial surveillance

1 Antibiotics Application in Aquaculture

Aquatic and marine produce and products drew significant attention in recent years owing to better taste and superior conformation of nutrients that resulted in enhanced demand both in intra and internal markets across the nations of the globe. The dire need and reliance on fish from open waters, and the depletion of sources therein, shifted to extremely exaggerated aquaculture practices to meet the high demand. Despite the fact, the intensification accommodates the necessity, it causes enormous pressure on fishes, resulting in disease outbreaks (Rottmann et al., 1992). For this reason, farmers are forced to use antibiotics to sustain stock survivability (Cabello et al., 2013). The administration of antibiotics is commonly done via medicated feed or by application right away into aquacultured ponds (Pham et al., 2015). As a part of containing mass mortalities from bacterial diseases, intense application of antimicrobials as a prophylactic and or metaphylactic measure by aquaculturists has become a quotidian practice (Cabello et al., 2013; Chi et al., 2017) or as a growth-boosting factor (WHO, 2014). Reports are available for the presence of USFDA-approved drugs (Table 1) as well as banned antimicrobial agents in food fishes (Chi et al., 2017). In some nations of Asia and Europe, fluoroquinolones bear legal permits for employment in fish culture (WHO, 2006). Unfortunately, overuse or misuse may contribute to the AMR emergence, which accelerates drug resistance towards both targeted and non-targeted microbes within the aquaculture system. In addition, there are several additional outside issues that initiate transmission of antimicrobial immunity of disease causing microbes in aquaculture, as the same is completely governed by external contributions, viz., inlet water, feed, manure, disinfectant, probiotics, etc. The possible sources of AMR contamination in fisheries and aquaculture are represented in Fig. 1.

Table 1 Approved drugs in aquaculture and fisheries (USFDA, 2017)

Antimicrobial agent	Commercial name	Approved species
Florfenicol	Aquaflor® type A	Channel catfish, salmonids
Oxytetracycline dihydrate	Terramycin® 200	Catfish, salmonids, lobster
Oxytetracycline hydrochloride	Oxymarine™, Terramycin 343, Phennoxy 343, Tetroxy Aquatic	Finfish fry and fingerlings
Sulfadimethoxine/ Ormetoprim	Romet-30®	Catfish, salmonids
Sulfamerazine	Sulfamerazine	Trout

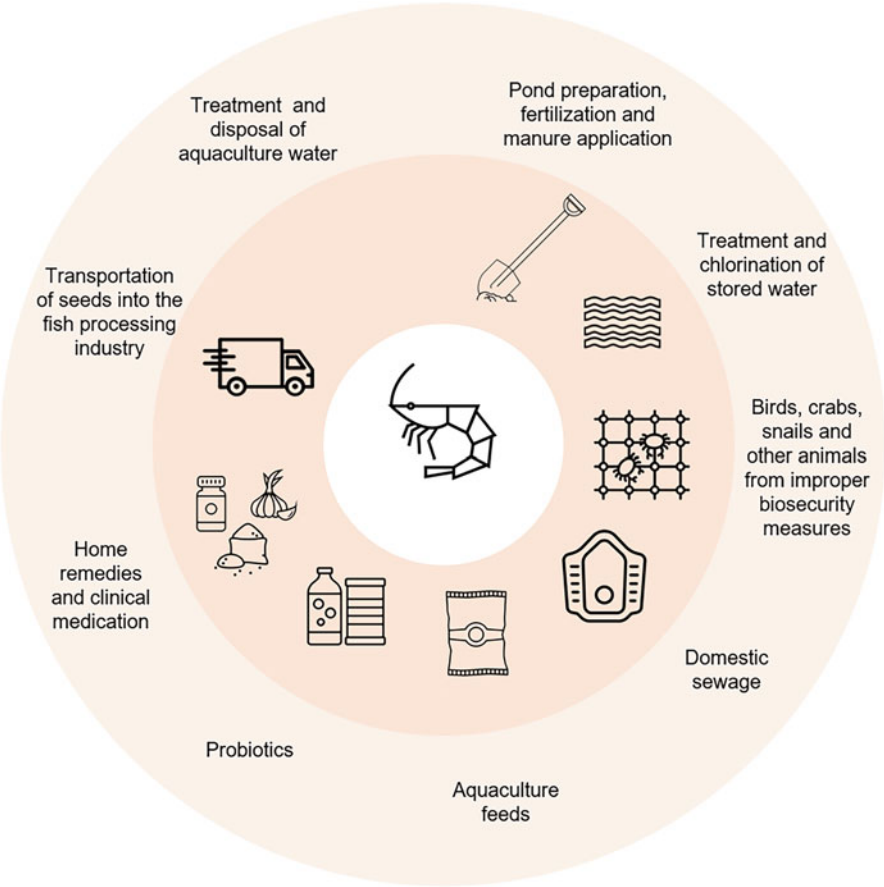


Fig. 1 Potential sources of AMR contamination into the aquaculture setting

2 Possible Sources for the AMR and Its Transmission

Integrated farming is a major and important source for the generation and transmission of antimicrobial obduracy owing to their combination of many systems. The incidence of very high intensity of microbes that are unresponsive to drugs both in water sources and also in manure was well documented, which may act as an input for various microbes (Zhao et al., 2020). In livestock, antibiotics cannot be completely absorbed or metabolized and are excreted in the form of exudates, namely fecal material and urine, and the degree of elimination is determined by the type of animal (Table 2). Bacteria such as *Acinetobacter* sp. and *Enterococcus* sp., from integrated fish farms of Thailand, showed significant resistance to many antibiotics (Su et al., 2011). Studies revealed that the lactobacilli intended for use in aquaculture as probiotics were harbingers of genes that are responsible of

Table 2 Excretion rate of antimicrobial agents in animal (Kumar et al., 2005; Kümmerer & Henninger, 2003)

Antimicrobial agent	Excretion rate (%)
Tetracycline	75–80
Lincosomides	60
Macrolides	50–90
Sulfamethazine	90
Chlortetracyclie	65
Tylosin	50–100
Norfloxacin	30
Ofloxacin	90
Sulfonamide	90
Sulfamethoxazole	85
Amoxicillin	10–20

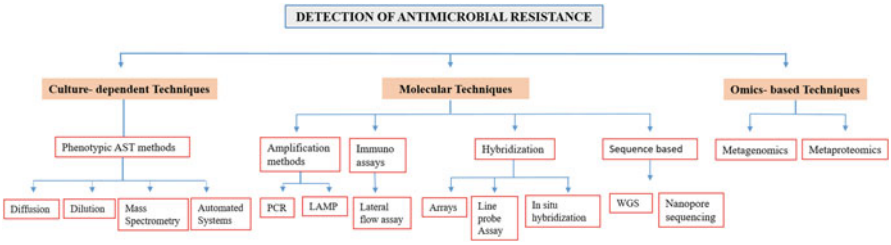


Fig. 2 Methods for the detection of AMR pathogens

antimicrobial unresponsiveness (Muñoz-Atienza et al., 2013). Ecological factors such as temperature, pH, salinity, concentration of antibiotics, etc. are playing a huge role in the development of AMR (MacFadden et al., 2018). Moreover, heavy metal contamination is one of the major possible channels for the drug's obdurate co-selection (Baker-Austin et al., 2006). The cradles of antimicrobial unresponsiveness in aquaculture are depicted in Fig. 2.

3 Report of Antimicrobial Resistance in Aquaculture Pathogens

The intended employment of drugs in aquaculture and the use of antimicrobials mainly aim to control the serious bacterial fish diseases. The development of resistance varies mostly by exchange of plasmids or mobile genetic elements. Plasmid-mediated resistance has been reported in most of the pathogens, like *Aeromonas* (Chenia, 2016), *Vibrio* (Xu et al., 2017), *Pseudomonas* (Magdy et al., 2014), *Edwardsilla tarda* (Nantongo et al., 2019; Ashraf et al., 2020), and *Yersinia ruckeri* (Duman et al., 2017).

Aeromonas salmonicida, an etiological agent of furunculosis, has showed acquired resistance to many antibiotics in temperate waters (WHO, 2006).

A. salmonicidia from Ireland showed transferable plasmid resistance to chloramphenicol, sulfonamides, streptomycin, trimethoprim, and tetracycline and non-transferable resistance to tetracycline in Japan (Aoki, 1997). The florfenicol unresponsiveness occurrence through plasmid and AmpC β-lactamase sequence related to plasmid was reported for the first time in *A. salmonicida* from north America (McIntosh et al., 2007). Resistance to β-lactam antibiotics, namely amoxicillin, carbenicillin, ticarcillin, and imipenem, in *A. hydrophila* from Rainbow trout was also detected (Saavedra et al., 2004). Lee and Wendy (2017) identified that *A. hydrophila* and *E. tarda* are unresponsive to antibiotics such as novobiocin, ampicillin, spiramycin, and chloramphenicol. The isolates were detected in red hybrid Tilapia from the cultured ponds. The *Yersinia ruckeri* isolates detected in trout cultured farms in Bulgaria were obdurate to permitted antimicrobials such as florfenicol, erythromycin, and oxytetracycline (Orozova et al., 2014). Scarano and others (2014) proved that *V. harveyi* possesses resistance to amoxicillin, ampicillin, erythromycin, and sulfadiazine from fish culture systems in Italy. The first report of *V. parahaemolyticus* that encodes the bla_{NDM-1} (carbapenem resistance) gene was obtained from imported shrimp, which also showed resistance to other nine antimicrobial agents (Briet and others, 2018). Oyelade and others (2018) observed the incidence of bla_{NDM-1} gene in pathogens such as *V. vulnificus*. Thus, as a food commodity, fish and shrimp create the possibility of transferring AMR to human gut flora through the food chain.

4 Possibility of Transfer from Clinical Settings to Aquaculture

The persistent contact and subsequent selection pressure will escalate the antimicrobial resistance (AMR) to the environmental microbes. This indicates the possible transmission of AMR from the environment to humans and reversal of the process. For instance, the studies of Furushita and others (2003) showed that tetracycline-unresponsive genes from the fish farm and clinical isolates were seen as similar in origin, confirming the transmission of AMR-obdurate genes between clinical settings and environments. Thus, it is very important to fasten the process of antimicrobial surveillance through effective diagnostic tools to prevent their transmission within the environment.

5 Detection Methods of AMR Pathogens

The unresponsiveness to drugs of the bacteria can be tested either phenotypically or genotypically. Phenotypic methods detect susceptibility through culture-dependent approaches, while molecular methods detect the genes responsible for their resistance.

5.1 Phenotypic Detection Methods

The phenotypic susceptibility tests mainly depend on the "MIC (*minimum inhibitory concentration*)" and interpretation by breakpoint values of "*CLSI* (*Clinical Laboratory Standards Institute*)" or "EUCAST (*European Committee on Antimicrobial Susceptibility Testing*)" strategies. The advantage of the conventional phenotypic method is simple and economical. Apart from this, the key to "Multiple Antibiotic Resistance (MAR)" can be derived in order to find the risk associated with antibiotic exposure. The MAR index of 0.2 and above, indicative of the contamination source, is highly hazardous, wherein intensive employment of antimicrobial drugs and the MAR index values of 0.2 or less signifies that antimicrobial drugs have been in use in rare occasions and the source is not of significant hazard (Krumperman, 1983). Recently, the World Health Organization (WHO) has developed the software, viz. WHONET, for the analysis of antibiotic sensitive test (AST) to derive multiple interpretations with a world unified protocol to support a clear and error-free concept. The phenotypic method can be tested by conventional methods (Diffusion and Dilution) or by advanced methods (Automated Systems and Mass spectrometry).

5.1.1 Conventional Methods

Disc Diffusion: In 1956, Bauer and Kirby have introduced Disc diffusion, and it is still the foremost technique for the phenotypic antibiotic sensitive test (AST). The inhibition zone diameter is an indication of obduracy pattern of a microbe to a specific antimicrobial drug (Varadi et al., 2017). Figure 3 represents Dodecca Disk Diffusion assay.

Dilution: Both agar and broth can be used as media for both micro- and macro-dilution techniques, where the antibiotics are incorporated in the media. Resistance

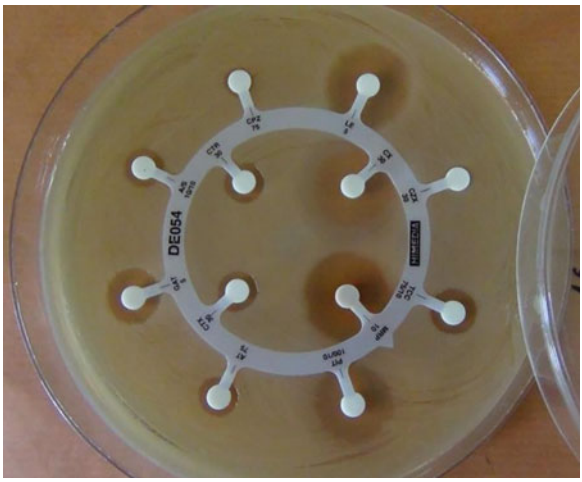


Fig. 3 Dodecca disk diffusion assay for the detection of Gram's negative bacteria

to antibiotics is measured as the MIC of the agent that inhibits the growth of particular bacteria. Compared to disc diffusion, the dilution technique is used for both antibiotic sensitivity as well as biomedical product evaluation.

5.1.2 Advanced Methods

Automated Systems: Numerous sample testing with precise determination of sensitivity urges to proceed with the automated method. It will be highly useful for clinical samples, where numerous samples have to be tested in a short duration. The major advantage is most of the automated methods have an inbuilt analysis system for identification as well as AST determination. As a result, the dual confusion can be sorted out clearly. Besides, it would be a more appropriate system for the sample containing multiple organisms responsible for the infection, facilitating the selection of antibiotic which is sensitive to those bacteria. Most of the automated instruments use turbidimetric, colorimetric, fluorometer, or photometer or its combination, which completely relies on the computer systems for result interpretation. The FDA approved analytical instruments are: *MicroScan WalkAway* (Beckman Coulter, Inc. Atlanta, Georgia, USA) (1980), *Micronaut* (Merlin, Berlin, Germany) (1990), the advantage test (Abbott Laboratories, Irving, Texas, USA) (1980), Vitek 2 (BioMe'rieux, Marcy-l'Étoile, France) (2000), Phoenix (BD Diagnostics, Franklin Lakes, New Jersey, USA) (2001), and Sensititre ARIS 2X (Trek Diagnostic Systems, Ohio, USA) (2004). The WalkAway utilizes micro-dilution and determines the growth either with a photometer or fluorometer. The BD Phoenix employs colorimetry and turbidimetry for detection of growth. The Vitek 2 employs turbidoimetric in monitoring bacterial growth. The fluorescence method is used in assessing bacterial growth when Sensititre ARIS 2X diagnostic system is under use. Ruzauskas et al. (2018) detected the MIC by "Sensititre" susceptibility testing plates (TREK Diagnostic Systems, UK) and Aris 2X (Thermo Fisher Scientific, UK) of Gram-negative bacteria in cultured fish.

Mass Spectrometry: *Matrix-assisted laser desorption ionization-time of flight mass spectrometry* in short "*MALDI-TOF MS*" is most penetrating and cutting-edge methods employed in detection of AMR by peak analysis of spectra.

In the context of carrying out the susceptibility tests for antimicrobials and assessing the levels of minimum inhibitory concentrations, an economically viable method was developed, known as MBT-ASTRA, which stands for "*MALDI Biotyper Antibiotic Susceptibility Test Rapid Assay*" (Burckhardt & Zimmermann, 2018). The benefit of this technique is that the conclusions can be drawn in 24 h. Even though phenotypic resistance pattern is regularly being used for the detection but in few occasions, owing to its limitation, genotypic confirmations are preferred.

5.2 Genotypic Detection Methods

Genotypic methods are accurate and rapid diagnostic techniques as they are based on nucleic acid hybridization or amplification. In this context, the gene of interest is

detected by using specific primers or probes. Genotypic methods can characterize drug obduracy and virulence genotypes. Important genotypic detection methods are discussed hereunder.

5.2.1 Polymerase Chain Reaction

The PCR method is extensively used in detection of genes of drug obduracy due to its fastidiousness. In order to avoid the lengthy biochemical screening of AST procedure, PCR is a better technique for rapid confirmation. For example, in screening of a voluminous number of samples for MRSA, it was possible by identifying the incidence of *mecA* gene (Visnuvinayagam et al., 2015; Sivaraman et al., 2016; Murugadas et al., 2016a, b). Most of the classical PCR needs gel electrophoresis for obtaining the results. In case of real-time PCR, a time-consuming gel electrophoresis step is not necessary, thereby shortening the diagnosis time. In addition, it can quantify the level of the gene in the sample; hence, it is also called as Quantitative PCR or Q-PCR. In recent periods, updated kits also available in identification of most of the genes that are obdurate to different drugs. The NucliSENSEasyQ® KPC platform (bioMérieux) (Spanu et al., 2012) and the Xpert® Carba-R cartridge from GeneXpert® (Findlay et al., 2015) are the automated real-time PCR kits available commercially for the detection of carbapenemases. Some of the PCR confirmations of antibiotic unresponsive genes associated with aquaculture and fisheries are shown in Table 3. Drug therapy promoting antimicrobial unresponsiveness in aquaculture setting was proved by Nonaka et al. (2007) by PCR detection of *tet*M genes after prolonged oxytetracycline (OTC) treatment. A novel class of tetracycline resistance gene, *tet*39, was identified in *Acinetobacter* spp. from freshwater trout farms using PCR (Agersø & Guardabassi, 2005). Even though these technologies provide greater advantages, novel know-hows, namely "*Ligase Chain Reaction, Nucleic Acid Sequence-Based Amplification, Strand Displacement Amplification and Loop-Mediated Isothermal Amplification,*" in short form known to be LCR, NASBA, SDA, and LAMP, respectively (Barany, 1991; Compton, 1991; Walker et al., 1992; Notomi et al., 2000), have been developed to reduce the need of expertise.

5.2.2 "Loop-Mediated Isothermal Amplification (LAMP)"

LAMP is a faster and stronger detection method which does not require an expensive thermocycler and electrophoresis for obtaining the results. Barnes et al. (2018) developed a smartphone-based LAMP technique called smaRT-LAMP technology. But all these techniques are less versatile and less developed for multiplex approaches than PCR.

5.2.3 Hybridization

In hybridization tests, nucleic acid probe-targeted gene sequences are allowed to attach for pairing. In situ hybridization helps to direct the detection of resistance markers in bacterial cells. The distribution of tetracycline resistance genes (*tet*) from freshwater ponds of Chile by DNA-DNA hybridization was well documented (Miranda et al., 2003).

Table 3 Antimicrobial-resistant genes associated with aquaculture

Antibiotic group	Resistance genes	Organism	References
Beta lactams	CTX-M	*Acinetobacter* sp.	Ruzauskas et al. (2018)
		Aeromonas sp.	Chenia and Vietze (2012)
		E. coli	Brahmi et al. (2018), Zhang et al. (2013)
		Klebsiella pneumoniae	Brahmi et al. (2018)
	TEM	*E. coli*	Sousa et al. (2011), Zhang et al. (2013)
		Vibrio sp.	Silvester et al. (2019)
	SHV	*E. coli*	Sousa et al. (2011), Zhang et al. (2013)
		Aeromonas sp.	Vega-Sanchez et al. (2014)
		Citrobacter sp.	Ruzauskas et al. (2018)
	OXA	*Pseudomonas* sp.	Ruzauskas et al. (2018), Zhang et al. (2013)
	AmpC	*Yersinia ruckeri*	Mammeri et al. (2006)
Betalactam-carbapenems	$bla_{\text{IMI-1}}$	*Enterobacter cloacae*	Brouwer et al. (2018)
	NDM-1	*Vibrio* sp.	Silvester et al. (2019)
	OXA 48	*Citrobacter* sp.	Ruzauskas et al. (2018)
Aminoglycosides	*aad*A	*E. coli*	Sousa et al. (2011)
	aac	*E. coli*	Brahmi et al. (2018)
Phenicols	*cml*A	*E. coli*	Sousa et al. (2011)
	*cat*A3	*Edwardsiella tarda*	Sun et al. (2009)
	*flo*R	*Yersinia ruckeri*	Duman et al. (2017)
Sulfonamide	*sul*1	*E. coli*	Sousa et al. (2011), Su et al. (2011), Zhang et al. (2013)
		Enterobacter aerogenes	Su et al. (2011)
		Salmonella sp.	
	*sul*2	*Yersinia ruckeri*	Duman et al. (2017)
		Aeromonas sp.	Ruzauskas et al. (2018)
		E. coli	Sousa et al. (2011), Su et al. (2011), Zhang et al. (2013)
		Serratia marcescens	Su et al. (2011)
	*sul*3	*E. coli*	Sousa et al. (2011), Zhang et al. (2013)
		Enterobacter aerogenes	Su et al. (2011)
		Yersinia ruckeri	Duman et al. (2017)
	dfr	*Citrobacter* sp.	Ruzauskas et al. (2018)
Tetracycline	*tet*A	*E. coli*	Sousa et al. (2011), Zhang et al. (2013)
		Pseudomonas sp.	Miranda et al. (2003)
		Edwardsiella tarda	Sun et al. (2009), Lo et al. (2014)

(continued)

Table 3 (continued)

Antibiotic group	Resistance genes	Organism	References
		Staphylococcus aureus	Fri et al. (2020)
	*tet*B	*Acinetobacter* sp.	Ruzauskas et al. (2018)
		E. coli	Zhang et al. (2013)
	*tet*C	*Yersinia ruckeri*	Duman et al. (2017)
	*tet*D		
	*tet*E	*Aeromonas* sp.	Miranda et al. (2003)
		Yersinia ruckeri	Duman et al. (2017)
	*tet*H	*Moraxella* sp.	Ruzauskas et al. (2018)
	*tet*M	*Staphylococcus aureus*	Fri et al. (2020)
		Streptococcus dysgalactiae	Nguyen et al. (2017)
	*tet*O	*Streptococcus dysgalactiae*	Nguyen et al. (2017)
	*tet*S		
	*tet*W	*E. coli*	Zhang et al. (2013)
Quinolones	*qnr*B	*Aeromonas* sp.	Chenia (2016)
	*qnr*S		
Macrolide	*erm*B	*Staphylococcus aureus*	Fri et al. (2020)
		Streptococcus dysgalactiae	Nguyen et al. (2017)
	*mef*A *msr*D	*Streptococcus dysgalactiae*	Nguyen et al. (2017)

5.2.4 DNA Microarrays

DNA microarray is an alternative to PCR, but labeled probe generation requires a PCR step. In the identification of genes that are virulent and unresponsive to various antimicrobials in *Salmonella* and *E. coli,* the Microarray Analysis is a relatively low-cost process (Chen et al., 2005). Hybridization-based techniques help to detect various molecular obdurate and species markers simultaneously.

5.2.5 Whole Genome Sequencing (WGS)

One of the important tools in assessing the unresponsiveness to antimicrobials is whole-genome sequencing, which can detect many resistance markers simultaneously; however, the major limitation is the generation of voluminous amount of data that is problematic for processing. Espedido et al. (2015) proved that WGS has a greater application in antimicrobial resistance profiling. Guo et al. (2019) carried out the genotypic description of *E. coli* that is unresponsive to many drugs isolated from food stuffs including fish by WGS methods.

5.2.6 Omics-Based Techniques

Omics technologies are aimed to detect the genes, mRNA, proteins, and metabolites from environmental samples. The antibiotic-unresponsive genes from coastline-based commercial mariculture structures were identified by the method of metagenomics (Wang et al., 2018). Further, the study identified that characteristic nitrifying bacteria, namely Nitrospinae, from the structures of mariculture with genes that are unresponsive to many drugs. The effects of perfluorooctane sulfonate on European eels and tiger shrimps (Giant) employing proteomics were assessed by Rodrigues et al. (2017) (Table 4).

All these sequence-based technologies rely on bioinformatics tool for data interpretation. And some of the online bioinformatics databases are AMRfinder, ARIBA, ARG-ANNOT, Genefinder, KmerResistance, MEGARes ResFinder, SRST2, etc. (Hendriksen & others, 2019). The accessible online bioinformatics implements and databanks for AMR detection are presented in Table 5. However, lacunae in expertise on bioinformatics are a serious impediment for carrying out surveillance on AMR. Genetic heterogeneity of AMR pathogens was determined by epidemiological typing methods, viz., "*Amplified Ribosomal DNA Restriction Analysis,*" "*Pulsed Field Gel Electrophoresis,*" "*Random Amplified Polymorphic DNA,*" and "*Restriction Fragment Length Polymorphism,*" in short ARDRA, PFGE, RAPD, and RFLP, respectively. Naviner et al. (2011) determined the clonal diversity of resistant strains from trout farms by PFGE typing. Recently, emerging genetic engineering tools, namely, "*Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR),*" have helped to tackle AMR by editing the genes that are obdurate to many antimicrobials.

6 Conclusion

It is a well-established fact that the unresponsiveness of microbes to various drugs is posing a significant hazard to human health across the world. And hence, the important measures of mitigation include recognizing the cradle and transmission of drug obduracy. The application of phenotypic as well as genotypic testing helps in identifying and addressing the AMR issue from various fields. It is also a well-recognized fact that the problem of microbial obduracy to drugs is widespread in animals, both cultured and wild, and in humans all over the world and environment, and one of the best approaches to tackle this problem is "one health." For this reason, the eradication of drug pollution and the obdurate genes therein in aquaculture are immediate need of the hour. The pragmatization of better aquaculture practices, operable biosecurity procedures, and application of disease deterrence dealings replacing the application of widespread chemotherapeutic agents can make a much-needed difference in handling the menace of resistance to antimicrobials.

Table 4 Summary of the selected emerging and future bacterial identification and AST technologies

Sl. no	Techniques	Working principle	Time for AST	Advantages	References
Emerging technologies for bacterial identification and AST					
Imaging based					
1	OCelloscope	Digital time-lapse microscopy scanning through a fluid sample, generating automatic graphs. Optical system measures the growth of bacterial cell	1–4 h	Fast, high sensitivity	Fredborg et al. (2013)
2	Bacterial Cytological Profiling (BCP)	Fluorescence microscopy to analyze the changes in cytological profiles of bacteria with antimicrobial application	<2 h	Rapid result and can be performed in microliter culture volumes	Nonejuie et al. (2013)
3	Multiplexed Automated Digital Microscopy (MADM)	Sample preparation by gel electrofiltration, cell immobilization by electrokinetic concentration identification by FISH, followed by dark-field microscopic time-lapse imaging	3–5 h	Rapid and fully automated platform for diagnosis	Chantell (2015)
Molecular- and imaging-based					
4	Accelerate pheno system	It combines gel electro filtration and fluorescence in situ hybridization for identification, automated dark-field microscopy for evaluating growth and MIC determination	7 h	Faster and valuable tool in blood culture diagnostics	Marschal et al. (2017)
Imaging-based and microfluidics					
5	Single-Cell Morphological Analysis (SCMA)	Analyzing morphological changes in single bacterial cells under various antimicrobial conditions	3–4 h	Fast and accurate method for AST	Choi et al. (2014)
Non-imaging-based					
6	BacterioScan™ FLLS	Electro-optical technology to measure bacterial growth	3–10 h	Rapid, flexible, and accurate substitute to broth microdilution	Hayden et al. (2016)

Non-imaging-based and microfluidics					
7	Electrochemical Sensor Array (GeneFluidics)	Electrochemical measurement of bacterial 16S rRNA	<4 h	Can be used for both nucleic acids and protein detection	Liao et al. (2006)
8	LifeScale	Microchannel resonators measures mass changes of the individual cells to evaluate antibiotic activity	>3 h	Sensitive mass and morphological analysis on single bacterial cells	Burg et al. (2006)
9	Nanodroplets/ nanoliter arrays	System combines the resazurin assay with a nanoliter well array, Fluorescence indicating the level of metabolic activity which is proportional to the amount of bacteria/ metabolism in the well	<6 h	Rapid, required fewer reagents than other liquid-phase tests	Avesar et al. (2017)
Molecular- and biochemical-based					
10	Pathogen specific bioparticles -Smarticles™	Non replicating phages carrying DNA probes, which specifically bind to particular bacteria that combined with plasmids with luciferase gene. Agent-resistant bacteria emit light	<4 h	Faster and reliable method	Opens Helix (2015)
Future technologies for bacterial identification and AST					
11	Plasmonic Imaging and Tracking (PIT)	Quantify nanometer motions of single bacterial cell and correlate with its metabolism and antibiotic action	<2 h	Quick, simple, and low cost, quantifying AST of individual cells in polymicrobial infections	Syal et al. (2016)
12	FASTest	Uses microfluidic chips and monitored bacterial growth with single-cell microscopic imaging	<30 min	Rapid technology showed the cell-shape, growth rates and the phenotypic resistance pattern	Baltekinet al. (2017)
13	Flow cytometry	Measurement of viable bacteria by fluorescent dyes that are capable to bind damaged cells, allowing to evaluate the effect of antimicrobial drugs	2–3 h	Enumerate morphological and physiological states of many cells within a short time	Gauthier et al. (2002)
14		Amplitude of the cantilever fluctuations sensed by the sensing chamber based on	<1 h		Longo et al. (2013)

(continued)

Table 4 (continued)

Sl. no	Techniques	Working principle	Time for AST	Advantages	References
	Atomic Force Microscopy (AFM) Cantilever	immobilized bacteria on the surface of cantilever		Detect low concentrations of bacteria, and quantitatively screen antibiotics response within minutes	
15	Nuclear Magnetic Resonance (NMR) spectroscopy	Identification and phenotypic resistance were determined by the analysis of bacterial metabolites and their magnetic properties	<6 h	Accurate and cost effective method	García-Álvarez et al. (2015)
16	Infrared Spectroscopy	Reflection techniques allows an IR spectrum by measuring light reflected by the sample	–	Fast, cheap method and does not require special preparation of samples	Lechowicz et al. (2013)
17	Magnetic Bead Spin Biosensor	Detects bacterial growth-based on the spin of magnetic microparticles. MIC were determined by using these self-assembled biosensors	<5 h	Rapid observation of bacterial growth	Kinnunen et al. (2012)
18	Microfluidic Agarose Channel (MAC) system	Immobilizes the bacteria in agarose and single cell growth under different antibiotic culture conditions can be tracked by microscopic time lapse imaging	4–10 h	Fast identification and accurate AST data	Choi et al. (2013)

19	Impedance Measurement	Samples were injected into non-functionalized chips and its impedance response was monitored in the presence and absence of antibiotics	<90 min	Rapid and real time monitoring of bacterial response to antibiotics	Safavieh et al. (2017)
20	Surface-Enhanced Raman Scattering (SERS) AST	The intensity of specific biomarkers like silver nanoparticles in SERS spectra was proportional to the antibiotic effectiveness	2 h	Rapid detection of bacterial activity and simultaneously gives spectroscopic specificity	Liu et al. (2016)
21	Isothermal Microcalorimetry (IMC)	Heat produced is proportional to the reaction rate in the bacterial suspension towards antibiotics and in turn determine MIC	~24 h	Apart from MIC, determined bacteriostatic and bactericidal effect of drugs	von Ah et al. (2009)
22	Electronic nose (E-nose)	An array of sensors, preprocessing electronics detects volatile organic compounds and a computer that reads signals by pattern detection	–	Affordable, basically maintenance-free, and does not require extensive sample preparation, distinguish MRSA from MSSA	Saviauk et al. (2018)

Table 5 Online bioinformatics tools and databases for AMR detection

Sl. no	Source	Link	Type	Year
1.	PATRIC	https://www.patricbrc.org/	Tool	2004
2.	RED-DB	http://www.fibim.unisi.it/REDDB/	Database	2007
3.	INTEGRALL	http://integrall.bio.ua.pt/?	Tool	2008
4.	ARDB	https://ardb.cbcb.umd.edu/	Database	2009
5.	LacED	http://www.laced.uni-stuttgart.de/	Database	2009
6.	TBDReaM	https://tbdreamdb.ki.se/Info/	Database	2009
7.	BLAD	http://www.blad.co.in/	Database	2012
8.	ResFinder	https://cge.cbs.dtu.dk/services/ResFinder/	Tools &Database	2012
9.	BacMet	http://bacmet.biomedicine.gu.se/	Database	2013
10.	CARD	https://card.mcmaster.ca/	Database	2013
11.	MUBII-TB-DB	https://umr5558-bibiserv.univ-lyon1.fr/mubii/mubii-select.cgi	Database	2013
12.	u-CARE	http://www.e-bioinformatics.net/ucare	Database	2013
13.	CBMAR	http://14.139.227.92/mkumar/lactamasedb	Database	2014
14.	Resfams	http://www.dantaslab.org/resfams/	Database	2014
15.	SRST2	http://katholt.github.io/srst2/	Tool	2014
16.	Mykrobe	http://www.mykrobe.com/	Tool	2015
17.	ShortBRED	http://huttenhower.sph.harvard.edu/shortbred	Tool	2015
18.	SSTAR	https://github.com/katholt/srst2	Tool	2015
19.	ABRICATE	https://github.com/tseemann/abricate	Tool	2016
20.	FARME	http://staff.washington.edu/jwallace/farme/	Database	2016
21.	KmerResistance	https://cge.cbs.dtu.dk/services/KmerResistance/	Tool	2016
22.	MEGARes	http://megares.meglab.org/	Database	2016
23.	ResFinderFG	https://cge.cbs.dtu.dk/services/ResFinderFG-1.0/	Tool&Database	2016
24.	SARG	http://smile.hku.hk/SARGs	Database	2016
25.	SCCmec Finder	https://cge.cbs.dtu.dk/services/SCCmecFinder/	Database	2016
26.	ABRES Finder	http://scbt.sastra.edu/ABRES/index.php	Database	2017
27.	AMRtime	https://github.com/beiko-lab/AMRtime	Tool	2017
28.	ARIBA	https://github.com/sanger-pathogens/ariba	Tool	2017
29.	BLDB	http://www.lahey.org/Studies/	Database	2017
30.	DeepArgs	http://bench.cs.vt.edu/deeparg	Tool	2017
31.	Galileo AMR	https://galileoamr.arcbio.com/mara/	Tool&Database	2017
32.	Mustard	http://mgps.eu/Mustard/	Database	2017
33.	NCBI-AMRFinder	https://www.ncbi.nlm.nih.gov/pathogens	Tool	2017

(continued)

Table 5 (continued)

Sl. no	Source	Link	Type	Year
34.	PointFinder	https://cge.cbs.dtu.dk/services/ResFinder/	Database	2017
35.	ResCap	https://github.com/valflanza/ResCap	Database	2017
36.	ARGDIT	https://github.com/phglab/ARGDIT	Tool&Database	2018
37.	ARG-miner	https://bench.cs.vt.edu/argminer/#/home	Database	2018
38.	GROOT	https://github.com/will-rowe/groot	Tool	2018
39.	Noradab	http://noradab.bi.up.ac.za/	Database	2018
40.	IRIDA plugin AMR detection	https://github.com/phac-nml/irida-plugin-amr-detection	Database	2019
41.	LREfinder	https://cge.cbs.dtu.dk/services/LREfinder/	Database	2019

References

Agersø, Y., & Guardabassi, L. (2005). Identification of Tet 39, a novel class of tetracycline resistance determinant in Acinetobacter spp. of environmental and clinical origin. *Journal of Antimicrobial Chemotherapy, 55*(4), 566–569.

Aoki, T (1997). Resistance plasmids and the risk of transfer. In *Furunculosis* (pp. 433-440). Academic.

Ashraf, A. A. E. T., Fatma, I. E.-H., Mohamed, S. E.-G., & Amany, A. S. (2020). Antibiotic resistance genes of Edwardsiella tarda isolated from *Oreochromis niloticus* and *Clarias gariepinus,* Benha. *Veterinary Medical Journal, 38*, 131–135.

Avesar, J., Rosenfeld, D., Truman-Rosentsvit, M., Ben-Arye, T., Geffen, Y., Bercovici, M., & Levenberg, S. (2017). Rapid phenotypic antimicrobial susceptibility testing using nanoliter arrays. *Proceedings of the National Academy of Sciences, 114*(29), E5787–E5795.

Baker-Austin, C., Wright, M. S., Stepanauskas, R., & McArthur, J. V. (2006). Co-selection of antibiotic and metal resistance. *Trends in Microbiology, 14*(4), 176–182.

Baltekin, Ö., Boucharin, A., Tano, E., Andersson, D. I, & Elf, J. (2017). Fast antibiotic susceptibility testing based on single cell growth rate measurements. *BioRxiv*, p. 071407. https://doi.org/10.1101/071407

Barany, F. (1991). Genetic disease detection and DNA amplification using cloned thermostable ligase. *Proceedings of the National Academy of Sciences, 88*(1), 189–193.

Barnes, L., Heithoff, D. M., Mahan, S. P., Fox, G. N., Zambrano, A., Choe, J., Fitzgibbons, L. N., Marth, J. D., Fried, J. C., Soh, H. T., & Mahan, M. J. (2018). Smartphone-based pathogen diagnosis in urinary sepsis patients. *eBioMedicine, 36*, 73–82.

Brahmi, S., Touati, A., Dunyach-Remy, C., Sotto, A., Pantel, A., & Lavigne, J. P. (2018). High prevalence of extended-spectrum β-lactamase-producing Enterobacteriaceae in wild fish from the Mediterranean Sea in Algeria. *Microbial Drug Resistance, 24*(3), 290–298.

Briet, A., Helsens, N., Delannoy, S., Debuiche, S., Brisabois, A., Midelet, G., & Granier, S. A. (2018). NDM-1-producing Vibrio parahaemolyticus isolated from imported seafood. *Journal of Antimicrobial Chemotherapy, 73*(9), 2578–2579.

Brouwer, M. S., Rapallini, M., Geurts, Y., Harders, F., Bossers, A., Mevius, D. J., Wit, B., & Veldman, K. T. (2018). Enterobacter cloacae complex isolated from shrimps from Vietnam carrying blaIMI-1 resistant to carbapenems but not cephalosporins. *Antimicrobial Agents and Chemotherapy, 62*(7).

Burg, T. P., Mirza, A. R., Milovic, N., Tsau, C. H., Popescu, G. A., Foster, J. S., & Manalis, S. R. (2006). Vacuum-packaged suspended microchannel resonant mass sensor for biomolecular detection. *Journal of Microelectromechanical Systems, 15*(6), 1466–1476.

Burckhardt, I., & Zimmermann, S. (2018). Susceptibility testing of bacteria using Maldi-Tof mass spectrometry. *Frontiers in Microbiology, 9*, 1744. https://doi.org/10.3389/fmicb.2018.01744

Cabello, F. C., Godfrey, H. P., Tomova, A., Ivanova, L., Dölz, H., Millanao, A., & Buschmann, A. H. (2013). Antimicrobial use in aquaculture re-examined: Its relevance to antimicrobial resistance and to animal and human health. *Environmental Microbiology, 15*(7), 1917–1942.

Chantell, C. (2015). Multiplexed automated digital microscopy for rapid identification and antimicrobial susceptibility testing of bacteria and yeast directly from clinical samples. *Clinical Microbiology Newsletter, 37*(20), 161–167.

Chen, S., Zhao, S., McDermott, P. F., Schroeder, C. M., White, D. G., & Meng, J. (2005). A DNA microarray for identification of virulence and antimicrobial resistance genes in *Salmonella serovars* and *Escherichia coli*. *Molecular and Cellular Probes, 19*(3), 195–201.

Chenia, H. Y., & Vietze, C. (2012). Tetracycline resistance determinants of heterotrophic bacteria isolated from a South African tilapia aquaculture system. *African Journal of Microbiology Research, 6*(39), 6761–6768.

Chenia, H. Y. (2016). Prevalence and characterization of plasmid-mediated quinolone resistance genes in Aeromonas spp. isolated from South African freshwater fish. *International Journal of Food Microbiology, 231*, 26–32.

Chi, T. T. K., Clausen, J. H., Van, P. T., Tersbøl, B., & Dalsgaard, A. (2017). Use practices of antimicrobials and other compounds by shrimp and fish farmers in Northern Vietnam. *Aquaculture Reports, 7*, 40–47.

Choi, J., Jung, Y. G., Kim, J., Kim, S., Jung, Y., Na, H., & Kwon, S. (2013). Rapid antibiotic susceptibility testing by tracking single cell growth in a microfluidic agarose channel system. *Lab on a Chip, 13*(2), 280–287.

Choi, J., Yoo, J., Lee, M., Kim, E. G., Lee, J. S., Lee, S., Joo, S., Song, S. H., Kim, E. C., Lee, J. C., & Kim, H. C. (2014). A rapid antimicrobial susceptibility test based on single-cell morphological analysis. *Science Translational Medicine, 6*(267), 267ra17.

Compton, J. (1991). Nucleic acid sequence-based amplification. *Nature, 350*(6313), 91–92.

Duman, M., Altun, S., Cengiz, M., Saticioglu, I. B., Buyukekiz, A. G., & Sahinturk, P. (2017). Genotyping and antimicrobial resistance genes of Yersinia ruckeri isolates from rainbow trout farms. *Diseases of Aquatic Organisms, 125*(1), 31–44.

Espedido, B. A., Dimitrijovski, B., van Hal, S. J., & Jensen, S. O. (2015). The use of whole-genome sequencing for molecular epidemiology and antimicrobial surveillance: Identifying the role of IncX3 plasmids and the spread of blaNDM-4-like genes in the Enterobacteriaceae. *Journal of Clinical Pathology, 68*(10), 835–838.

Findlay, J., Hopkins, K. L., Meunier, D., & Woodford, N. (2015). Evaluation of three commercial assays for rapid detection of genes encoding clinically relevant carbapenemases in cultured bacteria. *Journal of Antimicrobial Chemotherapy, 70*(5), 1338–1342.

Fredborg, M., Andersen, K. R., Jørgensen, E., Droce, A., Olesen, T., Jensen, B. B., Rosenvinge, F. S., & Sondergaard, T. E. (2013). Real-time optical antimicrobial susceptibility testing. *Journal of Clinical Microbiology, 51*(7), 2047–2053.

Fri, J., Njom, H.A., Ateba, C.N and Ndip, R.N (2020). Antibiotic resistance and virulence gene characteristics of methicillin-resistant *Staphylococcus aureus* (MRSA) isolated from healthy edible marine fish. *International Journal of Microbiology*, *2020*, 9803903. https://doi.org/10.1155/2020/9803903

Furushita, M., Shiba, T., Maeda, T., Yahata, M., Kaneoka, A., Takahashi, Y., Torii, K., Hasegawa, T., & Ohta, M. (2003). Similarity of tetracycline resistance genes isolated from fish farm bacteria to those from clinical isolates. *Applied and Environmental Microbiology, 69*(9), 5336–5342.

García-Álvarez, L., Busto, J. H., Avenoza, A., Sáenz, Y., Peregrina, J. M., & Oteo, J. A. (2015). Proton nuclear magnetic resonance spectroscopy as a technique for gentamicin drug

susceptibility studies with Escherichia coli ATCC 25922. *Journal of Clinical Microbiology, 53*(8), 2433–2438.

Gauthier, C., St-Pierre, Y., & Villemur, R. (2002). Rapid antimicrobial susceptibility testing of urinary tract isolates and samples by flow cytometry. *Journal of Medical Microbiology, 51*(3), 192–200.

Guo, S., Tay, M. Y., Thu, A. K., Seow, K. L. G., Zhong, Y., Ng, L. C., & Schlundt, J. (2019). Conjugative IncX1 plasmid harboring colistin resistance gene mcr-5.1 in Escherichia coli isolated from chicken rice retailed in Singapore. *Antimicrobial Agents and Chemotherapy, 63*(11).

Hayden, R. T., Clinton, L. K., Hewitt, C., Koyamatsu, T., Sun, Y., Jamison, G., Perkins, R., Tang, L., Pounds, S., & Bankowski, M. J. (2016). Rapid antimicrobial susceptibility testing using forward laser light scatter technology. *Journal of Clinical Microbiology, 54*(11), 2701–2706.

Hendriksen, R. S., Bortolaia, V., Tate, H., Tyson, G., Aarestrup, F. M., & McDermott, P. (2019). Using genomics to track global antimicrobial resistance. *Frontiers in Public Health, 7*, 242.

Kinnunen, P., McNaughton, B. H., Albertson, T., Sinn, I., Mofakham, S., Elbez, R., Newton, D. W., Hunt, A., & Kopelman, R. (2012). Self-assembled magnetic bead biosensor for measuring bacterial growth and antimicrobial susceptibility testing. *Small, 8*(16), 2477–2482.

Krumperman, P. H. (1983). Multiple antibiotic resistance indexing of Escherichia coli to identify high-risk sources of fecal contamination of foods. *Applied and Environmental Microbiology, 46*(1), 165–170.

Kumar, K., Gupta, S. C., Baidoo, S. K., Chander, Y., & Rosen, C. J. (2005). Antibiotic uptake by plants from soil fertilized with animal manure. *Journal of Environmental Quality, 34*(6), 2082–2085.

Kümmerer, K., & Henninger, A. (2003). Promoting resistance by the emission of antibiotics from hospitals and households into effluent. *Clinical Microbiology and Infection, 9*(12), 1203–1214.

Lechowicz, L., Urbaniak, M., Adamus-Białek, W., & Kaca, W. (2013). The use of infrared spectroscopy and artificial neural networks for detection of uropathogenic Escherichia coli strains' susceptibility to cephalothin. *Acta Biochimica Polonica, 60*(4), 713–718. PMID: 24432322.

Lee, S. W., & Wendy, W. (2017). Antibiotic and heavy metal resistance of Aeromonashydrophila and Edwardsiellatarda isolated from red hybrid tilapia (Oreochromis spp.) coinfected with motile aeromonas septicemia and edwardsiellosis. *Veterinary World, 10*(7), 803.

Liao, J. C., Mastali, M., Gau, V., Suchard, M. A., Møller, A. K., Bruckner, D. A., Babbitt, J. T., Li, Y., Gornbein, J., Landaw, E. M., & McCabe, E. R. (2006). Use of electrochemical DNA biosensors for rapid molecular identification of uropathogens in clinical urine specimens. *Journal of Clinical Microbiology, 44*(2), 561–570.

Liu, C. Y., Han, Y. Y., Shih, P. H., Lian, W. N., Wang, H. H., Lin, C. H., Hsueh, P. R., Wang, J. K., & Wang, Y. L. (2016). Rapid bacterial antibiotic susceptibility test based on simple surface-enhanced Raman spectroscopic biomarkers. *Scientific Reports, 6*(1), 1–15.

Lo, D. Y., Lee, Y. J., Wang, J. H, & Kuo, H. C. (2014). Antimicrobial susceptibility and genetic characterisation of oxytetracycline-resistant Edwardsiellatarda isolated from diseased eels. *Veterinary Record*. https://doi.org/10.1136/vr.101580

Longo, G., Alonso-Sarduy, L., Rio, L. M., Bizzini, A., Trampuz, A., Notz, J., Dietler, G., & Kasas, S. (2013). Rapid detection of bacterial resistance to antibiotics using AFM cantilevers as nanomechanical sensors. *Nature Nanotechnology, 8*(7), 522–526.

MacFadden, D. R., McGough, S. F., Fisman, D., Santillana, M., & Brownstein, J. S. (2018). Antibiotic resistance increases with local temperature. *Nature Climate Change, 8*(6), 510–514.

Magdy, I., El-Hady, M., Ahmed, H., Elmeadawy, S., & Kenwy, A. A. (2014). Contribution on *Pseudomonas aeruginosa* infection in African catfish (*Clarias gariepinus*). *Research Journal of Pharmaceutical, Biological and Chemical Sciences, 5*, 575–588.

Mammeri, H., Poirel, L., Nazik, H., & Nordmann, P. (2006). Cloning and functional characterization of the ambler class C β-lactamase of Yersinia ruckeri. *FEMS Microbiology Letters, 257*(1), 57–62.

Marschal, M., Bachmaier, J., Autenrieth, I., Oberhettinger, P., Willmann, M., & Peter, S. (2017). Evaluation of the accelerate pheno system for fast identification and antimicrobial susceptibility testing from positive blood cultures in bloodstream infections caused by Gram-negative pathogens. *Journal of Clinical Microbiology, 55*(7), 2116–2126.

Miranda, C. D., Kehrenberg, C., Ulep, C., Schwarz, S., & Roberts, M. C. (2003). Diversity of tetracycline resistance genes in bacteria from Chilean salmon farms. *Antimicrobial Agents and Chemotherapy, 47*(3), 883–888.

Muñoz-Atienza, E., Gómez-Sala, B., Araújo, C., Campanero, C., Del Campo, R., Hernández, P. E., Herranz, C., & Cintas, L. M. (2013). Antimicrobial activity, antibiotic susceptibility and virulence factors of lactic acid bacteria of aquatic origin intended for use as probiotics in aquaculture. *BMC Microbiology, 13*(1), 15.

Murugadas, V., Joseph, T. C., Reshmi, K., & Lalitha, K. V. (2016a). Prevalence of methicillin resistant Staphylococcus aureus in selected seafood markets and aquaculture farms in Kerala, south-west coast of India. *Indian Journal of Fisheries, 64*(3), 150–153.

Murugadas, V., Joseph, T. C., & Lalitha, K. V. (2016b). Distribution of pathotypes of Escherichia coli in seafood from retail markets of Kerala, India. *Indian Journal of Fisheries, 63*(1), 152–155.

Nantongo, M., Mkupasi, E. M., Byarugaba, D. K., Wamala, S. P., Mdegela, R. H., & Walakira, J. K. (2019). Molecular characterization and antibiotic susceptibility of Edwardsiella tarda isolated from farmed Nile Tilapia and African catfish from Wakiso, Uganda. *Uganda Journal of Agricultural Sciences, 19*(1), 51–64. https://doi.org/10.4314/ujas.v19i1.5

Naviner, M., Gordon, L., Giraud, E., Denis, M., Mangion, C., Le Bris, H., & Ganière, J. P. (2011). Antimicrobial resistance of Aeromonas spp. isolated from the growth pond to the commercial product in a rainbow trout farm following a flumequine treatment. *Aquaculture, 315*(3–4), 236–241.

Nguyen, T. T. T., Nguyen, H. T., Tsai, M. A., Byadgi, O., Wang, P. C., Yoshida, T., & Chen, S. C. (2017). Genetic diversity, virulence genes, and antimicrobial resistance of *Streptococcus dysgalactiae* isolates from different aquatic animal sources. *Aquaculture, 479*, 256–264.

Nonaka, L., Ikeno, K., & Suzuki, S. (2007). Distribution of tetracycline resistance gene, tet (M), in gram-positive and gram-negative bacteria isolated from sediment and seawater at a coastal aquaculture site in Japan. *Microbes and Environments, 22*(4), 355–364.

Nonejuie, P., Burkart, M., Pogliano, K., & Pogliano, J. (2013). Bacterial cytological profiling rapidly identifies the cellular pathways targeted by antibacterial molecules. *Proceedings of the National Academy of Sciences, 110*(40), 16169–16174.

Notomi, T., Okayama, H., Masubuchi, H., Yonekawa, T., Watanabe, K., Amino, N., & Hase, T. (2000). Loop-mediated isothermal amplification of DNA. *Nucleic Acids Research, 28*(12), e63–e63.

Opens, H. I. (2015). Roche gobbles Smarticles. *The New Yorker, 26*, 186.

Orozova, P., Chikova, V., & Sirakov, I. (2014). Diagnostics and antibiotic resistance of Yersinia ruckeri strains isolated from trout fish farms in Bulgaria. *IJDR, 4*(12), 2727–2733.

Oyelade, A. A., Adelowo, O. O., & Fagade, O. E. (2018). bla NDM-1-producing Vibrio parahaemolyticus and *V. vulnificus* isolated from recreational beaches in Lagos, Nigeria. *Environmental Science and Pollution Research, 25*(33), 33538–33547.

Pham, D. K., Chu, J., Do, N. T., Brose, F., Degand, G., Delahaut, P., De Pauw, E., Douny, C., Van Nguyen, K., Vu, T. D., & Scippo, M. L. (2015). Monitoring antibiotic use and residue in freshwater aquaculture for domestic use in Vietnam. *EcoHealth, 12*(3), 480–489.

Rodrigues, P. M., Campos, A., Kuruvilla, J., Schrama, D. and Cristobal, S. (2017). Proteomics in aquaculture: Quality and safety. In *Proteomics in food science* (pp. 279–295). Academic.

Rottmann, R. W., Francis-Floyd, R., & Durborow, R. (1992). *The role of stress in fish disease* (p. 474). Southern Regional Aquaculture Center.

Ruzauskas, M., Klimiene, I., Armalyte, J., Bartkiene, E., Siugzdiniene, R., Skerniskyte, J., Krasauskas, R., & Suziedeliene, E. (2018). Composition and antimicrobial resistance profile of Gram-negative microbiota prevalent in aquacultured fish. *Journal of Food Safety, 38*(3), e12447.

Saavedra, M. J., Guedes-Novais, S., Alves, A., Rema, P., Tacão, M., Correia, A., & Martínez-Murcia, A. (2004). Resistance to β-lactam antibiotics in *Aeromonas hydrophila* isolated from rainbow trout (Onchorhynchusmykiss). *International Microbiology, 7*(3), 207–211.

Safavieh, M., Pandya, H. J., Venkataraman, M., Thirumalaraju, P., Kanakasabapathy, M. K., Singh, A., Prabhakar, D., Chug, M. K., & Shafiee, H. (2017). Rapid real-time antimicrobial susceptibility testing with electrical sensing on plastic microchips with printed electrodes. *ACS Applied Materials & Interfaces, 9*(14), 12832–12840.

Saviauk, T., Kiiski, J. P., Nieminen, M. K., Tamminen, N. N., Roine, A. N., Kumpulainen, P. S., Hokkinen, L. J., Karjalainen, M. T., Vuento, R. E., Aittoniemi, J. J., & Lehtimäki, T. J. (2018). Electronic nose in the detection of wound infection bacteria from bacterial cultures: A proof-of-principle study. *European Surgical Research, 59*(1–2), 1–11.

Scarano, C., Spanu, C., Ziino, G., Pedonese, F., Dalmasso, A., Spanu, V., Virdis, S., & De Santis, E. P. (2014). Antibiotic resistance of Vibrio species isolated from Sparusaurata reared in Italian mariculture. *The New Microbiologica, 37*(3), 329–337.

Silvester, R., Pires, J., Van Boeckel, T. P., Madhavan, A., Balakrishnan Meenakshikutti, A., & Hatha, M. (2019). Occurrence of β-lactam resistance genes and plasmid-mediated resistance among Vibrios isolated from Southwest coast of India. *Microbial Drug Resistance, 25*(9), 1306–1315.

Sivaraman, G. K., Deesha, V., Prasad, M. M., Jha, A. K., Visnuvinayagam, S., Nadella, R. K., Chandni, V., & Basha, A. (2016). Incidence of community acquired methicillin-resistant *Staphylococcus aureus* (CA-MRSA) in seafood and its environment, Gujarat, India. *International Journal of Recent Scientific Research, 7*(11), 14279–14282.

Sousa, M., Torres, C., Barros, J., Somalo, S., Igrejas, G., & Poeta, P. (2011). Gilthead seabream (Sparusaurata) as carriers of SHV-12 and TEM-52 extended-spectrum beta-lactamases-containing Escherichia coli isolates. *Foodborne Pathogens and Disease, 8*(10), 1139–1141.

Spanu, T., Fiori, B., D'Inzeo, T., Canu, G., Campoli, S., Giani, T., Palucci, I., Tumbarello, M., Sanguinetti, M., & Rossolini, G. M. (2012). Evaluation of the new NucliSENSEasyQ KPC test for rapid detection of Klebsiella pneumoniae carbapenemase genes (bla KPC). *Journal of Clinical Microbiology, 50*(8), 2783–2785.

Su, H. C., Ying, G. G., Tao, R., Zhang, R. Q., Fogarty, L. R., & Kolpin, D. W. (2011). Occurrence of antibiotic resistance and characterization of resistance genes and integrons in Enterobacteriaceae isolated from integrated fish farms in South China. *Journal of Environmental Monitoring, 13*(11), 3229–3236.

Sun, K., Wang, H. L., Zhang, M., Xiao, Z. Z., & Sun, L. (2009). Genetic mechanisms of multi-antimicrobial resistance in a pathogenic Edwardsiellatarda strain. *Aquaculture, 289*(1–2), 134–139.

Syal, K., Iriya, R., Yang, Y., Yu, H., Wang, S., Haydel, S. E., Chen, H. Y., & Tao, N. (2016). Antimicrobial susceptibility test with plasmonic imaging and tracking of single bacterial motions on nanometer scale. *ACS Nano, 10*(1), 845–852.

Varadi, L., Luo, J. L., Hibbs, D. E., Perry, J. D., Anderson, R. J., Orenga, S., & Groundwater, P. W. (2017). Methods for the detection and identification of pathogenic bacteria: Past, present and future. *Chemical Society Reviews, 2017*(46), 4818–4832.

Vega-Sánchez, V., Latif-Eugenín, F., Soriano-Vargas, E., Beaz-Hidalgo, R., Figueras, M. J., Aguilera-Arreola, M. G., & Castro-Escarpulli, G. (2014). Re-identification of Aeromonas isolates from rainbow trout and incidence of class 1 integron and β-lactamase genes. *Veterinary Microbiology, 172*(3–4), 528–533.

Visnuvinayagam, S., Joseph, T. C., Murugadas, V., Chakrabarti, R., & Lalitha, K. V. (2015). Status on methicillin resistant and multiple drug resistant Staphylococcus aureus in fishes of Cochin and Mumbai coast, India. *Journal of Environmental Biology, 36*(3), 571.

Von Ah, U., Wirz, D., & Daniels, A. U. (2009). Isothermal micro calorimetry–a new method for MIC determinations: Results for 12 antibiotics and reference strains of *E. coli* and *S. aureus*. *BMC Microbiology, 9*(1), 1–14.

Walker, G. T., Fraiser, M. S., Schram, J. L., Little, M. C., Nadeau, J. G., & Malinowski, D. P. (1992). Strand displacement amplification—An isothermal, in vitro DNA amplification technique. *Nucleic Acids Research, 20*(7), 1691–1696.

Wang, J. H., Lu, J., Zhang, Y. X., Wu, J., Luo, Y., & Liu, H. (2018). Metagenomic analysis of antibiotic resistance genes in coastal industrial mariculture systems. *Bioresource Technology, 253*, 235–243.

World Health Organization. (2006). Report of a joint FAO/OIE/WHO expert consultation on antimicrobial use in aquaculture and antimicrobial resistance, Seoul, Republic of Korea, 13–16 June 2006.

World Health Organization (WHO). (2014). *Antimicrobial resistance: global report on surveillance*. https://www.who.int/antimicrobial-resistance/publications/surveillancereport/en/

Xu, Y., Wang, C., Zhang, G., Tian, J., Liu, Y., Shen, X., & Feng, J. (2017). ISCR2 is associated with the dissemination of multiple resistance genes among Vibrio spp. and Pseudoalteromonas spp. isolated from farmed fish. *Archives of Microbiology, 199*(6), 891–896.

Zhang R. Q., Ying G. G., Su H. C., Zhou L. J., & Liu Y. S. (2013). Antibiotic resistance and genetic diversity of Escherichia coli isolates from traditional and integrated aquaculture in South China. *Journal of Environmental Science and Health Part B, 48*, 999–1013. https://doi.org/10.1080/03601234.2013.816611

Zhao, S., Wei, W., Fu, G., Zhou, J., Wang, Y., Li, X., Ma, L., & Fang, W. (2020). Application of biofertilizers increases fluoroquinolone resistance in Vibrio parahaemolyticus isolated from aquaculture environments. *Marine Pollution Bulletin, 150*, 110592.

Molecular Tools for Characterizing AMR Pathogens

Karthika Raveendran, Murugadas Vaiyapuri, and Madhusudana Rao Badireddy

Contents

K. Raveendran
Microbiology, Fermentation and Biotechnology Division, ICAR-Central Institute of Fisheries Technology, Cochin, India
e-mail: karthikarr77@gmail.com

M. Vaiyapuri (✉)
Microbiology Fermentation and Biotechnology Division of ICAR-Central Institute of Fisheries Technology (ICAR-CIFT), Cochin, Kerala, India
e-mail: Murugadas.V@icar.gov.in

M. R. Badireddy
Visakhapatnam Research Centre of ICAR-CIFT, ICAR-Central Institute of Fisheries Technology (ICAR-CIFT), Visakhapatnam, Andhra Pradesh, India
e-mail: bm.rao@icar.gov.in

M. P. Mothadaka et al. (eds.), *Handbook on Antimicrobial Resistance*,
https://doi.org/10.1007/978-981-19-9279-7_32

Abstract

Antimicrobial resistance in bacterial pathogens is growing at a rapid pace. The problem of AMR has percolated to different sectors. The first and foremost task in the control of the AMR is providing the evidence for the type of AMR bacteria prevalent at the local level and ensuring that the data generated is amenable for global comparison. The local and global epidemiological depiction of the AMR pathogens is more accurate and appropriate with the use of molecular characterizing tools. Despite the fact that several molecular tools are used for characterizing AMR pathogens, there is a need for a one-stop research guide for the array of AMR pathogens prioritized by the World Health Organization. In this context, the present chapter provides insights on the molecular tools available for each AMR pathogen and selects the suitable tool for each purpose.

Keywords

AMR pathogens · Molecular tools · Sequencing methods and non-sequencing-based methods

1 Introduction

Antimicrobial resistance (AMR) is a global burden that poses great concerns regarding human health and is a matter that requires immediate attention from the international authorities. The World Health Organization (WHO) has prioritized a pathogens list that urgently requires focused research and development programs. The prioritizing of pathogens is based on the resolve to either develop new antibiotics or save the existing antibiotics to treat or control infections. The priorities are sorted as critical, high-, and medium-priority pathogens (Fig. 1; World Health Organization, 2017).

Among the pathogens in the priority list, the Gram-negative bacteria, viz., *Acinetobacter baumannii*; *Pseudomonas aeruginosa*; *Enterobacter*, namely, *Escherichia coli*, *Klebsiella*, *Serratia,* and *Proteus* that are responsible for ESBL production; *Campylobacter* sp.; *Helicobacter pylori*; *Salmonella* sp.; *Shigella* sp.;

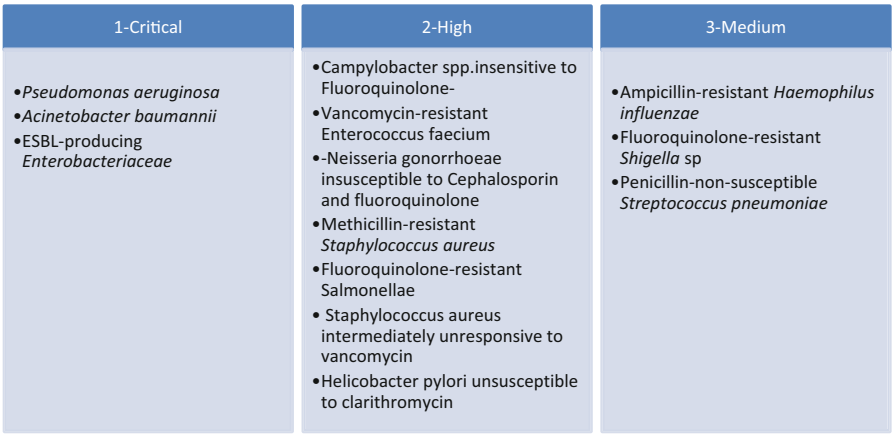


Fig. 1 Prioritized list of pathogens for AMR as per the WHO

Neisseria gonorrhoeae; *and Haemophilus influenzae* occupy the central role and are the primary targets for control. The other important Gram-positive bacteria that contribute to the AMR urgency are *Staphylococcus aureus*, *Enterococcus faecium*, *Streptococcus pneumoniae*, etc.

First and foremost, the task for controlling AMR is to provide evidence of their prevalence and also documenting the genotype of the prevalent pathogens. Molecular tools remain indispensable to understand the regional and global epidemiology and are essential to understand the point of emergence and spreading of the pathogens based on their clone relatedness and strain-level genetic diversity (Barrett et al., 2006; Vaiyapuri et al., 2019). Substantial evidence generated will be a useful parameter in designing strategies for the control of AMR pathogens (Ranjbar et al., 2014). Molecular methods for AMR resistance detect the presence of antibiotic-resistant genes (ARGs) or specific mutations associated with antibiotic resistance (WHO, 2019). Molecular methods can complement phenotypic methods by providing extra molecular details, viz., gene mutations pertaining the resistant phenotype identified, and thus improve the realization of extent of resistance and their underlying mechanisms behind the phenotypic resistance (World Health Organization, 2019).

In this chapter, molecular techniques that are widely used to characterize the important antimicrobial-resistant pathogens were assessed, and they include the ones that hold great promise in the near future.

2 Classification of Tools for Molecular Characterization

The genotypic methods were introduced during the 1970s for molecular epidemiology. Molecular characterization of AMR pathogens for understanding the local and global epidemiology falls into four categories, viz., methods based on amplification, sequencing, hybridization, and restriction digestion (Fig. 2).

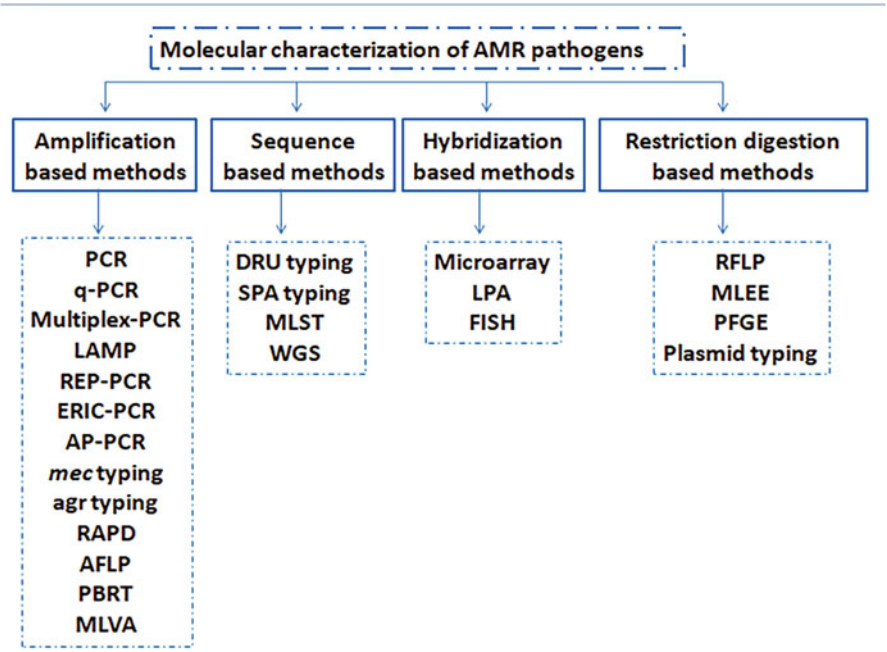


Fig. 2 Molecular tools for AMR detection and characterization

3 Amplification-Based Methods

In the methods that are based on amplification, polymerase chain reaction (PCR) is considered to be the main tool from which analysis will be carried out for further interpretations.

3.1 *AMR Gene Profiling Using PCR and Its Advancements*

The nucleic acid amplification enables the detection of number of target molecules by generating numerous copies of the target DNA, thus enhancing assay sensitivity. The PCR is a tool that has transformed molecular analysis by enabling target DNA is amplified exponentially using Taq DNA polymerase enzyme, which is highly thermostable (Mullis et al., 1986). PCR is a cyclic process and each cycle comprises of three steps: The first step is "denaturation" at 95 °C which separates the double-stranded (ds-DNA) into two individual DNA strands. The second step is "annealing" at 50–60 °C, in which the PCR primers are hybridized (annealed) to their complementary sequences on single-stranded template DNA. The third step is "extension" of the DNA by addition of nucleotides by *Taq* polymerase at 72 °C. The three steps of reaction cycle are repeated 30–35 times, which takes anything from 2 to 4 h

depending on the number of bases in the DNA template to be duplicated. The PCR gene products that have been amplified in number could now be visualized on agarose gels stained with fluorescent DNA chelating dyes such as ethidium bromide.

The PCR technique has become an integral part of microbiology laboratories to detect the existence of antibiotic-resistant genes as well as to characterize them. The technique is employed to monitor the incidence of bacteria that are unsusceptible to antimicrobials and also to determine the type of resistant genes they harbored, viz., *vanA* (insensitive to *vancomycin*), *mecA* (resisting methicillin), and *ampC* (unresponsive to ampicillin) were identified from waste, surface, and drinking waters (Schwartz et al., 2003). In addition to antibiotic susceptibility test (AST), the PCR techniques are commonly employed for the reason they are swift and ease in their application for detecting numerous ARGs in the bacterial isolates or environmental DNA the PCR assays have mostly been used in either pure and/or mixed cultures or environmental DNA materials for confirmation of ARGs against various classes of antibiotics such as aminoglycosides, sulfonamide, tetracycline, macrolides, chloramphenicol, fluoroquinolones, glycopeptides, and rifampin (Zhuang et al., 2021).

3.1.1 *Quantitative PCR*

Advances in PCR techniques include techniques like real-time PCR (qPCR), multiplex PCR, and isothermal amplification. In the qPCR method, the determination of DNA amplification occurs in real time by using non-specific fluorescent dyes or sequence-specific fluorescently labeled oligonucleotide probes (Higuchi et al., 1992). The qPCR technique is a highly sensitive and delivers instant data which saves substantial time. Since the technique is devoid of ethidium bromide, it is safer, as it does not require agarose gel electrophoresis. The disadvantage is that the machines are very expensive than traditional PCR machines. The conventional PCR assays could either identify the existing or the nonexisting nature of genes that are obdurate but cannot detect point mutations within the target genes. However, by using sequence-specific DNA probes, the qPCR technique could detect point mutations in any given gene. It is pertinent to note that qualitative endpoint PCR has an advantage over qPCR, in detecting larger fragments of target DNA, whereas qPCR method is more applicable for shorter fragments ranging between 150 bp and 250 bp.

Quantitative PCR has been used for monitoring ARGs such as *vanA*, *mecA*, *ampC*, *blaSHV-5*, *ermB,* and *tetO* in simulated revitalized arrangements of groundwater (Bockelmann et al., 2009). The qPCR assay was validated and applied to determine the rate of prevalence of multiple ARGs, namely, *fexA, intl1*, bla_{TEM}, *mcr-1*, *cfr*, *optrA*, and $bla_{CTX\text{-}M}$, in China (Chan et al., 2018). Of late, endpoint PCR and qPCR are being used for local and global epidemiological surveillance for AMR determinants in the archived bacterial pathogens. In Europe, a repository of bacterial pathogens were screened for colistin resistance genes which are mediated through plasmids harboring *mcr*-1 and *mcr*-2 gene variants (Doumith et al., 2016). These tools have taken a priority role in the outbreak investigation of diseases, and

now these tools are employed to more than single target genes in the assays (Chung et al., 2012).

3.1.2 *Multiplex PCR* (mPCR)

An advancement in the conventional PCR method is multiplex PCR (mPCR) where more than a single DNA fragment or genes are simultaneously targeted. The target DNA may be from a single bacteria or from a mixture of bacteria or environmental DNA (multiplex PCR has found its application in monitoring multiple AMR genes simultaneously by including different sets of primers in the same reaction mixture). The primary criterion for the multiplex PCR is that the amplicon size of diverse primers employed in the analyses are essentially of dissimilar proportions, so as to ensure proper separation of amplicons in agarose gel electrophoresis and clear visualization in gel documentation system/transilluminator. Quantitative multiplex PCR assays (qPCR) are probe-based assays wherein different probes are employed and each probe is labeled with a unique fluorescent dye, resulting in different observed colors for each assay that can be detected simultaneously. The strength of the signal from each dye quantifies the amount of each target separately in the same tube. The mPCR has been extensively used to identify predominantly occurring genes of ESBL, namely, bla_{TEM}, bla_{SHV}, $bla_{CTX\text{-}M}$, bla_{OXA}, etc. The mPCR format has been established to detect and characterize several AMR bacteria for resistance to a single group or sub-classes within the same group of antibiotics (Anjum et al., 2018). The significant and constructive point of performing mPCR is that an internal positive control for the genes can be included and there is a considerable reduction in time compared to running multiple singleplex PCR reactions. Optimization of multiplex PCR conditions can be performed with guidance (Elnifro et al., 2000).

3.1.3 *Loop-Mediated Isothermal Amplification* (LAMP)

In contrast to orthodox PCR, the isothermal PCR technique, namely, LAMP, necessitates no thermal cycling, and amplification of the target genes is carried out at a stable temperature ranging from 60 °C to 65 °C for about 60 min, the optimal temperature for *Bst* DNA polymerase that has a robust activity of transposition of strands (Notomi et al., 2000). The LAMP PCR amplification can also be carried out with the help of water bath and heating elements without dedicated PCR machine by measuring the development of turbidity with photometers (Mori & Notomi, 2009). A quantitative LAMP or real-time LAMP assay uses intercalating dyes that allows fluorescent detection and quantification of the amplicon in a real-time mode, which is considerably faster compared to PCR or qPCR. The LAMP assays have been modified since its discovery and are very useful in the detection of genes encoding antibiotic resistance (Chen et al., 2020). In *S. aureus*, the LAMP-based assays were employed for detecting *msrA*, *mecA*, or *mecC* genes responsible for macrolide–streptogramin-type B and methicillin resistance (Chen et al., 2020) and also in the detection of the bacterial strains that harbored genes that are insensitive to colistin from *mcr*-1 to *mcr*-5 (Zhong et al., 2019). These assays were established for the detection of genes responsible for ESBL and carbapenemase production and *AmpC* genes, in bacteria isolated from humans, poultry birds, and animals (Subramanya

et al., 2021). The eazyplexSuperBug CRE assay system of Amplexdiagnostics GmbH, Giessen, Germany, is a kit based on LAMP assay that targets CTX-M-type ESBLs and carbapenemase gene variants (Escriva et al., 2019).

The PCR, qPCR, mPCR, and LAMP assays are generally used for detection and characterization of AMR bacteria, and the profiles of AMR genes are used along with the other phenotyping or genotyping methods for virulence profiling.

3.2 *Virulence Factor Profiling*

In this approach, the virulence factors or determinants are profiled between the same species of pathogenic bacteria and categorized into a groups. This depends on occurrence or the non-occurrence of virulence attributes. The method was formulated in 2008 (Shayegh et al., 2008) and later on included many pathogens. The method was mainly used for *S. aureus* and MRSA for the detection and study of surface proteins associated with pathogenicity, toxins, enterotoxin genes, biofilm metabolomics, and several other virulence factor combinations (Adame-Gomez et al., 2020; Vaiyapuri et al., 2019). However, converting this profiling method of virulence as a complementary typing method was generated for MRSA (Nowrouzian et al., 2013). This method can be combined with other molecular typing or subtyping methods or profiling-based methods.

3.3 *"Repetitive Extragenic Palindromic-PCR"* (REP-PCR)

The rep-PCR is based on the amplification of repetitive extragenic palindromic (rep) elements (Versalovic et al., 1991) which generates fingerprints that are very specific in discriminating different strains within the species of bacteria. These repetitive rep elements were detected in several *Enterobacteriaceae* and closest non-*Enterobacteriaceae* bacteria, and the REP sequences are palindromic and hence form stem-loop structure. For molecular typing purposes in several bacteria, two sets of the primers targeting these rep elements are designed that are based on 38-bp sequences containing degenerate sequences in six positions with a variable loop of 5-bp amid both sides of a conserved palindrome stem. The REP element-based typing of AMR bacteria, viz., *E. coli*, *Salmonella* sp., *Citrobacter* sp. etc., were reported (Qian & Adhya, 2017).

3.4 *"Enterobacterial Repetitive Intergenic Consensus-PCR" (ERIC-PCR)*

Another set of highly conserved repetitive DNA sequences used for the typing of bacteria are the ERIC sequences that occurs in the intergenic (between genes coding for a protein) areas related to polycistronic operons or up−/downstreams of the untranslated regions of the bacterial genome. The ERIC sequences are 126-bp imperfect palindromic sequences that show similarity to REP sequences in many

features. They were detected mainly in *E. coli* and *Salmonella Typhimurium*. However, the typing method based on ERIC pattern is now expanded to the other bacteria within *Enterobacteriaceae* (Sharples and Lloyd, 1990).

3.5 *Arbitrary Primed-PCR*

In this method of PCR, a primer set with high stringency is used to generate a fingerprint from the nucleic acids. The polymorphism between the organisms is identified by the differences in the fingerprints. The method is comparably robust and reproducible, and the fingerprints represent the mutation, deletion, or insertions that occur in the genomic DNA. The benefit of the method is that prior information of the nucleic acid is not required, and the primer used is GTAAGGCCG (Ménard et al., 1992).

3.6 *Staphylococcal Cassette Chromosome mec Element Typing* (SCCmec)

The SCCmec gene complex is a combination of two different gene complexes, namely, the *mec* gene (methicillin insusceptible) and the *ccr* gene. The SCC*mec* typing of staphylococcal cassettes uses PCR to determine the type of combination of *mec* class and *ccr* gene subtype present, for molecular typing of MRSA, which is important in epidemiological studies. Initially, ten SCCmec elements (SCCmecI to SCCmecX) (Chongtrakool et al., 2006) were detected, and recently SCCmecXI (Petersdorf et al., 2015) was added. The SCC*mec*XII and XIII have been detected but are yet to be added to the public domain (Kaya et al., 2018). Different procedures of PCR were recognized and modified to detect and characterize the SCC*mec* elements. Among them, the methods developed by Oliveira et al. (2006), Milheirico et al. (2007), and Kondo et al. (2007) have been frequently used. The SCCmec types and several alternates are documented in the public domain (http://www.SCCmec.org/). Increasing number of variants in SCC*mec* elements has instrumented the researchers to implement software-based approaches and whole-genome sequencing data for SCC*mec* typing (SCCmec Finder) (Kaya et al., 2018).

3.7 *Accessory Gene Regulator* (agr) *Typing*

P2 operon of *agr* locus of the accessory gene regulator is used for accessory gene regulator typing in *S. aureus*. This locus has highly conserved and hypervariable regions called allelic groups. The primers designed for understanding the hypervariability within the *agr* categorize the organism into four allelic groups called *agrA*, *agrB*, *agrD*, and *agrC* (Robinson et al., 2005). The *agr* locus controls the expression of surface proteins and virulence factors in *S. aureus* and MRSA. Many PCR and real-time procedures were developed and employed to link the data on the

association between the *agr* locus and the existence of virulence features (Gilot et al., 2002; Vaiyapuri et al., 2019).

3.8 *Random Amplified Polymorphic DNA* (RAPD) *Assay*

The RAPD technique is a variant form of PCR that is geared up capriciously (William et al., 1990). The assay is similar to AP-PCR where primers of short random sequences (nine or ten bases) are used to anneal with template chromosomal DNA at an annealing temperature that is less than 35 °C (Franklin et al., 1999). The variations in the banding patterns determine the number of regions complimentary to the primer sequences and the distance between the primer binding sites. This method involves PCR, agarose gel electrophoresis, and investigation of the banding pattern variations between strains of bacterial populations. An important advantage of RAPD is that it is not essential to know the particular organism's target DNA sequences. Slowly the method has lost its importance due to several limitations, viz., highly sensitive method for annealing temperature, excellence and size of DNA template applied, $MgCl_2$ concentration, and primer quality and quantity used, and hence reproducibility between the laboratories is laboratory dependent (Panigrahi et al., 2019). More than 14 RAPD primers have been developed for microbial communities' structure analysis.

3.9 *Amplified Fragment Length Polymorphism* (AFLP)

AFLP is a technique based on PCR, where the genomic DNA of the bacteria is cut with restriction endonucleases into several fragments and the fragments are ligated with the restriction site-specific adapters. The fragments are amplified by PCR followed by their electrophoretic separation (Mueller & Wolfenbarger, 1999). Recent modification in AFLP uses primers labeled with fluorescent dyes resulting in the generation of DNA fragments with a fluorescent bar code followed by automated fragment analysis.

3.10 *PCR-Based Replicon Typing* (PBRT)

The PCR-based replicon typing (PBRT) technique was developed for understanding the transmission ability of the AMR pathogens of *Enterobacteriaceae* based on the possession of incompatibility plasmids (Inc plasmids). PCR identification of major plasmid incompatibility groups (18 types) has been potentially useful to monitor the spread of precise plasmids from diverse locations that are MDR in nature (Carattoli et al., 2005). Later on, revisions have been made for the PBRT method. The dissemination potential of β-lactamase and ESBL-producing *E. coli* was carried out with PBRT analysis (Sukmawinata et al., 2020).

3.11 *Multiple Locus Variable-Number Tandem Repeat* (MLVA)

MLVA analysis is a technique dependent on PCR wherein multiple loci of tandem repeat regions are targeted to generate a fingerprint. The MLVA analysis helps in species differentiation based on the length of the amplified region and the number of bands formed. Capillary electrophoresis increases the resolution of separation of banding pattern (Hyytia-Trees et al., 2007). Genotypic diversity studies of MDR *Salmonella* strains and ESBL-producing *Escherichia coli* were performed using MLVA (Kalai et al., 2018).

4 Sequencing-Based Methods

Rapid advances in the genome sequencing technologies over the last few decades have led to the rapid, efficient, and affordable sequencing of the genome, thus helping in efficient and quick diagnosis and surveillance in the field of microbiology. Sequence-based methods were mainly based on Sanger sequencing of the target genes and considering for allelic variations within the genes. Later on the Sanger sequencing procedure was further developed and improvised to target more than one gene for the same pathogen to improve the resolution. Sequencing-based methods help in accurate prediction of AMR, and for tracking the outbreak of drug-resistant strains in a hospital or the community (Hendriksen et al., 2019).

4.1 *Direct Repeat Unit* (DRU) *Typing*

DRU typing is established by the sequencing a 40 bp of direct repeat units (dru) in *variable-number tandem repeats (VNTR)* region near the SCC*mec* elements. In MRSA, *mec*A gene downstream end is very closer to IS431 in the SCC*mec* component of MRSA (Goering et al., 2008). The PCR amplification and sequencing of this region was used in the epidemiological analysis and subtyping of MRSA. The PCR protocol and cycling conditions of DRU typing can be obtained from a dedicated international working group (http://dru-typing.org/site/) and also automated analysis of the sequence by TRST plugin of bio-numeric.

4.2 *Staphylococcal Protein A* (SPA) *Typing*

The SPA gene has an X region which consists of 2–16 nucleotide repeat units that contributes to the variations in the gene length. This SPA region is the target for SPA typing in *S. aureus* or MRSA. It is normally used for typing of staphylococcal protein A, a virulence factor that acts as a binding site for IgG (Frenay et al., 1996). This technique is carried out by amplification and sequencing and SPA type assignment, and it is frequently used for local epidemiology which accumulates the genetic changes relatively very slower rate. Minimum spanning tree-based clustering of spa

types is carried out with open source or softwares belonging to StaphType software (Ridom GmbH, Wurzburg, Germany), and the grouping procedure is known as *Based Upon Repeat Pattern (BURP*) (Aires de Sousa et al., 2006).

4.3 *Multi-Locus Sequence Typing* (MLST)

MLST analysis was first employed in 1998 for discriminating *Neisseria meningitidis* (Maiden et al., 1998) and further extended to all the clinically important pathogens which include AMR bacteria. MLST analysis is based on amplification of inner segments of several housekeeping genes that vary with the type of the pathogens, followed by sequencing. Generally the PCR targets loci range between 450 and 500 bp; exemptions are for relatively less reproducible loci. This tool has high reproducibility and discriminatory power and portability of data, and it is a very well-known tool employed in global epidemiology analysis. Allele and sequence-type assignments are made based on the central public domain, i.e., https://pubmlst.org (Jolley et al., 2018). Housekeeping genes targeted for *E. coli* are *mdh*, *fumC*, *recA*, *adk*, *gyrB*, *purA*, and *icd*; for MRSA and *S. aureus*, *gmk*, *tpi, aroE*, *pta*, *arcC*, *glpF*, *and yqiL*; *Pseudomonas aeruginosa*, *mutL*, *aroE*, *ppsA*, *acsA*, *guaA*, *nuoD*, *and trpE*; *Campylobacter jejuni*, *tkt*, *glnA*, *glyA*, *aspA*, *gltA*, *pgm*, *and uncA*; and *Salmonella* sp., *sucA*, *dnaN*, *hisD*, *aroC*, *hemD*, *purE*, *and thrA* (Enright & Spratt, 1999; Murugadas et al., 2017). Minimum spanning tree development based on clonal complexes relatedness between STs was developed based on an algorithm called BURST (based upon related sequence types) to decipher the evolutionary variations that happened in solitary or multiple loci of the sequence. It also defines the clonal complexes (CCs), their distribution, and evolutionary events (Enright & Spratt, 1999).

4.4 *Whole-Genome Sequencing (WGS)*

Whole-genome sequencing (WGS) is a robust technique for sequencing and analyzing the complete genomes of bacterial isolates. There are several methods for sequencing the whole genomes. In the late 1970s, viral and bacterial genomes were sequenced by Maxam and Gilbert's chemical cleavage technique and Sanger sequencing by chain-termination method (Maxam and Gilbert, 1977; Sanger et al., 1977). In 2008, there was a shift to a more rapid, automated sequencing method facilitating the high-throughput sequencing of the larger genomes. The term "next-generation sequencing" (NGS) is given to sequencing technologies that emerged post-Sanger sequencing. It can produce voluminous sequencing data at incredibly low cost and time. The second-generation sequencing includes Roche/454, Ion Torrent Technology, pyrosequencing, Illumina/Solexa (HiSeq/MiSeq), and oligonucleotide ligation and detection (SOLiD). While Sanger's sequencing work is based on termination of the nucleotide chain by the incorporation of di-deoxynucleotides (ddNTPs) A, T, G, and C; output data are slightly less than one kilobase (kb) in

length. The dawn of NGS helped in simultaneous sequencing of millions of DNA fragments resulting in the production of millions of nucleotide short reads in parallel. The most common among the NGS methods are arguably the Illumina sequencing technology, which works on the principle "sequence by synthesis." The genomic DNA is randomly cleaved as fragments are passed to flow cell for sequencing. These shorter fragments were amplified to millions of clonal copies by solid-phase PCR which were attached to the flow cell. In the sequencing step, the photodetector will detect the addition of nucleotide to the newly added stranded with the help of excited fluorophores, and this step occurs in a cycle of reactions (Heather & Chain, 2016).

Limitations to the use of WGS are minimal. The whole-genome analysis is basically an arrangement of shorter fragments into overlapping bigger fragments as contigs and then to scaffold. The resistance gene presence will be predicted through reference databases. The presence of resistance genes could be predicted by comparing the complete genome sequence of bacteria to that of reference databases. WGS enables for voluminous information in the form of sequence reads, and adequate knowledge of software is essential to analyze and interpret results. WGS was used in the identification of the ESBL-producing *Enterobacteriaceae* (Jesumirhewe et al., 2020). WGS can be used to find and characterize pathogens more effectually, rapidly, and precisely. Epidemiological data on AMR pathogens are essential for an outbreak detection, and infection control. WGS has been used successfully to generate resistance profile for pathogens such as multidrug-resistant tuberculosis (Doyle et al., 2018) and high-priority foodborne pathogens, such as *Salmonella* (Cooper et al., 2020), *Campylobacter* spp. (Frazão et al., 2021), Shiga toxin-producing *E. coli* (Dallman et al., 2015), *Listeria monocytogenes* (Moura et al., 2017), and *S. aureus* (Egyir et al., 2020).

Along with epidemiological data on infectious diseases, WGS can guide the understanding of outbreaks, their transmission chains, and virulence of AMR strains, thus providing valuable insights in risk assessment and facilitating effective interventions. WGS is playing a remarkable role in the detection of outbreaks and quick removal of infection source and thereby reduction in expenses and health aids in terms of fewer cases and deaths. WGS allows genome-wide analysis and the uppermost possible resolution for pathogen subtyping, including characterization of AMR genes and plasmid subtypes (World Health Organization, 2020). WGS deals with greater interlaboratory comparability than the phenotypic challenge because of its elevated degree of reproducibility.

WGS is now used for many applications in clinical healthcare, viz., strain identification, predicting the phenotypic expression from the genotypes, and tracing the outbreaks (Parcell et al., 2021).

WGS has important application in the bacterial population genomics where the genome libraries are generated for bacterial pathogens. Depending on that, plentiful diagnostic tools were developed centered on the endpoint of the NGS data which contains bioinformatic channels (Joseph & Read, 2010). Infrastructure requirement as well as the recurring expenditure on consumables and also deciphering the molecular mechanism requires bioinformatic skills. Therefore, capacity building of bioinformatics and infrastructure development are highly essential in the era of WGS analysis.

5 Hybridization-Based Methods

This method works on the principle of hybridization of a labeled nucleic acid probe to a specific target AMR sequence. These probes can be labeled with either radio, fluorescent, or antigen bases, enzymes, or chemiluminescent compounds (Fluit et al., 2001). Depending on the probe used, autoradiography, fluorescence microscopy, or immunohistochemistry can be used for visualization.

Hybridization assays can be further sectioned into fluorescence in situ hybridization (FISH), DNA arrays, and line probe assays (LPAs). These hybridization assays are developed to detect or characterize enormous number of genes against a single pathogen or from single environmental sample based on the development of multiple probes for each target gene.

5.1 *Microarrays*

A microarray comprises of regularly arranged target DNA sequences bound to a solid support such as glass, nylon membrane, silicon wafers, or other functionalized substrates. The DNA in the sample is fluorescently labeled and added to the array (hybridization). An indicator of fluorescent microarray and a computer database can then detect and analyze many different AMR genes. Fink et al. (2019) developed a microarray-based AMR chip that detects enormous ARGs for β-lactams and vancomycin. An array chip was developed for 6 classes of important antimicrobials, namely, aminoglycosides, β-lactams, macrolides, sulfonamides & trimethoprim and tetracyclines with the help of 14 probes (Card et al., 2014) that detected 14 different resistance genes in total that convened resistance to the 6 antibiotic classes. Garneau et al. (2010) developed a microarray chip for screening 166 ARGs in important Gram positive and gram negative microbes.

5.2 Line Probe Assays (LPAs)

LPAs are DNA-based test strips that contain DNA probes specific to resistance genes or bacterial species markers. LPAs are based on the development of biotinylated probe in the amplification step which can detect the complementary sequences in the chip. Hence, it will bind to the strip containing test DNA samples. While processing, the unbound DNA will be washed off from the strip. The streptavidin alkaline phosphatase anchor will attach to the biotin. The alkaline phosphatase in the streptavidin cleaves the chromophores 5-bromo-4-chloro-3-indolyl-phosphate (BCIP) and nitro blue tetrazolium (NBT), thus leading to dark blue stain on the bound DNA. The patterns of the colored bands could be compared to that of a template strip to infer the presence of AMR genes. The LPA has been used to detect MRSA in the blood cultures (World Health Organization, 2019). Since LPA is a robust technique, it has been included in the control of drug-resistant TB in India (Desikan et al., 2017).

5.3 Fluorescence In Situ Hybridization (FISH)

FISH is a technique that employs hybridization of fluorescently labeled oligonucleotide probes to the complementary DNA sequences of resistance genes. Once the hybridization process completes, all the remaining probes are washed off. The epifluorescence or the microscopy that uses confocal laser scanning is used to capture the signal from the bounded probes for ARGs (Levsky & Singer, 2003). FISH probe was developed to detect ampicillin, macrolide, and chloramphenicol insusceptibility in *Escherichia coli*, *Helicobacter pylori*, and *Bacillus cereus*, respectively (Jüttner et al., 2004; Lee et al., 2019).

6 Restriction Digestion-Based Methods

6.1 *Restriction Fragment Length Polymorphism* (RFLP)

In RFLP technique, the chromosomal DNA of the microbes is processed with restriction enzymes, and the arrangements of the bands formed are compared to detect their relatedness. The method was first developed and used for constructing linkage in the genome of humans (Botstein et al., 1980). The same method can be used for any DNA, viz., PCR products, labeled probe with restriction sites for producing bands. The banding pattern or the fingerprint produced in the gel electrophoresis of agarose is based on the obtainability of restriction sites and their distribution across the chromosome. The RFLP method can be used for comparison of strains within the bacterial species. The use of rare-cutting enzymes decreases the number of bands produced compared to the use of the frequently cutting restriction enzymes. The banding pattern will be used in hybridization with probes. Subsequently, this method lost its importance due to limitations such as time and labor-intensive works for the extraction of pure DNA, restriction digestion, probe-based hybridization, documentation and analysis of bands (Ranjbar et al., 2014).

6.2 Multi-Locus Enzyme Electrophoresis

Prior to the development of MLST, the scheme developed with restriction enzymes for multiple loci of housekeeping genes is MLEE. In this method too, the banding pattern, viz., number and position of the bands, is analyzed. Once the MLST scheme is developed, MLEE method usage has greatly reduced (Kotetishvili et al., 2003).

6.3 Pulsed-Field Gel Electrophoresis (PFGE)

The PFGE is a “gold standard” method employed for molecular subtyping of bacterial pathogens. This technique employs macro-restriction of purified genomic DNA in an in situ condition in the agarose plug and digested with restriction

endonucleases (Barrett et al., 1994). Later on, the gold standard method was implemented for disease outbreak analysis from food under the PulseNet procedure (Swaminathan et al., 2001). Initially, the Tenover et al. (1995) guideline was used, and later on, software was developed for character-based analysis of the genomic DNA depending on the band number and positions that appeared in the gel electrophoresis (Barrett et al., 2006). Further, these procedures were used for source tracking local and global epidemiology (Vernile et al., 2009).

6.4 Plasmid Analysis

The plasmid is an extrachromosomal DNA present in bacteria, and its analysis was developed in 1981 (Schaberg et al., 1981). The descendant bacteria have the same plasmid, and hence, the plasmid analysis will be a useful tool for comparing bacterial clones. Particularly antimicrobial resistance and virulence determinant-related genes are carried in plasmid, and hence, it is very useful in AMR studies (Threlfall et al., 1990). Minor modifications are required in the plasmid DNA extraction based on the type of bacteria (Foley et al., 2009). Plasmid DNA is extracted from bacterial cultures and separated based on size by agarose gel electrophoresis. The analysis of plasmid profile depends on the number of plasmid bands and molecular weight of the plasmids, and from that similarity is calculated (Aktas et al., 2007). To overcome the conformational differences observed in the plasmid analysis, namely, linear and supercoiled, the plasmid analysis was incorporated with restriction digestion, and banding pattern was analyzed after digestion (Liu et al., 1996).

Other methods which are based on RFLP are ribotyping of ribosomal RNA and insertion sequence – RFLP which is primarily PCR based and followed by restriction digestion and fingerprint analysis (Dvorska et al., 2001).

7 Bioinformatics

Bioinformatics is a study that employs a range of computational techniques to analyze the genetic sequences and predict the biological activity/function. Bioinformatic methods and technologies are becoming more and more useful in analyzing the exponentially rising volumes of molecular data generated from genomics, proteomics, and transcriptomics. In addition, the quantity of information collated for molecular profile production and data collection in the form of different databases and scientific literatures has increased substantially in the area of microbial epidemiology. In the draft whole-genome sequence of *S. enterica* isolates, a bioinformatic approach was implemented to identify plasmids carrying antibacterial resistance genes (Kudirkiene et al., 2018). Recent progress in quick and inexpensive technology for DNA sequencing has pioneered diagnostic microbiology and microbial monitoring.

The bioinformatics tool compared different species based on the banding pattern produced in the gel electrophoresis. Nearly 1Kb–20Kb DNA fragments are generally

produced in the gel electrophoresis; all these are called character-based analysis, e.g., ribotyping, rep-PCR, ERIC-PCR, RAPD, AFLP, PFGE, PCR-RFLP, and MLVA (Li et al., 2009).

To date, a minimum of 47 openly accessible bioinformatics based diagnostic tools have been developed to detect AMR predictors in genomic and amino acid sequences, e.g., ResFinder, MEGARes, ARG-ANNOT, CARD, ARO, SRST2, GeneFinder, KmerResistance, ARIBA, AMRFinder, and Tetracycline MLS nomenclature (Hendriksen et al., 2019). In the present-day scenario, many bioinformatic tools are available in the public domain for determining the resistance from the sequences output.

The Comprehensive Antibiotic Resistance Database (CARD) has been expanding since 2017 by extensively curating the sequences, revising the ontological structure, reconstructing more than 500 new AMR model detections, developing a new classification paradigm, and expanding analytical instruments. Particularly, new Resistomes & Variants modules were developed to predict resistance in over 82 pathogens adopted from 100,000 genomes. The addition of these variants to CARD leads to the assessment of resistance prediction; in addition, AMR mobility and variants also could be assessed. Popular AMR databases are Antibiotic Resistance Genes Database (ARDB), ARG-ANNOT, Mykrobe, MvirDB, Antibiotic Resistance Genes Online (ARGO), DeepARG, KmerResistance, ResFinder, SRST2, ABRES FINDER, ARGminer, TBDReaM, U-CARE, MUBII-TB-DB, ARGDIT, SCCmec Finder, ResFams, ShortBRED, PointFinder, ARIBA, GROOT, IRIDA, Galileo AMR (MARA, RAC), MEGARes (AMRplusplus), NCBI AMRFinder, Noradab, Patric, SSTAR, INTEGRALL, BacMet, ResCap, RED-DB, SARG (ARGs-OAP; ARGpore), Mustard, FARMEDB, ResFinderFG, LREfinder, β-Lactamases Database, BLAD, BLDB, CBMAR, LacED, AMRtime, and Plugin AMR detection (Clausen et al., 2016).

Molecular tests offer vital, clinically applicable data, but also have limitations, i.e., it can be used for detection and identification of known resistance genes or mutations only. Hence, to confirm the correct classification of bacterial isolates, phenotypic resistance testing is always necessary. In some circumstances, the phenotypic test results and molecular tool results are not integrated properly. Some of the possible non-integration of results are false-positive and false-negative results, which could be due to the minor modification in AMR genes and/or cross-contamination of DNA.

8 Molecular Typing Tools Used in Characterizing AMR Pathogens

Characterizing AMR pathogens into types or subtypes or strain level was based on the micro-variation or macro-variation observed in the genotypes. Generally, the micro-variation analysis helps to determine the local level emergence and spread of the molecular types of the pathogens called local epidemiology. Macro-variation analysis is mostly carried out for identifying the pathogens to subtypes matching to

the global epidemic clones. These methods are used in outbreak investigations, endemicity analysis of pathogens, and also surveillance-related studies (Ranjbar et al., 2014).

The status of the particular molecular tools used for the epidemiological studies is based on several major factors, viz., typeability, repeatability, reproducibility, discriminatory power, stability, ease of generating and analysis of data, simplicity to perform, time and expenditure involved in implementation, robustness, and high throughput. The selection of methods for a particular pathogen depends on the reproducibility of the tool and the turnaround time available for the study (Foley et al., 2009; Ranjbar et al., 2014). Typing methods used for very important AMR pathogens of clinical importance and their utility are depicted in Table 1.

Molecular methods used for typing and subtyping of *E. coli* are multi-locus sequence typing (MLST), repetitive-element PCR (rep-PCR), pulsed-field gel electrophoresis (PFGE), fliC sequencing analysis, PCR-RFLP of fliC gene, MLVA, WGS, ribotyping, DNA microarray, AFLP, Clermont typing, resistance genes and integrons, virulence gene profiling, and plasmid analysis (Neamati et al., 2020).

Typing methods employed for *S. aureus* and/or MRSA are sequence-based methods such as *spa* typing, MLST, MLVA, restriction enzyme-based profiling by PFGE, multi-locus virulence analysis (MLVA), *coa* gene PCR–RFLP; *dru* typing, *SCCmec* typing, virulence factor profiling, *agr* typing, and *siru* (staphylococcal interspersed repeat unit) typing (Shopsin et al., 1999; Vaiyapuri et al., 2019). Recent advances involve next-generation sequence analysis, from which deciphering all the information inbuilt in the genome of the pathogens.

For *P. aeruginosa* typing methods used were ribotyping, ERIC PCR, RAPD, tDNA, virulence profiling, MLST, double-locus sequence typing, ERIC-PCR, insertion sequence analysis, and antimicrobial resistance gene profiling (Abdel-Rhman & Rizk, 2021).

For typing of *Salmonella* sp., commonly used methods are RAPD, PFGE, AFLP, MLST, clustered regularly interspaced short palindromic repeat (CRISPR) typing, PFGE analysis of plasmid DNA, antimicrobial resistance profiling, etc. (Gad et al., 2018).

In *K. pneumoniae*, molecular tools used for typing and subtyping are MLST, ERIC and RAPD, BOX-PCR, and antimicrobial resistance gene profiling (Lagha et al., 2021).

9 Restriction in the Use of Molecular AMR Tools

Only known genes or mutations can be detected by molecular testing. The cost efficiency of molecular AMR testing in both clinical and laboratory workflows is not sufficiently demonstrated. The testing is mainly used for public health monitoring (with a few exceptions, such as for tuberculosis) as opposed to clinical management. The ability of tests to detect mechanisms of resistance varies. Additional cost may be required for molecular testing. Practicable subsidized prototypes are obligatory for molecular AMR screening in economically underprivileged nations, especially those

Table 1 Comparison of typing methods

Tools/AMR pathogen	Discriminatory power	Portability	Robustness	Reproducibility	Throughput	Typeability	Basis for typing
PCR-virulence typing	3	2	3	4	3	2	Fragments/character
Rep-PCR	3	3	3	3	3	2	Fragments/character
ERIC-PCR	3	3	3	3	3	2	Fragments/character
Box-PCR	3	3	3	3	3	2	Fragments/character
Ribotyping	3	3	3	3	3	2	Fragments/ hybridization
AMR-genes and subtyping	3	3	3	4	3	2	Fragments/character
PFGE	3	3	2	3	1	3	Fragments/character
Plasmid replicon typing	2	3	3	2	2	3	Fragments/character
Multi-locus sequence typing	2	4	3	3	2	4	Sequence based
MLVA	3	3	3	3	3	3	Fragments/character
Microarray	3	3	3	3	3	3	Hybridization
WGS	4	4	2	2	1	4	Sequence based

Scale of 1–4, 1-weak, 2-good, 3-very good, 4-excellent

of Africa, Asia, and Latin American countries. The correlation of molecular test results and their clinical interpretation with phenotypical test results are imperfect, varying between bacterial species and antimicrobial agents. Lack of complete knowledge on the resistance mechanisms might lead to poor sensitivity to tests. Hence, it is pertinent to augment the understanding of molecular mechanisms and genetic factors underlying antimicrobial resistance. The testing of molecular AMR diagnoses for surveillance purposes may require proof of principle studies. Although WGS is beneficial, it has several public health limitations in terms of AMR monitoring in rapidly growing bacteria, which include significant initial and ongoing investments, as well as a complete knowledge of the molecular mechanisms that underpin it. WGS can currently not replace phenotypical AMR monitoring in fast-growing bacteria, as it has the ability to detect only known resistance mechanisms. Additional challenges before WGS are the lack of the capacity building, skilled technologists, laboratory infrastructure, bioinformaticians, and quality assurance protocol. Hence, each surveillance agency should initially focus on the establishing the infrastructure and capacity building activities followed with that selecting very few pathogens relevant to AMR evolution in public health. In order to extend the use of WGS for AMR surveillance, the initial thrust should be on selected bacterial pathogens of public health significance and antimicrobial agents with well-understood resistance mechanisms. Several phenotypical priority pathogens are monitored in the Global Antimicrobial Resistance and Use Surveillance System (GLASS). WGS for a subset of GLASS priority pathogens could initially be introduced for global AMR surveillance.

10 Advantages in Employing Molecular Tools

The advantage with cartridge tests, especially at clinical level, is the quicker pace of obtaining results than in culture-based tests. In the cartridge tests, the samples can be tested directly sans culture step, minimal laboratory space requirement, facilities, and training. Molecular testing can be more sensitive in detecting known markers than phenotypic tests; however, it doesn't always reliably predict phenotypical resistance to the presence of the resistance marker. Molecular AMR diagnostic data may provide additional data for monitoring and informing interventions.

11 Conclusions

Molecular tools involve not only the detection of AMR in pathogens but also help to type or subtype the bacterial pathogens to strain level. Several techniques have been evolved and modified accordingly in order to suit the requirement of typing. Recently developed sequence-based methods have surpassed the advantages of all the character-based analysis methods. However, the cost and infrastructure requirement needed for the sequence-based analysis limit their acceptance and usage across the globe. There is a drastic reduction in the cost per run of sequence-based methods;

however, they have not reached fully to the state of affordability to all the laboratories involved in the testing of bacterial pathogens.

12 Cross-References

- ▶ Avenues in the Determination of AMR in Human Health
- ▶ Detection of Antimicrobial Resistance in Veterinary Bacterial Pathogens
- ▶ Molecular Mechanisms of Antimicrobial Resistance
- ▶ Trends in the Determination of Antimicrobial Resistance in Aquaculture and Fisheries

References

Abdel-Rhman, S. H., & Rizk, D. E. (2021). Comparative assessment of different PCR-based typing methods of *Pseudomonas aeruginosa* isolates. *Infection and Drug Resistance, 14*, 1019–1035.

Adame-Gómez, R., Castro-Alarcón, N., Vences-Velázquez, A., Toribio-Jiménez, J., Pérez-Valdespino, A., Leyva-Vázquez, M. A., & Ramírez-Peralta, A. (2020). Genetic diversity and virulence factors of S. aureus isolated from food, humans, and animals. *International Journal of Microbiology, 2020*.

Aires-de-Sousa, M., Boye, K., De Lencastre, H., Deplano, A., Enright, M. C., Etienne, J., Friedrich, A., Harmsen, D., Holmes, A., Huijsdens, X. W., & Kearns, A. M. (2006). High interlaboratory reproducibility of DNA sequence-based typing of bacteria in a multicenter study. *Journal of Clinical Microbiology, 44*(2), 619–621.

Aktas, Z., Day, M., Kayacan, C. B., Diren, S., & Threlfall, E. J. (2007). Molecular characterization of *Salmonella typhimurium* and *Salmonella enteritidis* by plasmid analysis and pulsed-field gel electrophoresis. *International Journal of Antimicrobial Agents, 30*(6), 541–545.

Anjum, M. F., Zankari, E., & Hasman, H. (2018). Molecular methods for detection of antimicrobial resistance. In *Antimicrobial resistance in bacteria from livestock and companion animals* (pp. 33–50).

Barrett, T. J., Lior, H., Green, J. H., Khakhria, R., Wells, J. G., Bell, B. P., Greene, K. D., Lewis, J., & Griffin, P. M. (1994). Laboratory investigation of a multistate food-borne outbreak of *Escherichia coli* O157: H7 by using pulsed-field gel electrophoresis and phage typing. *Journal of Clinical Microbiology, 32*(12), 3013–3017.

Barrett, T. J., Gerner-Smidt, P., & Swaminathan, B. (2006). Interpretation of pulsed-field gel electrophoresis patterns in foodborne disease investigations and surveillance. *Foodborne Pathogens and Diseases, 3*, 20–31.

Böckelmann, U., Dörries, H. H., Ayuso-Gabella, M. N., de Marçay, M. S., Tandoi, V., Levantesi, C., Masciopinto, C., Van Houtte, E., Szewzyk, U., Wintgens, T., & Grohmann, E. (2009). Quantitative PCR monitoring of antibiotic resistance genes and bacterial pathogens in three European artificial groundwater recharge systems. *Applied and Environmental Microbiology, 75*(1), 154–163.

Botstein, D., White, R. L., Skolnick, M., & Davis, R. W. (1980). Construction of a genetic linkage map in man using restriction fragment length polymorphisms. *American Journal of Human Genetics, 32*(3), 314.

Carattoli, A., Bertini, A., Villa, L., Falbo, V., Hopkins, K. L., & Threlfall, E. J. (2005). Identification of plasmids by PCR-based replicon typing. *Journal of Microbiological Methods, 63*(3), 219–228.

Card, R. M., Warburton, P. J., MacLaren, N., Mullany, P., Allan, E., & Anjum, M. F. (2014). Application of microarray and functional-based screening methods for the detection of antimicrobial resistance genes in the microbiomes of healthy humans. *PLoS One, 9*(1), e86428.

Chan, W. S., Au, C. H., Ho, D. N., Chan, T. L., Ma, E. S. K., & Tang, B. S. F. (2018). Prospective study on human fecal carriage of Enterobacteriaceae possessing mcr-1 and mcr-2 genes in a regional hospital in Hong Kong. *BMC Infectious Diseases, 18*(1), 1–12.

Chen, X., Ma, K., Yi, X., Xiong, L., Wang, Y., & Li, S. (2020). The rapid and visual detection of methicillin-susceptible and methicillin-resistant *Staphylococcus aureus* using multiplex loop-mediated isothermal amplification linked to a nanoparticle-based lateral flow biosensor. *Antimicrobial Resistance & Infection Control, 9*(1), 1–12.

Chongtrakool, P., Ito, T., Ma, X. X., Kondo, Y., Trakulsomboon, S., Tiensasitorn, C., Jamklang, M., Chavalit, T., Song, J. H., & Hiramatsu, K. (2006). Staphylococcal cassette chromosome mec (SCCmec) typing of methicillin-resistant *Staphylococcus aureus* strains isolated in 11 Asian countries: A proposal for a new nomenclature for SCCmec elements. *Antimicrobial Agents and Chemotherapy, 50*(3), 1001–1012.

Chung, D. R., Baek, J. Y., Kim, H. A., Lim, M. H., Kim, S. H., Ko, K. S., Kang, C. I., Peck, K. R., Lee, N. Y., & Song, J. H. (2012). First report of vancomycin-intermediate resistance in sequence type 72 community genotype methicillin-resistant *Staphylococcus aureus*. *Journal of Clinical Microbiology, 50*(7), 2513–2514.

Clausen, P. T., Zankari, E., Aarestrup, F. M., & Lund, O. (2016). Benchmarking of methods for identification of antimicrobial resistance genes in bacterial whole genome data. *Journal of Antimicrobial Chemotherapy, 71*(9), 2484–2488.

Cooper, A. L., Low, A. J., Koziol, A. G., Thomas, M. C., Leclair, D., Tamber, S., Wong, A., Blais, B. W., & Carrillo, C. D. (2020). Systematic evaluation of whole genome sequence-based predictions of *Salmonella* serotype and antimicrobial resistance. *Frontiers in Microbiology, 11*, 549.

Dallman, T. J., Byrne, L., Ashton, P. M., Cowley, L. A., Perry, N. T., Adak, G., Petrovska, L., Ellis, R. J., Elson, R., Underwood, A., & Green, J. (2015). Whole-genome sequencing for national surveillance of Shiga toxin–producing *Escherichia coli* O157. *Clinical Infectious Diseases, 61*(3), 305–312.

Desikan, P., Panwalkar, N., Mirza, S. B., Chaturvedi, A., Ansari, K., Varathe, R., . . . & Pandey, M. (2017). Line probe assay for detection of Mycobacterium tuberculosis complex: An experience from Central India. *The Indian journal of medical research, 145*(1), 70.

Doumith, M., Godbole, G., Ashton, P., Larkin, L., Dallman, T., Day, M., Day, M., Muller-Pebody, B., Ellington, M. J., de Pinna, E., & Johnson, A. P. (2016). Detection of the plasmid-mediated mcr-1 gene conferring colistin resistance in human and food isolates of *Salmonella enterica* and *Escherichia coli* in England and Wales. *Journal of Antimicrobial Chemotherapy, 71*(8), 2300–2305.

Doyle, R. M., Burgess, C., Williams, R., Gorton, R., Booth, H., Brown, J., Bryant, J. M., Chan, J., Creer, D., Holdstock, J., & Kunst, H. (2018). Direct whole-genome sequencing of sputum accurately identifies drug-resistant *Mycobacterium tuberculosis* faster than MGIT culture sequencing. *Journal of Clinical Microbiology, 56*(8): e00666–18.

Dvorska, L., Bartos, M., Martin, G., Erler, W., & Pavlik, I. (2001). Strategies for differentiation, identification and typing of medically important species of mycobacteria by molecular methods. *Veterinární Medicína, 46*(11/12), 309–328.

Egyir, B., Hadjirin, N. F., Gupta, S., Owusu, F., Agbodzi, B., Adogla-Bessa, T., Addo, K. K., Stegger, M., Larsen, A. R., & Holmes, M. A. (2020). Whole-genome sequence profiling of antibiotic-resistant Staphylococcus aureus isolates from livestock and farm attendants in Ghana. *Journal of Global Antimicrobial Resistance, 22*, 527–532.

Elnifro, E. M., Ashshi, A. M., Cooper, R. J., & Klapper, P. E. (2000). Multiplex PCR: Optimization and application in diagnostic virology. *Clinical Microbiology Reviews, 13*(4), 559–570.

Enright, M. C., & Spratt, B. G. (1999). Multilocus sequence typing. *Trends in Microbiology, 7*(12), 482–487.

Escriva, B. F., Garcia, C. S., Palop, N. T., & Cardona, C. G. (2019). Eazyplex® SuperBug CRE system for the rapid detection of carbapenemase and extended spectrum beta-lactamase genes in gram-negative bacteria. *Clinical Microbiology and Infection, 4*, 1–3.

Fink, I. T., Palop, N. T., Salvador, R. B., Gómez, J. B., Cardona, C. G., & Ortega, D. N. (2019). Evaluation of the DNA microarray "AMR Direct Flow Chip Kit" for detection of antimicrobial resistance genes from Gram-positive and Gram-negative bacterial isolated colonies. *Enfermedades infecciosas y microbiologiaclinica, 37*(7), 454–457.

Fluit, A. C., Visser, M. R., & Schmitz, F. J. (2001). Molecular detection of antimicrobial resistance. *Clinical Microbiology Reviews, 14*(4), 836–871.

Foley, S. L., Lynne, A. M., & Nayak, R. (2009). Molecular typing methodologies for microbial source tracking and epidemiological investigations of Gram-negative bacterial foodborne pathogens. *Infection, Genetics and Evolution, 9*(4), 430–440.

Franklin, R. B., Taylor, D. R., & Mills, A. L. (1999). Characterization of microbial communities using randomly amplified polymorphic DNA (RAPD). *Journal of Microbiological Methods, 35*(3), 225–235.

Frazão, M. R., Cao, G., Medeiros, M. I. C., Duque, S. D. S., Allard, M. W., & Falcão, J. P. (2021). Antimicrobial resistance profiles and phylogenetic analysis of *Campylobacter jejuni* strains isolated in Brazil by whole genome sequencing. *Microbial Drug Resistance, 27*(5), 660–669.

Frenay, H. M. E., Bunschoten, A. E., Schouls, L. M., Van Leeuwen, W. J., Vandenbroucke-Grauls, C. M. J. E., Verhoef, J., & Mooi, F. R. (1996). Molecular typing of methicillin-resistant *Staphylococcus aureus* on the basis of protein A gene polymorphism. *European Journal of Clinical Microbiology and Infectious Diseases, 15*(1), 60–64.

Gad, A. H., Abo-Shama, U. H., Harclerode, K. K., & Fakhr, M. K. (2018). Prevalence, serotyping, molecular typing, and antimicrobial resistance of *Salmonella* isolated from conventional and organic retail ground poultry. *Frontiers in Microbiology, 9*, 2653.

Garneau, P., Labrecque, O., Maynard, C., Messier, S., Masson, L., Archambault, M., & Harel, J. (2010). Use of a bacterial antimicrobial resistance gene microarray for the identification of resistant *Staphylococcus aureus*. *Zoonoses and Public Health, 57*, 94–99.

Gilot, P., Lina, G., Cochard, T., & Poutrel, B. (2002). Analysis of the genetic variability of genes encoding the RNA III-activating components Agr and TRAP in a population of Staphylococcus aureus strains isolated from cows with mastitis. *Journal of clinical microbiology, 40*(11), 4060–4067.

Goering, R. V., Morrison, D., Al-Doori, Z., Edwards, G. F. S., & Gemmell, C. G. (2008). Usefulness of mec-associated direct repeat unit (dru) typing in the epidemiological analysis of highly clonal methicillin-resistant *Staphylococcus aureus* in Scotland. *Clinical Microbiology and Infection, 14*(10), 964–969.

Heather, J. M., & Chain, B. (2016). The sequence of sequencers: The history of sequencing DNA. *Genomics, 107*(1), 1–8.

Hendriksen, R. S., Bortolaia, V., Tate, H., Tyson, G. H., Aarestrup, F. M., & McDermott, P. F. (2019). Using genomics to track global antimicrobial resistance. *Frontiers in Public Health, 7*, 242.

Higuchi, R., Dollinger, G., Walsh, P. S., & Griffith, R. (1992). Simultaneous amplification and detection of specific DNA sequences. *Bio/Technology, 10*(4), 413–417.

Hyytiä-Trees, E. K., Cooper, K., Efrain, M. R., & Gerner-Smidt, P. (2007). Recent developments and future prospects in subtyping of foodborne bacterial pathogens. *Future Microbiology, 2*(2), 175–185.

Jesumirhewe, C., Springer, B., Allerberger, F., & Ruppitsch, W. (2020). Whole genome sequencing of extended-spectrum β-lactamase genes in Enterobacteriaceae isolates from Nigeria. *PLoS One, 15*(4), e0231146.

Jolley, K. A., Bray, J. E., & Maiden, M. C. (2018). Open-access bacterial population genomics: BIGSdb software, the PubMLST. org website and their applications. *Wellcome Open Research, 3*(124), 1–20.

Joseph, S. J., & Read, T. D. (2010). Bacterial population genomics and infectious disease diagnostics. *Trends in Biotechnology, 28*(12), 611–618.

Jüttner, S., Vieth, M., Miehlke, S., Schneider-Brachert, W., Kirsch, C., Pfeuffer, T., Lehn, N., & Stolte, M. (2004). Reliable detection of macrolide-resistant *Helicobacter pylori* via fluorescence *in situ* hybridization in formalin-fixed tissue. *Modern Pathology, 17*(6), 684–689.

Kalai, W., Martinez, I., Bikandi, J., Messadi, L., Khazri, I., Souissi, N., Saidani, M., Slim, A. F., Boubaker, I. B. B., & Garaizar, J. (2018). Antimicrobial susceptibility and MLVA analysis of S. Typhimurium strains isolated from human and poultry samples in Tunisia. *The Journal of Infection in Developing Countries, 12*(05), 313–320.

Kaya, H., Hasman, H., Larsen, J., Stegger, M., Johannesen, T. B., Allesøe, R. L., Lemvigh, C. K., Aarestrup, F. M., Lund, O., & Larsen, A. R. (2018). SCC mec Finder, a web-based tool for typing of staphylococcal cassette chromosome mec in *Staphylococcus aureus* using whole-genome sequence data. *Msphere, 3*(1), e00612–e00617.

Kondo, Y., Ito, T., Ma, X. X., Watanabe, S., Kreiswirth, B. N., Etienne, J., & Hiramatsu, K. (2007). Combination of multiplex PCRs for staphylococcal cassette chromosome mec type assignment: rapid identification system for mec, ccr, and major differences in junkyard regions. *Antimicrobial Agents and Chemotherapy, 51*(1), 264–274.

Kotetishvili, M., Stine, O. C., Chen, Y., Kreger, A., Sulakvelidze, A., Sozhamannan, S., & Morris, J. G., Jr. (2003). Multilocus sequence typing has better discriminatory ability for typing Vibrio cholerae than does pulsed-field gel electrophoresis and provides a measure of phylogenetic relatedness. *Journal of Clinical Microbiology, 41*(5), 2191–2196.

Kudirkiene, E., Andoh, L. A., Ahmed, S., Herrero-Fresno, A., Dalsgaard, A., Obiri-Danso, K., & Olsen, J. E. (2018). The use of a combined bioinformatics approach to locate antibiotic resistance genes on plasmids from whole genome sequences of *Salmonella enterica* serovars from humans in Ghana. *Frontiers in Microbiology, 9*, 1010.

Lagha, R., Abdallah, F. B., AlKhammash, A. A., Amor, N., Hassan, M. M., Mabrouk, I., Alhomrani, M., & Gaber, A. (2021). Molecular characterization of multidrug resistant *Klebsiella pneumoniae* clinical isolates recovered from King Abdulaziz Specialist Hospital at Taif City, Saudi Arabia. *Journal of Infection and Public Health, 14*(1), 143–151.

Lee, W. S., Lee, S., Kang, T., Ryu, C. M., & Jeong, J. (2019). Detection of ampicillin-resistant *E. coli* using novel nanoprobe-combined fluorescence in situ hybridization. *Nanomaterials, 9*(5), 750.

Levsky, J. M., & Singer, R. H. (2003). Fluorescence in situ hybridization: past, present and future. *Journal of Cell Science, 116*(14), 2833–2838.

Li, W., Raoult, D., & Fournier, P. E. (2009). Bacterial strain typing in the genomic era. *FEMS Microbiology Reviews, 33*(5), 892–916.

Liu, P. Y. F., Shi, Z. Y., Lau, Y. J., Hu, B. S., Shyr, J. M., Tsai, W. S., Lin, Y. H., & Tseng, C. Y. (1996). Use of restriction endonuclease analysis of plasmids and pulsed-field gel electrophoresis to investigate outbreaks of methicillin-resistant *Staphylococcus aureus* infection. *Clinical Infectious Diseases, 22*(1), 86–90.

Maiden, M. C., Bygraves, J. A., Feil, E., Morelli, G., Russell, J. E., Urwin, R., Zhang, Q., Zhou, J., Zurth, K., Caugant, D. A., & Feavers, I. M. (1998). Multilocus sequence typing: A portable approach to the identification of clones within populations of pathogenic microorganisms. *Proceedings of the National Academy of Sciences, 95*(6), 3140–3145.

Maxam, A. M., & Gilbert, W. (1977). A new method for sequencing DNA. *Proceedings of the National Academy of Sciences, 74*(2), 560–564.

Ménard, C., Brousseau, R., & Mouton, C. (1992). Application of polymerase chain reaction with arbitrary primer (AP-PCR) to strain identification of Porphyromonas (Bacteroides) gingivalis. *FEMS Microbiology Letters, 95*(2–3), 163–168.

Milheiriço, C., Oliveira, D. C., & de Lencastre, H. (2007). Update to the multiplex PCR strategy for assignment of mec element types in *Staphylococcus aureus*. *Antimicrobial Agents and Chemotherapy, 51*(9), 3374–3377.

Mori, Y., & Notomi, T. (2009). Loop-mediated isothermal amplification (LAMP): A rapid, accurate, and cost-effective diagnostic method for infectious diseases. *Journal of Infection and Chemotherapy, 15*(2), 62–69.

Moura, A., Tourdjman, M., Leclercq, A., Hamelin, E., Laurent, E., Fredriksen, N., Van Cauteren, D., Bracq-Dieye, H., Thouvenot, P., Vales, G., & Tessaud-Rita, N. (2017). Real-time whole-genome sequencing for surveillance of *Listeria monocytogenes,* France. *Emerging Infectious Diseases, 23*(9), 1462.

Mueller, U. G., & Wolfenbarger, L. L. (1999). AFLP genotyping and fingerprinting. *Trends in Ecology & Evolution, 14*(10), 389–394.

Mullis, K., Faloona, F., Scharf, S., Saiki, R. K., Horn, G. T., & Erlich, H. (1986). Specific enzymatic amplification of DNA in vitro: the polymerase chain reaction. In *Cold Spring Harbor symposia on quantitative biology* (Vol. 51, pp. 263–273). Cold Spring Harbor Laboratory Press.

Murugadas, V., Joseph, T. C., & Lalitha, K. V. (2017). Tracing contamination of Methicillin-resistant *Staphylococcus aureus* (MRSA) into seafood marketing chain by staphylococcal protein A typing. *Food Control, 78*, 43–47.

Neamati, F., Khorshidi, A., Moniri, R., & Hosseini Tafreshi, S. A. (2020). Molecular epidemiology of antimicrobial resistance of uropathogenic *Escherichia coli* isolates from patients with urinary tract infections in a tertiary teaching hospital in Iran. *Microbial Drug Resistance, 26*(1), 60–70.

Notomi, T., Okayama, H., Masubuchi, H., Yonekawa, T., Watanabe, K., Amino, N., & Hase, T. (2000). Loop-mediated isothermal amplification of DNA. *Nucleic Acids Research, 28*(12), e63–e63.

Nowrouzian, F. L., Karami, N., Welinder-Olsson, C., & Åhrén, C. (2013). Virulence gene typing of methicillin-resistant *Staphylococcus aureus* as a complement in epidemiological typing. *Journal of Microbiological Methods, 93*(3), 173–176.

Oliveira, D. C., Milheiriço, C., & de Lencastre, H. (2006). Redefining a structural variant of staphylococcal cassette chromosome mec, SCCmec type VI. *Antimicrobial Agents and Chemotherapy, 50*(10), 3457–3459.

Panigrahi, S., Velraj, P., & Rao, T. S. (2019). Functional microbial diversity in contaminated environment and application in bioremediation. In *Microbial diversity in the genomic era* (pp. 359–385). Academic Press.

Parcell, B. J., Gillespie, S. H., Pettigrew, K. A., & Holden, M. T. (2021). Clinical perspectives in integrating whole-genome sequencing into the investigation of healthcare and public health outbreaks–hype or help? *Journal of Hospital Infection, 109*, 1–9.

Petersdorf, S., Herma, M., Rosenblatt, M., Layer, F., & Henrich, B. (2015). A novel staphylococcal cassette chromosome mec type XI primer for detection of mecC-harboring methicillin-resistant *Staphylococcus aureus* directly from screening specimens. *Journal of Clinical Microbiology, 53*(12), 3938–3941.

Qian, Z., & Adhya, S. (2017). DNA repeat sequences: diversity and versatility of functions. *Current Genetics, 63*(3), 411–416.

Ranjbar, R., Karami, A., Farshad, S., Giammanco, G. M., & Mammina, C. (2014). Typing methods used in the molecular epidemiology of microbial pathogens: A how-to guide. *The New Microbiologica, 37*(1), 1–15.

Robinson, D. A., Monk, A. B., Cooper, J. E., Feil, E. J., & Enright, M. C. (2005). Evolutionary genetics of the accessory gene regulator (agr) locus in *Staphylococcus aureus*. *Journal of Bacteriology, 187*(24), 8312–8321.

Sanger, F., Nicklen, S., & Coulson, A. R. (1977). DNA sequencing with chain-terminating inhibitors. *Proceedings of the National Academy of Sciences, 74*(12), 5463–5467.

Schaberg, D. R., Tompkins, L. S., & Falkow, S. (1981). Use of agarose gel electrophoresis of plasmid deoxyribonucleic acid to fingerprint gram-negative bacilli. *Journal of Clinical Microbiology, 13*(6), 1105–1108.

Schwartz, T., Kohnen, W., Jansen, B., & Obst, U. (2003). Detection of antibiotic-resistant bacteria and their resistance genes in wastewater, surface water, and drinking water biofilms. *FEMS Microbiology Ecology, 43*(3), 325–335.

Sharples, G. J., & Lloyd, R. G. (1990). A novel repeated DNA sequence located in the intergenic regions of bacterial chromosomes. *Nucleic Acids Research, 18*(22), 6503–6508.

Shayegh, J., Atashpaz, S., & Hejazi, M. S. (2008). Virulence genes profile and typing of ovine Pasteurella multocida. *Asian Journal of Animal and Veterinary Advances, 3*(4), 206–213.

Shopsin, B., Gomez, M., Montgomery, S. O., Smith, D. H., Waddington, M., Dodge, D. E., . . . & Kreiswirth, B. N. (1999). Evaluation of protein A gene polymorphic region DNA sequencing for typing of Staphylococcus aureus strains. *Journal of clinical microbiology, 37*(11), 3556–3563.

Subramanya, S. H., Bairy, I., Metok, Y., Baral, B. P., Gautam, D., & Nayak, N. (2021). Detection and characterization of ESBL-producing Enterobacteriaceae from the gut of subsistence farmers, their livestock, and the surrounding environment in rural Nepal. *Scientific Reports, 11*(1), 1–13.

Sukmawinata, E., Uemura, R., Sato, W., Mitoma, S., Kanda, T., & Sueyoshi, M. (2020). IncI1 plasmid associated with blaCTX-M-2 transmission in ESBL-producing *Escherichia coli* isolated from healthy Thoroughbred racehorse, Japan. *Antibiotics, 9*(2), 70.

Swaminathan, B., Barrett, T. J., Hunter, S. B., Tauxe, R. V., & Force, C. P. T. (2001). PulseNet: the molecular subtyping network for foodborne bacterial disease surveillance, United States. *Emerging Infectious Diseases, 7*(3), 382.

Tenover, F. C., Arbeit, R. D., Goering, R. V., Mickelsen, P. A., Murray, B. E., Persing, D. H., & Swaminathan, B. (1995). Interpreting chromosomal DNA restriction patterns produced by pulsed-field gel electrophoresis: Criteria for bacterial strain typing. *Journal of Clinical Microbiology, 33*(9), 2233–2239.

Threlfall, E. J., Frost, J. A., Ward, L. R., & Rowe, B. (1990). Plasmid profile typing can be used to subdivide phage-type 49 of *Salmonella typhimurium* in outbreak investigations. *Epidemiology & Infection, 104*(2), 243–251.

Vaiyapuri, M., Joseph, T. C., Rao, B. M., Lalitha, K. V., & Prasad, M. M. (2019). Methicillin-resistant *Staphylococcus aureus* in seafood: Prevalence, laboratory detection, clonal nature, and control in seafood chain. *Journal of Food Science, 84*(12), 3341–3351.

Vernile, A., Giammanco, G., & Massa, S. (2009). PFGE: Importance in food quality. *Recent Patents in Food Nutrition and Agriculture, 1*(3), 248–251.

Versalovic, J., Koeuth, T., & Lupski, R. (1991). Distribution of repetitive DNA sequences in eubacteria and application to fingerprinting of bacterial genomes. *Nucleic Acids Research, 19*(24), 6823–6831.

Williams, J. G., Kubelik, A. R., Livak, K. J., Rafalski, J. A., & Tingey, S. V. (1990). DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. *Nucleic Acids Research, 18*(22), 6531–6535.

World Health Organization. (2017). WHO publishes list of bacteria for which new antibiotics are urgently needed.

World Health Organization. (2019). Global Antimicrobial Resistance Surveillance System (GLASS (: molecular methods for antimicrobial resistance (AMR) *diagnostics to enhance the global antimicrobial resistance surveillance system* (No. WHO/WSI/AMR/2019.1). World Health Organization.

World Health Organization. (2020). GLASS whole-genome sequencing for surveillance of antimicrobial resistance.

Zhong, L. L., Zhou, Q., Tan, C. Y., Roberts, A. P., & El-Sayed, M. A. E. G. (2019). Multiplex loop-mediated isothermal amplification (multi-LAMP) assay for rapid detection of mcr-1 to mcr-5 in colistin-resistant bacteria. *Infection and Drug Resistance, 12*, 1877.

Zhuang, M., Achmon, Y., Cao, Y., Liang, X., Chen, L., Wang, H., Siame, B. A., & Leung, K. Y. (2021). Distribution of antibiotic resistance genes in the environment. *Environmental Pollution*, 117402.

Part V

Mitigation Measures Across the Sectors

Antimicrobial Resistance (AMR) Surveillance Under One Health

P. Anand Kumar

Contents

Abstract

Antimicrobial resistance (AMR) has a significant effect on the lives of humans and animals, and it is emerging as a global scrouge. Increased antibiotic resistance in bacterial pathogens of medical and veterinary importance costs dearly to the lives of humans and animals. The AMR affliction must be addressed with appropriate surveillance, prevention, and control strategies. One Health approach involving human health, animal health, and environment sectors (multisectoral action) plays a crucial role in AMR surveillance. The objective behind the One Health surveillance is due to the microbial and genetic movements across human, animal, and environment sectors. As the antimicrobials usage (AMU) in humans and animals is one of the main drivers for AMR, it is quintessential to monitor them with efficient surveillance networks. However, One Health surveillance is a laborious task, requiring harmonization of protocols and collection of bacterial isolates from different sectors (human, veterinary/fishery, and environment). In veterinary and aqua sectors, AMR in foodborne bacteria is more focused as it poses public health threat. Zoonotic and indicator bacteria also assume relevance under One Health approach. AMR data integration and its analysis form the core section of the application and inference aspect of One Health surveillance.

P. Anand Kumar (✉)
Department of Veterinary Microbiology, NTR College of Veterinary Science, Sri Venkateswara Veterinary University, Gannavaram, Andhra Pradesh, India
e-mail: p_anandkumar@yahoo.com

M. P. Mothadaka et al. (eds.), *Handbook on Antimicrobial Resistance*,
https://doi.org/10.1007/978-981-19-9279-7_33

Prescription/use of certain antibiotics like doxycycline, azithromycin, etc., during the COVID-19 pandemic also necessitates the AMR surveillance under One Health during/after pandemic. Strong political will with sustained budgetary support is required for the implementation of AMR surveillance under One Health.

Keywords

Antimicrobial resistance (AMR) · Antimicrobial usage (AMU) · One Health · Surveillance · Human · Veterinary · Aqua and environment

1 Introduction

The unabated surge of AMR across the globe has disastrous effect on human, animal, and environment health with financial consequences (O'Neill, 2016). Though term AMR is generally used for resistance against all the antimicrobials such as antibacterial, antiviral, antifungal, antiprotozoal, and anthelmintic drugs, certain researchers use the more specific word antibiotic resistance (ABR) for addressing the resistance in bacteria against antibiotics. The objective of improving the health of humans and the health/productivity of animals necessitated the extensive use of antimicrobials (antibiotics) in low- and middle-income countries (LMICs), often without respecting the therapy guidelines many a times (Manyi-Loh et al., 2018; Robinson et al., 2016a, b). In animal husbandry and in aquaculture sectors, ignorance of the farmers about the hazards linked to misuse of antimicrobials on public health is also a notable factor (Landers et al., 2012). The AMR is poised to become a high-profile social issue, as it is personally relevant to the average citizen. Popular concern and support are required to take up the issue of AMR for framing new policies or initiatives to address the issue with sufficient funding for research. The AMR scourge must be addressed with appropriate surveillance, prevention, and control strategies. Formulation of public health policies usually requires evidence for action. This is vital for progress of any international/national programs and policies. Therefore, AMR surveillance especially under One Health initiative assumes great significance (Grundmann et al., 2011; Robinson et al., 2016a).

One Health is defined as "*the collaborative efforts of multiple disciplines working locally, nationally, and globally, to attain optimal health for people, animals and our environment*" (AVMA, 2008). Human health, animal health, and environment are interconnected. The AMR has vivid links with all the three domains, i.e., human, animal, and environment of One Health. Though many animal-associated bacterial infections are not found in humans, use of subtherapeutic doses of antibiotics for longer exposure periods of time facilitate ideal conditions for the bacteria in animal and fishery systems to fix the genetic determinants that confer antibiotic resistance. The evidence that links antibiotics use in agricultural/animal husbandry to AMR in people is reported to be substantiated (Robinson et al., 2016b). Hence, AMR

surveillance under One Health approach stands as a classical example for issues to be taken up under One Health (White & Hughes, 2019).

For prevention, control, and management of diseases in humans and animals, effective human and animal health surveillance systems are very important. Integration of the data sources from the human and animal health surveillance systems becomes a vital requisite for this task. For AMR surveillance, as the environmental health component also plays a highly significant role, surveillance activities under One Health approach yield benefits from "cross-fertilization" and pass the benefits to all the sectors to promote health for all. Due to these advantages, One Health approach is gaining momentum in this current decade (George et al., 2020).

World Health Organization (WHO), World Organization for Animal Health (OIE), and Food and Agriculture Organization (FAO) of United Nations joined hands for One Health approach (multisectoral action) towards AMR surveillance, prevention, and control strategies. Therefore, integrated surveillance of AMR is imperative. One Health High Level Expert Panel (OHHLEP) is an advisory panel on One Health for FAO, OIE, and WHO. According to OHHLEP, "*One Health is an integrated, unifying approach that aims to sustainably balance and optimize the health of people, animals, and ecosystems*." In 2021, the joint tripartite (FAO, OIE, and WHO) and United Nations Environment Protection (UNEP) supported the OHHLEP definition on "One Health" for all the activities under One Health approach (WHO, 2021).

The concept of One Health got a fillip with the meeting of scientists from 35 countries in September 2011 during the inauguration of the First International Congress on Pathogens at the Human, Animal Interface (ICOPHAI) at Addis Ababa, Ethiopia. In its second congress conducted at Porto de Galinhas, Brazil, during August 2013, representatives from 59 different countries attended. Many interconnected issues under One Health, including the AMR and capacity building needs for One Health approach, were presented (Gebreyes et al., 2014). The third congress of ICOPHAI was held in Chiag Mai, Thailand, during August 2015; the fourth congress was held in Doha, Qatar, during November 2017; and the fifth congress was held in Quebec City, Canada, during September 2019. The ICOPHAI congresses focused the necessity of national, international, interdisciplinary, and intersectoral cooperation for management of human health risks including those linked to AMR of bacteria in food chain, particularly with foods of animal origin (https://icophai.org).

The One Health coordination mechanisms are based on the objective of multisectoral coordination and for addressing the infectious diseases of zoonotic importance, as well as for other common concerns related to health at the interface of human-animal-environment sectors. This will be helpful for strengthening and developing collaboration, communication, and coordination across the sectors with effective leadership and its associated technical functions for achieving desired outcomes. The key factors for sustainable implementation of One Health approach include political will, resources (human and financial), designing goals, strong governance, routine coordination, and communication (WHO, FAO and OIE, 2019). Therefore, objectives of One Health approach are strengthening surveillance, laboratory capacity, IEC (information, education, and communication) activities, and capacity building through joint training of personnel from all the sectors.

Interrelationship exists among human health, animal health, and environment health sectors. Any change in climate will have impact on health of all the three sectors due to very complex and multidimensional interrelations among them. Though the climate change influences human and animal health including transmission of vector-borne diseases, the human and animal activities such as pollution and emission of gases, etc., also have equal influence on the climate (Otte et al., 2007; Saker et al., 2004). Therefore, the environment sector needs to be invariably included as a mandatory component in any study on human and animal health. Owing to the significance of the environment (soil and water) as a reservoir for antimicrobial resistance genes (ARGs) that are crucial in AMR transmission among bacteria and also due to the release of antimicrobial metabolites/residues into the environment from pharma industry effluents, hospitals, and farms wastes/discharges, the AMR surveillance in the environment sector under the One Health approach provides much needed significant inputs (Huijbers et al., 2015).

Among the diverse drivers for ABR in bacteria, the drivers such as antibacterial use that are responsible for selective pressure in generating the AMR fall under the category of "selection" component whereas the resistant bacteria between each linked compartment of human-animal-environment axis fall under the category of "transmission." The ABR bacteria are shared among humans, animals, and environment by human-to-human contacts, human-to-animal contact, animal-to-animal contact, human-to-environment-to-animal, and animal-to-environment-to-human interfaces (Booton et al., 2021). The ABR bacterial pathogens that are excreted through human stools and animal faces accumulate in the natural ecosystems, and they spread through the environment. Due to this, the wastewater treatment plants, water bodies for supply of drinking water, etc., are polluted with ABR bacteria and their ABR genes (Agramont et al., 2020). Therefore, as the AMR/ABR is inherently a complex field, a coordinated approach is required to tackle the AMR scourge for which the surveillance under One Health approach provides valuable information. The factors such as organized livestock, poultry, fishery and aqua farming with intensive farm practices, global distributions of foods (especially foods of animal origin) through imports and exports, international travel and trade, climate change, increased population and urbanization, etc., are responsible for global spread of ABR, especially in LMICs (Iskandar et al., 2020).

Not only the quadripartite of WHO, FAO, WOAH/ OIE, and UNEP but also other international organizations such as International Monetary Fund (IMF), World Bank, and G8 group of nations declared the AMR as the major global threat of the twenty-first century (World Bank Group, 2018). Since different ecosystems are involved in acquisition, emergence, and distribution of AMR, all these international organizations affirmed the need to have coordinated and interdisciplinary approach under One Health to address the AMR/ABR scourge (Hernando-Amado et al., 2019). The collaborative role of human health, animal health, and environmental sector researchers or professionals is indispensable to mitigate the global AMR, and such a collaborative role is obligatory in the national action plan to contain AMR in all the countries.

Due to involvement of all the pertinent components across the human-animal-environment interface under One Health approach, the health issues will be

addressed in a way that is more effective, efficient, and sustainable. The limited resources of money and personnel especially in LMICs may be utilized to the best possible level with the multisectoral One Health approach towards addressing zoonotic diseases and AMR. This ultimately helps to improve efficiency and effectiveness of zoonotic disease management that reduces the costs involved (Smith et al., 2019). Close collaboration among different organizations, institutions, and stakeholders is imperative to strengthen the One Health network. Understanding the national context and priorities is imperative for effective implementation of One Health approach in a country. Strategic planning and emergency preparedness are to be included in standard operating procedure (SOP) of implementation (WHO, FAO and OIE, 2019).

The Global Action Plan (GAP) released by the World Health Assembly in 2015 and the National Action Plan (NAP) of the Government of India released in 2017 emphasize the significance of robust AMR surveillance systems. Effective surveillance helps with monitoring the situation and also with evaluation. Different international and national organizations of human health, animal health, and environment sectors are involved. Therefore, the engagement in AMR surveillance is broadened. In the veterinary and fishery sectors, AMR in foodborne bacteria is more focused as it poses a public health threat (WHO, 2017).

The informed decisions on interventions for controlling any disease are based on the surveillance of that disease, and it is applicable to AMR too. The AMR surveillance includes collection, validation, analysis, and reporting of relevant microbiological and epidemiological data on AMR in humans, animals (foods), and environment, and on respective AMU in humans and animals (WHO, 2017). Increased antimicrobial use in LMICs is a serious cause of concern (Van Boeckel et al., 2015). However, lack of AMR expertise, poor laboratory infrastructure, and inadequate data management capacity are the hurdles for effective AMR surveillance systems. Spatiotemporal variation of AMR pattern in bacteria is assessed only through surveillance. Hence, capacity building in terms of expertise, laboratory infrastructure, and data management is crucial in AMR surveillance.

2 Value of One Health Surveillance

Antibiotics used in human and animal health by and large comprise of similar or the same molecules, which drives the spread of ABR between animals and humans directly or indirectly through the environment (Holmes et al., 2016). Microbial and genetic movements across the human, animal, and environment sectors constitute the basic objective behind the One Health surveillance. Horizontal gene transfer among the bacterial species is one of the significant key factors. The genetic determinants of AMR are transmitted/exchanged among bacterial pathogens of humans and animals. The adoption of bacteria due to antimicrobial use driving selective pressure in one sector is usually reflected in other sectors. On the same lines, the strategies adopted to contain AMR in one sector will affect the other sectors too (Heuer et al., 2009; Holmes et al., 2016; O'Neill, 2016; Woolhouse &

Ward, 2013). Integrated sampling from food animals, foods, environmental samples (e.g., water), and humans with harmonized protocols are crucial for AMR surveillance under One Health (Queenan et al., 2016). As majority of the available classes of antimicrobials are common in human and animal sector, implementation of AMR surveillance under One Health approach is the apt way of addressing the AMR (Collignon & McEwen, 2019).

The One Health surveillance approach has significant value across multiple dimensions. This will be quite useful to ascertain the baseline levels of bacteria and their ABR in reservoirs; it will also be helpful to explain the spread of ABR bacteria and their ARGs across the ecosystems. In this context, there is a need to identify temporal and spatial AMR trends. When such trends are identified, it will be useful to support the hypotheses on the sources and reservoirs of the resistant bacteria. Once linkage to specific antibiotic practices is done, targeted interventions through risk analysis can be modeled. AMR in pathogenic and commensal bacteria is equally important apropos AMR surveillance. Antimicrobial usage (AMU) in humans and animals is the main trigger for AMR; therefore, surveillance and monitoring of AMU and AMR in humans and animals are quintessential (ECDC/EFSA/EMA, 2015). This will help to assess the use of antimicrobials and antibiotic susceptibility pattern of bacteria in different populations. Zoonotic and indicator bacteria assume relevance for AMR surveillance under One Health approach.

Antibiotic resistance dynamics, influence of geographical origin, and management systems on antibiotic resistance genes flow within humans, animals, and the environment can be appreciated with the One Health approach (White & Hughes, 2019). One Health surveillance is fundamental to the National Action Plan (NAP). The spread of different bacterial strains and their genes across ecosystems can be described through an efficient surveillance mechanism. Critical data about the AMR pattern will be generated that can be used for mitigation purposes. Risk analysis of foodborne AMR hazards can be carried out. One Health surveillance data on AMR will be helpful for evaluating the achievements of evidence-based intervention. One Health approach helps to close the knowledge gaps due to involvement of multiple sectors. Comprehensive genetic information about antibiotic resistance-related genes in bacterial isolates of the different sectors (human, animal, and environment) coupled with conventional antibiotic susceptibility tests (ASTs) helps to understand the genotype-phenotype correlation, especially in foodborne bacterial pathogens of public health importance. A knowledge gap exists about the probable extent and mechanisms of transmission of ARGs between the normal gut flora of animals and humans.

One Health AMR surveillance shall also include bacterial pathogens of companion animals. This, coupled with AMR data of the bacterial pathogens from owners of the companion animals, provides a comprehensive picture with vital clues for mitigation and other relevant measures to address AMR. Games/sports animals and birds are also to be included in the One Health surveillance. Lately, in some countries, the AMR burden in migratory birds is also taken into consideration for AMR surveillance. The influence of different animal husbandry practices on AMR is also to be assessed (Mundaca-Shah et al., 2017).

In certain LMICs, antibiotics are used in agriculture for plant protection. The evolution of antibiotic resistance and its transmission to other sectors (human and animal) are the least understood, as the environmental component is not well studied in AMR surveillance. For all these reasons, One Health approach shall be considered as obligatory for AMR surveillance (Robinson et al., 2016a).

Keeping in view the significance of AMR globally with the multisectoral stakes, it is always apt to apply One Health approach to mitigate the AMR scourge, taking into consideration the costs and benefits associated with this approach verses the costs and responses associated with individual sector-isolated approach. Optimization of resources (financial, infrastructure, and human resources) can be achieved thorough appropriate planning exercises under the One Health approach. Overall strengthening of human, animal, and environmental health systems can be accomplished with the investments made under the One Health approach that facilitate readiness and competitiveness to face multisectoral hazards and yield broad societal benefits (Smith et al., 2019).

The effectiveness of models and outcomes and their efficiency are expected to improve with the One Health approach. In a low disease prevalence scenario, about US$ 184 million per year and in a high disease prevalence scenario, about US$ 506 million per year can be substantially saved with the "One Health" surveillance approach in LMICs, which excludes the savings generated from other activities such as planning, communication, education, natural resource benefits, training, and research (World Bank Group, 2018).

Intersectoral national surveillance systems on AMR under One Health in some countries are listed below (Queenan et al., 2016).

(a) The Danish Integrated Antimicrobial Resistance Monitoring and Research Programme (DANMAP)
(b) The Swedish Strategic Programme Against Antibiotic Resistance (STRAMA) and The Swedish Veterinary Antimicrobial Resistance Monitoring Programme (SVARM)
(c) The NethMap and Monitoring of Antimicrobial Resistance and Antibiotic Usage in Animals in the Netherlands (MARAN)
(d) The Canadian Integrated Program for Antimicrobial Resistance Surveillance (CIPARS)
(e) Observatoire National de l'Épidémiologie de la Résistance Bactérienne aux Antibiotiques (ONERBA, France)
(f) Norwegian Monitoring System for Antibiotic Resistance in Microbes (NORM) and NORM-Vet, Norway
(g) National Antimicrobial Resistance Monitoring System (NARMS), USA

In 2022, the strategic framework for collaboration on AMR was released by the quadripartite of WHO, FAO, WOAH/ OIE, and UNEP, with emphasis on "Together for One Health." This strategic framework document is considered as an important milestone in the collaboration among the important international organizations as these organizations assure jointly to support efforts of all the countries to increase

their national responses to AMR (WHO, FAO and OIE, 2022). The comparative advantage and catalytic role of these four international organizations to advance the One Health response to AMR are vividly described in this document.

3 Challenges to One Health Surveillance

Challenges for AMR surveillance under One Health approach include under-appreciation of financial and health benefits; silos in different sectors; competing interests among human, animal, and environment sectors and their respective organizations at national and regional levels; and imbalance in competence, ability, and resource allocation to conduct surveillance and information sharing across the sectors (FAO, 2020). Although at international level the joint tripartite of FAO, OIE, and WHO with UNEP are working together to address AMR under One Health, such a joint effort is still eluding at national and regional levels in many countries. Achieving unanimity on priorities to address AMR in all the sectors (humans, animals, and environment) is a challenging task in LMICs. Gaps in the AMR surveillance in individual sectors may seriously affect the AMR surveillance under One Health. Policy related to antimicrobial use in humans and animals in different countries, especially in LMICs, also poses a challenge (WHO, FAO and OIE, 2019).

One Health surveillance is laborious and expensive as bacterial isolates are to be collected from different sectors, protocols are to be harmonized, and samples are to be processed for AMR detection as per the standardized antibiotics panel and protocols. Data integration from all the participating sectors and harmonization of ways for analysis of the data are also crucial steps. A sound sampling system is crucial for successful surveillance. Though the AST is routinely carried out in human and veterinary medicine disease diagnostic laboratories, collection of bacterial isolates from food and environmental sectors is a challenging and arduous task. In addition, the silos in different sectors involved in One Health surveillance also hinder the execution. However, keeping in view the significance of One Health surveillance, national and state and federal public health authorities can effectively implement it by bringing together all the stakeholders under one platform (Mundaca-Shah et al., 2017).

In one of the case studies for AMR surveillance under One Health, the use of third generation of cephalosporins such as ceftiofur in the poultry sector and its time-related correlation with severe cases of human illness due to salmonellosis by consumption of poultry products were recognized in Canada (Dutil et al., 2010). Similarly, in rural Ecuador, Hedmann et al. (2019) reported the prevalence of CTX-M extended-spectrum beta-lactamase-secreting *Escherichia coli* in chickens from small-scale poultry farms and in the children living on the farms, under the One Health surveillance approach.

In surveillance studies under the One Health approach, data collected from different sectors needs integrated analysis, which can be achieved with teams having expertise across the sectors. Therefore, the teams of experts shall have the intersectoral knowledge, understanding, and shared abilities to work with the

resource persons of all other relevant sectors. The communication and networking across different sectors under One Health need to be improved through periodic shared meetings with multisectoral teams. To achieve this, a centralized program with centralized leadership and dedicated budgetary support is obligatory so that the capacity building at peripheral levels can also be achieved (Queenan et al., 2016).

4 Political Will for One Health Surveillance

Strong political will for capacity building in AMR surveillance under One Health is the major push for its implementation in any nation. Most of the countries have human health-focused AMR surveillance systems. The Sixty-eighth World Health Assembly in May 2015 endorsed a global action plan to tackle AMR. Member states of the United Nations adopted a political declaration of the high-level meeting on AMR via resolution A/RES/71/3 on 21 September 2016 during the 71st session of the UN General Assembly (UN, 2016). This political resolution highlighted the need to act on exponentially raising AMR and stressed the need for implementable actions for sustainable development. Since then, different countries have drafted their respective National Action Plans (NAP) on AMR, and One Health surveillance has assumed greater significance in implementing the action plan on AMR.

Paula Cray, Professor and Head, Department of Population Health and Pathobiology, North Carolina State University, Raleigh, USA, proposed Collective Antimicrobial Resistance Ecosystem (CARE) model for One Health surveillance on AMR (USDA, 2014). Based on constant exposures of various kinds of ABR determinants at the interface of humans, animals, and environment, this CARE paradigm is hypothesized. Samples from human specimens, retail food samples, and samples from food-processing animals are collected for processing and analysis. In human specimens, the bacteria isolated from the ill persons shall be monitored as the first and foremost priority. These specimens shall include the bacterial isolates from sporadic and outbreak foodborne cases. While collecting samples from human health care facilities, cautious approach is needed to differentiate the hospital-associated infections from the foodborne infections. The retail food samples constitute as the second most important samples next to human samples. Foods of animal origin are considered as the most significant source of foodborne AMR bacterial pathogens, with potential risk of human exposure. The types of foods of animal origin to be collected in the surveillance program depend on the consumer behavior for choice of foods in that geographical region. Samples from food-producing animals (healthy and sick) are the next most important specimens. Based on the retail meat samples collected, the species of food producing animals shall be chosen for collecting samples (Queenan et al., 2016). This, coupled with samples from the environment, provides an overall scenario of One Health surveillance on AMR (WHO, 2017).

The antibiotics and their metabolites excreted by humans and animals will find their way into drainage/wastewater treatment works, which subsequently empty into rivers and seawaters. Hence, in the countries where high volumes of antibiotics are used, environmental concentrations of antibiotics and their impact will be greatest,

and residues of these antibiotics will pass through wastewater treatment works, mostly undegraded. In view of these implications associated with the antibiotic residues, the pharmaceutical industry in developed countries had a developed database to provide predicted no effect concentrations (PNEC) as minimum inhibitory concentrations (MIC) for active ingredients that may select for ABR (Mundaca-Shah et al., 2017).

Important contentious issues with AMR surveillance are the heterogeneous data collected with numerous guidelines and data from different laboratories of different sectors. For appreciating the prevalence of AMR and AMU at a glimpse in the given time for human samples in a hospital, point prevalence surveys (PPS) are generally used. But the PPS for animal samples is different as samples from food animals are taken from the healthy animals at the time of their slaughter. The type of microbes and the magnitude of their resistance to antibiotics are different in sick animals, compared to healthy animals (Moore, 2019).

It is a known fact that policy decisions rely on economic and scientific evidence. Therefore, the fully integrated system of AMR surveillance under One Health concept is imperative. The costs associated with the integrated surveillance outweigh the costs associated with the unchecked resistance. This is in addition to the resultant benefits from the interventions and outcomes based on the integrated surveillance. A wider range of issues are assessed under the One Health AMR surveillance, viz., human health and welfare, animal health and welfare, consumer confidence in food safety with reference to AMU in food animals, and international trade (Queenan et al., 2016). It may take many years to realize the benefits of integrated AMR surveillance under One Health. However, improved synergies among different sectors of (human, animal, and environment), accurate risk identification, appropriate and effective prevention and control measures can be achieved with this approach.

The WHO Advisory Group on Integrated Surveillance of Antimicrobial Resistance (AGISAR) prepared a guidance document on "*Integrated Surveillance of AMR in food-borne bacteria: Application of a One Health Approach*" in collaboration with the FAO and OIE (WHO, 2017). This document was prepared for comparison of antibiotic susceptibility test results of bacteria isolated from food-producing animals, foods of animal origin and humans, with appropriate epidemiological methods under integrated surveillance.

With the integrated surveillance:

- Accurate estimates of AMR in different reservoirs can be ascertained.
- Spatiotemporal assessment of AMR trends can be studied.
- Spread of antibiotic-resistant bacteria and their genetic elements of resistance can be best described.
- Novel antibiotic-resistant bacteria across the different sectors can be identified.
- Hypothesis can be generated about the sources/origin and reservoirs of ABR bacteria.
- The efficiency of interventional measures to limit/contain the emergence and spread of ABR bacteria can be identified and evaluated.

- Risk analysis of foodborne antimicrobial resistance hazards can be done for collecting further information.
- Strategies and guidelines based on evidence can be devised for controlling AMU in human and veterinary hospitals, communities, livestock and poultry farms, aquaculture, and agriculture.

Many a times, the issue of AMR is discussed with reference to its impact on human health. However, AMR impact on animals and environmental health is also equally important. Veterinary epidemiologist Prescott refers the complex epidemiology of AMR as "*resistance anywhere is resistance everywhere*" (Prescott, 2014). While considering the methods for AMR containment it is highly essential to link AMU data with AMR surveillance data (Fig. 1). Identification of critical control points in a systems-based surveillance approach helps to improve the effectiveness. The AMU in animals shall be carefully judged keeping the issues such as restoring animal health and productivity, impact on livelihoods, hunger, and poverty alleviation.

Under One Health approach to address AMR, the guidelines on the use of antibiotics in humans and animals constitute as significant valuable component. However, a broader approach is required to address the issue of resistance to antibiotics in bacterial communities associated with wildlife and environment. The wildlife ecosystems constitute significant component in AMR surveillance under the One Health approach as they act as potentially important reservoirs for the AMR organisms (AROs) and antimicrobial resistance genes (ARGs). The *E. coli* species with AMR against multiple antibiotics have been isolated from many wildlife species and the mechanism of AMR was found to be through plasmid-mediated resistance that might be due to environment spread of AMR from humans and animals (Weiss et al., 2018). On the other hand, reports are available on the impact of antibiotic resistance in human strains of *Staphylococcus aureus* on wildlife such as chimpanzees in sanctuaries, as such drug-resistant *S. aureus* was isolated from these apes (Schaumburg et al., 2012). Therefore, the bacterial isolates from wildlife animals also act as important indicators of AMR that resulted from humans, animals,

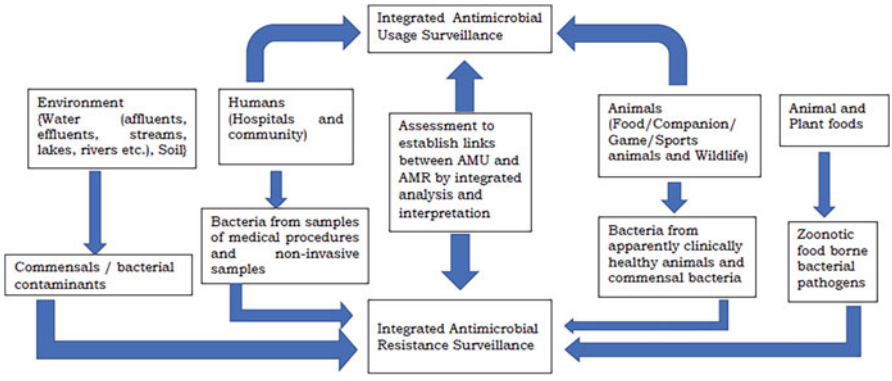


Fig. 1 An interconnected and integrated One Health surveillance framework

and environment sectors. Hence, the AMR surveillance under the One Health approach, which also includes the wildlife, is rightly useful in assessing the total AMR burden (White & Hughes, 2019). The additional monetary, social, and time investments made for AMR surveillance under One Health are likely to be recovered from the resulting benefits. Hence, initial constraints, if any, faced during AMR surveillance under One Health approach shall be addressed righteously and the program shall be carried forward as the benefits of the surveillance overweigh the constraints (Queenan et al., 2016).

Use of certain antibiotics such as doxycycline, azithromycin, etc., in humans is increased to several folds during COVID-19 pandemic due to certain antiviral and anti-inflammatory properties of these antibiotics. Medical physicians are used to prescribe these antibiotics to the SARS CoV2 positive and presumptive positive patients (Calderón-Parra et al., 2021; Hsu, 2020). However, many instances were reported about the indiscriminate use of the antibiotics by panic gripped public during this COVID-19 pandemic (Comber et al., 2020). Increase in use of antibiotics in such a scenario obviously increases the contamination of environment with antibiotics and their residues. As it is an established fact that ARGs flow within humans, animals, and environment systems, a specific AMR surveillance under One Health becomes a necessity during/after the pandemic. This will be helpful to assess the potential impact of antibiotic prescription or use during COVID-19 pandemic.

Based on the National Action Plan on AMR (NAP-AMR) of Government of India, National Centre for Disease Control (NCDC), Ministry of Health and Family Welfare, New Delhi, released Guidance for developing State Action Plans for Containment of Antimicrobial Resistance (SAPCAR), which rests on a One Health approach for AMR surveillance, in alignment with the NAP-AMR. The NCDC, New Delhi, is executing the National One Health program for prevention and control of zoonosis.

The Ministry of Health and Family Welfare (MoH&FW), Government of India, in association with the Government of Andhra Pradesh identified Krishna District of Andhra Pradesh to take up Indo-Dutch pilot project on AMR surveillance under One Health approach, involving human health, veterinary/animal health, and environment sectors. The bacterium *E. coli* was selected for AMR surveillance in the pilot project. Under the pilot project, the Department of Veterinary Microbiology, NTR College of Veterinary Science, Sri Venkateswara Veterinary University, Gannavaram, executed AMR surveillance in the veterinary/animal health and environment sectors, whereas the Department of Microbiology, Siddhartha Medical College, Vijayawada, executed AMR surveillance in human health sector. The *E. coli* isolates from urinary tract infections of patients under human health sector, from cloacal swabs of poultry under veterinary/animal sector, and from water samples under environment sector have been tested for their sensitivity/resistant patterns against harmonized panel of antibiotics in antibiotic sensitivity tests, and integrated analysis of the data from all these sectors was done (MoH&FW, 2019). With this pilot project, perhaps for the first time, MoH&FW, Government of India, had taken up AMR surveillance under One Health approach.

In October 2021, the Department of Biotechnology, Ministry of Science and Technology, Government of India, launched country's first One Health consortium

consisting of different medical and veterinary institutions with an objective to carry out surveillance of important bacterial, viral, and parasitic infections of zoonotic as well as transboundary pathogens in India.

5 Conclusion

In conclusion, AMR surveillance under One Health is very significant in addressing the AMR issues locally, nationally, and globally. Efforts shall be made to bring different stakeholders of human health, animal husbandry/health, agriculture, and environment sectors under one umbrella with the required budgetary support so that the most important creeping crisis of the world, i.e., AMR scourge can be addressed effectively.

References

Agramont, J., Gutiérrez-Cortez, S., Joffré, E., Sjöling, Å., & Calderon Toledo, C. (2020). Fecal pollution drives antibiotic resistance and class 1 integron abundance in aquatic environments of the Bolivian Andes impacted by mining and wastewater. *Microorganisms, 8*(8), 1122. https://doi.org/10.3390/microorganisms8081122

AVMA. (2008). *One health: A new professional imperative. One health initiative task force final report*. American Veterinary Medical Association.

Booton, R. D., Meeyai, A., Alhusein, N., Buller, H., Feil, E., Lamber, H., Mongkolsuk, S., Pitchforth, E., Reyher, K. K., Sakcamduang, W., Satayavivad, J., Singer, A. C., Sringernyuang, L., Thamlikitkul, V., Vass, L., Avison, M. B., & Turner, K. M. E. (2021). One Health drivers of antibacterial resistance: Quantifying the relative impacts of human, animal and environment use and transmission. *One Health, 12*, 100220, DOI. https://doi.org/10.1016/j.onehlt.2021.100220

Calderón-Parra, J., Muiño-Miguez, A., Bendala-Estrada, A. D., Ramos-Martínez, A., Muñez-Rubio, E., Carracedo, E. F., Montes, J. T., Rubio-Rivas, M., Arnalich-Fernandez, F., Pérez, J. L. B., Bruñén, J. M. G., Beamonte, E. C., Fontan, P. M. P., Carmona, M. M., Martínez, R. F. M., García, A. G., Mosteiro, C. S., Almeida, C. T., Moraleja, J. G., Deodati, F., Escalante, M. D. M., Tomás, M. L. A., Huelgas, R. G., Rojo, J. M. C., & Núñez-Cortés, J. M. (2021). Inappropriate antibiotic use in the COVID 19 era: Factors associated with inappropriate prescribing and secondary complications. Analysis of the registry SEMI-COVID. *PLOS One*. https://doi.org/10.1371/journal.pone.0251340

Collignon, P. J., & McEwen, S. A. (2019). One Health – Its importance in helping to better control of antimicrobial resistance. *Tropical Medicine and Infectious Disease, 4*(22), 2–21. https://doi.org/10.3390/tropicalmed4010022

Comber, S. D. W., Upton, M., Lewin, S., Powell, N., & Hutchinson, T. H. (2020). COVID 19, antibiotics and one health: A UK environmental risk assessment. *Journal of Antimicrobial Chemotherapy*. https://doi.org/10.1093/jac/dkaa338

Dutil, L., Irwin, R., Finley, R., Ng, L. K., Avery, B., Boerlin, P., Bourgault, A. M., Cole, L., Daignault, D., Desruisseau, A., Demczuk, W., Hoang, L., Horsman, G. B., Ismail, J., Jamieson, F., Maki, A., Pacagnella, A., & Pillai, D. R. (2010). Ceftiofur resistance in *salmonella enterica* serovar Heidelberg from chicken meat and humans, Canada. *Emerging Infectious Diseases, 16*(1), 48–54.

ECDC/EFSA/EMA. (2015). ECDC/EFSA/EMA (European Centre for Disease Prevention and Control /European Food Safety Authority Europeans Medicines Agency) first joint report on the integrated analysis of the consumption of antimicrobial agents and occurrence of

antimicrobial resistance in bacteria from humans and food-producing animals. *European Food Safety Authority Journal, 13*(1), 4006–4114.

FAO. (2020). *National framework for One Health, Food and Agriculture Organization of United Nations*. India Office.

Gebreyes, W. A., Dupouy-Camet, J., Newport, M. J., Oliveira, C. J. B., Schlesinger, L. S., Saif, Y. M., Kariuki, S., Saif, L. J., Saville, W., Wittum, T., Hoet, A., Quessy, S., Kazwala, R., Tekola, B., Shryock, T., Bisesi, M., Patchanee, P., Boonmar, S., & King, L. J. (2014). The global one health paradigm: Challenges and opportunities for tackling infectious diseases at the human, animal, and environment Interface in low-resource settings. *PLoS Neglected Tropical Diseases, 8*(11), e3257. https://doi.org/10.1371/journal

George, J., Häsler, B., Mremi, I., Sindato, C., Mboera, L., Rweyemamu, M., & Mlangwa, J. (2020). A systematic review on integration mechanisms in human and animal health surveillance systems with a view to addressing global health security threats. *One Health Outlook, 2*, 11. https://doi.org/10.1186/s42522-020-00017-4

Grundmann, H., Klugman, K. P., Walsh, T., Ramon-Pardo, P., Sigauque, B., & Khan, W. (2011). A framework for global surveillance of antibiotic resistance. *Drug Resistance Updates, 14*, 79–87.

Hedmann, H., Eisenberg, J., Trueba, G., Berrocal, V., & Zhang, L. (2019). High prevalence of extended spectrum beta-lactamase CTX-M producing Escherichia coli in small-scale poultry farming in rural Ecuador. *American Journal of Tropical Medicine and Hygiene, 100*, 374–376.

Hernando-Amado, S., Coque, T. M., Baquero, F., & Martínez, J. L. (2019). Defining and combating antibiotic resistance from One Health and global health perspectives. *Nature Microbiology, 4*(9), 1432–1442.

Heuer, O. E., Kruse, H., Grave, K., Collignon, P., Karunasagar, I., & Angulo, F. J. (2009). Human health consequences of use of antimicrobial agents in aquaculture. *Clinical Infectious Diseases, 49*(8), 1248–1253.

Holmes, A. H., Moore, L. S. P., Sundsfjord, A., Steinbakk, M., Regmi, S., Karkey, A., Guerin, P. J., & Piddock, L. J. V. (2016). Understanding the mechanisms and drivers of antimicrobial resistance. *Lancet, 387*(10014), 176–187.

Hsu, J. (2020). How COVID 19 is accelerating the threat of antimicrobial resistance. *Biomed Journal*. https://doi.org/10.1136/bmj.m1983

Huijbers, P. M., Blaak, H., de Jong, M. C., Graat, E. A., Vandenbroucke-Grauls, C. M., & de Roda Husman, A. M. (2015). Role of the environment in the transmission of antimicrobial resistance to humans: A review. *Environmental Science and Technology, 49*(20), 11993–12004.

Iskandar, K., Molinier, L., Hallit, S., Sartelli, M., Catena, F., Coccolini, F., Hardcastle, T. C., Roques, C., & Salameh, P. (2020). Drivers of antibiotic resistance transmission in low- and middle-income countries from a "one health" perspective-a review. *Antibiotics (Basel Switzerland), 9*(7), 372. https://doi.org/10.3390/antibiotics9070372

Landers, T. F., Cohen, B., Wittum, T. E., & Larson, E. L. (2012). A review of antibiotic use in food animals: Perspective, policy and potential. *Public Health Reports, 127*(1), 4–22.

Manyi-Loh, C., Mamphweli, S., Meyer, E., & Okoh, A. (2018). Antibiotic use in agriculture and its consequential resistance in environmental sources: Potential public health implications. *Molecules, 23*(4), 795–843.

MoH&FW. (2019). D.O. Letter Z. 18025/60/2018-Micro/NCDC Dt; 15th May 2019 of the Joint Secretary, Ministry of Health and Family Welfare, Government of India, New Delhi.

Moore, C. E. (2019). Changes in antibiotic resistance in animals. *Science, 365*(6459), 1251–1252.

Mundaca-Shah, C., Ayano Ogawa, V., & Nicholson, A. (2017). *Combating antimicrobial resistance: A One Health approach to a global threat. Proceedings of a workshop of the National Academy of Sciences*. Engineering and Medicine. The National Academies Press.

O'Neill, J. (Ed.). (2016). *Review on antimicrobial resistance. Tackling a global health crisis: Initial steps. Tackling drug resistant infections globally: Final report and recommendations*. Welcome Trust - HM Government.

Otte, J., Roland-Holst, D., Pfeiffer, D., Soares-Magalhaes, R., Rushton, J., Graham, J., & Sibergeld, E. (2007). *Industrial livestock production and global health risks. Pro-poor livestock policy initiative research report*. FAO.

Prescott, J. F. (2014). The resistance tsunami, antimicrobial stewardship, and the golden age of microbiology. *Veterinary Microbiology, 171*, 273–278.

Queenan, K., Hasler, B., & Rushton, J. (2016). A One health approach to antimicrobial resistance surveillance: Is there a business case for it? *International Journal of Antimicrobial Agents, 48*(4), 422–427.

Robinson, T. P., Bu, D. P., Carrique-Mas, J., Fevre, E. M., Gilbert, M., Grace, D., Hay, S. I., Jiwakanon, J., Kakkar, M., Kariuki, S., Lakshminarayan, R., Lubroth, J., Magnusson, U., Thi Ngoc, P., Van Boeckel, T. P., & Woolhouse, M. E. J. (2016a). Antibiotic resistance is the quintessential one health issue. *Transactions of the Royal Society of Tropical Medicine and Hygiene, 110*, 377–380.

Robinson, T. P., Wertheim, H. F. L., Kakkar, M., Kariuki, S., Bu, D., & Price, L. B. (2016b). Animal production and antimicrobial resistance in the clinic. *The Lancet, 387*(10014), e1–e3.

Saker, L., Lee, K., Cannito, B., Gilmore, A., & Campbell-Lendrum, D. H. (2004). *Globalization and infectious diseases: A review of the linkages*. World Health Organization. https://apps.who.int/iris/handle/10665/68726

Schaumburg, F., Mugisha, L., Peck, B., Becker, K., Gillespie, T. R., Peters, G., & Leendertz, F. H. (2012). Drug-resistant human *Staphylococcus aureus* in sanctuary apes pose a threat to endangered wild ape populations. *American Journal of Primatology, 74*, 1071–1075.

Smith, K. M., Machalaba, C. C., Seifman, R., Feferholtz, Y., & Karesh, W. B. (2019). *Infectious disease and economics: The case for considering multi-sectorial impacts, 7*, 1–6. https://doi.org/10.1016/j.onehlt.2018.100080.

UN. (2016). *United Nations: Draft political declaration of the high-level meeting of the General Assembly on Antimicrobial resistance*. http://www.un.org/pga/71/wp-content/uploads/sites/40/2016/ 09/DGACM_GAEAD_ESCAB-AMR-Draft-PoliticalDeclaration-1616108E.pdf

USDA. (2014). *Antimicrobial resistance action plan*. Washington, DC.

Van Boeckel, T. P., Brower, C., Gilbert, M., Grenfell, B. T., Levi, S. A., Robinson, T. P., Teillant, A., & Laxminarayan, R. (2015). Global trends in antimicrobial use in food animals. *Proceedings of National Academy of Sciences, 112*(18), 5649–5654.

Weiss, D., Wallace, R. M., Rwego, I. B., Gillespie, T. R., Chapman, C. A., Singer, R. S., & Goldberg, T. L. (2018). Antibiotic-resistant Escherichia coli and class 1 integrons in humans, domestic animals, and wild primates in rural Uganda. *Applied and Environmental Microbiology, 84*, e01618–e01632.

White, A., & Hughes, J. M. (2019). Critical importance of one health approach to antimicrobial resistance. *Eco Health, 16*, 404–409.

WHO. (2017). *Integrated surveillance of antimicrobial resistance in food-borne bacteria: Application of a One health approach*. Guidance from the WHO Advisory Group on Integrated Surveillance of Antimicrobial Resistance (AGISAR). World Health Organization.

WHO. (2021). *Tripartite and UNEP support OHHLEP's definition of "One Health"*. https://www.who.int/news/item/01-12-2021-tripartite-and-unep-support-ohhlep-s-definition-of-one-health

WHO, FAO, & OIE. (2019). *Taking a multisectoral, One Health Approach: A Tripartite Guide to Addressing Zoonotic Diseases in Countries*. https://www.who.int/initiatives/tripartite-zoonosis-guide

WHO, FAO, OIE, & UNEP (2022). *Strategic framework for collaboration on antimicrobial resistance together for One Health*. World Health Organization, Food and Agriculture Organization of the United Nations and World Organization for Animal Health. www.who.int/publications/i/item/9789240045408

Woolhouse, M. E. J., & Ward, M. J. (2013). Sources of antimicrobial resistance. *Science, 341*, 1460–1461.

World Bank Group. (2018). *Operational framework for strengthening human, animal, and environmental public health systems at their interface*. International Bank for Reconstruction and Development/The World Bank. http://documents.worldbank.org/curated/en/703711517234402168/pdf/12

Therapeutic Rationalization of Antibacterial Drug Doses in Aquaculture by Using Pharmacokinetic (PK)–Pharmacodynamic (PD) Indices to Contain the Antimicrobial Resistance

G. S. Rao

Contents

Abstract

The aquaculture sector is identified as a potential source to provide livelihood to mankind in terms of high animal protein food and working employable opportunities for the growing global population of the world. The Food and Agricultural Organization (FAO) has indicated that fish and shrimp production is continuing to increase at global level with rapid growth. Since there is a rapid growth and demand for aquaculture, it is facing challenges in terms of disease outbreaks due to the increase in intensive farming. Freshwater as well as marine fish farming, viz., bass, carp, catfish, eel, perch, salmon tilapia, trout, and also cultured shrimp, are vulnerable to bacterial infections. These fish varieties and shrimp are distressed by microbial diseases, resulting in extreme financial losses. Outbreaks of various infections in fish/shrimp farms necessitate the application of antibacterial drugs, namely, oxytetracyclines, sulfonamides, fluoroquinolones, and other important drugs that are common both in human and veterinary practice. As there is rampant usage of antibacterial drugs to deter or deal with diseases besides

G. S. Rao (✉)
Department of Veterinary Pharmacology & Toxicology, NTR College of Veterinary Science, Sri Venkateswara Veterinary University, Gannavaram, Andhra Pradesh, India
e-mail: raogs64@gmail.com

M. P. Mothadaka et al. (eds.), *Handbook on Antimicrobial Resistance*,
https://doi.org/10.1007/978-981-19-9279-7_34

their application as supporters of growth in farm animals comprising aquaculture too, the advent of strong unresponsiveness microbes to antimicrobial drugs is becoming a serious and difficult-to-deal-with problem across the world. Hence, the situation has warranted an urgent pragmatic approach to improve or refresh in treating infections of aquaculture by using old and new antibiotics rationally with optimization of dosages.

Optimizing dosing schedules for different antimicrobial agents used in fish/aquatic animals for various bacterial diseases must consider the anatomical and physiological differences in the mammals and the husbandry practices as well. Using medicated feed is the most general and practicable method for treating farmed fish. Once an antimicrobial agent is administered to an aquatic animal, the drug is subjected to absorption, distribution, metabolism, and elimination (ADME) processes by which its concentration reaches maximum in blood and tissue of the animal to produce the therapeutic response. The change in concentrations of the antimicrobial agent in the blood compartment of the aquatic animal after drug administration is understood by pharmacokinetic studies. Pharmacokinetic (PK) parameters are obtained from pharmacokinetic studies, such as "*total body clearance* (Cl_B)," "*volume of distribution* (V_d)," "*area under plasma concentration–time curve*" ($AUC_{0–24h}$), "*elimination half-life*" ($t_{1/2\beta}$), and "*peak plasma concentration* (C_{max})" of antimicrobial agent post-ingestion in aquatic animal species. Pharmacokinetic characterization of sulfonamides, oxytetracyline, quinolones, amoxicillin, and florfenicol in aquatic species was determined and well documented. The determining factor of antibacterial drugs that produce their effect on the bacteria responsible for the disease either by inhibiting or killing microorganism is the "*minimum inhibitory concentration (MIC)*," which is known to be "pharmacodynamic (PD)" limit. Integration of pharmacokinetic (PK) parameters like C_{max} and $AUC_{0–24h}$ that are obtained for an antimicrobial drug in the host and a pharmacodynamic (PD) parameter, namely, MIC determined for a bacterial pathogen is known as "*PK/PD index.*" The effect of a particular antimicrobial drug challenged against a specific microbe is assessed by the abovementioned index. The treatment achievement of different antimicrobial drugs varies with associated PK/PD indices. For critical breakpoints of PK-PD surrogate markers for different groups of antimicrobial agents, the minimum magnitude should be for "$C_{max}/MIC > 8–10$, $AUC_{0–24h}/MIC > 125$, *and* $T > MIC > 50\%$" to get better therapeutic outcomes. These surrogate markers are guidelines for optimization of dosage schedules for established doses of antimicrobial agents in aquatic species. These established guidelines will be helpful to minimize the development of resistance in the microorganisms.

Keywords

Aquaculture · Antimicrobial agent · Pharmacokinetics · Pharmacodynamics · MIC · PK-PD indices · C_{max} · $AUC_{0–24h}$ · $T > MIC$

1 Introduction

Aquaculture is a growing sector with rapid progress providing monetary benefits and high-quality protein diet. This sector has also been identified as a potential source to provide livelihood to millions of people with working and employable opportunities. By the year 2050, the world population is predicted to reach nine billion. In this context, fish/shrimp farming is going to play a pivotal role in supplying animal protein to the growing population (Godfray et al., 2010). The "Food and Agricultural Organization (FAO)" of the United Nations indicated that fish and shrimp production is continuing to increase at the global level. It is likely to be led by China, followed by South Asian, Latin American, the Caribbean, and European countries with freshwater species and mollusks dominating aquaculture production in the coming years.

India has taken the second position after China in global fish production in which aquaculture constitutes over a third of the country's total fish production (Jayasankar, 2018). With the rapid growth and demand for aquaculture, this sector is facing challenges in the form of disease outbreaks by virtue of enhanced intensifications in farming practices. Freshwater farmed as well as marine fish, viz., bass, carp, catfish, eel, salmon tilapia, trout, and also cultured shrimp, are often found devastated by bacterial diseases. It is estimated that the annual economic loss of more than US$120 million is linked to major common fish bacterial pathogens such as *Aeromonas hydrophila*, *Yersinia ruckeri,* and *Vibrio fluvialis* between 1990 and 1992 in China (Subasinghe, 2005; Wei, 2002). The outbreak of bacterial, parasitic, or viral infections in fish/shrimp farms is one of the main risks identified, and, accordingly, major emphasis is now given to the prevention and treatment of these diseases in aquatic species by using certain identified antimicrobial agents or developing plausible vaccines.

The outbreaks of various infections in fish/shrimp farms require the application of antibacterial drugs such as oxytetracyclines, sulfonamides, fluoroquinolones, and other important drugs that are common both in human and veterinary practice. In the present-day scenario, it is accepted that rampant usage of antibacterial drugs for the prevention or treatment of diseases apart from their use as growth promoters in farmed animals, namely, aquatic organisms, cattle, goats, pigs, poultry, and sheep, is in practice. It has also been observed that nontherapeutic use of these antimicrobial drugs is more common in poultry and aquaculture (Kirchhelle, 2018).

2 Aquaculture–Antimicrobial Agents

Antimicrobial agents are in routine use in the inhibition and handling of bacterial diseases in cultured fish and shrimp across the globe (Cabello, 2006; Cabello et al., 2013). Animal farming for food purpose with intensity at industry pace has enhanced food productivity at a very low cost for which heavy price is being paid now in the form of increased antimicrobial resistance in the world.

The aquaculture sector is identified as a major antimicrobial consumer in many countries in Southeast Asia (Le et al., 2005). Both Chile and Vietnam have witnessed a heavy consumption of antimicrobial agents in salmon fish and shrimp farming, respectively (Cabello, 2006). As this sector is moving to intensive production system, it may become another main source of contamination of the aquatic field with antimicrobial agents in the near future (Aly & Albutti, 2014; Van Boeckel et al., 2015; World Bank, 2013). Reports indicate that 90% of the total world aquaculture production areas are located in economically emerging nations, which lack regulatory infrastructure and enforcing legal frame on the use of antibacterial drugs that lead to variability in their use (Schar et al., 2020). The number of antibiotics approved in various countries is different, and their order can be arranged in ascending order like 1 in Norway, 3 in the United States, 6 in China, 11 in Thailand, 12 in Japan, 14 in Taiwan, and 29 in the European Union as per available published data. However, the following antibiotics are approved in most countries: florfenicol, oxytetracycline, sulfonamides, oxolinic acid, and amoxicillin.

Pharmaceuticals, including antimicrobial drugs, are generally dosed in aquaculture farms by mixing the medicines in feed, which may lead to leaching of the drugs into both water and the environment, leading to public health hazards (Miller & Harbottle, 2017). In this era of increased administration of antibacterial agents in animal agriculture and aquaculture, the emergence of drug resistance in the microorganisms is possible, which is becoming a new challenge to humans and resource constraints in developing fresh and novel antimicrobial agents. Hence, situation has warranted an urgent approach to improve or refresh modalities in treating infections of aquaculture by using old and new antibiotics rationally with optimization schedule.

The Office International des Epizooties (OIE) has set the "*Aquatic Animal Health Code (AAHC)*" for appropriate application of pharmaceuticals in aquatic animals. The OIE has enlisted important antimicrobial agents used in the veterinary field. Till now, there is no regulated system of supervision in the world and the source of antimicrobial agents for aquaculture is mainly from both human and veterinary fields. Different classes of antibacterial agents, which are generally used in aquaculture, are shown in Table 1 as per the OIE list (OIE, 2018). The uses of different antimicrobial agents in various aquatic species for the bacterial disease are listed in Table 2 (Ibrahim et al., 2020).

3 Antimicrobial Agents: Pharmacokinetics

Establishing dosing schedules for different antimicrobial agents in fish/aquatic species for different bacterial diseases must consider the anatomical and physiological differences in the animals and the husbandry practices as well. Different routes of drug usage are to be followed. Using medicated feed is the most general and practicable method for treating farmed fish. This requires spontaneous ingestion of

Table 1 Antimicrobial agents of veterinary importance that are used in aquaculture

Class	Subclass/group	Substance	Therapeutic use
Aminocoumarin	-	Novobiocin	Septicemic conditions in fish
Aminoglycosides	Aminocyclitols	Spectinomycin, streptomycin	The wide range of applications and the nature of the diseases treated make aminoglycosides extremely important for veterinary medicine
	Aminoglycosides + Dehydroxystreptamine	Kanamycin	
Aminopenicillins		Ampicillin Amoxicillin	Furunculosis, pseudotuberculosis, and vibriosis
Amphenicols		Florfenicol Thiamphenicol	These compounds are particularly important in treating some bacterial diseases in fish especially salmonellosis and other Gram-negative bacterial diseases
Bicyclomycin		Bicozamycin	Septicemias in fish
Carboxypenicillins		Tobicilin	Enterococcicosis
Licosamides		Lincomycin	Lincosamides are essential in the treatment of Mycoplasmal pneumonia and hemorrhagic enteritis
Macrolides		Josamycin Kitasamycin Spiramycin Mirosamycin Erythromycin	Gram+ve organisms
Phosphonic acid		Fosfomycin	Essential in fish diseases
Quinolones	First generation	Flumequine Miloxin Oxolinic acid	Furunculosis, atypical furunculosis, classical vibriosis, yersiniosis
	Second generation	Enrofloxacin Sarafloxacin	
Sulfonamides		Sulfadimethoxine Sulfafurazole Sulfamerazine Sulfamethoxine	Broad-spectrum antimicrobial agents
	Potentiated sulfonamides	Trimethoprim+ Sulfonamide Ormetoprim+ Sulfonamide	
Tetracyclines		Doxycycline Oxytetracycline Tetracycline	Broad-spectrum antimicrobial agents

Table 2 Antimicrobial agents used for bacterial disease in aquatic species

Antimicrobial agent	Aquatic animal	Disease	Causative organism
Tetracyclines, trimethoprim sulfonamides, and fluoroquinolones like broad-spectrum antibacterial	Fish	Tenacibaculosis (bacteria)	*Tenacibaculum maritimum*
Broad-spectrum antibacterial drugs	Pisces	Vibriosis (bacteria)	*Vibrio anguillarum, Vibrio ordalii*
Broad-spectrum antimicrobial agents	Pisces	Epitheliocystis	*Chlamydia* sp.
Florfenicol, oxytetracycline, chloramine	Salmonid	Gills infection	*Flavobacterium branchiophila*
Broad-spectrum antibacterial drugs	Salmonid	Piscirickettsiosis	*Piscirickettsia salmonis*
Sulfadiazine, trimethoprim, oxolinic acid, flumequine	Salmonid	Furunculosis	*Aeromonas salmonicida*
Antimicrobial agents with broad-spectrum activity	Rainbow-trout	Enteritis in *Oncorhynchus mykiss*	*Candidatus arthromitus*
Sulfadiazine, trimethoprim, old quinolones	Rainbow trout, salmonids, catfish	Enteric red mouth infection (ERM)	*Yersinia ruckeri*
Antimicrobial agents with broad-spectrum activity	Rainbow trout	Red mark syndrome	*Flavobacterium psychrophilum*
Sulfamerazine	Turbout	Furunculosis	*Aeromonas salmonicida*
Amoxicillin, gentamicin, ofloxacin	Catfish	Hemorrhagic septicemia	*Aeromonas veronii*
Oxytetracycline (OTC)	Shrimp	Bacterial shell infection	*V. anguillarum, Aeromonas* spp.
OTC	Shrimp	Vibrio disease	*V. parahaemolyticus*
OTC	Prawns	Bacterial shell infection	*Vibrio anguillarum, Pseudomonas* spp., *Aeromonas* spp., *Vibrio* spp.
OTC	Prawns	Vibrio disease	*Vibrio parahaemolyticus*
OTC	Crustaceans	Necrotizing hepato-pancreatitis (NHP)	Intracellular proteobacteria
OTC, quinolones	Crustaceans	Luminous *Vibrio* spp. (*V. harveyi*)	Luminous *Vibrio* spp. *(V. harveyi)*

medicated feed in the disease condition. Since feed consumption in fish will be very less in diseased conditions, antibiotic therapy through medicated feed should be prophylactic, whereas bath treatment and pulse dosing methods should be used in diseased conditions. Gastric emptying in fish is not rapid compared to mammals. Absorption of drugs like weak bases takes place in the stomach. Hepatic first-pass metabolism for drugs after absorption from oral route is a possibility in the aquatic species due to the existence of liver portal system like in mammals, and hence, the bioavailability of drugs depends on the extent of metabolism of the drug in liver. Similar to mammals, biliary excretion and enterohepatic recycling of drugs also take place in fish. Drugs that are directly administered to fish in water are absorbed via the gills; hence, water hydrogen ion concentration (pH) and its composition affect drug absorption since only unionized portions are absorbed. Initially a significant amount of drug is absorbed by the gills and transported to the kidneys, which may undergo first-pass metabolism in renal tubules. In this context, it has to be observed that enzyme induction to foreign compounds occurs first in the kidney in fish before hepatic induction sets in. Renal portal system does exist in fish kidney unlike in the mammals, where portal vein circulation reaches the tubules and exposes them to a much higher amount of cardiac output than in mammals. Further, drugs can reach directly the tubules by the caudal vein in fish. Osmoregulation is an important process for aquatic animals, and it is a function of the kidney in freshwater fish. However, marine species are hypo-osmotic to salt water. As a part of maintaining osmoregulation, the freshwater fish do not take water in brackish conditions; however, the same is not the case with marine fish. Biliary concentrations of xenobiotics in fish are common. Both endogenous and exogenous compounds are enterohepatically recirculated in fish. The features of high xenobiotic concentrations in bile, enterohepatic circulation, and delayed elaboration will influence the apparent residence time of drug residues in fish. On the other hand, shrimps that represent the class of shellfish/crustaceans concentrate drug or its metabolites in hepatopancreas. Further, temperature has a great influence on every aspect of fish physiology, including drug disposition kinetics like absorption, metabolism, and elimination. Retaining higher amounts of antibiotics for longer periods in cold-acclimatized animals compared to warm-acclimated animals has been reported. As the plasma protein content is low, the plasma protein binding of drugs tends to be low in fish compared with mammals.

Once an antimicrobial agent is given to aquatic species, the drug is subject to absorption, distribution, metabolism, and elimination (ADME) processes by which its concentration reaches maximum in blood and tissue and, therefore, the therapeutic response will be attained. The variations in the levels of antimicrobial agent upon administration in the liquid compartments of aquatic animal during the course of time are called pharmacokinetics. In other words, it is "what the body does to the drug." Once, an antimicrobial agent is administered, it undergoes ADME processes shortly known as pharmacokinetics of antimicrobial agent that is characterized after analyzing the concentration–time profile of the agent to obtain the pharmacokinetic (PK) parameters, such as "*total body clearance (Cl_B), volume of distribution (V_d),*

area under the plasma concentration–time curve ($AUC_{0–24\ h}$), elimination half-life ($t_{1/2\beta}$), peak plasma concentration" of antimicrobial agent post its application in aquatic animal (C_{max}) and bioavailability (F) of the agent especially after extravascular administration in aquatic species. Pharmacokinetic characterization of sulfonamides, oxytetracyline, quinolones, amoxicillin, and florfenicol in aquatic species was determined after their administration (Grondel et al., 1986; Kverme et al., 2019; Park et al., 2015; Rigos et al., 2003; Rigos et al., 2020; Rigos & Smith, 2015; Samuelsen, 2006).

4 Antimicrobial Agents: Pharmacodynamics

After the agent reaches therapeutic concentration at the site of action in the body to give its effect, it emits the beneficial consequence by its mode of action that is assessed by the pharmacodynamics (PD), better described as "*what the drug does to the body.*" Antibacterial drugs have consequence on the bacteria responsible for the disease either by inhibiting or killing the same. The minimum inhibitory concentration (MIC) is the determining factor for assessing the impact of the antimicrobial agent. It delivers evidence on the susceptibility of the pathogen against the antimicrobial drug. MIC is defined as the minimum concentration of the antimicrobial drug necessitated to impede the growth of bacteria. Different methods are employed to determine MIC, including disk diffusion, E-test, microdilution, and macrodilution in in vitro by assessing the growth of organism underscreening with various dilutions of the antimicrobial agent. MIC data were published in the recent past for different bacterial pathogens of aquatic animals against different antimicrobial agents (Kum et al., 2008; Smith et al., 2014; Wayne, 2020)**.**

It is very pertinent to augment the doses of drugs considering the increase in antimicrobial resistance to available drugs and the present-day paucity of new antimicrobial agents to treat infections in humans, animals, and aquatic species. Much progress has been made recently in determining the relationship between the exposure to the concentrations of antimicrobials vs. the growth responses of the microorganisms, with regard to pharmacokinetic (PK) and pharmacodynamic (PD) principles in both human and veterinary medicine. Optimizing therapy is aimed at maximizing the therapeutic outcome and minimizing the development of antimicrobial resistance either in the infective microorganism or normal flora of the host during therapy.

Optimization of doses of antimicrobial agents involves selecting the effective drug or combination of drugs, the correct dose and route of administration, and the right duration, followed by decreasing population of pathogen-directed therapy once culture results are known. Subtherapeutic dosing of antimicrobial agents is associated with poor therapeutic outcomes and increased incidences of drug resistance. Optimal dosing of antimicrobial agents based on PK/PD principles has the potential to improve therapeutic efficiency and preventing the resistance development in microorganisms.

5 Pharmacokinetic (PK)–Pharmacodynamic Integration

Integration between pharmacokinetic (PK) parameters such as C_{max} and $AUC_{0–24h}$ that are obtained for antimicrobial drug in host and a pharmacodynamic (PD) parameter, namely, MIC, determined for a bacterial pathogen is known as index of PK/PD. This measures the effect of an antimicrobial drug against certain bacterial infections. In the therapeutic assessment of antimicrobial treatment employed for PK/PD analysis, there are three important guiding principles of PK/PD that are directly related to the consequence of antimicrobial agents. The three components are

1. The time during which the absorption of the drug was completed or directly above MIC (T > MIC) in the blood post administration of drug
2. The highest concentration to MIC ratio (C_{max}/ MIC)
3. The proportion of the 24-h area below the curve of concentration–time to MIC (AUC/MIC) (Papich, 2014)

The same is depicted in Fig. 1, where T (time after drug administration in hours) on X axis vs. concentration of antimicrobial drug on Y axis in micro- or nanograms per ml is depicted to elucidate the PK–PD relationship.

The reliable indicators of antimicrobial agent efficacy in therapeutics based on the activity arrangement of respective agents are PK/PD indices. Three important types

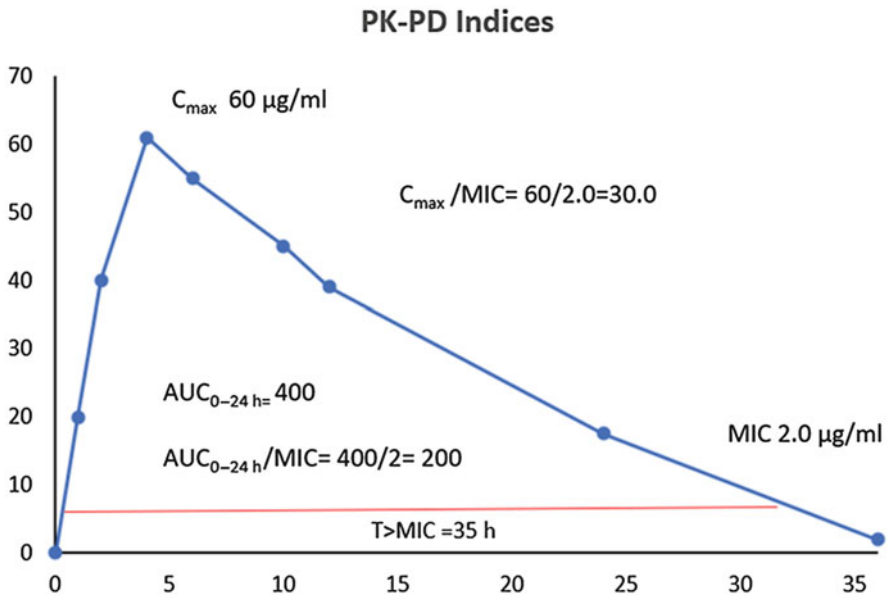


Fig. 1 Schematic representation of time (h, x-axis) vs antimicrobial drug concentration (ug/ml, y-axis) in blood of aquqtic animal post drug administration

Table 3 Classification of antimicrobials based on PK–PD indices

Antimicrobial group	Type of effect	PK–PD index
Aminoglycosides, fluoroquinolones, polymyxins, daptomycin, or metronidazole	Prolonged persistent bacterial killing effect with concentration dependency	Ratios of either C_{max}/MIC or $AUC_{0–24h}$/MIC (note: persistent effects for prolonged time with ability to prevent regrowth of bacteria even drug concentration falls below the MIC, post-antibiotic effect [PAE])
Beta-lactam antibiotics (penicillins, cephalosporins, carbapenems, and monobactams)	Short duration effect on bacterial killing with time dependency	T > MIC, time period of exposure of microorganism to above MIC of agents in 24 h period, e.g., 50–70% of time in 24 h above MIC
Tetracyclines, tigecycline, macrolides (azithromycin, clindamycin), linezolid and other oxazolidinones, chloramphenicol, trimethoprim-sulfonamides, and vancomycin	Bacterial-killing effect with concentration independency with prolonged time persistence	C_{max}/MIC or $AUC_{0–24h}$/MIC

of antibacterial effects were identified. The first type shows the elimination of antibacterial activity that relies on concentration with effects that are firm and extended. The second removes antimicrobial activity, which is based on time sans or short period effects that are persistent. The third exhibits removal of antimicrobial activity that is not based on concentration but tenaciously protracted effects (Table 3.)

6 PK–PD Indices

While considering PK/PD indicators, one must choose proper PK/PD index to optimize antimicrobial efficacy that represents a surrogate marker for clinicians in practice. It is noteworthy observing that the PK/PD guides connected to the accomplishment of treatment for various antibacterial drugs are indeed different and presented in Table 4 (Asin-Prieto et al., 2015). As indicated in the table, for the critical break points of PK–PD surrogate markers for different groups of antimicrobial agents, the minimum magnitude should be for "$C_{max}/MIC > 8–10$, $AUC_{0–24h}/MIC > 125$ *and* $T>MIC > 50\%$" to get better therapeutic outcomes. These surrogate markers will be the guidelines for optimization of dosage schedules with the doses established for the antimicrobial agents in aquatic species. Following these guidelines will be helpful to minimize the development of resistance in microorganisms.

Table 4 PK–PD target surrogate markers for therapeutic outcome

Class of antimicrobials	Antimicrobial agent	PK–PD surrogate marker	Therapeutic outcome determining indicative magnitude
Beta-lactams	Penicillins	T > MIC (percentage)	50–60%
	Cephalosporins		60–70%
	Carbapenems		40–50%
Aminoglycosides		C_{max}/MIC (ratio)	10
Quinolones		$AUC_{0–24h}$/MIC (ratio)	125
Tetracyclines		$AUC_{0–24h}$/MIC	25
Glycopeptides	Vancomycin	$AUC_{0–24h}$/MIC	400
Macrolides	Clarithromycin	$AUC_{0–24h}$/MIC	25
	Azithromycin	$AUC_{0–24h}$/MIC	25
	Daptomycin	$AUC_{0–24h}$/MIC	666
	Tigecycline	$AUC_{0–24h}$/MIC	17.9
	Linezolid	$AUC_{0–24h}$/MIC	100
	Colistin	$AUC_{0–24h}$/MIC	27.6–45.9

7 PK–PD Indices: Optimization of Dosages

The PK–PD markers describe the relationships between the microorganism's exposure to the unbound protein concentration (Fu of antibacterial drug). According to Lees et al. (2008), a noted veterinary pharmacologist, "*It is the exposure, and especially exposure to sub-optimal drug concentrations which is the most important single factor in resistance emergence and its subsequent spread*." Hence, strategies were aimed to give antibacterial drugs to animals employing quantities that achieve appropriate pharmacokinetic–pharmacodynamic (PK–PD) substitute indicators. The use of antibacterial drug concentration vs. bacterial killing relationships and PK–PD indices for evaluating antibacterial agents has become common in medicine of humans and the veterinary sectors. A number of publications on pharmacokinetics of enrofloxacin, an antimicrobial fluoroquinolone, and also other antibacterial agents that give the idea in the recent years in journals of veterinary/aquatic animal health offered the pharmacokinetic limitations and pharmacodynamics of the enrofloxacin and how both pharmacokinetics and pharmacodynamics relate to balanced prescription schedules of enrofloxacin in aquatic animals. The obtained PK–PD indices for enrofloxacin and other antimicrobial agents are presented in Table 5. Further, a Microsoft Access data "Phish-Pharm" of different drugs and chemicals used in aquaculture is updated that include pharmacokinetic data of antibiotics published to assist investigators or researchers in developing new animal drugs for aquatic animal species (Crosby et al., 2022).

Applying PK–PD indices has become a necessary strategy for regulatory bodies while planning antimicrobial usage. The PK–PD markers have now been applied to

Table 5 PK–PD indices of some of the antimicrobial agents in aquatic species

Antimicrobial agent	Aquatic species	Bacterial pathogen	Pharmacokinetic parameters (PK)		Pharmacodynamic parameter (PD)	PK–PD ratio	Reference
			C_{max} (μg/ml) / AUC_{0-24h} (μg/ml.h		MIC	Cmax/ MIC, or $AUC_{0-24h/MIC}$ (μg/ml)	
Enrofloxacin	Tilapia	*Aeromonas salmonicida*	1.92	57.91	0.16	12 or 362	Estrada-San Agustín et al., 2019
		Vibrio sp.	1.92	57.91	0.08	24 or 724	
Enrofloxacin	Grass carp	*Aeromonas hydrophila*	5.68	51.68	0.25	22.72 or 206.72	Xu et al., 2013
Enrofloxacin			11.37	135.17		45.78 or 540.68	
Enrofloxacin			14.3	180.97		57.2 or 723.88	
Sarafloxacin	Pacific whiteshrimp	*Vibrio parahaemolyticus*	12.24	101.15	1.0	12.24 or 101.15	Ma et al., 2022
Enrofloxacin	Crucian carp	*Aeromonas hydrophila, Aeromonas salmonicida, Vibrio salmonicida Yersinia ruckeri*	18.4	362	1.84	10 or 196.74	Shan et al., 2022
Doxycycline	Yellow cat fish	*Edwardsiella ictaluri*	4.67	192.102	0.5	9.34 or 384.20	Xu et al., 2021
Florfenicol	Salmon	*Piscirickettsia salmonis*	-	257.0	2.0	99.9	San Martin et al., 2019

regulate breakpoints by the "*Clinical and Laboratory Standards Institute (CLSI; formerly NCCLS)*" *Subcommittee on Antimicrobial Susceptibility Testing of Bacteria* sourced from aquatic animals (CLSI, 2020). In the recent past, CLSI has massively spread the list of agents for which there are breakpoints specific for veterinary. Species-specific MIC breakpoints in companion animals have now been established and a committee is now establishing breakpoints for aquatic species too.

The employment of antimicrobial drugs is avoidable when in cognizance the conditions are not conducive to optimal outcomes. The application of appropriate dosages and rational regimens that suit markers of PK–PD for every antimicrobial as discussed above in aquatic species is ensured to minimize the emergence of resistance in microorganisms.

8 Conclusions

PK/PD indices are being extensively and successfully used for the past few years both in medical settings of human and animals for good therapeutic outcomes. This kind of methodology has been accepted since the last decade and has proven its usefulness for appropriate dosing of antimicrobial agents in clinical practice though this concept is dynamically evolving in the use of combination of antibiotics. Establishing PK/PD indices in aquaculture for various antibacterial drugs against various bacterial infections is the need of the hour to optimize doses of antibacterial drugs used in aquatic species, thereby minimizing the emergence of antimicrobial resistance.

References

Aly, S. M., & Albutti, A. (2014). Antimicrobials use in aquaculture and their public health impact. *Journal of Aquaculture Research & Development, 5*(4), 1.

Asin-Prieto, E., Rodriguez-Gascon, A., & Arantxazu Isla, A. (2015). Applications of the pharmacokinetic/pharmacodynamic (PK/PD) analysis of antimicrobial agents. *Journal of Infection and Chemotherapy, 21*, 319–329.

Cabello, F. C. (2006). Heavy use of prophylactic antibiotics in aquaculture: A growing problem for human and animal health and for the environment. *Environmental Microbiology, 8*(7), 1137–1144.

Cabello, F. C., Godfrey, P. H., Tomova, A., Ivanova, L., Dölz, H., Millanao, A., & Buschmann, A. H. (2013). Antimicrobial use in aquaculture re-examined: Its relevance to antimicrobial resistance and to animal and human health. *Environmental Microbiology.* https://doi.org/10.1111/1462-2920.12134

CLSI. (2020). *Performance standards for antimicrobial susceptibility testing of bacteria isolated from aquatic animals* (3rd ed.). CLSI supplement, VET04. Clinical and Laboratory Standards Institute.

Crosby, T. C., Kittel, E. C., & Gieseker, C. M. (2022). Phish-pharm: A searchable data base of pharmacokinetics and drug residue literature in Fish-2022 update. *The AAPS Journal, 24*, 105.

Estrada-San Agustín, E., Gutiérrez, L., Bernad, M., Castillo-Juarez, H., Sanchez, S., & Sumano, H. (2019). Pharmacokinetics of two dosing forms of a recrystallized enrofloxacin as

hydrochloride dihydrate in tilapia (*Oreochromisniloticus* × *Oreochromismossambicus*). *Aquaculture International, 27*, 849–857.

Godfray, H. C. J., Beddington, J. R., Crute, I. R., Haddad, L., Lawrence, D., Muir, J. F., Pretty, J., Robinson, S., Thomas, S. M., & Toulmin, C. (2010). Food security: The challenge of feeding 9 billion people. *Science, 327*(5967), 812–818. https://doi.org/10.1126/science.1185383

Grondel, J. L., Nouws, J. F. M., & Haenen, O. L. M. (1986). Fish and antibiotics: Pharmacokinetics of sulphadimidine in carp (*Cyprinuscaprio*). *Veterinary Immunology and Immunopathology, 12*, 281–286.

Ibrahim, M., Ahmad, F., Yaqub, B., Ramzan, A., Imran, A., Afzaal, M., Ali Mirza, S. A., Mazhar, I., Younus, M., Akram, Q., Taseer, M. S. A., Ahmad, A., & Ahmed, S. (2020). Current trends of antimicrobials used in food animals and aquaculture in antibiotics and antimicrobial resistance genes in the environment. In *Antibiotics and antimicrobial resistance genes in the environment* (pp. 39–69). Elsevier.

Jayasankar, P. (2018). Presentstatus of aquaculture in India – A review. *Indian Journal of Fisheries, 65*, 157–165.

Kirchhelle, C. (2018). Pharming animals: A global history of antibiotics in food production (1935–2017). *Palgrave Communications, 4*, 96. https://doi.org/10.1057/s41599-018-0152-2. www.nature.com/palcomms

Kum, C., Kirkan, S., Sekkin, S., Akar, F., & Boyacioglu, M. (2008). Comparison of *in vitro* antimicrobial susceptibility in *Flavobacteriumpsychrophilium* isolated from Rainbowtroutfry. *Journal of Aquatic Animal Health, 20*, 245–251.

Kverme, K. O., Haughland, G. T., Hannisdal, R. T., Kallekleiv, M., Colquhoun, D. J., Lunestad, B. T., Wergeland, H. I., & Samuelsen, O. B. (2019). Pharmacokinetics of Florfenicol in lump fish (*Cyclopteruslumpus*L.) after single oral administration. *Aquaculture, 512*, 1–7. (734279).

Le, T. X., Munekage, Y., & Kato, S. (2005). Antibiotic resistance in bacteria from shrimp farming in mangrove areas. *The Science of the Total Environment, 349*, 95–105.

Lees, P., Svendsen, O., & Wiuff, C. (2008). Strategies to minimize the impact of antimicrobial treatment on the selection of resistant bacteria. In L. Guardabassi, L. B. Jensen, & H. Kruse (Eds.), *Guide to Antimicrobial Use in Animals* (pp. 77–101). Blackwell Publishing. (Chapter 6).

Ma, R., Wu, Y., Li, G., Zhao, S., Li, L., & Fang, W. (2022). Pharmacokinetics of sarafloxacin hydrochloride in the pacific white shrimp, *Litopenaeus vannamei*, following multiple -dose oral administration. *Aquaculture, 548*. https://doi.org/10.1016/j.aquaculture.2021.737662

Miller, R. A., & Harbottle, H. (2017). Antimicrobial drug resistance in fish pathogens. *Microbiology Spectrum, 6*(1), ARBA-0017.

OIE. (2018). List of antimicrobial agents of veterinary importance. The World Assembly of OIE delegates. Resolution no. XXVIII. Updates.

Papich, M. G. (2014). Pharmacokinetic–pharmacodynamic (PK–PD) modelling and the rational selection of dosage regimes for the prudent use of antimicrobial drugs. *Veterinary Microbiology, 171*, 480–486.

Park, J. Y., Awji, E. G., Suh, J. W., & Park, S. C. (2015). Pharmacokinetics, pharmacokinetic-pharmacodynamic relationship and withdrawal period of amoxicillin sodium in olive flounder (*Paralichthysolivaceus*). *Xenobiotica, 46*, 522–529.

Rigos, G., & Smith, P. (2015). A critical approach on pharmacokinetics, pharmacodynamics, dose optimization and withdrawal times of oxytetracyclinein aquaculture. *Reviews in Aquaculture, 7*, 77–106.

Rigos, G., Nengas, L., Tyrpenouc, A. E., Alexis, M., & Troisi, G. M. (2003). Pharmacokinetics and bioavailability of oxytetracycline in gilthead sea bream (*Sparusaurata*) after a single dose. *Aquaculture, 221*, 75–83.

Rigos, G., Kogiannou, D., Padros, F., Cristofol, C., Florio, D., Fioravanti, M., & Zarza, C. (2020). Best therapeutic practices for the use of antibacterial agents in finfish aquaculture: A particular view on European Sea bass (*Dicentrarchus labrax)* and giltheadseabream (*Sparus aurata)* in Mediterranean aquaculture. *Reviews in Aquaculture, 13*, 1–39.

Samuelsen, O. B. (2006). Pharmacokinetics of quinolones in Fish. *Aquaculture, 255*, 55–75.

San Martin, B., Fresno, M., Cornejo, J., Godoy, M., Ibarra, R., Vidal, R., Araneda, M., Anadon, A., & Lapierre, L. (2019). Optimization of florfenicol dose against *Piscirickettsia salmonis* in Salmo Sagar through PK/PD studies. *PLoS One, 14*(5), e0215174.

Schar, D., Klein, E. Y., Laxminarayan, R., Gilbert, M., & Van Boeckel, T. P. (2020). Global trends in antimicrobial use in aquaculture. *Scientific Reports, 10*, 21878. https://doi.org/10.1038/s41598-020-78849-3

Shan, Q., Huang, H., Zheng, G., Yin, Y., Zhu, X., Ma, L., Zhou, H., Xi, W., Li, L., Liu, S., & Wang, J. (2022). Pharmacokinetics and tissue residue profiles of enrofloxacin in Crucian carp (*Carassius auratus gibelio)*following single and multiple oral administration. *Frontiers in Veterinary Science, 9*, 1–11. https://doi.org/10.3389/fvets.2022.872828

Smith, P., Endris, R., Kronvall, G., Thomas, V., Verner, D., Wilhelm, C., & Dalsgaard, I. (2014). Epidemiological cut-off values for *Flavobacterium psychrophilum* MIC data generating by standard test protocol. *Journal of Fish Diseases, 1–12*. https://doi.org/10.1111/jfd.12336

Subasinghe, R. P. (2005). Epidemiological approach to aquatic animal health management: Opportunities and challenges for developing countries to increase aquatic production through aquaculture. *Preventive Veterinary Medicine, 67*, 117–124.

The World Bank. (2013). Fish to 2030: Prospects for fisheries and aquaculture. World Bank. https://reliefweb.int/report/world/fish-2030-prospects-fisheries-and-aquaculture. Accessed March 10, 2015.

Van Boeckel, T. P., Brower, C., Gilbert, M., Grenfella, B. T., Simon, A., Levina, S. A., Robinsoni, T. P., & Laxminarayan, R. (2015). Global trends in antimicrobial use in food animals. *PNAS, 112*(18), 5649–5654.

Wayne, P. A. (2020). *CLSI. Performance standards for antimicrobial susceptibility testing of bacteria isolated from aquatic animals* (3rd ed.) CLSI supplement Vet04. Clinical and Laboratory Standards Institute. ISBN 978-1-68440-075-1.

Wei, Q. (2002). Social and economic impacts of aquatic animal health problems in aquaculture in China. *FAO Fisheries Technical Paper, 406*, 55–61.

Xu, L., Wang, H., Yang, X., & Lu, L. (2013). Integrated pharmacokinetics-pharmacodynamics parametersbased dosing guidelines of enrofloxacin in grass carp *Ctenopharyngodonidella* to minimize selection of drug resistance. *BMC Veterinary Research, 9*, 126.

Xu, N., Li, M., Ai, X., & Lin, Z. (2021). Determination of pharmacokinetic and pharmacokinetic-pharmacodynamic parameters of doxycycline against *Edwardsiella ictalurid* in yellow cat fish *(Pelteobargus fulvidraco*). *Antibiotics, 10*, 329. https://www.mdpi.com/2079-6382/10/3/329

Antibiotic Residues in Aquatic Foods and Their Methods of Detection

Satyen Kumar Panda and Niladri Sekhar Chatterjee

Contents

Abstract

Antibiotic residues are often found in the aquatic foods due to direct use in aquaculture or arising from pollution of waterbodies from nonpoint sources. Exposure to these antibiotic residues through aquatic food has far-reaching health implications, including the advent of antibiotic resistance microorganisms. Hence, it is important to screen for remnants of antimicrobial drugs in foods to establish regulatory control and help prevent public health emergencies. "*Liquid chromatography-tandem mass spectrometry (LC-MS/MS)"*- based estimation is the pivotal process for accurate quantitative estimation and confirmation of the rest of the drugs in food. This chapter provides an overview of antibiotic residue analysis in fish and fish products employing "LC-MS/MS." A brief description of the principle of analysis using "*LC-MS/MS"* has been provided. Further, discussion is centered on the analysis of proscribed antimicrobials such as nitrofuran

S. K. Panda (✉) · N. S. Chatterjee
ICAR-Central Institute of Fisheries Technology, Kochi, Kerala, India
e-mail: Niladri.Chatterjee@icar.gov.in

M. P. Mothadaka et al. (eds.), *Handbook on Antimicrobial Resistance*,
https://doi.org/10.1007/978-981-19-9279-7_41

metabolites and chloramphenicol, and analysis of multiclass antibiotics in a multiresidue analysis approach.

Keywords

Mass spectrometry · Antibiotics · Nitrofurans · Chloramphenicol · Multiresidue analysis · Method validation

1 Introduction

More than 200 veterinary drugs are known to be used in aquaculture for prophylactic and therapeutic purposes to prevent bacterial diseases in large-scale aquaculture production and processing (Love et al., 2011). These widely used antibiotics are natural or synthetic compounds with broad-spectrum bactericidal and bacteriostatic properties to destroy bacterial action or inhibit their growth (Cañada-Cañada et al., 2009). Often such antibiotics and veterinary drugs are applied through medicated feed. However, the unregulated and improper application of antimicrobials in aquaculture drives the growth of strong unresponsiveness to antimicrobials in foodborne pathogens and its impact on humans has emerged as a global concern (Santos & Ramos, 2018). Accumulation of residual antibiotics may lead to antimicrobial-resistant gene dissemination or bacterial infections in humans (Jian et al., 2021). It is likely that the residues of antibiotics could accumulate in wild fish, captured in waterbodies in vicinities of aquacultured regions. Regulatory agencies such as the "European Union (EU), FAO-CODEX, USDA, Health Canada," etc., have formulated safe "maximum residue limits (MRLs)" for veterinary medications and drugs in various food stuffs (EU, 2010; FAO, 2021; Health Canada, 2023; USDA, 2023). EU regulation (EU) 2017/625 provides the technical guidance and performance criteria for drug residue detection and control (EU, 2017). Beyond concerns of antibiotic resistance development, some of the antibiotic drugs are restricted for use in aquaculture due to their cancer-causing potential and other toxicological consequences. Residues of such prohibited substances in aquatic foods are strictly controlled and "minimum required performance limits (MRPLs)" or "reference point of action (RPA)" has to be established as per the guidelines in the EU Regulation (EU) 2019/1871 (EU, 2019). Since these are prohibited compounds, the RPA for these compounds is often fixed at the lowest possible concentration that can be accurately quantified as per the present state of the art for analysis of such compounds. Chloramphenicol and nitrofuran metabolites fall under the category of prohibited compounds in fish commodity. The first MRPL for chloramphenicol was published in 2003 as per "Commission Decision 2003/181/EC, 13 March 2003*"* and was subsequently revised (EU, 2003). Table 1 presents a list of prohibited antibiotics in aquatic foods and their RPA [Commission Regulation (EU) 2019/1871] (EU, 2019). Several other global regulatory agencies, including CODEX and *"Food Safety and Standards Authority of India (FSSAI),"* have proposed tolerance limits or MRL for antibiotics in aquatic food products. Table 2 presents the lowest

Table 1 List of antibiotics and drugs prohibited to be used in aquaculture food production and their corresponding RPA/tolerance limit

Prohibited antibiotics/ drugs	Reference point of action (RPA)/tolerance limit (ng/g)
Furaltadone	0.50
Furazolidone	0.50
Nitrofurantoin	0.50
Nitrofurazone	0.50
Chloramphenicol	0.15
Chlorpromazine	1
Clenbuterol	1
Colchicine	1
Crystal violet	1
Dapsone	1
Diethylstilbestrol	1
Dimetridazole	1
Glycopeptides	1
Ipronidazole	1
Metronidazole	1
Ronidazole	1
Stilbenes and steroids	1
Sulfamethoxazole	1
Malachite Green	0.5

global MRL/tolerance limit prescribed by any of the regulatory agencies and that of FSSAI for prominent antibiotics found in aquatic food products (FSSR, 2011).

This chapter briefly describes the classification of major antibiotics and their uses, elaborates the concept, and elucidates "LC-MS/MS" for evaluating remnants of antimicrobials. The chapter also touches upon the method validation protocol as per the "European Union Guideline EC/2021/808 (EU, 2021)."

Incidence of the remainders of antimicrobials in aquatic foods could be due to misuse of antibiotic treatment in aquaculture and from nonpoint sources such as discharge of hospital, municipal, and animal husbandry effluents in waterbodies. Antibiotics are often administered in aquaculture through feed, oral, and injection route for therapeutic, subtherapeutic, and prophylactic uses (Chen et al., 2020); however, their use in aquaculture needs to be regulated stringently. For example, the USFDA approved the use of only "chloramine-T, formalin, hydrogen peroxide, oxytetracycline hydrochloride, tricaine methanesulfonate, chorionic gonadotropin, florfenicol, oxytetracycline dihydrate, sulfadimethoxine/ormetoprim, and sulfamerazine" **(**USFDA, 2022). In Norway, purchase of antimicrobials for aquaculture purposes needs a veterinarian's prescription, and only therapeutic use is ensured in this manner. The Norwegian Medicines Agency controls the sale of antimicrobials through pharmacies and feed plants. The agency mandates that the amount of antibiotics used needs to be mandatorily reported and related prescriptions must be retained (Directorate of Fisheries Norway, 2001).

Table 2 The lowest global MRL/tolerance limit and FSSAI MRL for prominent antibiotic compounds

Compound	Regulated marker	Fish and fishery products	
		Lowest global MRL	FSSAI MRL
Albendazole	Albendazole sulfone	–	100
-do-	-do-	–	
-do-	Albendazole 2-aminosulfone	–	
Albendazole oxide	Albendazole oxide	–	
-do-	-do-	–	
Albendazole oxide	Albendazole 2-aminosulfone	–	
Amoxicillin	Amoxicillin	50	–
Ampicillin	Ampicillin	50	10
Chlortetracycline	Chlortetracycline	100	200 (Prawn)
Chlortetracycline	4-epi-chlrotetracycline		
Cloxacillin	Cloxacillin	300	10
Colistin	Colistin	150	–
Danofloxacin	Danofloxacin	100	–
Dicloxacillin	Dicloxacillin	300	–
Difloxacin	Difloxacin	300	–
Doxycycline	Doxycycline	100	–
Emamectin (B1a)	Emamectin (B1a)	100	–
Enrofloxacin	Ciprofloxacin	100	–
Enrofloxacin	Enrofloxacin		–
Erythromycin	Erythromycin	200	–
Florfenicol	Florfenicol	100	–
Florfenicol	Florfenicol-amine		–
Flumequine	Flumequine	500	500
Gentamicin(s)	Totality of C1, C1a, C2, 2a	50	–
Lincomycin	Lincomycin	100	–
Neomycin	Neomycin B	500	–
Oxacillin	Oxacillin	300	–
Oxolinic acid	Oxolinic acid	100	300
Oxytetracycline	Oxytetracycline	100	100, 200 (Prawn)
Oxytetracycline	4-epi-oxytetracycline		
Penicillin G (Benzylpenicillin)	Penicillin G	50	–
Sarafloxacin	Sarafloxacin	10	–
Spectinomycin	Spectinomycin	300	
Sulfonamides	Total of all compounds that fit into sulfonamide	100	
Sulfanilamide	Sulfanilamide		10
Sulfadiazine	Sulfadiazine	100	10
Tetracycline	Tetracycline	100	100, 200 (Prawn)
Tetracycline	4-epi-tetracycline		

(continued)

Table 2 (continued)

Compound	Regulated marker	Fish and fishery products	
		Lowest global MRL	FSSAI MRL
Thiamphenicol	Thiamphenicol	50	
Tilmicosin	Tilmicosin	50	
Trimethoprim	Trimethoprim	100	50

Sources for Remnants of Antimicrobials in Aquatic Foods

However, intensive aquaculture has resulted in higher incidence of bacterial diseases and ever-increasing employment of drugs in fish farms (Defoirdt et al., 2011). Assessing the antimicrobial application in aquaculture is a difficult task due to the wide disparity in data sharing by the aquaculture-practicing countries (Burridge et al., 2010). An estimate suggests 500–600 MT of antimicrobial drugs were employed in shrimp aquaculture in Thailand during the year 1994, and there was large variation in the quantum of antibiotic use between countries (Defoirdt et al., 2011). For example, the antibiotic use in Norway is 1 g per MT production, while in Vietnam the amount of antibiotic used can be as high as 700 g per MT production.

In India, use of antibiotics in aquaculture is not approved. The Coastal Aquaculture Authority (CAA) regularly publishes a list of aquaculture inputs that are free from antibiotics (CAA, 2020). Institutions and agencies, viz., "ICAR-Central Institute of Fisheries Technology (ICAR-CIFT)," "Export Inspection Council (EIC)," and "Marine Product Export Development Agency (MPEDA)," through the respective laboratory networks strictly monitor the incidence of the rest of the antimicrobials in aquatic foods. This food safety system has prevented the inappropriate use of antibiotics in Indian aquaculture to a large extent as evidenced by the fewer export consignment rejections due to antibiotic residues.

However, antibiotic and veterinary drug residues in aquatic food through nonpoint sources such as hospital, municipal, and animal husbandry effluents are difficult to control and a major concern worldwide. Antibiotic residue from nonpoint source may often not present in the target list for food safety monitoring system of a country. Such antibiotic residues may escape the food safety net and affect consumer health. Such residues from nonpoint source necessitate untargeted monitoring using high-resolution mass spectrometry systems.

2 Antibiotic Classes and Significance for Antibiotic Residue Analysis

The chemical structures and mechanisms of action are the basis for the classification of antibiotics**.** A particular antibiotic class contains compounds that are similar in structure and have a similar mode of action. Compounds within a class often share a core structure and have variations in the side chain. Such side-chain modifications result in various physicochemical properties, pharmacokinetics, and pharmacodynamic

properties of the compounds. The major classes of antibiotics, prominent member compounds, and spectrum of activity are presented in Table 3 (Wang et al., 2011).

The classification of antibiotics as per chemical class suggests that these compounds have diverse polarity ranges and physicochemical properties. These diverse physicochemical properties often make it challenging to solubilize, extract, and analyze these compounds in a single strategy. A ready reckoner of solvents used for solubilizing analytical standards for prominent antibiotic compounds is presented in Table 4.

3 Principle for "Liquid Chromatography–Tandem Mass Spectrometry (LC-MS/MS)" Assessment of Antibiotic Remnants

Modern "LC-MS/MS" instruments are the ultra-sophisticated equipment that are employed in unambiguous detection and accurate amounts of trace levels of rest antimicrobials, drug residues, and residues of other pharmacologically active substances in food (Desmarchelier et al., 2022). These are precision analytical equipment where a liquid chromatograph front end is connected with an ambient ionization mass spectrometer. While the liquid chromatograph separates the compounds from matrix co-extractives, the mass spectrometer provides accurate identification and quantification.

Ionization of the analyte molecules to a charged state (positively or negatively charged) is the first step in mass spectrometers. Subsequently, parent ions and their descendant fragment ions that are analyzed on the base of their mass to charge ratio (m/z) and identified on a photomultiplier tube detector. Hence, a "mass spectrometer" has an ion source that produces the analyte ions; a "mass analyzer" that further analyzes the analyte ions and fragment ions based on m/z ratio; and a detector that detects the analyte ions and fragment ions. Based on the different technology combinations used for ionization in the ion source and mass analysis in "mass analyzer," there are various commercial mass spectrometers available for versatile bioanalysis applications (Jongedijk et al., 2023; Wang et al., 2013). Figure 1 presents different types of ion source and mass analyzer combinations schematically.

"LC-MS/MS" with "electron spray ionization (ESI)" source and "triple quadrupole mass analyzer" is the ultimate standard for confirmative and measureable detection of trace levels of remnants of antimicrobial drugs in farmed aquatic foods (Robert et al., 2015). The proficiency of ESI technology as a popular robust ion source that can interface with an LC and is suitable for ionizing a number of mid-polar compounds was established during the 1990s (Fenn et al., 1989). Sample solution is pumped through a fine metal capillary tube and nebulized at the tip with the help of nebulizer gas. Either positive or negative voltage (3–5 kV) is applied at capillary tip, producing positively and negatively charged well-formed droplets, respectively. The capillary is usually placed orthogonally or off-axis from the orifice

Table 3 Major antibiotic classes, prominent member compounds, and spectrum of activity

Class	Members	Spectrum
Aminoglycosides	"Amikacin, apramycin, dihydrostreptomycin, gentamicin, kanamycin, neomycin B, streptomycin A, tobramycin"	Aerobic Gram-ve and few Gram +ve microbes (*Staphylococcus aureus)*
Aminocyclitols	Spectinomycin	Aerobic Gram-ve and Gram +ve microbes
β-Lactams	Amoxicillin, ampicillin, penicillin G, cloxacillin, clavulanic acid, ceftiofur, cephalexin	Gram +ve microbes viz., Corynebacteria, Streptococci and Staphylococci, particularly *Staphylococcus aureus* and Gram-ve bacteria, viz., *Escherichia coli, Klebsiella pneumonia, Haemophilus influenza*, *Pasteur Ella,* and *Salmonella* Active against anaerobic bacteria, apart from ESBL Bacteroides and *Clostridium difficile*
Quinoxalines	Carbadox	Gram +ve and few Gram-ve bacteria; besides, few chlamydiae and protozoa
Lincosamides	Lincomycin, pirlimycin	Entire *Staphylococcus* species, *Streptococcus* species (except *Streptococcus faecalis*), *Erysipelothrix insidiosa, Leptospira pomona*, and Mycoplasma species
Macrolides	Erythromycin, tilmicosin	Aerobic and anaerobic Gram +v bacteria & Gram-ve- cocci, besides "*Haemophilus, Actinobacillus, Bordetella, Pasteurella, Campylobacter,* and *Helicobacter*"
Phenicols	Florfenicol, thiamphenicol	All obligate anaerobes and suppress the growth of rickettsia and chlamydia species
Polypeptide	Bacitracin A, colistin (polymyxin E), novobiocin, polymyxin B	Gram +ve bacteria but exhibits little activity against Gram-ve microbes
Glycopeptide	Vancomycin	Gram+ve cocci
Streptogramins	Virginiamycin	Gram+ve bacteria such as *Clostridium perfringens*
Quinolones	"Ciprofloxacin, danofloxacin, difloxacin, enrofloxacin, flumequine, nalidixic acid, norfloxacin, oxolinic acid, sarafloxacin"	Many Gram-ve bacteria and Gram +ve bacteria
Sulfonamides	"Sulfacetamide, sulfachloropyridazine, sulfadiazine, sulfadimethoxine,	Gram +ve and -ve bacteria, few chlamydia, *Nocardia* and *Actinomyces*

(continued)

Table 3 (continued)

Class	Members	Spectrum
	sulfamerazine, sulfamethazine, sulfamethoxazole, sulfanilamide, sulfapyridine"	species, and few protozoa including coccidia and Toxo*plasma* species
Diaminopyrimidines	Trimethoprim	Acts synergistically with sulfonamides
Tetracyclines	Tetracycline and its epimer, oxytetracycline, and its epimer, chlortetracycline, and its epimer, doxycycline	Broad-spectrum antibiotic

of mass spectrometer cone plate to avoid uncharged particle and solvent droplets from entering the mass spectrometer.

The solvent in the droplets is rapidly evaporated by the heated drying gas (nitrogen), which leaves only a cloud of charged analytes. This ion cloud enters the region of high vacuum levels of mass spectrometer through the orifice of the cone plate and a series of focusing lens, which applies a focusing voltage to focus the maximum possible number of charged ions. ESI is a soft ionization technique where pseudo molecular weight ions or adducts are produced and can deliver significant data on the MW of the compound. The application of +ve voltage on the capillary results in the formation of adducts "$[M+H]^+$, $[M+Na]^+$, $[M+NH_4]^+$, $[M+CH_3OH+H]^+$." When operated in negative mode $[M-H]^-$, $[M + acetate]^-$, $[M + formate]^-$ adducts are most common. Such adducts are selected as parent ion or precursor ion of the target analyte (Varghese et al., 2012).

A combination of four metal rods in parallel is known as "quadrupole mass analyzer" with an ion movement path between them. In triple quadrupole mass analyzer, three sets of quadrupoles are placed next to each other either in a linear or curved ion path. While the first and third quadrupoles scans m/z ratio-dependent ions, the remaining quadrupole performs as a collision cell for producing fragments, which are daughter ions or product ions from the parent ions/precursor ions. Essentially the first quadrupole scans for the precursor ions and the third quadrupole scans for the product ions.

"Triple quadrupole MS systems" are also employed along with MS/MS assay termed "selected reaction monitoring (SRM)." The SRM type is also known as "multiple reaction monitoring (MRM)" mode. The SRM mode provides high selectivity to the analysis by removing all other interfering ions. When a particular direct current (DC) and radio frequency (RF) voltages is applied, the RF/DC ratio in the quadrupole 1 (Q1) allows one particular parent ion (m/z) to have a stable trajectory and pass through. The parent ion then reaches quadrupole 2 (Q2), where product ions are formed by "collision-induced dissociation (CID)" using an inert gas (generally nitrogen or argon). The resultant ions are then filtered by the quadrupole 3 (Q3) and peaks are observed only for those specific product ions filtered by Q3. The fragment

Table 4 Solvents used for solubilizing analytical standards for prominent antibiotic compounds analyzed in aquatic products

	Antibiotics CRM	Purity (%)	Soluble in
	Amoxicillin trihydrate	96.68	Water
	Ampicillin trihydrate	85.70	0.2 M HCl
	Cloxacillin sodium	94.40	Water
	Tetracycline hydrochloride	97.50	Methanol
	Oxytetracycline	91.30	Methanol
	Chlortetracycline hydrochloride	90.90	Methanol
	Doxycycline hyclate	91.50	Water
	4 epi tetracycline	94.40	Methanol
	Trimethoprim	99.50	DMSO
	Sulfacetamide	98.80	Methanol
	Sulfadiazine	99.80	Methanol
	Sulfathiazole	99.21	DMSO
	Sulfapyridine	99.46	DMSO
	Sulfamerazine	99.70	DMSO
	Sulfamethoxypyridazine	99.71	DMSO
	Sulfaethoxypyridazine	98.60	Methanol
	Sulfamethoxazole	99.60	DMSO
	Sulfaquinoxaline	96.33	0.5 m NaOH
	Sulfadimethoxine	99.80	DMSO
	Sulfadoxine	98.90	DMSO
	Sulfanilamide	99.70	0.5 m NaOH
	Sulfachlorpyridazine	99.11	Methanol
	Ciprofloxacin	99.50	0.2% acetic acid
	Enrofloxacin	99.40	DMSO
	Norfloxacin	99.00	0.2% acetic acid
	Danofloxacin mesylate	95.28	DMSO
	Difloxacin hydrochloride	97.13	Water
	Sarafloxacin hydrochloride	85.82	Water
	Oxolinic acid	98.20	0.5 m NaOH
	Nalidixic acid	99.61	Chloroform
	Flumequine	99.03	Methanol
	Ofloxacin	96.49	0.2% acetic acid
	“Albendazole	99.40	DMSO
	Albendazole sulfone	96.12	DMSO
	Albendazole sulfoxide	97.50	DMSO
	Albendazole 2 aminosulfone”	98.70	DMSO

ions provide unambiguous identification in addition to identification with molecular weight. Fig. 2 presents the schematic working of a triple quadrupole mass analyzer in MRM mode.

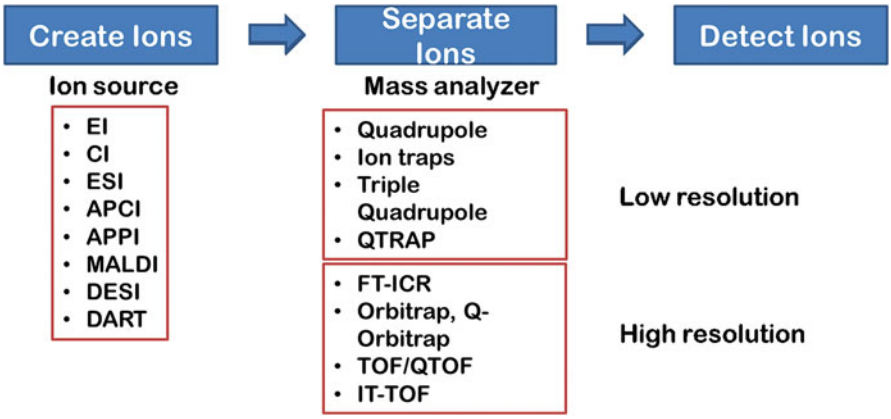


Fig. 1 Different ion source and mass analyzer combinations in mass spectrometers

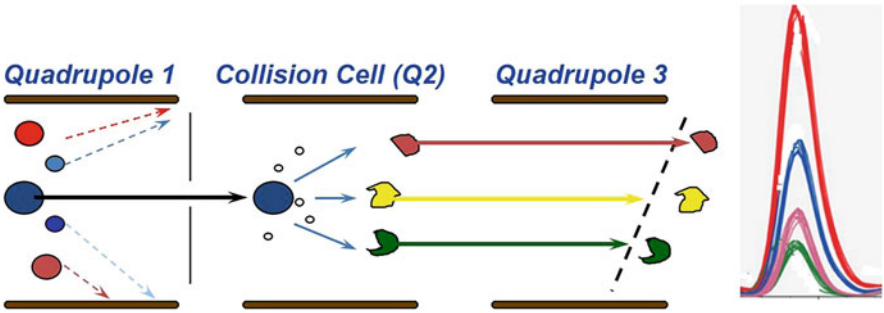


Fig. 2 Schematic working principle of MRM mode in triple quadrupole mass spectrometer

4 Sample Preparation/Processing for Fishery Products

After bringing to the laboratory, the samples are immediately frozen. The samples should be taken out of the freezer at least 2 h before sample preparation so that the samples are thawed but still cold. The risk of evaporation of extracting solvent can be nullified by using cold matrices. The sample should be thawed by placing it inside a zip lock bag and dipping the bag in lukewarm water (~35 °C). There should not be any hardcore or ice crystal in the thawed sample. This can be ensured by gently squeezing the sample bag intermittently. Caution should be exercised while squeezing so as not to damage the sample texture. The thawed sample needs to be processed by removing the nonedible parts and cut into small pieces. The pieces are then homogenized in a food processor for 60–120 s until there are no lumps. The food processor should be thoroughly cleaned between two different sample units to avoid cross-contamination.

5 LC-MS/MS-Based Examination of Chloramphenicol

Chloramphenicol (CAP), an antibiotic with wide-ranging antimicrobial properties against most of the microbes, was employed in earlier times in faunal farming. This antibiotic is produced by *Streptomyces venezuelae* and some other Actinomycetes. The commercial CAP is, however, produced by chemical synthesis. Use of CAP in animal husbandry is prohibited by the United States, the EU, India, Canada, Australia, China, Japan, and other countries. The compound is known to induce aplastic anemia and suspected as a carcinogen. The "Reference Point of Action (RPA)" assigned to chloramphenicol by the European Commission and USFDA for products of animal origin is 0.3 μg kg^{-1} (EU, 2019).

"A triple quadrupole HPLC-MS/MS" with RP-18 end-capped analytical column, of dimensions 250/150/100×4.6/3.0/2.1 mm, 3–5 μm particle dimension, or similar type can be used for chloramphenicol analysis. Water and acetonitrile gradient, at a flow rate of 0.3–1.0 mL per min, depending on column ID and length can be used. The typical run time comes to 6–12 min. The analysis is done in the ESI-negative MRM mode. The MRM transitions for chloramphenicol are 321 > 152, 321 > 257, and 326 > 156 for quantitation, validation, and MRM transitions for in-house standard (CAP-d5), respectively.

Precisely 2.0 g of homogenized edible portion of tissue is weighed and further skewed with 200 μL of CAP-d5 as per local calibration ((20 μg L^{-1}). The sample is then extracted with ethyl acetate (5 mL) by vortexing for 10 min. Following extraction, the sample containing tubes is centrifuged at about 8000 rpm for 15 min. The upper layer harboring ethyl acetate is then shifted to a thoroughly washed Turbovap tube accumulator under nitrogen. The decanting process is repeated with another 5 mL ethyl acetate and all the ethyl acetate layers are collected in the same Turbovap tube and dried under nitrogen at about 45 °C. The dried residue is dissolved in 1 mL of hexane:carbon tetrachloride (1:1, v/v) by vortex mixing, to which 1 mL of water is added and mixed with vortex agitation. This solvent mixture is then centrifuged at about 8000 rpm for 15 min at 40 °C for parting of stratums. The aqueous upper layer is quantitatively transferred into an LC vial for injection in HPLC-MS/MS (Rønning et al., 2006). Matrix-fortified calibration standards are prepared in a similar manner for developing calibration curve. The linearity of calibration curve can be improved significantly with employment of local standards. The concentration in the sample is determined using this calibration curve in instrument software. Since matrix-fortified calibration standards are processed in the same manner as sample, dilution factor is not necessary.

6 LC-MS/MS Employment in the Detection of Nitrofurans Metabolites

Nitrofurans (NFs) are broad-spectrum antibiotics and show antiparasitic activity. These antibiotics are applied widely in animal husbandry recently. However, NFs are metabolized into potential carcinogenic compounds and transformable metabolites

in vivo with bioaccumulation in faunal-sourced foods. Considering the serious health hazard, many countries banned NFs in the production of food of animal origin. Despite regulatory prohibition, NFs are still being detected in foods of faunal source both in developed and developing nations, indicating incessant application of NFs. Stringent analytical method for analyzing NFs is very important since the RPA has recently been revised to 0.50 μg/kg (EU, 2019).

At present, four NFs metabolites, viz., "3-amino-5-morpholinomethyl-1,3-oxazolid-2-one (AMOZ), semicarbazide (SEM), 1-aminohydantoin (AHD), and 3-amino-2-oxazolidinone (AOZ)," are extensively scrutinized worldwide for incidence in faunal sourced foodstuffs. A triple quadrupole LC-MS/MS with RP-18 chromatography column and operating in ESI-positive MRM mode is the gold standard for trace-level quantification of these nitrofuran metabolites (Melekhin et al., 2022). A gradient program of the variable phase consisting of 0.1% 5 mM ammonium formate in water and acetonitrile is used at the flow rate of 0.3–1 mL per min depending upon column length and inner diameter. Apart from the analytical standards of the four NF metabolites, their local calibrations, viz., "AHD-13C3, SCA-HCl – 13C, 15N2, AMOZ-d5 & AOZ-d4," are necessitated. The analytical standards and internal standards are derivatized using 2-nitrobenzaldehyde (2-NBA) for quantification in LC-MS/MS. The MRM transitions of the nitrophenyl (NP) derivatives are shown in Table 5.

Sample preparation involves hydrolysis of bound residue. Briefly, shrimp/fish tissue (4 g) sample is weighed and spiked with 50 ppb internal standard solution (200 μL) containing all the four internal standards of the metabolites. This relates to 2.5 ppb in the concluding 1 mL of the extract capacity in LC vial. The bound residues of the NF metabolites are hydrolyzed with 0.2 M HCl (10 mL) and the released metabolites are derivatized with 100 mM, 2-NBA solution in methanol (300 μL). The derivatization requires overnight (minimum16 h) incubation at 37 °C ± 2 °C under shaking conditions. Recent studies have tried to shorten the derivatization time using microwave-assisted derivatization (Luo et al., 2019). Following overnight incubation, the sample tubes are transported to ambient temperatures and 0.3 M tri-sodium-phosphate liquid is supplemented (300 μL). If needed, 2 M NaOH solution is used to adjust the pH to neutral. The derivatized NF metabolites are then distributed into ethyl acetate by vortex mixing and centrifugation for 10 min at 8000 rpm. The ethyl acetate extraction is repeated once more and the extracts are pooled in a concentrator tube. The extracts are evaporated at 45 °C under nitrogen in a Turbovap concentrator. In case of observation of color or

Table 5 MRM transitions of the nitrophenyl (NP) derivatives

S. no.	MRM of NP	MRM transitions
1	AMOZ	335 > 291, 335 > 100
2	AMOZd5	340 > 296
3	AOZ	236 > 134, 236 > 104
4	AOZd4	240 > 134
5	AHD	249 > 134, 249 > 178
6	SEM	209 > 166, 209 > 192

fat content, then hexane:carbon tetrachloride (v/v: 50/50) wash is required. The aqueous layer is subjected to quantitative LC-MS/MS analysis (Guichard et al., 2021).

7 Simultaneous Analysis of Multiclass Antibiotic Residue Using LC-MS/MS

Apart from banned antibiotics, multiclass antibiotics belonging to quinolones, sulfonamides, and tetracyclines can be analyzed in a single multiresidue method. A typical "LC-MS/MS multiresidue detection technique" for antimicrobial drugs in fish/shrimp tissue encompassing a minimum of nine quinolones (including fluoroquinolones), ten sulfonamides, and three tetracyclines. Multiresidue method can save sample analysis time and consumables by analyzing multiple compounds of different classes in a single experiment. However, achieving satisfactory recovery of all the antibiotics is challenging because of the widely varying chemical structures and physicochemical properties among different classes. Multiresidue methods involving aminoglycosides in the same extraction are rare. Recently published methods have suggested four sample preparation workflows to cover all the multiclass antibiotics. However, a typical multiresidue method that covers most of the important antibiotics in fish/shrimp is described here briefly.

The choice of chromatography column is critical for achieving satisfactory retention and separation of all the target antibiotics. The commonly used columns are Waters XBridge BEH C18 column, Waters Acquity HSS T3 column, Phenomenx Kinetex C18, Restek Raptor C18, etc. Gradient program of water and methanol together with 0.1% formic acid is typically used flow @ of 0.3–1 mL, depending on column dimensions. Typical chromatographic separation time is no more than 15 min. The typical MRM parameters for LC-MS/MS analysis in ESI-positive mode are presented in Table 6.

Different extraction strategies are reported in the literature. A typical sample preparation procedure involves extraction with acetonitrile. Briefly, 2.0 ± 0.1 g of sample is extracted with acetonitrile (8 mL) and acidified water (0.5 mL water containing 5% formic acid) in a centrifuge tube (50 mL PP). The sample is extracted by vortex agitation (20 s) followed by centrifugation at $18 \times 10^2 \times g$ for 10 min and the acetonitrile supernatant is collected in a glass test tube (5 mL). The decanting method is performed twice, and both the extracts are pooled. The pooled extract is then dried using a Turbovap nitrogen generator at 40 °C. The dehydrated concentrate is transformed with 1 mL aqueous methanol (95 parts methanol and 5 parts water containing 0.1% formic acid). The reconstituted extract is filtered using a 0.20 μm PTFE syringe filter in an LC ampule for LC-MS/MS investigation (Saxena et al., 2018).

8 Method Validation

Analytical methods play an important role in ensuring monitoring regulator of rest antibiotic in faunal-originated products. Appropriate and suitable method must be employed for assessment purposes. The "European Council (EC) Directive 96/23/

Table 6 MRM transitions for analysis of multiclass antibiotics in LC-MS/MS

Group	Compounds/Molecular formulae	Mol. mass (g/mol)	Forerunner mass (m/z)	Product mass (m/z)
β lactams	Amoxicillin trihydrate $C_{16}H_{19}N_3O_5S.3H_2O$	419.45	398.00	381.00
				349.20
	Ampicillin trihydrate $C_{16}H_{19}N_3O_4S.3H_2O$	403.5	382.20	223.00
				333.20
	Cloxacillin sodium $C_{19}H_{17}ClN_3NaO_5S$	475.88	436.20	277.00
				160.00
Tetracyclines	Tetracycline hydrochloride $C_{22}H_{25}ClN_2O_8$	480.9	445.20	410.10
				154.10
	Oxytetracycline $C_{22}H_{24}N_2O_92H_2O$	496.5	461.20	201.10
				426.10
	Chlortetracycline hydrochloride $C_{22}H_{23}ClN_2O_8HCl$	515.3	479.00	462.00
				444.00
	Doxycycline hyclate $(C_{22}H_{24}N_2O_8).2HCl.C_2H_6O.H_2O$	512.94	445.00	428.00
				410.00
	4 epi tetracycline $C_{22}H_{25}ClN_2O_8$	480.9	445.10	410.20
				428.20
Sulphonamides	Trimethoprim $C_{14}H_{18}N_4O_3$	290.32	291.10	230.20
				261.20
	Sulfacetamide $C_8H_{10}N_2O_3S$	214.24	215.00	156.00
				92.00
	Sulfadiazine $C_{10}H_{10}N_4O_2S$	250.3	251.10	108.00
				156.10
	Sulfathiazole $C_9H_9N_3O_2S_2$	255.32	256.10	156.10
				92.10
	Sulfapyridine $C_{11}H_{11}N_3O_2S$	249.29	250.10	156.10
				108.10
	Sulfamerazine $C_{11}H_{12}N_4O_2S$	264.3	265.10	156.00
				108.10
	Sulfamethoxypyridazine $C_{11}H_{12}N_4O_3S$	280.3	281.10	155.90
				92.10
	Sulfaethoxypyridazine $C_{12}H_{14}N_4O_3S$	294.33	295.00	156.00

(continued)

Table 6 (continued)

Group	Compounds/Molecular formulae	Mol. mass (g/mol)	Forerunner mass (m/z)	Product mass (m/z)
				108.00
	Sulfamethoxazole $C_{10}H_{11}N_3O_3S$	253.28	254.10	156.10
				92.10
	Sulfaquinoxaline $C_{14}H_{12}N_4O_2S$	300.34	301.10	156.10
				92.10
	Sulfadimethoxine $C_{12}H_{14}N_4O_4S$	310.3	311.10	156.10
				92.10
	Sulfadoxine $C_{12}H_{14}N_4O_4S$	310.33	311.10	140.10
				65.00
	Sulfanilamide $C_6H_8N_2O_2S$	172.2	156.00	108.00
				92.00
	Sulfachlorpyridazine $C_{10}H_9ClN_4O_2S$	284.72	285.00	156.00
				108.10
Fluoroquinolones	Ciprofloxacin $C_{17}H_{18}FN_3O_3$	331.34	332.10	231.10
				288.20
	Enrofloxacin $C_{19}H_{22}FN_3O_3$	261.25	360.30	342.20
				316.40
	Norfloxacin $C_{16}H_{18}FN_3O_3$	319.3	320.00	302.00
				231.00
	Danofloxacin mesylate $C_{20}H_{24}FN_3O_6S$	453.48	358.20	340.20
				314.10
	Difloxacin hydrochloride $C_{21}H_{19}F_2N_3O_3HCl$	435.85	400.10	382.10
				356.10
	Sarafloxacin hydrochloride $C_{20}H_{17}F_2N_3O_3HCl$	421.83	386.00	368.00
				342.00
	Flumequine $C_{14}H_{12}FNO_3$	261.25	262.10	202.10
				174.10
	Ofloxacin $C_{18}H_{20}FN_3O_3$	361.37	362.00	261.00
				317.90
Quinolones	Oxolinic acid $C_{13}H_{11}NO_5$	261.23	262.10	244.00
				216.10
	Nalidixic acid $C_{12}H_{12}N_2O_3$	232.24	233.10	215.20
				187.10
Albendazole	Albendazole $C_{12}H_{15}N_3O_2S$	265.3	266.00	234.00

(continued)

Table 6 (continued)

Group	Compounds/Molecular formulae	Mol. mass (g/mol)	Forerunner mass (m/z)	Product mass (m/z)
				191.00
	Albendazole sulfone $C_{12}H_{15}N_3O_4S$	297.33	298.20	266.10
				159.10
	Albendazole sulfoxide $C_{12}H_{15}N_3O_3S$	281.33	282.20	240.10
				208.10
	ABZ 2 amino sulfone $C_{10}H_{13}N_3O_2S$	239.29	240.10	198.10
				133.20

EC" provides guidelines on quality criteria of any analytical method that will be used to test antibiotic remnants in food products of animal origin. The directive was followed by a legislation, named "Commission Decision 2002/657/EC," and provides a detailed acceptance criteria parameter ensuring the performance of an analytical method intended for antibiotic residue testing in food. The legislation also provides a guideline on the interpretation of results.

Analytical method for antibiotic residue testing must be confirmatory, providing information on the molecular structure and mass of the compound. Ideally chromatographic separation followed by mass spectrometry detection should be used. Chromatographic separation becomes important for isobaric compounds. Use of internal standards in quantification provides better calibration curve, selectivity, and recovery correction. Hence, it should be added to the samples and matrix-fortified standards at the same concentration before extraction. External standard addition method is followed when no internal standard is available for use. After standard spiking in the sample, the corresponding increase in peak area should be proportional to the amount of spiked analyte. A retention time variation of ± 0.1 min is allowed. The analysis must include quality control sample (spiked at RPA level), matrix blank, and reagent blank. A typical sequence list in LC-MS/MS is as follows:

> Reagent blank, compliant control sample, sample to be confirmed, compliant control sample again, and finally known noncompliant control sample. Any variation from the above protocol should be justified.

Determination of "relative retention time (RRT)" is an important criterion. The RRT of the analyte is the degree of the chromatographic retaining period time of the analyte to that of the local calibrations. RRT should be similar to the measured solution/skewed sample, where a tolerance of $\pm 2.5\%$ is allowed. The MS detection of the antibiotic residues is carried out in MRM mode. The ratio of the quantifier and qualifier ion is known as ion ratio and should not vary more than 30% than the ion ratio observed in calibration standards/spiked standards.

To guarantee that the investigative method is fit for purpose for the presentation in regular screening, the method has to be authenticated for the following parameters:

- *Specificity*: Specificity is the capacity of an analytical technique to distinguish among target analyte and co-extractives. If an analyte peak is detected in the matrix blank, it should be $\leq$30% of the reporting limit.
- *Stability*: Stability provides a measure of compounds' degradation behavior under different storage and handling conditions. Degraded analytical standards may introduce serious error in the result; hence, stability of analyte in calibration solution should be monitored stringently.
- *Calibration curve*: Linearity is determined by linear regression of analyte response to concentration of calibration standards. Matrix effect from the co-extractives determined the type of calibration curve that could be using solvent "standard, matrix-matched standard or matrix-fortified standard." Matrix-fortified standard should ideally be used when the matrix effect is more than 15%. Calibration curve should have a minimum of 5 points, including zero.
- *Recovery*: Recovery is the determined concentration expressed as the percentage of the spiked concentration. When "Certified Reference Material" is unavailable, the average recovery of the analyte using the process should be confirmed through authentication tests. Recovery ranges of 50–120% are acceptable when the skewed concentration is less than 1 μg kg^{-1}. However, for skewed concentration that varies from 1 to 10 μg kg^{-1}, the adequate recovery is from 70% to 110%.
- *Repeatability*: The percentage comparative SD of six independent measurements performed by an analyst in a particular laboratory provides the repeatability of the method.
- *Within-laboratory reproducibility*: The percentage relative standard deviation of independent measurements (six samples each time) carried out on three dissimilar days by three diverse analysts in a particular laboratory provides the internal laboratory reproducibility.
- *Reproducibility*: Reproducibility of the method is determined in a similar manner through inter-laboratory comparison studies.
- *Decision limit (CCα)*: CCα is the statistically determined concentration from the validation data, which is used for determining compliance or noncompliance of a sample with an error possibility α. A model with analyte concentration above this decision limit is considered noncompliant even if the value is lower than RPA.
- *Detection capability (CCβ)*: CCβ is a measure of detection capability of the method with an error probability of β.
- *Ruggedness (minor change)*: A rugged analytical method does not get affected by minor but reasonable changes in the analytical method. Such changes could be different analysts, different batches of chemicals and consumables, extraction time, centrifugation speed, etc.
- *Measurement uncertainty (MU)*: MU is the measure by which a reported result can deviate from laboratory to laboratory compared to the true concentration. Similar to CCα and CCβ, MU is determined statistically using the validation data and is a requirement of regulatory agencies such as the "National Accreditation Board for Testing and Calibration Laboratories (NABL)."

9 Conclusion

Antibiotic residues in aquatic food may contribute to the AMR development. Residues of banned antibiotics in the aquatic food are still found worldwide and are an extremely serious human well-being apprehension. Regulatory agencies and national food safety bodies worldwide have strictly implemented residue control regime for antibiotics in products of animal origin. Liquid chromatograph with "triple quadrupole mass spectrometer" is identified to be the most advanced and appropriate standard for unambiguous detection and accurate quantification of antibiotics in aquatic food. The analytical methods for antibiotic estimation have to be proved fit for purpose by validation as per the parameters mentioned in internationally recognized method validation guidelines. For banned antibiotics, single-residue analytical method is preferred. Nevertheless, efforts have been made to advance multiresidue analytical procedures that can analyze multiple antibiotics in a single method. But diverse chemical nature of the antibiotic classes makes it difficult to extract and analyze them in a single method.

References

Burridge, L., Weis, J. S., Cabello, F., Pizarro, J., & Bostick, K. (2010). Chemical use in salmon aquaculture: A review of current practices and possible environmental effects. *Aquaculture, 306*, 7–23. https://doi.org/10.1016/j.aquaculture.2010.05.020

CAA. (2020). *List of certified antibiotic-free aquaculture inputs*. https://www.caa.gov.in/uploaded/doc/. Accessed Jan 2023.

Cañada-Cañada, F., Muñoz de la Peña, A., & Espinosa-Mansilla, A. (2009). Analysis of antibiotics in fish samples. *Analytical and Bioanalytical Chemistry, 395*, 987–1008. https://doi.org/10.1007/s00216-009-2872-z

Chen, J., Sun, R., Pan, C., Sun, Y., Mai, B., & Li, Q. X. (2020). Antibiotics and food safety in aquaculture. *Journal of Agricultural and Food Chemistry, 68*, 11908–11919. https://doi.org/10.1021/acs.jafc.0c03996

Defoirdt, T., Sorgeloos, P., & Bossier, P. (2011). Alternatives to antibiotics for the control of bacterial disease in aquaculture. *Current Opinion in Microbiology, 14*, 251–258. https://doi.org/10.1016/j.mib.2011.03.004

Desmarchelier, A., Savoy, M. C., Delatour, T., & Mottier, P. (2022). Extended coverage of veterinary drug residues in food by LC-HRMS to ensure food compliance and prevent the spread of antimicrobial resistance. *Microchemical Journal, 183*, 108057. https://doi.org/10.1016/j.microc.2022.108057

Directorate of Fisheries Norway. (2001, August 14). *Monitoring of medicine residues and undesirable substances in cultivated fish*. http://www.fiskeridir.no/english/pages/news/facts/monitoring.html. Accessed Jan 2023.

EU. (2003). 2003/181/EC: Commission decision of 13 March 2003 amending decision 2002/657/EC as regards the setting of minimum required performance limits (MRPLs) for certain residues in food of animal origin (Text with EEA relevance) (notified under document number C (2003) 764). http://data.europa.eu/eli/dec/2003/181(1)/oj. Accessed Jan 2023.

EU. (2010). Commission regulation (EU) No 37/2010 of 22 December 2009 on pharmacologically active substances and their classification regarding maximum residue limits in foodstuffs of animal origin. http://data.europa.eu/eli/reg/2010/37(1)/oj. Accessed Jan 2023.

EU. (2017). Regulation (EU) 2017/625 of the European Parliament and of the Council of 15 March 2017 on official controls and other official activities performed to ensure the application of food

and feed law, rules on animal health and welfare, plant health and plant protection products, amending Regulations (EC) No 999/2001, (EC) No 396/2005, (EC) No 1069/2009, (EC) No 1107/2009, (EU) No 1151/2012, (EU) No 652/2014, (EU) 2016/429 and (EU) 2016/2031 of the European Parliament and of the Council, Council Regulations (EC) No 1/2005 and (EC) No 1099/2009 and Council Directives 98/58/EC, 1999/74/EC, 2007/43/EC, 2008/119/EC and 2008/120/EC, and repealing Regulations (EC) No 854/2004 and (EC) No 882/2004 of the European Parliament and of the Council, Council Directives 89/608/EEC, 89/662/EEC, 90/425/EEC, 91/496/EEC, 96/23/EC, 96/93/EC and 97/78/EC and Council Decision 92/438/EEC (Official Controls Regulation)Text with EEA relevance. http://data.europa.eu/eli/reg/2017/625/oj. Accessed Jan 2023.

EU. (2019). Commission regulation (EU) 2019/1871 of 7 November 2019 on reference points for action for non-allowed pharmacologically active substances present in food of animal origin and repealing Decision 2005/34/EC (Text with EEA relevance). http://data.europa.eu/eli/reg/2019/1871/oj. Accessed Jan 2023.

EU. (2021). Commission implementing regulation (EU) 2021/808 of 22 March 2021 on the performance of analytical methods for residues of pharmacologically active substances used in food-producing animals and on the interpretation of results as well as on the methods to be used for sampling and repealing Decisions 2002/657/EC and 98/179/EC. http://data.europa.eu/eli/reg_impl/2021/808/oj. Accessed Jan 2023.

FAO. (2021). *Maximum residue limits (MRLs) and Risk management recommendations (RMRs) for residues of veterinary drugs in Foods-CXM2*. https://www.fao.org/fao-who-codexalimentarius/codex-texts/maximum-residue-limits/en/. Accessed Jan 2023.

Fenn, J. B., Mann, M., Meng, C. K., Wong, S. F., & Whitehouse, C. M. (1989). Electrospray ionization for mass spectrometry of large biomolecules. *Science, 246*, 64–71. https://doi.org/10.1126/science.2675315

FSSR. (2011). *Food safety and standards (contaminants, toxins and residues) regulation*. https://fssai.gov.in/upload/uploadfiles/files/Compendium_Contaminants_Regulations_28_01_2022.pdf. Accessed Jan 2023.

Guichard, P., Laurentie, M., Hurtaud-Pessel, D., & Verdon, E. (2021). Confirmation of five nitrofuran metabolites including nifursol metabolite in meat and aquaculture products by liquid chromatography-tandem mass spectrometry: Validation according to European Union decision 2002/657/EC. *Food Chemistry, 342*, 128389.

Health Canada. (2023). *List of maximum residue limits (MRLs) for veterinary drugs in foods*. https://www.canada.ca/en/health-canada/services/drugs-health-products/veterinary-drugs/maximum-residue-limits-mrls/list-maximum-residue-limits-mrls-veterinary-drugs-foods.html. Accessed Jan 2023.

Jian, Z., Zeng, L., Xu, T., Sun, S., Yan, S., Yang, L., Huang, Y., Jia, J., & Dou, T. (2021). Antibiotic resistance genes in bacteria: Occurrence, spread, and control. *Journal of Basic Microbiology, 61*, 1049–1070. https://doi.org/10.1002/jobm.202100201

Jongedijk, E., Fifeik, M., Arrizabalaga-Larrañaga, A., Polzer, J., Blokland, M., & Sterk, S. (2023). Use of high-resolution mass spectrometry for veterinary drug multi-residue analysis. *Food Control, 145*, 109488. https://doi.org/10.1016/j.foodcont.2022.109488

Love, D. C., Rodman, S., Neff, R. A., & Nachman, K. E. (2011). Veterinary drug residues in seafood inspected by the European Union, United States, Canada, and Japan from 2000 to 2009. *Environmental Science & Technology, 45*, 7232–7240. https://doi.org/10.1021/es201608q

Luo, X., Yu, Y., Kong, X., Wang, X., Ji, Z., Sun, Z., & You, J. (2019). Rapid microwave assisted derivatization of nitrofuran metabolites for analysis in shrimp by high performance liquid chromatography-fluorescence detector. *Microchemical Journal, 150*, 104189.

Melekhin, A. O., Tolmacheva, V. V., Apyari, V. V., & Dmitrienko, S. G. (2022). Current trends in analytical strategies for the chromatographic determination of nitrofuran metabolites in food samples. An update since 2012. *J Chromatogr A, 1685*, 463620. https://doi.org/10.1016/j.chroma.2022.463620

Robert, C., Gillard, N., Brasseur, P. Y., Ralet, N., Dubois, M., & Delahaut, P. (2015). Rapid multiresidue and multi-class screening for antibiotics and benzimidazoles in feed by ultra high performance liquid chromatography coupled to tandem mass spectrometry. *Food Control, 50*, 509–515.

Rønning, H. T., Einarsen, K., & Asp, T. N. (2006). Determination of chloramphenicol residues in meat, seafood, egg, honey, milk, plasma and urine with liquid chromatography–tandem mass spectrometry, and the validation of the method based on 2002/657/EC. *Journal of Chromatography. A, 1118*, 226–233. https://doi.org/10.1016/j.chroma.2006.03.099

Santos, L., & Ramos, F. (2018). Antimicrobial resistance in aquaculture: Current knowledge and alternatives to tackle the problem. *International Journal of Antimicrobial Agents, 52*, 135–143. https://doi.org/10.1016/j.ijantimicag.2018.03.010

Saxena, S. K., Rangasamy, R., Krishnan, A. A., Singh, D. P., Uke, S. P., Malekadi, P. K., Sengar, A. S., Mohamed, D. P., & Gupta, A. (2018). Simultaneous determination of multi-residue and multi-class antibiotics in aquaculture shrimps by UPLC-MS/MS. *Food Chemistry, 260*, 336–343.

USDA. (2023). *Maximum residue limit database*. https://bcglobal.bryantchristie.com/. Accessed Jan 2023.

USFDA. (2022). *Approved aquaculture drugs*. https://www.fda.gov/animal-veterinary/aquaculture/approved-aquaculture-drugs. Accessed Jan 2023.

Varghese, R. S., Zhou, B., Nezami Ranjbar, M. R., Zhao, Y., & Ressom, H. W. (2012). Ion annotation-assisted analysis of LC-MS based metabolomic experiment. *Proteome Science, 10*, 1–9. https://doi.org/10.1186/1477-5956-10-S1-S8

Wang, J., MacNeil, J. D., & Kay, J. F. (2011). *Chemical analysis of antibiotic residues in food*. John Wiley & Sons.

Wang, X., Wang, S., & Cai, Z. (2013). The latest developments and applications of mass spectrometry in food-safety and quality analysis. *TrAC Trends in Analytical Chemistry, 52*, 170–185. https://doi.org/10.1016/j.trac.2013.08.005

Antimicrobial Stewardship in India: Success and Challenges

Sangeeta Sharma and Renu Gupta

Contents

Abstract

India has developed National Action Plan to combat AMR (NAP-AMR), and activities to implement the action plan are gaining momentum. Most of the antimicrobial stewardship (AMS) strategies are implemented in single organization or healthcare sector, and progress has been limited due to gaps in implementation strategies and research. Vertical integration within each sector and horizontal integration between sectors is essential to consolidate gains through "One Health" approach along with assessment of degree of integration of AMS across the whole health economy and its impact.

S. Sharma
Neuropsychopharmacology, Institute of Human Behaviour and Allied Sciences, Delhi, India

R. Gupta (✉)
Microbiology, Institute of Human Behaviour and Allied Sciences, Delhi, India
e-mail: renugupta2929@gmail.com

M. P. Mothadaka et al. (eds.), *Handbook on Antimicrobial Resistance*,
https://doi.org/10.1007/978-981-19-9279-7_35

Implementation and monitoring of Schedule H1 is weak since majority of healthcare is provided through the unregulated private sector. Regulation can be made effective only if these are implemented. Engagement with sector-specific regulatory organizations and state governments is required to strengthen mechanisms for production, sale, and use of antimicrobials.

Implementing full-scale AMS program (AMSP) is a resource-intensive exercise requiring investments to build in AMS structures (governance, human resources, laboratory support, information technology) and alignment of processes. Education on fundamental AMS principles also requires to be integrated into preclinical and clinical curriculum of medical and allied streams.

For AMS at healthcare facility level, active involvement of clinical leaders and cross specialty engagement are crucial for its success. Particularly role of the clinical pharmacist and nurses in AMS is untapped. Pre-authorization and/or prospective audit and feedback are core components of any AMSP for optimization of antimicrobial use. The decisions to implement one or the other AMS strategy or a combination of strategies should be based on the availability of facility-specific resources for consistent implementation. Surveillance of antimicrobial use and AMR is the key component to enable designing of facility specific interventions and monitoring success of AMSP.

Keywords

AMS · Antimicrobial stewardship · AMSP · Antimicrobial stewardship program · One Health · Human sector · Nonhuman sector · AMR

1 Introduction

The threat of antimicrobial resistance (AMR) is rapidly progressing and intensifying. AMR is directly linked with inappropriate antimicrobial use (AMU). Antimicrobials are used inappropriately in both human and nonhuman sectors (animals, fish, agriculture) for therapeutic, prophylactic, metaphylactic purposes and for growth promotion in animals, thereby leading to accelerated rates of AMR (O'Neill, 2014; Pokharel et al., 2020). It is estimated that 20–50% of human use is unnecessary and 40–80% of animal use is highly questionable (BSAC, 2018). AMR pathogens may arise in any setting utilizing antibiotics in any form, i.e., human health, animal health, agriculture, and environment (Gandra et al., 2017). AMR besides being detrimental to the species in which they arise also poses a risk to fellow humans, animals, and environment as AMR pathogens/genes can travel across human-animal-environment interface ultimately risking the ecosystem.

The key factors for rising AMR in human sector are continuing inappropriate prescribing, inadequate infection prevention and control practices, drug promotions, and limited access to essential lifesaving antimicrobials when needed vis-à-vis easy access to all antimicrobials as over-the-counter medicines without a prescription.

Self-medication, use of leftover medicines and sharing of medicines, and informal consultations further add to the problem. Global travel facilitates cross-border transfer of resistance (Gandra et al., 2017). Other reasons for inappropriate prescribing are inadequate knowledge among prescribers, diagnostic uncertainties combined with nonavailability or non-utilization of diagnostic services, lack or non-utilization of standard treatment guidelines/formularies, fear of litigations and poor patient outcomes, dispensing prescribers, and lastly inappropriate real or perceived patient demand.

AMR is a multidimensional and multi-sectoral problem, and the only possible solution to combat this escalating threat of AMR is to reduce antibiotic use in all the sectors by adopting "One Health" approach with mutual coordination, collaboration, and communication (Bhatia, 2017). The rapid rise of AMR with its associated technical, economic, and social impact has made it essential to rigorously implement antimicrobial stewardship (AMS) for containment of AMR. A high level of political, social, and individual commitment is urgently required for containment of this unstoppable devastating biological phenomenon (Bhatia et al., 2019).

Understanding the magnitude of the problem of AMR, India developed its National Action Plan to contain AMR (NAP-AMR) in 2017 following "One Health" approach after extensive, multi-sectoral consultative process (MoHFW, 2017). The overarching goal of NAP is to effectively combat AMR and contribute toward the global efforts to tackle this public health problem. In fact, the work toward tackling this menace of AMR had begun much before the NAP-AMR with constitution of National Task Force on AMR Containment in 2010 followed by the development of National Policy for Containment of AMR, the Jaipur Declaration, and the inclusion of antimicrobial containment in the 12th 5-year plan in 2011 (WHO, 2011a; MoHFW, 2011). Also, infection management in reproductive, maternal, and child health program, 2013; National Guidelines on Clean Hospitals (Kayakalp Guidelines), 2015, were rolled out in cognizance of infection prevention being a key component to combat AMR (MoHFW, 2013, 2015). The NAP-AMR has integrated several such initiatives in the last decade.

Strategy 4 of NAP-AMR is specifically directed to optimize the use of antimicrobial agents in human and nonhuman sectors. Antimicrobial stewardship (AMS) is defined as "coordinated interventions designed to improve and measure the appropriate use of antimicrobials by promoting the selection of the optimal drug regimen including dosing, duration of therapy, and route of administration" (Barlam et al., 2016). The gains from AMS are improved patient outcomes, quality of care, and reduced adverse events. AMS also leads to savings due to unnecessary healthcare costs by higher cure rates and reduced treatment failures due to correct prescribing for correct indications, and optimized resource utilization (Nathwani et al., 2019).

AMS programs hold promise to prolong the lifespan of existing antimicrobials besides limiting the adverse economic impacts. This chapter briefly discusses the overarching principles of AMS programs with its goals, core structures, tools, and mechanisms for implementation, success, challenges, and future directions in India.

2 AMS Framework in India

NAP-AMR requires coordination among multiple government agencies involving health, education, environment, livestock, and legislative to combat emergence of AMR and linkages with infection control and antimicrobial use and resistance surveillance. The Indian Council of Medical Research (ICMR), National Centre for Disease Control (NCDC), Central Drugs Standard Control Organisation (CDSCO), Food Safety and Standards Authority of India (FSSAI), and National Health Mission (NHM) have been identified as key stakeholders along with 11 ministries and other organizations. The NCDC has been designated as the focal point for implementation, coordination, and monitoring and evaluation (M&E) of the NAP-AMR program.

Development of NAP-AMR is a major advancement, but this top-level action plan needs implementation at the state level and supplementation at the health facility level. Considering India's diverse health sector, mix of public and private providers, and health being a state subject, each state government has to set its own legislative, administrative, financing, and healthcare delivery models as per their priorities and context. Therefore, implementing a pan-India antimicrobial stewardship program (AMSP) is a challenging and gigantic task and requires endeavors at policy, administrative, and service provision level (Walia et al., 2019a). So far only three states (Kerala, Madhya Pradesh, and Delhi) have rolled out their state action plans based on their current situation, issues, and challenges. A few other states are in process in developing their action plan.

The essential AMSP components at national and facility level are discussed below (Table 1).

2.1 Regulations

Enactment and enforcement of AMR regulatory framework is necessary for ensuring the optimization of AMU in humans, animals, and consequent environmental protection. To curb and control indiscriminate use of antimicrobials in both human and nonhuman sectors, several regulations mentioned below have been enacted:

- In India, although most of the antimicrobials are prescription drugs under Schedule H and require dispensing only against a valid prescription, but in reality almost all drugs can be obtained freely over-the-counter without a prescription. To reduce the over-the-counter sale of select third- and fourth-generation antibiotics like carbapenem, ertapenem, meropenem, tigecycline, daptomycin, some antitubercular drugs, etc., Schedule H1 was enacted in 2013 under the Drugs and Cosmetics Act, 1945 (MoHFW, 2013). Schedule H1 combines some of the requirements under Schedule X (i.e., requirement for a duplicate prescription, a separate license, and maintenance of meticulous storage and dispensing records) with Schedule H (i.e., retail dispensing only against a valid prescription) (Hazra, 2014).

Table 1 The core components of antimicrobial stewardship program

Core components	National/state/regional level	Facility level
Plans and strategies	National multi-sectoral coordination group Technical working group National AMS action plan Dedicated funds	Leadership commitment AMS leadership committee Multidisciplinary AMS team Dedicated AMS leader/champion Defined responsibility and lines of accountability
Education and training	Public awareness campaigns School education programs Undergraduate curriculum Standards for staffing, training, accreditation, and secured funding	AMS team training In-service training of all health professionals Access to updated educational resources
Regulations and guidelines	Legislation/regulations Essential medical list Access, watch, reserve list of antibiotics Standard treatment/infection control/AMSP guidelines	Institutional formulary with access, watch, & restricted categorization AMS action plan Institutional treatment & infection control guidelines Policies/formats for documentation
Supporting technologies and data	National surveillance system Access to essential diagnostic test	Access to microbiology laboratory and imaging Financial, human, and IT support
Monitoring	Dashboard for monitoring key performance indicators	Review/audits or point prevalence surveys Advice/feedback with specific action points
Research	Secured funding	Basic and operational research

Schedule H1 requires a warning printed in red on the box that these medicines are to be sold only on the prescription of a registered medical practitioner and a separate register recording patient's and prescriber's details along with the quantity dispensed. The drugs' control authority has the responsibility to enforce the regulation and conduct surprise checks to monitor sale of medicines under Schedule H1. Even after having this Schedule H1 for several years, awareness about this Schedule among the prescribers, pharmacists, and public is generally inadequate. Further, since this schedule does not provide any disincentives for prescribers, and its enforcement remains poor (Hazra, 2014).

- The regulation of antimicrobial sale is another challenge. Although prescription of all medicines is required to be in generic name, in reality, most drugs are prescribed and dispensed by the brand names due to incentives given by pharmaceutical companies (Porter et al., 2020). Moreover, a large component of antimicrobials is not prescription based (use by informal prescribers, AYUSH practitioners) and dispensed over-the-counter without a prescription. Learning from experience of government's restriction on bedaquiline (a new antitubercular class in 50 years for multidrug-resistant tuberculosis released for use under closely monitored conditions to enable optimal drug effectiveness and safety), similar restrictions may be considered on sale of critical antimicrobials.

- To preserve the effectiveness of the existing antimicrobials, use of certain medically important classes of antimicrobials (critically important antimicrobials (CIAs) the highest priority for human medicine), needs to be restricted to clearly defined indications while ensuring accessibility and affordability to low-income populations (WHO, 2018a). Immediate measures are required to ban or reduce the prophylactic use of these CIAs and their use in sub-therapeutic concentrations in animals as a growth promoter. Banning of mixing colistin (a drug of last resort), in a feed of the animals, is a welcome step in this direction (Singh, 2019). Presence of antimicrobial residues in animal food is a concern among the public and medical health professionals. Tolerance limits in foods of animal origin for some 43 antibiotics and veterinary drugs have been notified by the FSSAI by amending Food Safety and Standards Act, 2006 in 2019. However, there is a long way to go; a schedule needs to be enacted to phase out all antimicrobials of high importance being used for growth promotion.
- Drugs and Cosmetic Act 1945 was amended in 2012 in order to make it mandatory to mention the time to withhold food produced from animals (withdrawal period) on drug label itself after administration of drugs (such as 7 days for eggs and milk, 28 days for meat from poultry and mammals, and 500 days for fish meat). Its effective implementation in practice would play an important role in curbing misuse of antimicrobials.
- Biomedical Waste Management Rules, 1988, amended in 2016 provide recommendations for segregation, treatment, and disposal of expired or discarded medicines to prevent environment contamination (Ministry of Environment, 2016).
- The draft standards for antibiotic residues in pharmaceutical effluents and common effluent treatment plants developed by the Central Pollution Control Board under Environment (Protection) Amendment Rules, 2019, provide stringent limits for 121 antibiotics but are yet to be notified. India being one of the largest manufacturers of antibiotics, a large amount of these medicines finds its way in the waste without pre-treatment which can contaminate the environment. Therefore, stringent regulation is immediately required to limit antibiotic residues in wastewaters from all sources including healthcare, slaughterhouses, and pharmaceutical industry.

The compliance to regulatory mechanisms is highly fragmented and patchy in India, partly because of variable willingness and capacity of state governments in implementation of regulatory mechanisms (Porter et al., 2020). The Federal Government must engage with sector-specific regulatory organizations and state governments to strengthen and expand regulatory mechanisms for production, sale, and use of antimicrobials in all the sectors utilizing antimicrobials in any form. Only select antibiotics which do not induce cross-resistance in humans should be allowed for therapeutic purposes, and use of critically important antimicrobials as a growth promoter in animals should be restricted. There is need to co-design innovative implementable solutions rather than historical approach of reprimanding (legal

threats/penalties) considering the stakeholders' perspective and dynamics of production, profit, and prescribing practices.

2.2 Leadership Commitment and Governance

Gaining leadership/management commitment for AMS is a priority as without strategic input and lack of integration from higher level, the gains may be very limited despite existence of other structures like presence of excellent policies, infectious disease specialists with expertise on antibiotic use, etc.

AMSP involves commitment from many departments, and best performance requires active engagement of senior hospital executives. Senior leadership support is critical in achieving clinical provider's buy-in, creating a culture of clinical excellence and interdisciplinary team involvement, empowering AMS team, and establishing reinforcement structures (such as reporting mechanisms and measurement). Hospital leadership can ensure the program has sufficient budget, time, technology, and resources to achieve the program goals. Also, full-scale AMSP in any setting requires leadership support and commitment for ensuring availability of manpower (such as microbiologist, clinical pharmacologists/clinical pharmacist, infectious disease specialists), budget for AMS activities, IT-enabled information systems, etc.

A review of the published studies on implementation of AMSP at facility level in human healthcare revealed that only 6 of the 17 studies (35.2%) reported the implementation of all AMSP components whereas 11 studies revealed implementation of only one or more components (Bahl et al., 2020). Leadership support at health facility level was identified in only 9 of the 20 hospitals (45%) surveyed under Global Health Security Agenda-funded work on capacity building for AMR-HAI (Purva et al., 2019). The implementation is almost nonexistent in veterinary and animal agriculture facilities.

Leadership support can be leveraged by demonstrating the impact of AMSP on patient outcomes (improved results of publicly reported HAI), patient satisfactions, higher quality of care, and cost savings by reduction in cost of antimicrobials, and mortality due to resistant infections.

2.3 Accountability and Responsibility

A multidisciplinary AMS team consisting of healthcare professionals with adequate expertise and motivation is essential to perform AMS activities and to generate descriptive activity reports/outcomes and give feedback to continuously improve AMS program (Carling et al., 2003). Though membership of the AMS team is flexible, core members are an infectious diseases (ID) physician (or lead doctor or physician champion), a clinical microbiologist, a clinical pharmacist, and a nurse with expertise in IPC, along with other members as considered appropriate.

Identification of an AMS leader/champion with clearly defined responsibilities and collaboration with other committees such as drugs and therapeutics committee, IPC, and antibiotic policy committees is essential to establish lines of accountability and reporting. However, situation at present in India is dismal. The ICMR 2013 survey found notable shortage of ID physicians and clinical pharmacists in tertiary healthcare institutions (Walia et al., 2015). The survey of 20 hospitals by Purva and others found that accountability score and key support for AMSP were 53% and 58%, respectively (Purva et al., 2019).

AMSP team members must be chosen based on their expertise, credibility, and leadership skills to convince and influence seniors as well as frontline healthcare staff about the benefits of the AMSP. Active involvement of clinicians in AMS and cross-specialty engagement is crucial for the success of the stewardship program. The role of clinical leaders is to develop multidisciplinary treatment guidelines with criteria for use of specific antimicrobials, routine tracking, and monitoring of the prescribing practices. Further, leader's role is to provide feedback and guidance to take corrective actions based on the observed gaps, evaluation of cost benefit/effectiveness of stewardship initiatives (e.g., savings due to reduced healthcare-associated infection, savings on purchasing higher-end antimicrobials), and developing outcome measure matrix. It is important to understand the determinants or "unwritten rules" that influence antibiotic prescribing behaviors such as clinical autonomy and hierarchies within clinical peer specialties which may overrule policies, guidelines, and expert input (Charani et al., 2013).

Potential of the nurses is less recognized in India. Nurses being the largest workforce and being in position as team leaders can significantly contribute to the success of the AMSP. Studies have shown that nurse led AMS interventions significantly improved prescribing practices by questioning an antimicrobial order and application of their organizational and collaboration skills (Gillespie et al., 2013; Rout & Brysiewicz, 2017).

Pharmacists are an important pillar for any AMSP and can undertake multiple roles from procuring, dispensing, and monitoring of antimicrobials to implementation of targeted AMS interventions (Parente & Morton, 2018).

2.4 Infection Prevention and Control

Infection prevention and control (IPC) is an important pillar for any AMS program to reduce dependence on antibiotics and thus emergence and spread of AMR. IPC is one of the core elements in National Patient Safety Implementation Framework 2018–2025 (MoHFW, 2018–2025). IPC practices also decrease the transmission of pathogens (both sensitive and antibiotic resistant) and genes from one person/animal. The broad principles of IPC in humans and animals are the same (Table 2) (MOHFW, 2020; Byers, 2020).

Infection control and prevention supplements other strategies to tackle AMR. National guidelines for infection prevention control in healthcare facilities were developed by the NCDC in 2020 (MoHFW, 2020). ICMR has integrated IPC with AMS and enrolled nearly 40 surveillance sites to initiate and improve AMS

Table 2 Principles for reducing the requirement of antimicrobials

Category	Humans	Animals
Prevention of infection (to reduce the introduction of microorganisms)	Standard precautions (must for *all* patients): Hand hygiene Use of personal protective equipment Respiratory hygiene/cough etiquette Safe injection practices/ sharp safety Biomedical waste management Cleaning, disinfection, sterilization process Environmental cleaning and spill management Bundle approach for insertion and maintenance of devices	External biosecurity (bioexclusion): Minimize introduction of new animals with the already established animals in farm Minimize the number of herds from which new animals are chosen Select genetically resistant populations Consider individual animal resilience (adaptive capacity to changing environment) Clean and disinfect transport vehicles and containers All-in-all-out system, i.e., cleaning and disinfection of building/unit whenever new batch of animals is introduced Isolate sick animals before introduction Provide clean water, feed, air Exclude pests Barriers for human access (clean boots, clothing, etc.) to animal housing Environmental control, i.e., ventilation, exhaust pathogen-laden air after filtering to reduce pathogen load outside the farm building
	Vaccination and herd immunity	
Control of infection spread (to reduce transmission or spread of pathogen once an infection or disease is suspected or diagnosed)	Isolation precautions Contact Airborne Droplet	Internal biosecurity (biocontainment): Reduce stocking density, segregation of sick animals Housing (ventilation, temperature, drainage litter, bedding, stocking rate) Hygiene Infection control protocols
	Early identification of infectious pathogen Microbiological risk assessment Rapid reaction to stop/reduce transmission	
Surveillance and audits	To assess the burden, monitor trends and develop evidence-based polices to contain infections and address AMR	Flock or herd health surveillance program

concurrently with hospital infection prevention and control program (Walia et al., 2019b). However, these efforts need to be expanded to include a much larger number of institutions in both public and private sectors. Implementation of hospital infection control (HIC) program has also become a mandatory requirement for healthcare facilities under different accreditation schemes such as National Accreditation Board for Hospitals & Healthcare Providers (NABH) and National Quality Assurance Standards (NQAS). Further, creating a public demand would drive hospitals to adopt effective infection control practices.

2.5 Optimizing Antimicrobial Use

AMR is directly linked to the usage of antimicrobials. Overuse and misuse of antimicrobial are continuing because of diagnostic uncertainty, influence from medical representatives, and inadequate knowledge. The broad principles of optimizing AMU are listed in Table 3 (ICMR, 2018).

Key measures to optimize AMU are discussed below.

2.5.1 Awareness, Education, and Training

Antimicrobials are used and misused in all the sectors; therefore, targeted awareness, training, and education programs are required for public, prescribers, regulators, and all other stakeholders to bring out behavioral change and reduce inappropriate antimicrobial use. Prescribing is a complex, dynamic social process, influenced by many determinants. Inadequate training on prescribing antimicrobials combined with the lack of a functional policy on antimicrobial use is leading to unchecked growth of AMR in Indian hospitals.

Community Awareness

Emergence of community-associated resistant organisms (e.g., *Streptococcus pneumoniae*, *Staphylococcus aureus*, *and Escherichia coli*) in the last decade has also highlighted the need of community-based education and awareness programs as antimicrobials are consumed in much larger proportion in community compared to hospitals. Mass media campaigns can raise awareness and address misbeliefs and attitudes with regard to use of antibiotics. Several mass awareness campaigns to optimize AMU in community are being undertaken by different agencies such as Swachh Bharat Abhiyan initiative, redline campaign for Schedule H1 drugs for rationalizing AMU, and curb self-medication. "World Antibiotic Awareness Week" is celebrated every November with the objective to raise awareness about antimicrobials, infection control, and appropriate antibiotic use. It is being used as an opportunity to bring all stakeholders (policy makers, managers, academia, patients, communities, patient's groups, and nongovernment organizations) together towards raised awareness about this major public health problem. However, these activities require concerted efforts by all stakeholders and need to be organized regularly with greater public-private partnership with academia and professional organizations.

Table 3 Pearls for optimizing antimicrobial use

Justify the need of antimicrobials before initiating treatment
Collect necessary specimens for microbiological culture and sensitivity testing
Order other relevant tests to establish accurate diagnosis
Choose the appropriate antimicrobial agent based on facility guidelines considering:
Infectious agent/disease presentation
Spectrum of activity
Evidence of safety and efficacy
Risk of antimicrobial resistance selection and dissemination
Availability and cost
Optimize route and dosage regimens based on pharmacokinetic and pharmacodynamic principles
Initiate empiric therapy in life-threatening/serious illness with in 1 hour
Prefer narrow-spectrum over broad-spectrum, generic over branded, lower-generation over higher-generation antimicrobials
De-escalate empiric antimicrobial regimen based on antibiotic susceptibility results, other laboratory/diagnostic tests, and the clinical response
Reserve critically important antimicrobial agents for difficult/refractory infections and for human use
Restrict off-label use of antimicrobials
Minimize duration of antibiotic treatment in accordance with the laid therapeutic objectives
Document in drug chart:
Indication for antibiotic initiation, treatment protocol, and outcome objectives (clinical or microbiological cure)
Details of route of administration, dosage, frequency, rate of administration, duration of treatment, precautions, and withholding period (for animals)
Review or stop date on the drug chart for all antibiotics
Indication if any restricted antibiotics are prescribed, authorization obtained and from whom (full advice, time, and name of consultant microbiologist/ID physician to be recorded in the notes)
Record of all known allergies or no known allergy prominently in red ink in the allergy box on the front page of the patient's case sheet
Monitor patient daily for compliance and response to treatment
Review the need to continue antimicrobial therapy on a daily basis
Note all the adverse reaction and failure to respond
Consultation with microbiologist/ID physician where treatment is apparently failing

Education and Training

Curriculum: Earlier there was inadequate emphasis on providing training in prescribing antibiotics, and infection control in undergraduate medical curriculum with no emphasis on AMSP. Recently, in 2019 competence-based curriculum in undergraduate medical training has been introduced by the National Medical Commission (NMC) erstwhile Medical Council of India to inculcate necessary skills for prescribing. This new competency-based curriculum with horizontal (across basic sciences) and vertical (basic sciences embedded in a clinical context) integration is expected to foster collaboration between various disciplines to establish a coherent curriculum and bridge the gap between theory and practice; however, committed faculty trained in this new educational approach is crucial for its success (WHO, 2018b).

Further, the examination system in India is largely based on assessment of knowledge on subjective parameters, rather than assessment of core and soft skills acquired during their training. Examination systems should incorporate explicitly defined objective measures to ensure medical graduates are fit to practice and know prudent prescribing.

Pre-/In-service training: Many studies have reported inadequate knowledge and awareness about appropriate antimicrobial use among prescribers as the main reason for their misuse or overuse (Salsgiver et al., 2018). Pre-service training can be utilized to educate and build skills of new recruits on the correct prescribing practices based on evidence-based guidelines/antibiotic policy, etc. Compulsory in-service training with periodic assessments and retrainings, utilizing common educational methods like seminars and offline/online e-learning modules, problem-based learning modules, and case-based, bedside discussions result in improved prescribing practices. Other unique strategies such as social media platforms and educational video games snippets could also be employed to bring perceivable change in behavior (Satterfield et al., 2020). Frequent reiteration/reminders of key messages could be employed for sustained behavior modification. ICMR has developed concise AMS guidelines for improving antibiotic use for healthcare facilities so as to develop their capacity to design strategies, interventions, assessment, and monitoring of AMS practices (ICMR, 2017). ICMR is also conducting workshops for tertiary care facilities including medical colleges to educate healthcare workers on the principles of judicious antibiotic use, applying guidelines and algorithms to ensure improved patient outcomes, prevent antibiotic misuse, and minimize AMR.

Team approach: Most of these programs on AMS are directed toward prescribers. Strengthening capacity and human resource skills across all sectors is crucial for implementation of AMSP. A team-based approach with multi-stakeholder engagement and utilization of existing infrastructure and human resources for managing all aspects of infections needs to be developed (Carling et al., 2003). The AMS team can be expanded to leverage community medicine, public health experts, clinical pharmacist, and nurses. Further, need-based targeted trainings are required at all levels focusing on all the aspects of AMS including diagnosing infectious diseases; general principles of prescribing antimicrobials, dispensing, and administration; ordering correct tests; collecting appropriate samples; interpretation of reports and antibiograms; and appropriate documentation. In addition, for effective use of information technology, trainings on use of IT to audit, monitor, and track progress of AMS initiatives are required for specific group of the workers.

Creating an adequately trained workforce will require public-private partnerships and collaboration across professional associations/bodies. The tertiary care institutions should not only provide support to remote and resource-constrained settings and peripheral health centers but also could mentor and educate alternative medicine (AYUSH) practitioners.

2.5.2 Standard Treatment Guidelines (STGs)

Availability and easy access to up-to-date guidelines for antimicrobial usage is also an important prerequisite for appropriate prescribing. National Standard Treatment

Guidelines for AMU in infectious diseases was developed by the NCDC in 2016 followed by ICMR in 2017 (revised in 2022) (MoHFW, 2016; ICMR, 2017). These guidelines based on syndromic approach allow clinicians to choose the most appropriate antimicrobials according to the presenting signs and symptoms, commonly implicated pathogens and susceptibility data collected through national networks. However, availability of guidelines alone does not change behavior, as huge gaps exist between the production of evidence and its uptake in clinical practice settings. Therefore, these guidelines need to be introduced to the clinicians by means of repeated training program. Regular review and updation of guidelines along with dissemination to all cadres of staff are also essential to ensure trust and compliance.

Translating guidelines into practice requires developing and implementing facility-specific guidelines for AMSP, antibiotic formulary, antimicrobial prescribing, and hospital infection control. Frontline professionals have greater acceptance, if guidelines are developed based on local context and allow flexibility of prescribing. The facility-specific guidelines are also useful to ensure availability of recommended antimicrobials in formulary and to decide the empiric and definitive therapy based on syndromic approach, local epidemiology, susceptibility pattern, AMS principles, safety, and efficacy in the specific patient population. The ICMR survey in 2013 found encouraging results regarding availability of HIC guidelines in health facilities (75%); however, prescription guidelines and AMSP document were found in only 40% and 65% of healthcare facilities, respectively (Walia et al., 2015). The major difficulty in developing facility-specific antibiotic use guidelines could be largely because of inadequate AMR data and limited microbiology laboratory support. Moreover, development of guidelines requires considerable skills for critically appraising evidence.

Besides guidelines, standardized prescription chart, medical records, and transfer notes with demarcated space for documenting the indication and antibiotics prescribed (agent, dose, route, interval, duration, allergies, and review dates) serve as an important aid to review, de-escalate, and stop antibiotics in optimum timeframe.

Though antimicrobial stewardship has been included as an important quality accreditation standard, its implementation might be a long way off. Also, there is limited progress in the field of nonhuman sector. A booklet for reducing AMR in animals entitled *Drivers, Dynamics and Epidemiology of Antimicrobial Resistance in Animal Production* was released by FAO in 2016. However, national guidelines for animal sector are yet to be developed.

2.6 Diagnostic Stewardship

A robust microbiology laboratory is a prerequisite for any AMSP to identify pathogens, capturing the magnitude and trend of AMR across human, animal, and environment sectors. Access to microscopy, culture, and sensitivity reports not only allows for accurate diagnosis and targeted antibiotic treatment but also enables formulation of local treatment guidelines based on cumulative antibiograms and surveillance of resistant pathogens with integration into IPC. Automation of culture

and sensitivity testing allows early identification and susceptibility reports with resultant decrease in duration of broad-spectrum empirical therapy. Molecular diagnostic support facilitates detection of difficult to culture pathogens in useful timeframe. Besides microscopy/culture support, access to other quality assured point of care, rapid diagnostic tests (biomarkers), therapeutic drug monitoring (TDM), and imaging services is essential to establish alternate diagnosis and escalate or de-escalate treatment as culture-based test results are often delayed or negative. Available evidence shows that point-of-care diagnostic testing by rapid antigen and rapid immunoassay are associated with reduced antibiotic use and unnecessary investigations such as chest radiographs or other blood and urine tests (Messacar et al., 2016).

Unfortunately, the capacity of many health facilities to provide diagnostic support is still inadequate, and, in the absence, or limited diagnostic services, the clinicians are compelled to rely on clinical judgement for prescribing just-in-case antimicrobials (Bhattacharya et al., 2019). There is gross shortage of microbiologists and ID specialists in the country for standardized laboratory workup, creation of antibiograms, and analysis of surveillance data (Walia et al., 2015). Creation of postdoctoral training courses in infectious diseases in all major teaching hospitals with training of microbiologists in infection control and antimicrobial stewardship is urgently required. ICMR began supporting laboratory strengthening by providing necessary resources to build infrastructure and recruit manpower for generating AMR data. National Essential Diagnostics List for different health care levels has also been rolled out by ICMR toward laboratory strengthening and diagnostic stewardship (ICMR, 2019).

Besides improving access to basic diagnostic support, investments toward establishing antifungal and antiviral diagnostic facilities, automated interpretive diagnosis with integration into machine learning, and host gene expression studies also need to be explored.

2.6.1 Antibiograms

Antibiograms allow initiation of appropriate empiric therapy. A single hospital-wide antibiogram is not sufficient as there may be differences in type of pathogens implicated and susceptibility patterns in different patient care areas and communities served (AMR pathogens may be more common in an intensive care unit compared to a low-risk unit). Antibiograms should be stratified based on patient risk status (community-acquired infection/healthcare-associated infection/immunocompromised), type of infection, and population age group (pediatrics/adult) (WHO, 2011b).

Microbiology laboratory should ensure selective reporting or cascade reporting of antimicrobials as reporting of all the tested antimicrobials leads to inappropriate choices by the prescribers (Liao et al., 2020). Selective reporting refers to reporting of susceptibility results of a limited number of antibiotics rather than whole panel of antibiotics tested. Preferential reporting of first-line antibiotics should be encouraged with release of results of second-line antibiotics only if an organism is resistant to the first-line antibiotic or patient clinical condition does not allow use of certain

antibiotics, e.g., linezolid and daptomycin results should be released only when enterococci are non-susceptible to ampicillin and vancomycin.

2.6.2 Therapeutic Drug Monitoring (TDM)

Monitoring the serum concentration of antibiotics allows adjustments of the dose of antibiotics in patients based on pharmacokinetic (PK) and pharmacodynamics (PD) parameters. Accordingly, the dose of the antibiotic can be adjusted to achieve the desired concentrations of antibiotics for the defined time periods to bring in the maximal antimicrobial effect. TDM of antibiotics is useful for optimal adjustment of daily dose of concentration-dependent antibiotics. Dosing strategies based on PK/PD principles for once-daily dosing aminoglycosides and continuous infusion with vancomycin are effective to reduce nephrotoxicity, length of hospital stay, and mortality (Barlam et al., 2016; Onufrak et al., 2016). TDM technology can be utilized to deliver personalized antibiotic dosing schemes. However, availability of the TDM facility is limited, currently available at select tertiary care facilities only, which needs to be expanded.

2.7 AMSP Strategies

Formulary restriction and/or pre-authorization, automatic stop orders, selective susceptibility reporting from microbiology laboratory, and antibiotic cycling are some of the active strategies to reduce the overall antibiotic use. Among these strategies, *front-end strategies*, i.e., formulary restriction with pre-authorization, and *back end strategies*, i.e., prescription audit and feedback, are core components of any AMSP. Any of these strategies or a combination may be initiated depending upon the available resources (Barlam et al., 2016). ICMR 2013 survey reported that in India, only 30% of healthcare institutes implemented AMSP strategies (Walia et al., 2015). AMR-HAI survey revealed that prescription-specific and broad interventions to improve antibiotic use were implemented in 52% and 33% of facilities, respectively (Purva et al., 2019). Indian hospitals though have started with low-hanging fruits such as developing prescription policies, restricting the usage of higher antibiotics, enforcing education, but there is a need to expand it gradually to encompass a maximum number of strategies. The major AMSP strategies identified are as follows.

Formulary restrictions, is placing restrictions on the prescribing of certain antimicrobials available in the hospital's formulary according to approved criteria such as indications, prescribers, services (OPD, IPD, emergency, and ICUs), patient populations, or any combination of these.

Pre-authorization is a strategy to reduce use of certain antibiotics by imposing requirement of seeking prior approval from a designated person before prescribing. Pre-authorization is usually implemented for use of empirical broad-spectrum antibiotics and combination therapy for initial 48–72 hours until culture reports are available. These restrictions can be incorporated into antibiotic ordering forms (either paper or electronic format) to trigger or prompting review once culture report is available. Pre-authorization brings significant reduction in the use of the restricted

antibiotics and associated costs. Real-time availability of the person providing approval and communication with the requesting clinician and the AMSP team are some of the challenges in the implementation of this strategy. However, unwanted negative impact of this strategy is delay in initiation of treatment with corresponding increase in use of not-restricted antimicrobials; therefore, changes in usage patterns of alternative treatment modalities in the wake of restrictions on antibiotics need monitoring. To avoid the unnecessary delays in initiation of life-saving but restricted antibiotics, institutions often allow administration of the restricted antibiotic overnight or for 48–72 hours until approval can be obtained. Further, it has been shown that direct chart reviews for optimization by AMS teams are more effective than off-hour approvals by authorized personnel (Parente & Morton, 2018).

In automatic stop orders, antibiotic administration is stopped automatically after a designated time, if duration of treatment is not specified, e.g., automatic stop orders after a single dose of surgical antibiotic prophylaxis or empirical therapy with broad-spectrum antibiotics will be stopped after 48 hours if review to escalate, de-escalate, or continue same treatment is not documented. It reduces the duration of unnecessary treatment and prompts prescriber to review and reorder therapy, if indicated.

Antimicrobial cycling is scheduled rotation of antimicrobials used in a particular setting (hospital or unit OPD, ICU) to reduce the selection pressure and propagation of AMR pathogens. Antibiotic cycling involves withdrawal of an antibiotic/antibiotic class from use (within a ward or an institution) for a designated period and replacement with another antibiotic from a different class having a similar spectrum of activity.

AWaRe classification: There is a potential for misuse and overuse of second- and third-line antimicrobials in primary/secondary care settings with underutilization of first-line drugs (including penicillins). While on one hand restriction on availability of newer-generation antimicrobials according to the service level or providers is required, on the other hand there is a need to ensure regular availability and accessibility of first-line antibiotics. AWaRe classifies antibiotics into Access, Watch, and Reserve category to assist the policy makers to set performance targets and guide its optimal use (WHO 2019). Access group generally contains narrow-spectrum antibiotics used for most common clinical infection syndromes whose access should be improved. Watch group comprises of generally broader-spectrum antibiotic classes (highest priority agents on the list of critically important antimicrobial drugs for human medicine) which have potential for development of resistance (WHO, 2018a). The Reserve group consists of last-resort antibiotics for targeted use in MDR infections. Overall quality of antibiotic use can be assessed by measuring consumption by quantifying the use of antibiotics according to the AWaRe categories. AWaRe classification could potentially be used as a simple traffic light metric of appropriate antibiotic use for its easy implementation at the lower levels.

2.8 Surveillance

Mechanisms for assessing the overall burden of AMU, AMR, and HAIs at national level and facility level are essential for the formulation of evidence-based policies/

programs, to monitor progress and impact of interventions on reducing AMR (Bhatia, 2017; Fridkin et al., 2002).

National level: A surveillance system to capture standardized data on AMR at national level was a challenge, but now surveillance networks have been initiated in both human and nonhuman sectors. Antimicrobial Resistance Surveillance Research Network (AMRSN) was initiated by ICMR in 2013 to generate a nationally representative reliable data on AMR to guide treatment strategies and rationalize AMSP in India (Walia et al., 2019b). The network started with 6 reference labs located in 4 tertiary care medical institutions each for 6 priority pathogens and 16 regional centers in tertiary care hospitals. ICMR is handholding all the network sites by providing teaching and trainings, developing resources in the form of standard operating procedures (SOPs) for isolation and antimicrobial susceptibility testing besides supporting laboratory infrastructure.

Recently the AMRSN of ICMR has expanded its horizon to collect data on HAI in addition to AMR in collaboration with All India Institute of Medical Sciences (AIIMS), New Delhi, and Centers for Disease Control and Prevention (CDC). HAI-AMR surveillance network currently includes ~40 hospitals, representing almost all regions and states of India. NCDC also initiated a National AMR Surveillance Network in 2017 for capturing AMR which currently has around 29 sites across the country (ICMR). The NCDC network sites have also started capturing AMU data. NCDC as the national coordinating center for AMR surveillance is reporting aggregated AMR surveillance data to the Global Antimicrobial Surveillance System (GLASS) to contribute toward global understanding of the AMR trends.

Similarly, a nationwide network for AMR in livestock and fisheries, i.e., Indian Network for Fishery and Animal Antimicrobial Resistance (INFAAR), has been initiated as a collaborative effort of Indian Council of Agriculture Research (ICAR), and Food and Agriculture Organization (FAO) with technical support from the National Institute of Veterinary Epidemiology and Disease Informatics, Bengaluru (NIVEDI), and National Bureau of Fish Genetic Research (BFGR) since 2017 (FAO, 2017). The network laboratories are generating AMR data on veterinary pathogens.

Although these surveillance sites are active and considerable progress has been made in data capture for AMR and AMU, the system is inadequate to understand complex dynamics of AMU and AMR, considering the huge population and diverse cultural determinants. Further, ICMR's surveillance network currently only includes tertiary medical centers, and data from these centers is representative of the AMU/AMR in community. There is an urgent need for strengthening laboratory services and integration of data collection at multi-sectoral level. Further understanding of the holistic picture of AMU and AMR in various sectors human, animal, environmental, and food samples is essential to identify most appropriate interventions and opportunities for controlling AMR in the country.

Integrated surveillance system from human and nonhuman sectors will also help in collecting uniform nationwide data on AMR in real time to assist in formulating policies and actions. Besides, convergence of the data arising from surveillance, infection control, and stewardship activities is essential to handle AMR crisis (Walia et al., 2019b).

Facility level: Every facility implementing AMS must measure antibiotic use and AMR rates, analyze trends, and communicate the data for action to the team. Days of therapy (DOTs) and defined daily dose (DDDs) are standardized methods for measurement of antibiotic use and can be useful for facility-level monitoring and inter-facility comparisons (Monnet, 2006). Point prevalence surveys (PPS) are very effective tool for assessment of appropriateness of prescribing, identification of targeted interventions, and measuring its impact on antimicrobial use (Ansari et al., 2009; Seaton et al., 2007). PPS also supports the development of national prescribing indicators for monitoring of antimicrobial use (Malcolm et al., 2012).

Monitoring the trends of AMR rates is a common approach to measure the impact of AMSP activities as decreased AMR rates suggest declined antimicrobial use. However, linking AMR with decline in AMU is not as straightforward as several other factors govern the AMU rates such as infection with multiple pathogens, host factors (immunocompromised), and longtime frame (months to years) required for AMR to revert to susceptibility status.

Measuring processes like documented indications for antimicrobial use, timely switching from IV to oral antibiotics or timely stoppage of unnecessary IV antibiotics, length of therapy, interventions designed to increase compliance to guidelines are other metrics can also be utilized to assess the effectiveness of AMSP (Gasparetto et al., 2019). Measurement of mortality rates, disease-or pathogen-specific mortality, or cure rates are commonly used patient outcome measures. Though these outcome measures are objective and reliable method, they are less suitable for mild infections (such as uncomplicated UTI). Outcome measures are difficult to measure, and there is a need to strengthen measuring of the outcomes. An Indian study revealed that measurement of outcomes has been initiated in only 49% of the 20 hospitals surveyed with feedback in 53% of facilities (Purva et al., 2019).

Besides, risk-benefit analysis by measuring the treatment failures, unintended negative effects such as hospital readmission, or increase in healthcare-associated infection is also essential. It is also important to balance these by monitoring program costs (e.g., salary for stewardship personnel; therapeutic drug monitoring) for continued administrative support for AMSP activities. Continuous monitoring and feedback to the prescribing teams is essential to define areas for improvement.

2.9 Information Technology (IT)

IT can assist in stewardship activities by providing innovative applications for information, education, measurement, and implementing AMS program. Besides, it will allow correlating changes in AMU with AMR over time, comparison of facilities, and generate data for regional and national databases (Bremmer et al., 2018). Availability and adaptation of computer-based system have been found useful to assist in decisions related to antimicrobial prescribing, dosage, drug interactions based on drug, patient's factors, treatment guidelines, antibiograms, and microbiology susceptibility reports. Computer support for stewardship activities can help in

monitoring of adherence to dosing guidelines as well as extent of adverse effects. However, absence of hospital/laboratory information systems in most of the public and private institutions in India impedes the data capturing mechanisms with difficulty in correlating AMR with antimicrobial consumption rates and clinical outcomes.

Computerization of facilities with IT support in designing apps/programs with incorporation of rules and algorithms, guidelines, and notification of restricted antibiotics can assist clinicians in decision-making while ordering antimicrobials and have great potential to improve the prescribing processes. Further, IT can aid in designing apps for surveillance of antimicrobial consumption by tracking antimicrobial use right from production to sale and finally consumption by users and aid in stricter implementation of regulations like Schedule H1.

2.10 Research

Research to find implementable solutions to contain AMR requires a multi-sectoral, multidimensional approach with focus on several aspects driving AMU and resistance. The fact that AMR is increasing at a rapid pace in the country combined with the realization that few new antimicrobials are being developed makes it imperative for a more directed and focused research. Research is being conducted by ICMR to understand the molecular mechanism of resistance, clonality of drug-resistant pathogens, and transmission dynamics and create data management systems. Other options to enable AMR-related clinical trials for new antimicrobials through existing network sites are also being explored. Research is also needed to find out alternatives to antimicrobials (i.e., immune modulators, probiotics, enzymes, trace elements, etc.) which can be used in conflicting clinical indications.

Keeping in view that human, animal, and environmental health are interdependent, formative research beyond the biosciences into social factors and practices governing antimicrobial use in specific regional and local contexts is indispensable to achieve change. Some of the possible research areas which need prioritization are:

(a) Improved surveillance and infection control
(b) Judicious use of antibiotics
(c) New preventive measures
(d) New therapeutic strategies
(e) Basic research for understanding the genetic basis of resistance
(f) Preclinical toxicology methodology research and clinical trials
(g) Pharmacovigilance studies
(h) Research on patients and the community
(i) Research on prescribers and dispensers
(j) Research in hospital settings
(k) Factors driving antibiotic use for food animals and health system research

Scientists, academia, pharmaceutical industry, and government need to come together to identify, promote, and support innovations and research encompassing human, veterinary, and environment sectors as One Health approach. Cross-sectoral collaboration and funding support from apex research bodies like ICMR and Indian Council of Agricultural Research (ICAR) are essential to enhance understanding of epidemiological linkages between AMU and AMR in human, veterinary, and environment sectors, and development of indigenous cost-effective diagnostic tools/interventions (Metlay et al., 2006). Harnessing newer technologies like genome sequencing, proteomics, and bioinformatics may pave the way for newer diagnostics, alternatives to antibiotics, and novel stewardship strategies and tools for surveillance and management of antimicrobial usage.

2.11 Monitoring, Evaluation, and Feedback

Efficient monitoring and evaluation (M&E) system to gauge the progress made through activities is an integral part of the AMR containment program. Regular time-to-time assessment of AMS program is essential to understand the existing structures, processes, and outcomes to decide whether to continue, modify, or discontinue the interventions. The key metrics to assess AMS program are listed in Table 4. Tools for M&E, skills, and institutional support need to be integrated with SMART (Specific, Measurable, Attainable, Relevant, and Timely) indicators and targets for continual improvement.

Prospective review/audits and feedback is the most promising strategy to bring in behavioral modification toward appropriate antibiotic use and improved patient outcomes (Elligsen et al., 2012). The audit and review can also be accomplished by a scheduled post-prescription clinical review by AMS team member(s) and also provide direct and timely feedback to the prescriber for a range of point-of-care stewardship interventions especially regarding choice or appropriateness of antimicrobials, route of administration, timeliness, duration of therapy, use of investigation, and their interpretation with a view to escalate, de-escalate, or stop therapy at the time of prescription itself.

Communicating, sharing, and learning from data is also important. Feedback to the prescribers, multidisciplinary team consultations, discussions, and conferences promote learning about prudent prescribing and redesigning of ineffective or discontinuation of harmful interventions which do not have a significant impact on antibiotic use. Several studies using this approach have shown decreased antimicrobial usage, better acceptance, and improved clinician satisfaction (Elligsen et al., 2012; DiazGranados, 2012; Newland et al., 2012). In India, not much literature is available in this regard. Educational interventions are most effective in positively impacting the prescribing behavior when used in combination compared to when used alone (Satterfield et al., 2020).

Table 4 Key indicators to assess AMS programs

Structural	Multidisciplinary team Guidelines for empiric treatment and surgical prophylaxis Continuing education and trainings Surveillance of resistance and antibiotic use
Process	Proportion of patients with documented indication for antibiotic use Proportion of patients with documentation of stop/review date Proportion of patients with documentation of length of therapy by indication Proportion of patients with 48-hour review Proportion of patients with de-escalation to narrow-spectrum antibiotic based on microbiology data Proportion of patients converted to oral therapy Proportion of patients with surgical prophylaxis within the previous 60 minutes Excess days of therapy (i.e., unnecessary days of therapy avoided based on accepted targets and benchmarks) Proportion of patients compliant with facility-based guideline or treatment algorithm
Outcome	Defined daily dose (DDD) per 1000 patient-days or DDD per admission Duration of therapy (DOTs) per 1000 patient-days Proportion of DDDs in AWaRe and other groups 30-day mortality Length of hospital stay Unplanned hospital readmission within 30 days Proportion of patients diagnosed with hospital-acquired *Clostridium difficile* infection or other adverse event(s) related to antibiotic treatment Proportion of patients with clinical failure (e.g., need to broaden therapy, recurrence of infection) Broad−/narrow-spectrum antimicrobial ratio Proportion of patients receiving appropriateness of therapy

3 Conclusions

NAP-AMR symbolizes the government recognition of antibiotic "crises and their commitment to action." Buy-in of relevant stakeholders in both human and non-human sectors is crucial. Several initiatives to build capacity on rational prescribing practices, basic identification and susceptibility testing in bacteriology, surveillance of antibiotic consumption and AMR, quality assurance, data capture, and data management have begun with engagement of national and state levels, professional bodies, and civil societies. There is an urgent need for accelerating multi-sectoral engagement for improving access to effective antibiotics and a strong and enforceable regulatory mechanism. For comprehensive implementation of NAP-AMR, both structural and functional systems need to be operationalized at all levels. Implementation of AMS programs has been initiated at tertiary care hospitals focusing on some core elements; there is an urgent need to implement comprehensive AMSP programs at these centers and to also include secondary level hospitals where most AMU takes place. At a facility level, efforts should be focused on implementation of all AMS components including pre-authorization, antimicrobial cycling, computerized data collection for surveillance, and appropriate utilization of microbiology labs to further

consolidate the benefits of AMSP. Understanding the holistic picture of AMU and AMR human, animal, environmental, and food samples is essential to identify interventions and opportunities for controlling AMR in the country. Making quality accreditation mandatory may be a promising approach. Formative research into social factors and practices governing antibiotic use is indispensable to achieve change. Uninterrupted funds to support establishment of quality assured microbiology laboratory across all the sectors, development of human resource, enabling environment and IT support, surveillance, and program monitoring are required for uniform implementation across all sectors.

References

Ansari, F., Erntell, M., Goossens, H., & Davey, P. (2009). The European Surveillance of Antimicrobial Consumption (ESAC) Point Prevalence Survey of Antibacterial Use in 20 European Hospitals in 2006. *Clinical Infectious Diseases, 49*(10), 1496–1504.

Bahl, A., Sahni, A., Martolia, R., Jain, S., & Singh, S. (2020). Implementation of antimicrobial stewardship activities in India. *Indian Journal of Medical Specialties, 11*(1), 5–9.

Barlam, T. F., Cosgrove, S. E., Abbo, L. M., et al. (2016). Implementing an Antibiotic Stewardship Program: Guidelines by the Infectious Diseases Society of America and the Society for Healthcare Epidemiology of America. *Clinical Infectious Diseases, 62*(10), e51–e77.

Bhatia, R. (2017). Feasibility of combating AMR using One Health approach. *Bulletin of the Department of Medical Sciences Thailand, 59*, 209–215.

Bhatia, R., Katoch, V., & Inoue, H. (2019). Creating political commitment for antimicrobial resistance in developing countries. *Indian Journal of Medical Research, 149*(2), 83–86.

Bhattacharya, S., Joy, V. M., Goel, G., et al. (2019). Antimicrobial stewardship programme – From policies to practices: A survey of antimicrobial stewardship programme practices from 25 centres in India. *The Journal of Academy of Clinical Microbiologists, 21*, 4–9.

Bremmer, D. N., Trienski, T. L., Walsh, T. L., & Moffa, M. A. (2018). Role of technology in antimicrobial stewardship. *Medical Clinics of North America, 102*(5), 955–963. https://doi.org/10.1016/j.mcna.2018.05.007. Epub 2018 Jul 14. PMID: 30126584.

British Society for Antimicrobial Chemotherapy. (2018). Antimicrobial stewardship from principles to practice. Available from: https://bsac.org.uk/antimicrobial-stewardship-from-principles-to-practice-e-book/. Accessed 16 July 2020.

Byers, C. G. (2020). Biosecurity measures in clinical practice. *Vet Clin North Am Small Anim Pract, 50*(6),1277–1287.

Carling, P., Fung, T., Killion, A., Terrin, N., & Barza, M. (2003). Favorable impact of a multidisciplinary antibiotic management program conducted during 7 years. *Infection Control & Hospital Epidemiology, 24*, 699–706.

Charani, E., Castro-Sanchez, E., Sevdalis, N., et al. (2013). Understanding the determinants of antimicrobial prescribing within hospitals: The role of "Prescribing Etiquette". *Clinical Infectious Diseases, 57*(2), 188–196.

DiazGranados, C. A. (2012). Prospective audit for antimicrobial stewardship in intensive care: Impact on resistance and clinical outcomes. *American Journal of Infection Control, 40*, 526–529.

Elligsen, M., Walker, S. A., Pinto, R., et al. (2012). Audit and feedback to reduce broad-spectrum antibiotic use among intensive care unit patients: A controlled interrupted time series analysis. *Infection Control Hospital Epidemiology, 33*, 354–361.

Food and Agriculture Organization. (2017). *FAO facilitates operationalization of India's Antimicrobial Resistance (AMR) surveillance network*. Available at: http://www.fao.org/india/news/detail-events/en/c/1026526/

Fridkin, S. K., Lawton, R., Edwards, J. R., Tenover, F. C., McGowan, J. E., Jr., & Gaynes, R. P. (2002). Monitoring antimicrobial use and resistance: Comparison with a national benchmark on reducing vancomycin use and vancomycin-resistant enterococci. *Emerging Infectious Diseases, 8*, 702–707.

Gandra, S., Joshi, J., Trett, A., Lamkang, A. S., & Laxminarayan, R. (2017). *Scoping report on antimicrobial resistance in India*. Center for Disease Dynamics, Economics & Policy. Available at: Cddep.org. https://cddep.org/wp-content/uploads/2017/11/AMR-INDIA-SCOPING-REPORT.pdf. Accessed 19 Sept 2020.

Gasparetto, J., Tuon, F. F., Dos Santos Oliveira, D., et al. (2019). Intravenous-to-oral antibiotic switch therapy: A cross-sectional study in critical care units. *BMC Infectious Diseases, 19*(1), 650. https://doi.org/10.1186/s12879-019-4280-0

Gillespie, E., Rodrigues, A., Wright, L., Williams, N., & Stuart, R. L. (2013). Improving antibiotic stewardship by involving nurses. *American Journal of Infection Control, 41*, 365–367.

Hazra, A. (2014). Schedule H1: hope or hype? *Indian J Pharmacol. 46*(4), 361–2.

Indian Council of Medical Research. (2017). *Treatment guidelines for antimicrobial use in common clinical syndromes*. Available at: http://bic.icmr.org.in/iamrsn/images/pdf/STG270217.pdf. Accessed 19 Sept 2020.

Indian Council of Medical Research. (2018). *Antimicrobial Stewardship Program Guidelines*. Available at: https://main.icmr.nic.in/sites/default/files/guidelines/AMSP_0.pdf. Accessed 30 Jan 2021.

Indian Council of Medical Research. (2019). *National essential diagnostic list*. Available at: https://main.icmr.nic.in/sites/default/files/guidelines/NEDL_2019.pdf. Accessed 14 Jan 2022.

Indian Council of Medical Research. *Antimicrobial resistance surveillance and research network*. Available at: http://iamrsn.icmr.org.in/index.php/amrsn/amrsn-network. Accessed 20 Sept 2020.

Liao, S., Rhodes, J., Jandarov, R., DeVore, Z., & Sopirala, M. (2020). Out of Sight—Out of Mind: Impact of Cascade Reporting on Antimicrobial Usage. *Open Forum Infectious Diseases, 7*(2).

Malcolm, W., Nathwani, D., Davey, P., et al. (2012). From intermittent antibiotic point prevalence surveys to quality improvement: Experience in Scottish hospitals. *Antimicrobial Resistance and Infection Control, 2*, 3. https://doi.org/10.1186/2047-2994-2-3

Messacar, K., Parker, S., Todd, J., & Dominguez, S. (2016). Implementation of rapid molecular infectious disease diagnostics: The role of diagnostic and antimicrobial stewardship. *Journal of Clinical Microbiology, 55*(3), 715–723.

Metlay, J. P., Powers, J. H., Dudley, M. N., Christiansen, K., & Finch, R. G. (2006). Antimicrobial drug resistance, regulation, and research. *Emerging Infectious Diseases, 12*(2), 183–190.

Ministry of Environment, Forest and Climate Change, Government of India. (2016). *Bio-Medical Waste Management Rules*. 2016 Published in the Gazette of India, Extraordinary, Part II, Section 3, Sub-Section (i), Notification; New Delhi, the 28th March, 2016. Available at: https://dhr.gov.in/sites/default/files/Bio-medical_Waste_Management_Rules_2016.pdf. Accessed 14 Jan 2022.

Ministry of Health & Family Welfare, Government of India. (2013). *A strategic approach to reproductive, maternal, new born, child and adolescent health (RMNCH A+) in India*. Available at: https://nhm.gov.in/images/pdf/RMNCH+A/RMNCH+A_Strategy.pdf. Accessed 2 Jan 2022.

Ministry of Health and Family Welfare, Government of India. (2011). *National policy for containment of antimicrobial resistance.* Available at: https://ncdc.gov.in/. Accessed 20 Sept 2020.

Ministry of Health and Family Welfare, Government of India. (2013). Gazette notification G.S.R. 588(E). The Gazette of India dated August 30, 2013. pp. 4–8.

Ministry of Health and Family Welfare, Government of India. (2015). *Guidelines for Implementation of "Kayakalp" Initiative*. Available at: http://www.nhm.gov.in/images/pdf/infocus/Implementation_Guidebook_for_Kayakalp.pdf. Accessed 29 Mar 2019.

Ministry of Health and Family Welfare, Government of India. (2016). *National Treatment Guidelines for Antimicrobial Use in Infectious Diseases*. Available at: https://ncdc.gov.in/WriteReadData/l892s/File622.pdf. Accessed 16 April 2023.

Ministry of Health and Family Welfare, Government of India. (2017). *National Action Plan on Antimicrobial Resistance*. Available at: https://www.ncdc.gov.in/WriteReadData/linkimages/AMR/File645.pdf. Accessed 19 Sept 2020.

Ministry of Health and Family Welfare, Government of India. (2018–2025). National patient's safety implementation framework. Available at: https://www.nhp.gov.in/national-patient-safety-implementation-framework-(npsif)_pg. Accessed 5 Feb 2020.

Ministry of Health and Family Welfare, Government of India. National Centre for Disease Control. (2020). *National Guidelines for Infection Prevention and Control in Healthcare Facilities*. Available at: https://ncdc.gov.in/showfile.php?lid=431. Accessed 22 May 2020.

Monnet, D. L. (2006). *ABC Calc - Antibiotic consumption calculator ABC Calc Version 3.1. 18*. Statens Serum Institute.

Nathwani, D., Varghese, D., Stephens, J., et al. (2019). Value of hospital antimicrobial stewardship programs [ASPs]: A systematic review. *Antimicrobial Resistance & Infection Control, 8*(1), 1–13.

National Accreditation Board for Hospitals and Healthcare Providers. (2020). Accreditation Standards for Hospitals, fifth edition.

Newland, J. G., Stach, L. M., DeLurgio, S. A., et al. (2012). Impact of a prospective-audit-with feedback antimicrobial stewardship program at a children's hospital. *Journal of Pediatric Infectious Diseases, 1*, 179–186.

O'Neill, J. (2014). *Review on Antimicrobial Resistance: Tackling a crisis for the health and wealth of nations*. Review on Antimicrobial Resistance. Available at: https://amr-review.org. Accessed 9 Aug 2020.

Onufrak, N. J., Forrest, A., & Gonzalez, D. (2016). Pharmacokinetic and pharmacodynamic principles of anti-infective dosing. *Clinical Therapeutics, 38*(9), 1930–1947.

Parente, D. M., & Morton, J. (2018). Role of the pharmacist in antimicrobial stewardship. *Medical Clinics of North America, 102*(5), 929–936. https://doi.org/10.1016/j.mcna.2018.05.009

Pokharel, S., Shrestha, P., & Adhikari, B. (2020). Antimicrobial use in food animals and human health: Time to implement 'One Health' approach. *Antimicrobial Resistance and Infection Control, 9*, 181. https://doi.org/10.1186/s13756-020-00847-x

Porter, G., Joshi, J., Bhullar, L., & Kotwani, A. (2020). Smart Regulation of Antibiotic Use in India: Understanding, Innovating and Improving Compliance Project Group. Using 'smart regulation' to tackle antimicrobial resistance in low-income and middle-income countries. *BMJ Global Health, 5*(1), e001864.

Purva, M., Randeep, G., Rajesh, M., et al. (2019). Assessment of core capacities for antimicrobial stewardship practices in Indian Hospitals: Report from a Multicentric Initiative of Global Health Security Agenda. *Indian Journal of Medical Microbiology, 37*(3), 309–317.

Rout, J., & Brysiewicz, P. (2017). Exploring the role of the ICU nurse in the antimicrobial stewardship team at a private hospital in KwaZulu-Natal, South Africa. *Southern African Journal of Critical Care, 33*, 46–50.

Salsgiver, E., Bernstein, D., Simon, M. S., et al. (2018). Knowledge, attitudes, and practices regarding antimicrobial use and stewardship among prescribers at acute-care hospitals. *Infection Control and Hospital Epidemiology, 39*(3), 316–322. https://doi.org/10.1017/ice.2017.317

Satterfield, J., Miesner, A. R., & Percival, K. M. (2020). The role of education in AMS stewardship. *Journal of Hospital Infection, 105*, 130–141.

Seaton, R. A., Nathwani, D., Burton, P., et al. (2007). Point prevalence survey of antibiotic use in Scottish hospitals utilizing the Glasgow Antimicrobial Audit Tool (GAAT). *International Journal of Antimicrobial Agents, 29*, 693–699.

Singh M. K.. (2019). *Central government bans use of Colistin in livestock to tackle anti-microbial resistance*. Available at: https://www.mondaq.com/india/food-and-drugs-law/853840/central-government-bans-use-of-colistin-in-livestock-to-tackle-anti-microbial-resistance. Accessed 14 Jan 2022.

Swachh Bharat Mission. (2014). Available at: https://swachhbharatmission.gov.in/SBMCMS/about-us.htm. Accessed 20 Sept 2020.

Walia, K., Ohri, V. C., & Mathai, D. (2015). Antimicrobial Stewardship Programme of ICMR. Antimicrobial stewardship programme (AMSP) practices in India. *Indian Journal of Medical Research, 142*, 130–138.

Walia, K., Ohri, C., Madhumathi, J., & Ramasubramanian, V. (2019a). Policy document on antimicrobial stewardship practices in India. *Indian Journal of Medical Research, 149*(2), 180–184. https://doi.org/10.4103/ijmr.IJMR_147_18

Walia, K., Madhumathi, J., Veeraraghavan, B., et al. (2019b). Establishing antimicrobial resistance surveillance & Research network in India: Journey so far. *Indian Journal of Medical Research, 149*(2), 164.

World Health Organization. (2011a). *Jaipur declaration on antimicrobial resistance*. Available at: http://origin.searo.who.int/entity/antimicrobial_resistance/rev_jaipur_declaration_2014.pdf. Accessed 20 Sept 2020.

World Health Organization. (2011b). *Step-by-step approach for development and implementation of hospital and antibiotic policy and standard treatment guidelines*. Available at: https://apps.who.int/iris/handle/10665/205912. Accessed 31 Jan 2021.

World Health Organization. (2018a). *Critically Important Antimicrobials list (CIA) for human medicine*. Available at: https://apps.who.int/iris/bitstream/handle/10665/312266/9789241515528-eng.pdf?ua=1. Accessed 20 Sept 2020.

World Health Organization. (2018b). *WHO competency framework for health workers' education and training on antimicrobial resistance*. WHO/HIS/HWF/AMR/2018.1. Licence: CC BY-NC-SA 3.0 IGO.

World Health Organization. (2019). *WHO AWaRe Classification Database of Antibiotics for evaluation and monitoring of use*. Available at: https://www.who.int/publications/i/item/WHOEMPIAU2019.11. Accessed 21May 2021.

Phages and Their Derived Proteins as Promising Alternatives to Mitigate MDR *Salmonellae*

K. S. Sritha and Sarita G. Bhat

Contents

Abstract

Antibiotics are considered as "wonder drugs" to combat microbial infections for decades ever since their discovery. Unfortunately, the arrival of Multi Drug Resistant (MDR) superbugs due to inappropriate and excessive use of antibiotics is a global threat accountable for high death tolls and lethal infections. Antibiotic resistance in gram-negative bacteria is well documented, especially their occurrence in aquaculture and livestock. Multidrug-resistant *Salmonella* are becoming progressively recalcitrant to treatment in human patients. This widespread decline in the effectiveness of antibiotics has prodded the scientific world to explore and investigate natural antimicrobial agents or other alternatives in the prophylaxis of several microbial infections. Among them, bacteriophage-mediated therapeutic

K. S. Sritha · S. G. Bhat (✉)
Department of Biotechnology, Cochin University of Science and Technology, Cochin University, Cochin, Kerala, India
e-mail: sgbhat@cusat.ac.in

M. P. Mothadaka et al. (eds.), *Handbook on Antimicrobial Resistance*,
https://doi.org/10.1007/978-981-19-9279-7_36

approaches are promising alternatives. Phage proteins as well as engineered phages are the latest arsenal being explored to combat AMR in bacteria. Lytic phages with therapeutic potential against these pathogens require characterization in terms of morphology, growth parameters, stability under various conditions, host range, and capability to infect host under nutrient-exhausted conditions. Current approaches involve using phage-encoded enzymes in fighting against multidrug-resistant bacterial infections instead of the whole phages principally endolysins that are responsible for interrupting the synthesis of peptidoglycan. These are potent bacteriolytic agents at a low concentration and have been extensively reported targeting various MDR strains identified *in vitro*. Genomic investigation of bacteriophages is a prerequisite to reveal the presence of globular endolysins. These are a promising alternative methods that will possibly reduce impact of AMR.

Keywords

Antibiotics · AMR · *Salmonella* · Bacteriophages · Endolysins

1 Introduction

Food-borne illness is a serious issue and an important global cause of death and morbidity (Majowicz et al., 2010). In 2015, "Food-borne Disease Burden Epidemiology Reference Group (FERG)," established by the "World Health Organization (WHO)," estimated 0.6 billion incidences of food-borne infections in 2010, leading to more than 400,000 mortalities (WHO, 2015). The global impact of "Food Borne Disease (FBD)" is corresponding to those of key communicable illnesses *viz*., "Tuberculosis, HIV/AIDS and Malaria" (Havelaar et al., 2015)." In developing countries, absence of infrastructure and poor sanitation, etc., augment the chance of illness, and despite awareness, food safety remains relegated (Grace, 2015; WHO, 2015). FBD are also significant barriers to socioeconomic development worldwide (WHO, 2015). In India, food-borne diseases cost about 28 billion USD or nearly 0.5% of the nation's "Gross Domestic Product (GDP)" (Kristkova et al., 2017). The causes of FBD are mainly due to diarrheal disease agents. According to the reports, viruses account for major cause of 59%, 39%, and 2% illnesses which are associated with foods of viral, bacteria, and parasites, respectively. But severe cases are mostly related to bacterial origin leading to hospitalizations (63.9%) and further deaths (Scallan et al., 2011).

2 Salmonellosis

Salmonella, a facultative gram-positive bacterium is a major human pathogen. Salmonellosis is the gastrointestinal disorder triggered through *Salmonella* of non-typhoidal type and is regarded as the second most prevalent microorganism leading to foodborne infection. Salmonellosis, recognized as one of the leading contributors to

ailments, is associated with consumption of contaminated foods resulting in 35% and 28% of hospital confinement and mortalities, respectively (Scallan et al., 2011). It is projected that yearly salmonellosis accounts for more than 78 million cases of food-borne illnesses world over, with nearly 59,000 deaths (Havelaar et al., 2015). Infection starts with the consumption of food contaminated with feces or urine carrying *Salmonella* and subsequent passage of bacteria to stomach. Since gastric pH act as a barrier for bacterial infection, *Salmonella* exhibits acid tolerance response, a complex adaptive system, which requires the production of more than 50 acid shock proteins. Those *Salmonella* organisms surviving in the low-pH environment proceed to the intestine. Upon entry into the small intestine, the *Salmonellae* attach to the mucosa and with support of fimbriae and flagella located on exterior of the bacteria lead to incursion of epithelial cells. The incubation period generally is 6–72 h but will depend upon the host and the inoculum size. The symptoms of *Salmonella* infection include diarrhea (that can be bloody), fever, and stomach cramps. Nausea, vomiting, and headache can also occur. Some effector proteins contribute significantly to the activation of secretory pathways and altering ion balances within cells, leading to diarrhea, and few have role in gastroenteritis (Wallis & Galyov, 2000). Epithelial cells get invaded by Salmonella causing the release of proinflammatory cytokines that induce an inflammatory reaction leading to ulceration and destruction of the mucosa. Symptoms can last up to 4–7 days. However, the duration and consequence of the contagion depend on different dynamics such as the number of cells ingested in the form of inoculum and the invulnerable condition of the host, which include susceptible elderly groups, vicissitudes in the intestine's endogenous flora, diabetes, cancer, rheumatological illnesses, HIV infection, and immunosuppression. The infection is usually self-limiting. Drinking plenty of water is indorsed. However, it is endorsed to obtain antimicrobial therapy for the patients who are extremely ill and with hazardous factors. Antimicrobial agents are very much required when quick disruption of fecal shedding of *Salmonella* should be necessitated to prevent the outbreaks of salmonellosis in institutions.

Salmonella diagnosis involves identification of organism through plating of samples on media specific for *Salmonella.* MacConkey agar is the most commonly used medium (low-selective) in which *Salmonella* displays colorless colonies due to the absence of lactose fermentation. However, for specific isolation laboratories employ media of highly selectivity kind, namely, "*Salmonella-Shigella* (SS) agar, xylose-lysine-deoxycholate (XLD) agar, and Hektoen enteric (HE) agar plates." Metabolic characteristic of hydrogen sulfide production by *Salmonella* is utilized in theses media thus showing colonies with black centers and inability to utilize lactose (Fàbrega & Vila, 2013).

3 Sources of Infection: *Salmonella*

Salmonella transmission takes place through the path of oral by fecal and is, consequently, spread mainly by food and water, direct contact with animals, and rarely by contact with human to human. An estimated 94% of salmonellosis is

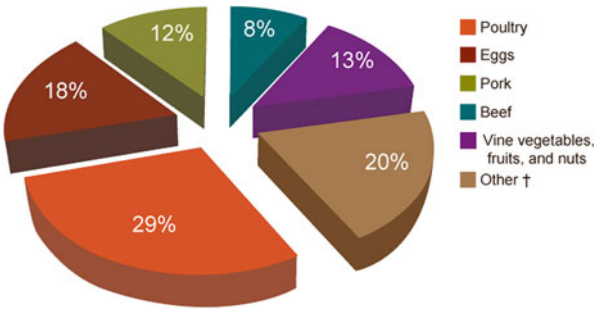


Fig. 1 Food associated with *Salmonella* outbreaks

transmitted by food (CDC, 2013). *Salmonella* exists in most food of production settings, and *Salmonella* contamination takes place at different stages of harvest, postharvest handling from farm to fork, or pond to plate in case of aquatic products.

One of the important sources of infections is faunal originated foodstuffs. Among these sources, poultry and associated food products remain well-recognized cradles of *Salmonella* contamination. Raw or improperly cooked eggs, mayonnaise made in domestic conditions, and ice creams are high-risk products. Besides, meats such as beef and pork have been reported to be important cradles of salmonellosis.

Application of contaminated food origin pathogenic bacteria for irrigation purposes contributes to the eventual infection of fruits and vegetables. Subsequently, innumerable food-originated epidemics have been related to farm produce of fresh harvests.

Pasteurized milk and raw milk have been involved in salmonellosis contaminations and occurrences. *Salmonella* epidemics were recorded in various sources of food as chocolate, peanut butter, tuna fish, bean sprouts, etc. (Carrasco et al., 2012). Cross-contamination is high among personnel involved in food handling, foodstuffs, and surfaces in which food comes in touching base that possibly results in epidemics connected with restaurant food as well (Fig. 1).

4 Antibiotic Resistance in *Salmonella*

Ever since the serendipitous Alexander Fleming's detection of Penicillin in 1928, the world has witnessed a plethora of antimicrobials used as arsenal to combat bacterial infections. Most were discovered from soil microorganisms, and today there are several antibiotics known which can disrupt most biological processes in the bacterial cell.

There has been a drastic increase of unresponsiveness to drugs in *Salmonellae* ranging from 20% to 30% in the last decade of the previous century to an alarming situation of two-thirds at the advent of this millennium (Su et al., 2004). This increase in antimicrobial resistance in developing countries is an unavoidable consequence of the employment of drugs in food-producing faunae either as therapeutic or prophylactic agent or for growth promotion. Antibiotic resistance is higher in

animal isolates, and a high frequency of *Salmonella* is being detected in supply chains of foods with strong unresponsiveness to drugs (Nair et al., 2018). The antibiotic resistance is different among numerous serotypes of nontyphoidal *Salmonella*, and in some of them it is considerably significant. A much higher rate of resistance was found in the globally prevalent serotype, *S. enteric typhimurium*. Drug-resistant *Salmonella* strains are more virulent than susceptible strains. Likewise, compared to infections caused by susceptible strains, MDR *Salmonella* strains have been connected with a higher risk of aggressive infection, higher incidence and period of hospitalization, increased duration of illness, and risk of death (Prestinaci et al., 2015; Eng et al., 2015).

5 Alternative Control Measures

The control measures taken so as to minimize the risk of *Salmonella* incidence are applied either as preharvest or as postharvest procedures. Certain safety measures which condition sustained personnel and equipment practices have been recommended by FDA, protecting against cross contamination. Additional prevention and control measures involved are grouped into biological, chemical, and physical procedures (Table 1).

6 Bacteriophages, an Attractive Alternative to Antibiotics

Currently, bacteriophages are widely being reassessed as a substitute to antimicrobial drugs. Phage therapy involves simple concept of managing microbial infections through bacteriophages by exploiting its ability to kill a specific pathogenic bacterium.

Table 1 Antibiotic alternative control measures of *Salmonella*

Control method	Technique	References
Physical	Irradiation	Bruhn (1995)
	Electrolyzed water	Venkitanarayanan et al. (1999)
	Ultrasound	Morild et al. (2011)
	Microwaves	Galis et al. (2013)
Chemical	Chlorinated compounds	Coppen et al. (1998)
	Trisodiumphosphate (TSP)	Kim and Slavik (1994)
	Organic acids	Mani-López et al. (2012)
	Ozone	Møretro et al. (2012)
Biological	Prebiotics Probiotics Competitive exclusion Essential oils Bacteriocins Bacteriophages	Eeckhaut et al. (2008) Vila et al. (2009) Galiş et al. (2013) Nair et al. (2014) Silva et al. (2018)

Morphologically, most of bacteriophages exhibit distinct three-dimensional structure by means of a crown and appendage bound by a connector. Nevertheless, "cubic, pole, lemon-shaped, filamentous or pleomorphic viruses" are also reported. Virion consists of a protein coat called capsid (polyhedral, filamentous, or connected to a tail) encapsulating a genome comprising either of DNA or RNA which may be double or single stranded (Fig. 2) (Rossmann et al., 2005; Harada et al., 2018).

6.1 Phage Therapy

The first documented therapeutic case occurred in 1919, where phages were used to cure patients of dysentery by d'Herelle and met with success. However, in 1921 the first application of phages was published when phages were employed to manage skin ailments of *Staphylococcal* by Bruynoghe and Maisin (Hermoso et al., 2007). Encouraged by results, d'Herelle used phages to contain cholera epidemics in India (1927) and the Egyptian plague, decreasing mortality rate from 62.8% to 8.1%, in control clusters to experimental phage-remedied clusters, respectively (Summers, 2001). The early success of d'Herelle drove other scientists to use phages therapeutically and prophylactically. Later, an expansion in phage therapy occurred leading to varying degrees of success for curing numerous bacterial infections including cholera, dysentery, and *Staphylococcal* infections. In 1930s, companies like "Fu Eli Lilly (Indianapolis, IN, USA)" and "E.R. Squibb and Sons (Princeton, NJ, USA)" started commercialization of phages against various bacterial pathogens. Two centers were established for beneficial phage investigation and creation, *viz*., "Eliava Institute of Bacteriophage, Microbiology and Virology (EIBMV) of the Georgian

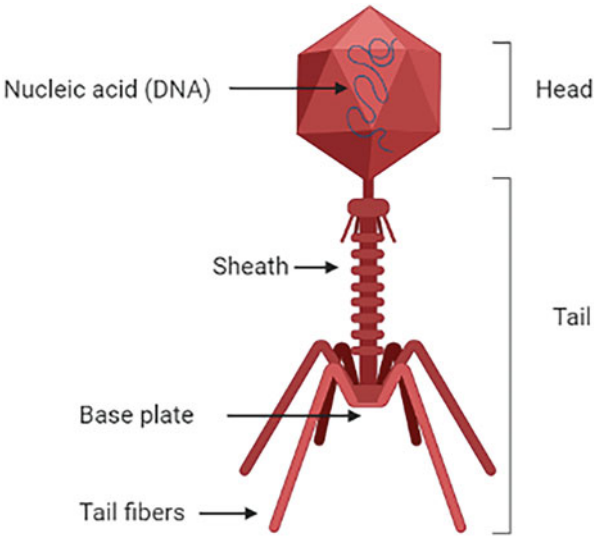


Fig. 2 Structure of a typical bacteriophage

Academy of Sciences, Tbilisi, Georgia" established in 1923 by Giorgi Eliava, a reputable bacteriologist from Georgia, in collaboration with Felix d'Herelle and "HIIET of the Polish Academy of Sciences, Wroclaw, Poland" established in 1952 (Sulakvelidze et al., 2001; Fischetti et al., 2006; Moelling et al., 2018). The advent of across-the-board antimicrobial drugs in the 1940s and lacunae on understanding of biology of bacteriophages was the cause of indifference to phage therapy in the twentieth century. However, the then Soviet researchers continued their work on phage development at the EIBMV. Nevertheless, the dangerous upsurge of "Antibiotic Resistant Bacteria (ARB)" and the pursuit of "substitutes for antimicrobial drugs given rise to" resurfaced attention in phage therapy (Moelling et al., 2018).

6.1.1 Recent Advancements

Remarkably, bacteriophages after an interlude have encountered a revival of interest for therapeutic and prophylactic application (Fortuna et al., 2008). The USA authorized application of phage merchandises to prevent bacterial contamination in the commercial sold foods (meat, cheese, etc.). Important developments took place in the year 2006 when "*USFDA*" permitted a phage research ListShield,™ an amalgamation of six individually purified phages manufactured by Intralytix Inc., for application as an antibacterial preservative of foods to reconnoitre *L. monocytogenes* in precooked meats and fowl-based foodstuffs (Goodridge & Bisha, 2011). Several commercial phage formulations for biocontrol of bacterial pathogens were subsequently developed and FDA-approved. The stepping up phage application in agriculture has exposed an impending advantage for managing numerous plant pathogens. The company "OmniLytics, Inc. Salt Lake City, USA" received United States "Environmental Protection Agency (EPA)" authorization for applying its invention "Agriphage" to battle plant pathogenic microbes (Garcia et al., 2008). These advances imply the acknowledgment of phage as biological control agents in food production.

In modern era, Western European countries (Belgium, France) have permitted the therapeutic use of bacteriophages in the USA; however, clinical application is not yet permitted by "US Food and Drug Administration" although a number of bodies are emerging with phage therapeutics.

A scientific finding published in the year 2009 revealed that placebo managed with double-barreled clinical trials (Phase I/II) of phage treatment against ailments is associated with prolonged otitis of *P. aeruginosa* that leads to condensed microbial counts (Wright et al., 2009). Most strikingly, a well-documented case is that of a patient who was in treatment with phages for *A. baumannii* strain recovering from mortal ailment (Schooley et al., 2017). In 2013, the "European Commission under the 7th Framework Program called PhagoBurn" started using phages in managing the *P. aeruginosa* infections of burn wounds in clinical trials that led to successful reduction of the bacterial load, nonetheless at a reduced stride in relation to existing conventional treatments of care. Elizabeth Kutter successfully treated diabetic foot ulcers that were unresponsive to antibiotics by topical administration of a *Staphylococcal* phage (Fish et al., 2016).

Although, several trials have been reported, sound clinical trials are required for final acceptance. Some researchers recommend a combination of phage therapy and

antibiotics as phages may increase the uptake by cells. A compassionate application of phage therapy is also in discussion for the plausible reason of absence of adverse effects, its historical recognition in application in clinical settings, publications, and a solid support by means of fundamental research (McCallin et al., 2019). In the intervening time, similar recommendation indicated that contemporary clinical trials need rational planning to be safer and more comprehensive, possibly to produce appreciable amount of data in contrast to past efforts. However, in recent years, because of massive advancement in methods for characterizing and evaluating phage for antibacterial treatments, researchers could conduct supplementary laboratory studies as well as WGS of phage and bacteria garnered during the process of treatment, thus supporting better designing of clinical trials. Though challenges remain for bacteriophage therapy, it is an attractive model due to several advantages unlike antibiotics (Fig. 3). Nevertheless, solutions overcoming challenges are being sought (Table 2).

Fig. 3 Advantages of phage therapy over antibiotics

Table 2 Solutions for challenges in phage therapy

Challenge	Solutions	Reference
Narrow host range	1.Use a cocktail of phages 2. Use phages that use bacterial receptors that are more evolutionarily conserved at the molecular level	Tanji et al. (2005) Goodridge (2010)
Lysogeny of phages causing horizontal exchange of genetic material causing transfer of virulence genes	1. Using obligate lytic phages 2. Whole genome sequencing of strong candidate phages for clinical trials for the confirmation of the absence of lysogenic genes	Cheetham and Katz (1995) Pirnay et al. (2015)
Bacterial resistance to phages	1. Using phage that uses bacterial receptors that are more evolutionarily conserved at the molecular level 2. Utilizing phages with fast adsorption rate and large burst size 3. Cocktail of several phages each binding to different surface receptors	Nilsson (2014)
Complex pharmacokinetics	Virulent phages with large burst size and short latent period and having ability to bind host rapidly	Nilsson (2014)
Immunogenicity	Phage preparation should be devoid of endotoxins and/or should follow the current pharmacopeia requirements based on the type of application	Hietala et al. (2019)
Lack of correctly designed clinical trials supporting its effectiveness:	1. Clinical trials are recommended to be planned based on unique features of phages 2. Clinical trials should be carried out in a scientifically correct, transparent, properly controlled manner using large sample sizes	Cooper et al. (2016) Parracho et al. (2012) Reindel and Fiore (2017)
Lack of a dedicated regulatory frame work that recognizes clinical use of bacteriophage	Current drug approval processes are less suitable for phages, and an alternative pathway for approval of phages is recommended	Verbeken et al. (2014) Aminov et al. (2017) Abedon et al. (2017)

6.2 *Salmonella* Bacteriophages As Biological Control Agents in Food Production and Processing

Lytic phages, as a biological control agent of food-borne pathogen, are of growing interest in the present-day scenario. Natural phage-based biocontrol agents are

increasingly being accepted due to the enhanced demand for nonchemical, green antimicrobials. The basic "farm to fork" approach can be used for application of phages throughout the entire food chain. During various phases of food manufacturing, phages are used to eliminate food pathogens without affecting food quality or safety.

Phage application can be classified into: (1) preharvest regulation by reducing the colonization of pathogens of food origin in food-generating fauna and fowls (phage therapy); application of phages through faunal feeds or spewed on bodies prior to animal detriment/slaughter to avert microbial contamination of meat at harvest locations. Bacteriophages can also be employed to avoid spoilage of fruits and vegetables prior to harvesting; (2) employment of phage to sterilize surface areas in which foods under preparation come in contact in food industry (phage biosanitation and biocontrol); studies have confirmed the effectiveness of phages in eradication of biofilms; (3) postharvest restriction of pathogenic microbes associated with food stuffs by phage employment straight onto the harvested/postharvest processed types (biopreservation) (Greer, 2005; Sillankorva et al., 2012; O'Sullivan et al., 2019) (Tables 3 and 4). Phages are applied either as mono-phage or as phage

Table 3 A summary of studies on preharvest application of *Salmonella* phages

Phage	Animal	Reference
φ25, φ151, and φ10	Ceca of broiler chickens	Atterbury et al. (2007)
Phage cocktail	Swine	Wall et al. (2010)
UAB_Phi20, UAB_Phi78, and UAB_Phi87	Mouse, White leghorn, and chicken	Bardina et al. (2012)
ΦCJ07	1-day-old chicks	Lim et al. (2012)
Φ st1	Chicken	Wong et al. (2014)
PSE phage	Quail	Ahmadi et al. (2016)

Table 4 A summary of studies on direct application of *Salmonella* phages onto a variety of foods

Phage	Food	Reference
Phage P22, Phage 29C	Chicken skin	Goode et al. (2003)
Phage P7	Cooked or raw meat	Bigwood et al. (2008)
Cocktail (SCPLX-1)	Fresh-cut apple, honeydew melon slices	Leverentz et al. (2001)
SJ2	Milk cheese	Modi et al. (2001)
F01-E2	Turkey deli meat, chocolate milk, hot dogs, and seafood	Guenther et al. (2012)
UAB_Phi 20, UAB_Phi78, and UAB_Phi87	Pig skin, chicken breast, eggs, and lettuce	Spricigo et al. (2013)
P22	Whole and skimmed milk, apple juice, liquid egg, and energy drink	Zinno et al. (2014)
SE07	Fruit juice, fresh eggs, beef, and chicken meat	
LPST10	Lettuce, tofu	Huang et al. (2018)

Table 5 Commercial *Salmonella* phage preparations

Product	Description	Website
Phage guard S	Micreos	https://phageguard.com/meat-poultry/
SalmoFresh™	Intralytix	http://www.intralytix.com/index.php?page=prod&id=3
SalmoPro™	OmniLytics, Inc	http://www.fda.gov/downloads/Food/IngredientsPackagingLabeling/GRAS/Notice Inventory/UCM624100.pdf

concoction. In phage therapy, new vistas were opened in the year 2006 when "USFDA" permitted a phage preparation called "ListShield," which is a concoction of six individually purified phages manufactured by Intralytix Inc., against *Listeria monocytogenes as* an antimicrobial food preservative in precooked and poultry foodstuffs (Bren, 2007). Concomitant to the development, acceptance of phages as food additives by FSIS directive 7120.1 is the most important stepping stone.

Numerous commercial *Salmonella* phage provisions employed for biocontrol of hazardous microbes that are accepted by the FDA are shown in Table 5.

7 Phage Proteins As an Alternative to Antibiotics

Bacteriophages are extraordinary source of proteins and can be tapped for biotechnological applications. Bacteriophage functional genomics through genome sequencing offers exciting possibility of phages being explored for detection, typing, and control of bacteria (Santos et al., 2018). Antimicrobial phage-encoded polypeptides are harnessed at all stages in the bacteriophage life cycle. Interestingly, polypeptides encoded by temperate phages can also be exploited. Upon sequencing of 26 *Staphylococcus aureus* phages, Liu et al. (2004) identified 31 polypeptide families showing antimicrobial activity against *S. aureus*.

Bacteriophages, during the infection process (adsorption and lysis steps), use different enzymes to degrade different host cell barriers, and these enzymes interestingly have a potential to be used as antimicrobial proteins. Major group of enzymes involved are depolymerases and endolysins. Additional phage-encoded enzymes identified to have potential applications as antibacterial weapons are holins, anti-CRISPR proteins, and "Virion Associated Peptidoglycan Hydrolases (VAPGHs)" (Santos et al., 2018).

Depolymerases are tail-related enzymes produced by bacteriophages that may degrade extracellular polysaccharides (EPS), present on the bacterial surface, thereby facilitating access to the host receptors. Depolymerases either act on capsular polysaccharides or exopolysaccharides of biofilm environments or "cleave structural polysaccharides" such as "LPS" or the "PG glycan strands" (Latka et al., 2017). Based on the mode of action of polysaccharide cleavage, depolymerases are classified into different types: (1) Endosialidases; (2) Endorhamnosidases; (3) Alginate Lyases; and (4) Hyaluronate Lyases. These protease and SDS-resistant monomeric enzymes exhibit parallel right-handed β-helices and are demonstrated to remain

stable at an acidic condition (pH 5.0) and upstretched temperatures of up to 80 °C (Steinbacher et al., 1994; Knecht et al., 2020). The hallmark of depolymerization is formation of halos surrounding the plaque that enhances in diameter during the course of incubation, while plaque size remains constant and can be used to detect capsule-degrading phages (Cornelissen et al., 2011). One of the striking features of depolymerases is to destroy biofilms by degrading EPS. Disruption of the biofilm facilitates the penetration of antibiotics, disinfectants, and those phages that are ineffective in biofilm but can act on planktonic cells. Thus, the recommended strategy to treat bacterial biofilms is with amalgamation of diverse phages/depolymerases acting on dissimilar receptors/structures. For developing an efficient method, it is suggested to recognize mode of action of interaction between depolymerases and additional agents in knocking out biofilms of bacteria.

Encoded by bacteriophages, endolysins popularly known as lysins are involved in the degradation of peptidoglycan during the progeny release of bacteriophages at the lytic reproductive cycle termination phase (Nelson et al., 2012). Peptidoglycan structures endure barometric hydrostatic pressure in cells of bacteria ranging from 20 to 50, and this collapsing of the peptidoglycan (PG) cell wall polymer leads to instantaneous consequences of osmotic shock, rupture of cell, and at times decease of the host microbe (Seltmann and Holst, 2013). When applied exogenously, lysins could destroy peptidoglycan from outside, leading to the quick disintegration lysis of gram-positive bacteria (Loessner et al., 1995). Nevertheless, with gram-positive bacteria, the outer membrane impairs direct access of peptidoglycan by lysins limiting its application (Loessner, 2005; Fischetti, 2005). This potential of using endolysin as enzybiotic against pathogenic bacteria is being exploited. Endolysin was unfolded as therapeutic "enzybiotic" by Nelson and coworkers in 2001 by demonstrating the ability of purified recombinant endolysin to prevent or reduce establishment of Group A Streptococci in mice mucosal exteriors (Nelson et al., 2001). In 2015, the USA recognized endolysin in the "National Action Plan" as a part of tackling AMR microbes (Love et al., 2018).

The fundamental configuration of lysins contains of two domains disconnected by a short linker region: "N-terminal catalytic domain (CD)" and the "C-terminal cell wall binding domain (CBD)"catalyzes peptidoglycan hydrolysis and binds to a specific substrate in cell wall repectively (Nelson et al., 2012; Oliveira et al., 2012). In few cases, lysins with multiple CDs are found in bacteriophages. The catalytic area of endolysin signifies the actual enzyme, catalyzing disintegration of cell wall, and can be categorized with regard to mode of action on cleaving locations in "peptidoglycan; muramidase, glycosaminidases, lytic transglucosylases, amidases, endopeptidases." Phage glycosidase are a superfamily of lysozyme-related enzymes that imbue the glycolytic breakdown of O-glycosidic link of bacterial peptidoglycan. Glycosidases split into "N-acetyl-β-D glycosaminidases (glycosaminidases), N-acetyl-β-D-muramidase (muramidases or lysozymes)." Glucosaminidase slashes the glycan component on the decreasing side of NAG, whereas muramidase grazes the glycan component of the PG on the diminishing side of NAM. Similar to muramidases, lytic transglycosylase cleaves the β (1 → 4) linkages between N-acetylmuramyl and N-acetylglucosaminyl residues of peptidoglycan but adds a

new glycosidic bond forming an N-acetyl-1,6- anhydro-muramyl moiety and does not require water molecule for hydrolysis, in contrast to lysozyme. Phage amidase and N-acetylmuramyl-L-alanine amidases are endolysins that hydrolyze the Peptidoglycan link amid N-acetylmuramoyl and L-amino acid deposits. It is hypothesized that this bond of hydrolysis is more disruptive to the PG in comparison to hydrolysis of other bonds, and that bacteriophages that need rapid lysis of host cells may have evolved to favor it. "Phage carboxy/endopeptidase PGHs" form the imposing group of ECDs that slice peptide bond-related amino acids in the small peptides that associate with sugar polymers. Peptidoglycan peptidases are again classified based on their specificity as "carboxypeptidases (removal of C-terminal amino acid) or endopeptidases (cleavage within the peptide)." "LD-or DL-peptidases" slash amid an L- and a D-amino acid, while DD-peptidases cut amid two D-amino acids. With the increased number of endolysin sequences being deposited into the protein data base, the enzymatically active domains are further divided into families and subfamilies.

Some critical properties that consider endolysin as alternatives include host specificity and high refractory toward developing resistance. These enzymes are specific so that without disturbing the often-desired commensal microflora they destroy the target pathogen, thus giving them a benefit over many commonly used antibiotics (Oliveira et al., 2012). There is not much indication of resistance toward endolysin. The exposure of *S. pneumoniae* to lysin in low concentrations that was grown on agar plates as well as in the liquid culture (for several cycles) did not result in reemergence of drug obdurate strains (Loeffler et al., 2001; Fischetti, 2005). Comparable outcomes were acquired with lysins for *S. pyogenes* (PlyC) and *B. anthracis* (PlyG) (Schuch et al., 2002; Schmelcher et al., 2012). A possibility of resistance toward endolysins could be the modification of cell wall causing stearic hindrance; however, the coevality of bacteriophages and their hosts will presumably make the development of unresponsiveness an uncommon occasion to facilitate the phage survival in the environment (Fischetti, 2010; Schmelcher et al., 2012). Antimicrobial efficacy of endolysins, in negligible quantities of "ng," could annihilate microbes from culture suspension in few seconds (Loeffler et al., 2001; Nelson et al., 2001) and is superior to other known biological compounds in terms of time required to eliminate microorganisms this quickly. Another interesting fact is the synergetic effect that is demonstrated by endolysin in blend with other endolysins or antibiotics by increasing the efficacy to eliminate bacteria less accessible to antibiotic or reducing the doses required for certain applications. Coming to safety, no toxicity was demonstrated in pharmacology studies in single and repeated-doses of endolysin in rodents and dogs.

7.1 Endolysins Challenging GramPositive Microbes

Even though phage endolysins have been used mostly against gram-positive bacteria, there is a challenge in using endolysins for gram-positive bacteria as the outer membrane prevents exogenous endolysins contacting with the peptidoglycan.

Efforts are made by using different strategies to overcome the outer membrane (OM) which have enhanced research on endolysins capable of lysing and eliminating gram-positive microbes. These stratagems comprise (1) documentation of endolysins with inherent membrane-passaging characteristics; (2) applying a combination of endolysins and outer membrane-permeabilizing mediators or usages; and (3) production of blending proteins amid endolysins and antibacterial peptides, facilitating self-promoted acceptance of enzyme over the OM (Briers et al., 2015). PG layer in gram-positive microbes is far slender in comparison to PG of gram-positive microbes, thus signifying the requirement of only fewer molecules to degrade the PG, if endolysins could pass the OM. Some gram-positive endolysins have intrinsic membrane-passing capability, and the antibacterial activity of endolysins is enhanced when in union with chemical permeabilizers. One of the most widely used is EDTA. Other permeabilizing agents include organic acids such as "citric, malic, and lactic acids." Cationic peptides, namely, "PGLa peptide or Poly-L arginine," were shown to be effective outer membrane permeabilizers. A novel method has been developed by merging a polycationic nanopeptide and a modular endolysin recognized as "Artilysin." Later, innolysins (combining the endolysins with phage receptor binding proteins) and chimeolysins (fusing two spheres of influence from numerous endolysins to acquire an upgraded endolysin with lytic action, increased solubility, and extended spectrum) were developed. Through investigation, it is required to govern the appropriate external membrane permeabilizers aimed at advanced *in vivo* investigation. Although the nanoparticles can be considered a potent outer membrane-disrupting agent, the studies are still in infancy and is a promising field for further research.

Numerous recombinant endolysins derived from *Salmonella* phage have been reported. The lytic spectrum of endolysins will become broader when jointly employed with cell membrane-permeabilizing compounds. *Salmonella* phage SPN1S endolysin expressed and purified Lim et al. (2012) presented disintegration motion countering *E. coli* and *S. typhimurium* in a buffer through EDTA in cell membrane disruption. The SPN1S exhibited antimicrobial action against "*Pseudomonas*, *Shigella*, *Cronobacter*, *Salmonella*, and *Vibrio* species." In another related study, a modular "*Salmonella* phage endolysin Gp110," with an undefined sphere of C terminus, exhibited lytic activity of extraordinarily high kind against *Salmonella* and other gram-positive microbes. This endolysin with reliable and predictable effectiveness in food and medical applications is of interest for future research. The synergetic effect of endolysin has made it a promising antimicrobial in food industry. Further studies elucidating combination approaches with different outer membrane permeabilizing agents and synergism with different antimicrobial agents will be of interest.

8 Engineered Bacteriophages

Sometimes the natural phages cannot be used due to certain barriers such as phages with desired host range which may have lysogenic gene or phages that may not be able to kill the bacterium effectively. It would be beneficial to bring in modifications

to enhance the therapeutic values, safety, and host range. Recent advances in technologies and tools have resulted in development of modular designer-phages with enhanced features that could control multidrug-resistant bacteria efficiently and can act as innovative tools for detecting pathogens, development of drug, and more (Pires et al., 2016; Tao et al., 2019). Phages are engineered to enhance the antibacterial activity as well to enlarge the host range (Pires et al., 2016). Phage-engineering potentials to create modified phages with exceptional properties for prophylactic and therapeutic applications. Phage-engineering strategies involve Homologous Recombination, BRED (Bacteriophage Recombineering with Electroporated DNA), CRISPR-Cas technique, etc.

Bacteriophage Recombineering with "Electroporated DNA" method comprises coalesce amid coelectroporated viral DNA and PCR yields with arms of affinity. Efficiency of recovery varies, and unassuming slicing is recuperated at greater rate of recurrence than gene additions or substitutions. The method can be used to produce strict lytic phages removing precise genes for lysogeny. PCR analysis can confirm the presence of desired progeny.

CRISPR-Cas-genome technology is a simple and powerful technology utilized to efficiently edit phages. The technology necessitates creation of a host-recombinant strain capable of active Cas protein expression as well as a guide RNA aimed toward the parental phage. The CRISPR/Cas9 editing mechanism can advance by opting for powerful crRNAs that can be a major limiting factor.

Since editing and engineering of phages are significantly simpler nowadays compared to previous years, researchers are now focusing on constructing synthetic phages which are advantageous when natural phages are not available or if there is a need for phages with a particular gene addition. The method involves combining phage genome remains amplified using PCR and/or built employing artificial oligonucleotides; human made phage genomes are assembled into a vector. These improvements with the combination of other technologies mentioned can broaden phage therapy by producing phages on demand by tackling evolved phage resistance as well as narrow host range.

9 Conclusion

With the inevitable and frequent occurrence of antibiotic-resistant *Salmonella* and the urgent call for novel classes of antimicrobials, bacteriophages and bacteriophage encoded proteins act as promising candidates to combat the food-borne pathogen *Salmonella.* Phage therapy, however, is currently being used only in combination with antibiotic treatment or during complex cases in patients where therapeutic failure occurs. The major hindrance in growth of phage therapy is lacunae for well-designed clinical trials and critical analysis of relevant factors leading to failure and success of the trails. A centralized initiative for clinical evaluation of bacteriophages is appropriate. Another limiting factor is the number of phages available for the pathogen to be treated. Considering the high specificity of bacteriophages, a centralized facility with extensive diversity of phages, enough and readily available

in treating the infection of a given patient, becomes obligatory. Moreover, a standardized protocol is obligatory for methods and preparation of formulations.

References

Abedon, S. T., García, P., Mullany, P., & Aminov, R. (2017). Phage therapy: Past, present and future. *Frontiers in Microbiology, 8*, 981.

Ahmadi, M., Karimi Torshizi, M. A., Rahimi, S., & Dennehy, J. J. (2016). Prophylactic bacteriophage administration more effective than post-infection administration in reducing *Salmonella enterica* serovar Enteritidis shedding in quail. *Frontiers in Microbiology, 7*, 1253.

Aminov, R., Caplin, J., Chanishvili, N., Coffey, A., Cooper, I., De Vos, D., Doškař, J., Friman, V. P., Kurtböke, İ., Pantucek, R., & Pirnay, J. P. (2017). Application of bacteriophages. *Microbiology Australia, 38*(2), 63–66.

Atterbury, R. J., Van Bergen, M. A. P., Ortiz, F., Lovell, M. A., Harris, J. A., De Boer, A., & Barrow, P. A. (2007). Bacteriophage therapy to reduce *Salmonella* colonization of broiler chickens. *Applied and Environmental Microbiology, 73*(14), 4543–4549.

Bardina, C., Spricigo, D. A., Cortés, P., & Llagostera, M. (2012). Significance of the bacteriophage treatment schedule in reducing *Salmonella* colonization of poultry. *Applied and Environmental Microbiology, 78*(18), 6600–6607.

Bigwood, T., Hudson, J. A., Billington, C., Carey-Smith, G. V., & Heinemann, J. A. (2008). Phage inactivation of food-borne pathogens on cooked and raw meat. *Food Microbiology, 25*(2), 400–406.

Briers, Y., & Lavigne, R. (2015). Breaking barriers: expansion of the use of endolysins as novel antibacterials against Gram-negative bacteria. *Future Microbiology, 10*(3), 377–390. https://doi.org/10.2217/fmb.15.8

Bruhn, C. M. (1995). Consumer attitudes and market response to irradiated food. *Journal of Food Protection, 58*(2), 175–181.

Carrasco, E., Morales-Rueda, A., & García-Gimeno, R. M. (2012). Cross-contamination and recontamination by *Salmonella* in foods: A review. *Food Research International, 45*(2), 545–556.

Centers for Disease Control and Prevention. (2013). CDC's outbreak net foodborne outbreak online database. http://wwwn.cdc.gov/foodborneoutbreaks

Cheetham, B. F., & Katz, M. E. (1995). A role for bacteriophages in the evolution and transfer of bacterial virulence determinants. *Molecular Microbiology, 18*(2), 201–208.

Cooper, C. J., Khan Mirzaei, M., & Nilsson, A. S. (2016). Adapting drug approval pathways for bacteriophage-based therapeutics. *Frontiers in Microbiology, 7*, 1209.

Coppen, P., Fenner, S., & Salvat, G. (1998). Antimicrobial efficacy of AvGard carcase wash under industrial processing conditions. *British Poultry Science, 39*(2), 229–234.

Cornelissen, A., Ceyssens, P. J., T'syen, J., Van Praet, H., Noben, J. P., Shaburova, O. V., Krylov, V. N., Volckaert, G., & Lavigne, R. (2011). The T7-related *Pseudomonas putida* phage φ15 displays virion-associated biofilm degradation properties. *PLoS One, 6*(4), e18597.

Eeckhaut, V., Van Immerseel, F., Dewulf, J., Pasmans, F., Haesebrouck, F., Ducatelle, R., Courtin, C. M., Delcour, J. A., & Broekaert, W. F. (2008). Arabinoxylooligosaccharides from wheat bran inhibit *Salmonella* colonization in broiler chickens. *Poultry Science, 87*(11), 2329–2334.

Eng, S. K., Pusparajah, P., Ab Mutalib, N. S., Ser, H. L., Chan, K. G., & Lee, L. H. (2015). *Salmonella*: A review on pathogenesis, epidemiology and antibiotic resistance. *Frontiers in Life Science, 8*(3), 284–293.

Fàbrega, A., & Vila, J. (2013). Salmonella enterica serovar Typhimurium skills to succeed in the host: virulence and regulation. *Clinical Microbiology Reviews, 26*(2), 308–341.

Fischetti, V. A. (2005). Bacteriophage lytic enzymes: Novel anti-infectives. *Trends in Microbiology, 13*(10), 491–496.

Fischetti, V. A. (2010). Bacteriophage endolysins: A novel anti-infective to control Gram-positive pathogens. *International Journal of Medical Microbiology, 300*(6), 357–362.

Fischetti, V. A., Nelson, D., & Schuch, R. (2006). Reinventing phage therapy: Are the parts greater than the sum? *Nature Biotechnology, 24*(12), 1508–1511.

Fish, R., Kutter, E., Wheat, G., Blasdel, B., Kutateladze, M., & Kuhl, S. (2016). Bacteriophage treatment of intransigent diabetic toe ulcers: A case series. *Journal of Wound Care, 25*(Suppl 7), S27–S33.

Fortuna, W., Międzybrodzki, R., Weber-Dąbrowska, B., & Górski, A. (2008). Bacteriophage therapy in children: Facts and prospects. *Medical Science Monitor, 14*(8), RA126–RA132.

Galiş, A. M., Marcq, C., Marlier, D., Portetelle, D., Van, I., Beckers, Y., & Théwis, A. (2013). Control of *Salmonella* contamination of shell eggs – Preharvest and postharvest methods: A review. *Comprehensive Reviews in Food Science and Food Safety, 12*(2), 155–182.

Garcia, P., Martinez, B., Obeso, J. M., & Rodriguez, A. (2008). Bacteriophages and their application in food safety. *Letters in Applied Microbiology, 47*(6), 479–485.

Goode, D., Allen, V. M., & Barrow, P. A. (2003). Reduction of experimental *Salmonella* and *Campylobacter* contamination of chicken skin by application of lytic bacteriophages. *Applied and Environmental Microbiology, 69*(8), 5032–5036.

Goodridge, L. D. (2010). Designing phage therapeutics. *Current Pharmaceutical Biotechnology, 11*(1), 15–27.

Goodridge, L. D., & Bisha, B. (2011). Phage-based biocontrol strategies to reduce food-borne pathogens in foods. *Bacteriophage, 1*(3), 130–137.

Grace, D. (2015). Food safety in low and middle income countries. *International Journal of Environmental Research and Public Health, 12*(9), 10490–10507.

Greer, G. G. (2005). Bacteriophage control of food-bornebacteria. *Journal of Food Protection, 68*(5), 1102–1111.

Guenther, S., Herzig, O., Fieseler, L., Klumpp, J., & Loessner, M. J. (2012). Biocontrol of *Salmonella* Typhimurium in RTE foods with the virulent bacteriophage FO1-E2. *International Journal of Food Microbiology, 154*(1–2), 66–72.

Harada, L. K., Silva, E. C., Campos, W. F., Del Fiol, F. S., Vila, M., Dąbrowska, K., Krylov, V. N., & Balcão, V. M. (2018). Biotechnological applications of bacteriophages: State of the art. *Microbiological Research, 212*, 38–58.

Havelaar, A. H., Kirk, M. D., Torgerson, P. R., Gibb, H. J., Hald, T., Lake, R. J., & Speybroeck, N. (2015). World Health Organization global estimates and regional comparisons of the burden of food-borne disease in 2010. *PLoS Medicine, 12*(12), e1001923.

Hermoso, J. A., García, J. L., & García, P. (2007). Taking aim on bacterial pathogens: From phage therapy to enzybiotics. *Current Opinion in Microbiology, 10*(5), 461–472.

Hietala, V., Horsma-Heikkinen, J., Carron, A., Skurnik, M., & Kiljunen, S. (2019). The removal of endo-and enterotoxins from bacteriophage preparations. *Frontiers in Microbiology, 10*, 1674.

Huang, C., Shi, J., Ma, W., Li, Z., Wang, J., Li, J., & Wang, X. (2018). Isolation, characterization, and application of a novel specific *Salmonella* bacteriophage in different food matrices. *Food Research International, 111*, 631–641.

Kim, J. W., & Slavik, M. F. (1994). Trisodium phosptiate (TSP)treatment of beef surfaces to reduce *Escherichia coli* O157: H7 and *Salmonella typhimurium*. *Journal of Food Science, 59*(1), 20–22.

Knecht, L. E., Veljkovic, M., & Fieseler, L. (2020). Diversity and function of phage encoded depolymerases. *Frontiers in Microbiology, 10*, 2949.

Kristkova, Z. S., Grace, D., & Kuiper, M. (2017). *The economics of food safety in India: A rapid assessment*. Wageningen University and Research.

Latka, A., Maciejewska, B., Majkowska-Skrobek, G., Briers, Y., & Drulis-Kawa, Z. (2017). Bacteriophage-encoded virion-associated enzymes to overcome the carbohydrate barriers during the infection process. *Applied Microbiology and Biotechnology, 101*(8), 3103–3119.

Leverentz, B., Conway, W. S., Alavidze, Z., Janisiewicz, W. J., Fuchs, Y., Camp, M. J., Chighladze, E., & Sulakvelidze, A. (2001). Examination of bacteriophage as a biocontrol method for *Salmonella* on fresh-cut fruit: A model study. *Journal of Food Protection, 64*(8), 1116–1121.

Lim, T. H., Kim, M. S., Lee, D. H., Lee, Y. N., Park, J. K., Youn, H. N., Lee, H. J., Yang, S. Y., Cho, Y. W., Lee, J. B., & Park, S. Y. (2012). Use of bacteriophage for biological control of *Salmonella Enteritidis* infection in chicken. *Research in Veterinary Science, 93*(3), 1173–1178.

Liu, J., Dehbi, M., Moeck, G., Arhin, F., Bauda, P., Bergeron, D., Callejo, M., Ferretti, V., Ha, N., Kwan, T., & McCarty, J. (2004). Antimicrobial drug discovery through bacteriophage genomics. *Nature Biotechnology, 22*(2), 185–191.

Loeffler, J. M., Nelson, D., & Fischetti, V. A. (2001). Rapid killing of *Streptococcus pneumoniae* with a bacteriophage cell wall hydrolase. *Science, 294*(5549), 2170–2172.

Loessner, M. J. (2005). Bacteriophage endolysins – Current state of research and applications. *Current Opinion in Microbiology, 8*(4), 480–487.

Loessner, M. J., Wendlinger, G., & Scherer, S. (1995). Heterogeneous endolysins in *Listeria monocytogenes* bacteriophages: A new class of enzymes and evidence for conserved holin genes within the siphoviral lysis cassettes. *Molecular Microbiology, 16*(6), 1231–1241.

Love, M. J., Bhandari, D., Dobson, R. C., & Billington, C. (2018). Potential for bacteriophage endolysins to supplement or replace antibiotics in food production and clinical care. *Antibiotics, 7*(1), 17.

Majowicz, S. E., Musto, J., Scallan, E., Angulo, F. J., Kirk, M., O'Brien, S. J., Jones, T. F., Fazil, A., & Hoekstra, R. M. (2010). International Collaboration on Enteric Disease "Burden of Illness" Studies, 2010. The global burden of nontyphoidal *Salmonella gastroenteritis*. *Clinical Infectious Diseases, 50*(6), 882–889.

Mani-López, E., García, H. S., & López-Malo, A. (2012). Organic acids as antimicrobials to control *Salmonella* in meat and poultry products. *Food Research International, 45*(2), 713–721.

McCallin, S., Sacher, J. C., Zheng, J., & Chan, B. K. (2019). Current state of compassionate phage therapy. *Viruses, 11*(4), 343.

Modi, R., Hirvi, Y., Hill, A., & Griffiths, M. W. (2001). Effect of phage on survival of *Salmonella Enteritidis* during manufacture and storage of cheddar cheese made from raw and pasteurized milk. *Journal of Food Protection, 64*(7), 927–933.

Moelling, K., Broecker, F., & Willy, C. (2018). A wake-up call: We need phage therapy now. *Viruses, 10*(12), 688.

Møretrø, T., Heir, E., Nesse, L. L., Vestby, L. K., & Langsrud, S. (2012). Control of *Salmonella* in food related environments by chemical disinfection. *Food Research International, 45*(2), 532–544.

Morild, R. K., Christiansen, P., Sørensen, A. H., Nonboe, U., & Aabo, S. (2011). Inactivation of pathogens on pork by steam-ultrasound treatment. *Journal of Food Protection, 74*(5), 769–775.

Nair, D. V., Nannapaneni, R., Kiess, A., Schilling, W., & Sharma, C. S. (2014). Reduction of *Salmonella* on turkey breast cutlets by plant-derived compounds. *Food-bornePathogens and Disease, 11*(12), 981–987.

Nair, D. V., Venkitanarayanan, K., & Kollanoor Johny, A. (2018). Antibiotic-resistant *Salmonella* in the food supply and the potential role of antibiotic alternatives for control. *Food, 7*(10), 167.

Nelson, D., Loomis, L., & Fischetti, V. A. (2001). Prevention and elimination of upper respiratory colonization of mice by group A Streptococci by using a bacteriophage lytic enzyme. *Proceedings of the National Academy of Sciences, 98*(7), 4107–4112.

Nelson, D. C., Schmelcher, M., Rodriguez-Rubio, L., Klumpp, J., Pritchard, D. G., Dong, S., & Donovan, D. M. (2012). Endolysins as antimicrobials. *Advances in Virus Research, 83*, 299–365.

Nilsson, A. S. (2014). Phage therapy – Constraints and possibilities. *Upsala Journal of Medical Sciences, 119*(2), 192–198.

O'Sullivan, L., Bolton, D., McAuliffe, O., & Coffey, A. (2019). Bacteriophages in food applications: From foe to friend. *Annual Review of Food Science and Technology, 10*, 151–172.

Oliveira, H., Azeredo, J., Lavigne, R., & Kluskens, L. D. (2012). Bacteriophage endolysins as a response to emerging food-bornepathogens. *Trends in Food Science and Technology, 28*(2), 103–115.

Parracho, H. M., Burrowes, B. H., Enright, M. C., McConville, M. L., & Harper, D. R. (2012). The role of regulated clinical trials in the development of bacteriophage therapeutics. *Journal of Molecular and Genetic Medicine, 6*, 279.

Pires, D. P., Oliveira, H., Melo, L. D., Sillankorva, S., & Azeredo, J. (2016). Bacteriophage-encoded depolymerases: Their diversity and biotechnological applications. *Applied Microbiology and Biotechnology, 100*(5), 2141–2151.

Pirnay, J. P., Blasdel, B. G., Bretaudeau, L., Buckling, A., Chanishvili, N., Clark, J. R., Corte-Real, S., Debarbieux, L., Dublanchet, A., De Vos, D., & Gabard, J. (2015). Quality and safety requirements for sustainable phage therapy products. *Pharmaceutical Research, 32*(7), 2173–2179.

Prestinaci, F., Pezzotti, P., & Pantosti, A. (2015). Antimicrobial resistance: A global multifaceted phenomenon. *Pathogens and Global Health, 109*(7), 309–318.

Reindel, R., & Fiore, C. R. (2017). Phage therapy: Considerations and challenges for development. Clin Infect Dis. 2017;64(11):1589–1590. https://doi.org/10.1093/cid/cix188. Google Access 10 March 2023.

Rossmann, M. G., Morais, M. C., Leiman, P. G., & Zhang, W. (2005). Combining X-ray crystallography and electron microscopy. *Structure, 13*(3), 355–362.

Santos, S. B., Costa, A. R., Carvalho, C., Nóbrega, F. L., & Azeredo, J. (2018). Exploiting bacteriophage proteomes: The hidden biotechnological potential. *Trends in Biotechnology, 36*(9), 966–984.

Scallan, E., Hoekstra, R. M., Angulo, F. J., Tauxe, R. V., Widdowson, M. A., Roy, S. L., & Griffin, P. M. (2011). Foodborne illness acquired in the United States – Major pathogens. *Emerging Infectious Diseases, 17*(1), 7.

Schmelcher, M., Donovan, D. M., & Loessner, M. J. (2012). Bacteriophage endolysins as novel antimicrobials. *Future Microbiology, 7*(10), 1147–1171.

Schooley, R. T., Biswas, B., Gill, J. J., Hernandez-Morales, A., Lancaster, J., Lessor, L., Barr, J. J., Reed, S. L., Rohwer, F., Benler, S., & Segall, A. M. (2017). Development and use of personalized bacteriophage-based therapeutic cocktails to treat a patient with a disseminated resistant *Acinetobacter baumannii*infection. *Antimicrobial Agents and Chemotherapy, 61*(10).

Schuch, R., Nelson, D., & Fischetti, V. A. (2002). A bacteriolytic agent that detects and kills *Bacillus anthracis*. *Nature, 418*(6900), 884–889.

Seltmann, G., & Holst, O. (2013). *The bacterial cell wall*. Springer Science & Business Media.

Sillankorva, S. M., Oliveira, H., & Azeredo, J. (2012). Bacteriophages and their role in food safety'. *International Journal of Microbiology, 2012*, 863945.

Silva, C. C., Silva, S. P., & Ribeiro, S. C. (2018). Application of bacteriocins and protective cultures in dairy food preservation. *Frontiers in Microbiology, 9*, 594.

Spricigo, D. A., Bardina, C., Cortés, P., & Llagostera, M. (2013). Use of a bacteriophage cocktail to control *Salmonella* in food and the food industry. *International Journal of Food Microbiology, 165*(2), 169–174.

Steinbacher, S., Seckler, R., Miller, S., Steipe, B., Huber, R., & Reinemer, P. (1994). Crystal structure of P22 tailspike protein: Interdigitated subunits in a thermostable trimer. *Science, 265*(5170), 383–386.

Su, L. H., Chiu, C. H., Chu, C., & Ou, J. T. (2004). Antimicrobial resistance in nontyphoid *Salmonella* serotypes: A global challenge. *Clinical Infectious Diseases, 39*(4), 546–551.

Sulakvelidze, A., Alavidze, Z., & Morris, J. G. (2001). Bacteriophagetherapy. *Antimicrobial Agents and Chemotherapy, 45*(3), 649–659.

Summers, W. C. (2001). Bacteriophage therapy. *Annual Reviews in Microbiology, 55*(1), 437–451.

Tanji, Y., Shimada, T., Fukudomi, H., Miyanaga, K., Nakai, Y., & Unno, H. (2005). Therapeutic use of phage cocktail for controlling *Escherichia coli* O157: H7 in gastrointestinal tract of mice. *Journal of Bioscience and Bioengineering, 100*(3), 280–287.

Tao, P., Chen, Y., Batra, H., Dong, J., Chen, C., & Rao, V. B. (2019). Genetic engineering of bacteriophages against infectious diseases. *Frontiers in Microbiology, 10*, 954.

Venkitanarayanan, K. S., Ezeike, G. O., Hung, Y. C., & Doyle, M. P. (1999). Efficacy of electrolyzed oxidizing water for inactivating *Escherichia coli* O157: H7, *Salmonella enteritidis*, and *Listeria monocytogenes*. *Applied Environmental Microbiology, 65*(9), 4276–4279.

Verbeken, G., Pirnay, J. P., Lavigne, R., Jennes, S., De Vos, D., Casteels, M., & Huys, I. (2014). Call for a dedicated European legal framework for bacteriophage therapy. *Archivum Immunologiae et TherapiaeExperimentalis, 62*(2), 117–129.

Vila, B., Fontgibell, A., Badiola, I., Esteve-Garcia, E., Jiménez, G., Castillo, M., & Brufau, J. (2009). Reduction of *Salmonella enterica* var. Enteritidis colonization and invasion by *Bacillus cereus* var. toyoi inclusion in poultry feeds. *Poultry Science, 88*(5), 975–979.

Wall, S. K., Zhang, J., Rostagno, M. H., & Ebner, P. D. (2010). Phage therapy to reduce preprocessing *Salmonell*a infections in market-weight swine. *Applied and Environmental Microbiology, 76*(1), 48–53.

Wallis, T. S., & Galyov, E. E. (2000). Molecular basis of Salmonella-induced enteritis: micro-Review. *Molecular Microbiology, 36*(5), 997–1005.

Wong, C. L., Sieo, C. C., Tan, W. S., Abdullah, N., Hair-Bejo, M., Abu, J., & Ho, Y. W. (2014). Evaluation of a lytic bacteriophage, Φ st1, for biocontrol of *Salmonella enterica* serovar Typhimurium in chickens. *International Journal of Food Microbiology, 172*, 92–101.

World Health Organization. (2015). *WHO estimates of the global burden of foodborne diseases: Food-borne disease burden epidemiology reference group 2007–2015*. World Health Organization.

Wright, A., Hawkins, C. H., Änggård, E. E., & Harper, D. R. (2009). A controlled clinical trial of a therapeutic bacteriophage preparation in chronic otitis due to antibiotic-resistant *Pseudomonas aeruginosa*; A preliminary report of efficacy. *Clinical Otolaryngology, 34*(4), 349–357.

Zinno, P., Devirgiliis, C., Ercolini, D., Ongeng, D., & Mauriello, G. (2014). Bacteriophage P22 to challenge *Salmonella* in foods. *International Journal of Food Microbiology, 191*, 69–74.

Marine Bioactive Compounds: An Alternative to Antibiotics

P. Amruth, Rosemol Jacob M., and Suseela Mathew

Contents

Abstract

Antimicrobial resistance (AMR) remains as a critical concern of modern medicine today. Medical practitioners face difficulty in treating various microbial-associated ailments as a consequence of acquired antibiotic resistance and evolution of pathogens. Therefore, requisite to alternate antibiotics or alternatives to antibiotics becomes a necessity. Oceanic realm remains to be the repository of several bioactive compounds with pronounced biological activities. There remains aplenty of compounds isolated with antibacterial, antiviral, anticancer, antirheumatic, anti-inflammatory, and anticoagulant and numerous other properties. Owing to its structural diversity, marine environments offer scope to identify unique compounds with potential biological functionality. In this section, the exploitation of marine organisms for the isolation of potential antimicrobials, its characteristics, chemistry,

P. Amruth (✉) · R. Jacob M. · S. Mathew
Biochemistry and Nutrition Division, ICAR-Central Institute of Fisheries Technology, Cochin, India
e-mail: amrithprakash.edu@gmail.com; rosemol529@gmail.com; suseela.mathew@icar.gov.in

M. P. Mothadaka et al. (eds.), *Handbook on Antimicrobial Resistance*,
https://doi.org/10.1007/978-981-19-9279-7_37

and mode of action are briefly described. The chapter aims to induce insights to researchers and scientists to formulate novel drugs by exploring these potential compounds that undoubtedly act as alternate antibiotics or alternate to antibiotics and nutraceutical compounds with potential antimicrobial activities.

Keywords

AMR · Nutraceutical · Antimicrobial · Antibiotic

1 Introduction

Antimicrobial resistance (AMR) remains as a key obstacle in instituting effective treatment for infectious diseases. A death rate of two million people each year has been observed globally as a result of the infection triggered by bacteria. In view of this treacherous scenario, the World Health Organization (WHO) has systematized the "World Antimicrobial Awareness Week" for raising awareness about the rational use of antimicrobials. To tackle these problems, there exists demand to discover novel antimicrobials with remarkable biological functionality. The marine realm serves as a prospective deposit of numerous natural compounds with imminent scope which has to be explored to derive novel antimicrobials in future.

The earth's surface covers 70 percentiles with oceanic realm that constitute nearly 87% of life on earth. The marine ecosystem encompasses immense collection of distinctive valuable components with potential bioactivities (Kang et al., 2015). More than 200,000 species of algae and invertebrates and 12 exclusive phyla of marine organism are found in the sea. Abundant natural products are seen to instigate from marine faunae like sponges, cnidarians, mollusks, and microbes, notably bacteria. Dated from 1985 to 2008, there are records of nearly 12,322 novel compounds isolated from marine species. A record of around 6948 natural compounds has been sequestered from marine environment, dated from 2009 (Hu et al., 2011). These records signify the prosperity and diversity of the existing natural compounds in ocean. Therefore, it is very evident that ocean undoubtedly serves as a repository of unexplored bioactive compounds.

From the investigations carried in the preceding years, it is foreseen that the bioactive compounds derived from oceanic source outperform to a great extent in terms of the structural diversity and uniqueness compared to ones that have been derived from terrestrial origin. In view of the remarkable biological functionality offered by the marine-derived bioactive compounds, they are undoubtedly regarded as important molecules in the formulation of novel and desirable agents in the fields of biomedicine and pharmaceutics (Aneiros & Garateix, 2004). Microorganisms residing at extreme habitats generate antibiotics posing enormous inhibitory actions against detrimental Gram +ve and Gram -ve bacteria (Fenical, 1993). Chlorthiamide, an organic compound isolated from C*lostridium cellulolyticum* (Lincke et al., 2010),

apart from its antibacterial role, generates compounds such as salinosporamide, which functions as a proteasome inhibitor in clinical trials (Ahn et al., 2007). Other compounds such as marinomycins function as antitumor antibiotics (Kwon et al., 2006), and apratoxin A isolated from marine kingdom has reported excellent anticancer capabilities (Luesch et al., 2001).

As a consequence of the inconvenience in the cultivation and isolation in the laboratory compared to those being isolated from terrestrial environment, the marine realm offers unique and distinctive bioactive compounds that are underexplored (Kanagasabapathy et al., 2011). In light of the research outcome from the previous studies, it is manifested that factors including unavailability of sophisticated methodologies, increased research expenses, lack of expertise, and manpower remain as threats to the research in this area. Moreover, as a result of regulatory barriers, the identification of novel promising compounds remains to be ineffective due to the lack of liberty to conduct investigation, and thus there exists an unceasing decline in the isolation of antibacterial compounds from sea or ocean. In the evolutionary process, acquisition of metabolic power is also ascertained by pathogens due to mutations that results in modifying its genetic materials (Bérdy, 2012).

This chapter provides a brief description on the antibiotics from oceanic environment, their related chemistry and antibacterial characteristics, and their mechanisms of action. Thus, for therapeutic and medical requirements, it evokes insights to researchers to separate antibacterial compounds which might be used in new formulations of drugs and can be used after clinical trials.

2 Potential Antibiotics from Marine Realm and Their Prospective Exploration as Nutraceuticals

The ocean remains as an unexplored cradle of scaffolds with distinctive products that motivated scientists and researchers to explore them for research and developmental activities, especially in the area of antimicrobial resistance. Marine creatures are categorized into three important groups, i.e., nekton, plankton, and benthos, which hold a broader molecular diversity compared to terrestrial organisms as a result of their prolonged evolutionary background (Belarbi et al., 2003). Research on natural products of marine origin has led to identification of abundant assorted composites by way of powerful medicinal significance. These composites are categorized into phenols, alkaloids, tannins, quinones, glycosides, xanthones, polyketides, lactones, terpenes, macrocycles, peptides, and fatty acids (Nweze et al., 2020).

Bioactive compounds resulting from marine invertebrates include 60% of marine fauna which are advantageous therapeutic agents for humans. From marine invertebrates, according to the investigations of Leal et al. (2012), nearly 10,000 new natural compounds were isolated. A specific mechanism has been ascertained in marine invertebrates for defending them against intrusive microbes by the innate immune system (Wright, 1981) that leads to the formation of an antimicrobial

peptide Myticusin-1, an antimicrobial peptide isolated from *Mytilus* of the Molluscan family has been proved to act against pathogenic microorganisms.

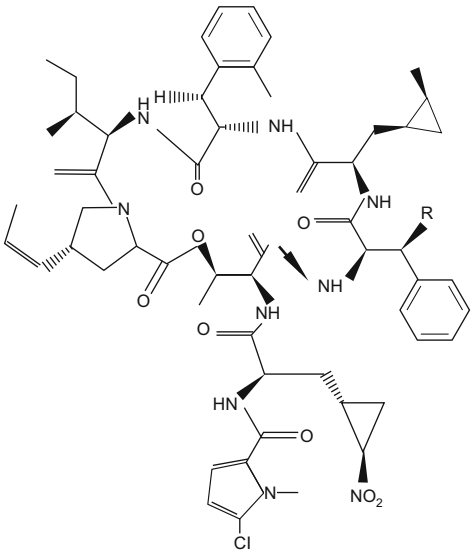
The terrestrial microorganisms have been anticipated as the chief focus of research from the innovation of penicillin by Alexander Fleming in 1929. Marine organisms have now become the center of researchers worldwide due to the increased duplication rate of metabolites isolated from the soil which leads to augmented resistance of pathogens and a surge in communicable ailments. Soil Actinomycetes are demonstrated to generate unique and distinctive natural products, among many are antibiotics (Berdy, 2005). In the midst, the fruitful generator of effective natural products originated from marine *Actinobacteria* (Manivasagan et al., 2014) significantly becomes advantageous to pharmaceutical industry. As a result of the extremity in environmental profile shown by marine domain, a wider genetic and metabolic diversity is postured by marine Actinomycetes which have paved the way for the generation of novel metabolites. Secondary metabolites derived from marine bacteria remained efficacious against certain infectious microbes. Diazepinomicin produced from ascidian, *Micromonospora* (Charan et al., 2004), lobophorins E and F, an actinobacterial strain from sea sediment (Riedlinger et al., 2004), hormaomycins B and C, an *Actinomycetes* strain from marine sediment, and abyssomicin C, a *Verrucosispora* strain from marine sediment (Bae et al., 2015), are few examples to cite the antimicrobial efficacies of these metabolites.

To the scientific world, Kingdom fungi has also significantly contributed to potential antibiotics. From *Phaeophyta Rosenvingea* sp., a potential antibiotic termed pestalone has been derived (Cueto et al., 2001). From *Aspergillus* sp., an antibiotic terretonin G is isolated and OPMF00272 from *Porifera* (Fukuda et al., 2014). The fungal strain *Aspergillus ostianus* is the source for the antimicrobial agent, namely, 9-chloro-8-hydroxy-8, 9-deoxyaspyrone, 8-chloro-9-hydroxy-8, 9-deoxyasperlactone, and 9-chloro-8-hydroxy-8,9-deoxyasperlactone (Namikoshi et al., 2003). In view of these prospective bioactive compounds offered by marine organisms, they can also be potentially explored in the formulation of novel nutraceuticals apart from their usage in the field of biomedicine.

3 Biochemical Functionality of Marine Antibiotics

Antibiotics derived from terrestrial kingdom are known to exhibit different actions against diverse group of pathogens, and now researchers worldwide are apprehensive with regard to the specific mechanism of compounds isolated from marine bacteria. Studies conducted earlier have engrossed on the identification of structural characteristics of novel antibacterial compounds from oceanic territory. At present, there exists very limited research progression with regard to the complete mechanism of action of antibiotics derived from the aquatic environment.

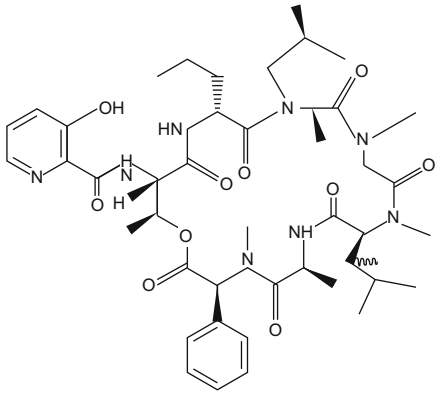
3.1 Protein/Polypeptide



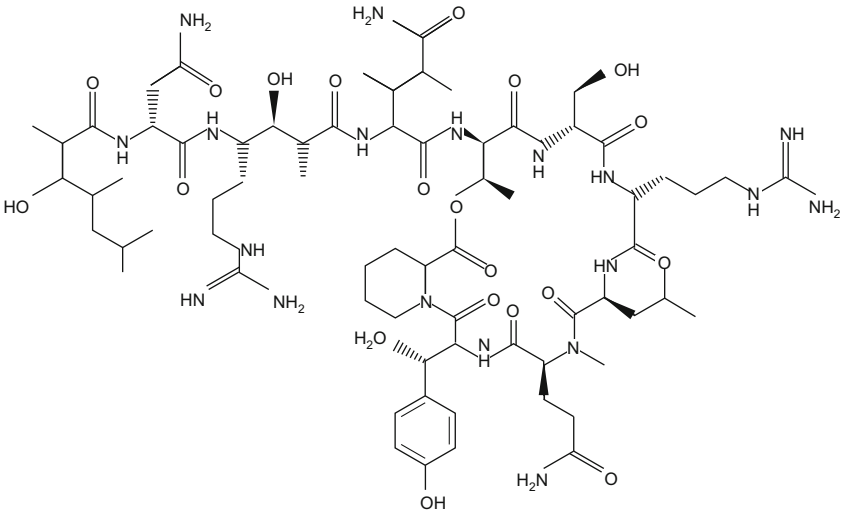
Hormaomycin B

The external of marine yellow perch fish *Perca flavescens* is abundant in a protein bacteriocin PSY2, which exhibits antibacterial activities against Gram +ve and -ve bacteria. The inactivity of this strain against trypsin treatment has confirmed it as protein, and this turns to be an antibiotic that could be used for seafood preservation (Sarika et al., 2012). *Hydrophis cyanocinctus*, a sea snake, became a rich source of cathelicidin which has exemplary antimicrobial activities (Wei et al., 2015). This functionality was aided by disrupting the pathogens' cell membrane by eliminating the cells of bacteria. The isolated compound has also shown decreased cytotoxicity against mammalian cells making it a better ingredient by advancing research on them to considerably formulate these to antibiotics. An 11 KDa peptide constituting 104 amino acid residues termed Myticusin-1 isolated from adult mussel's hemolymph was observed to possess antagonistic properties toward *B. subtilis*, *S. aureus*, *Sarcina lutens*, and *Bacillus megaterium* (Gram +ve), and a mild effect was noticed against Gram -ve strains including *P. aeruginosa*, *V. parahaemolyticus*, *E. coli*, and *Vibrio harveyi* (Liao et al., 2013). Hormaomycin B and C, from an Actinomycetes strain (SNMSS), was isolated from Mohary. These peptide-derived components with highly modified amino acid residue possess remarkable inhibitory properties against pathogenic bacterial strain *Kocuria rhizophila* NBRC12, 708 (Bae et al., 2015). A depsipeptide called etamycin remained among the first to be isolated from a CNS-575 *Actinomycete* strain. This compound falls in streptogramin class of antibiotics and was demonstrated to possess significant antibacterial activities against a

spectrum of hospital-associated methicillin-resistant *S. aureus* (Haste et al., 2010). A cyclic depsipeptide derived from *Neamphius specius* termed as nemphamide B was demonstrated to possess antimycobacterial abilities against mycobacterium *S. megmatis*. The structures of hormaomycin B, etamycin, and neamphamide B are depicted below.



Etamycin



Neamphamide B

The bioactive compounds quinomycin G, dipeptide, cyclo-(L-Pro-4-OH-L-Leu), and echinomycin derived from sponge (*Gelliodes carnosa*) *Streptomyces* sp. LS298 and cyclic dipeptide class have shown pronounced resistance against pathogen MRSA, MRSE, vancomycin obdurate *E. faecium* ATCC 700221, and vancomycin-insensitive *E. faecalis* ATCC 51299 (Zhen *et al*.,2015). Clavanin A, from the marine tunicate *Styela clava*, showed pronounced antimicrobial activity

against *E. coli* and *S. aureus* in vitro, and it is an AMP that is used for the management of wound and sepsis infections (da Silva et al., 2015).

3.2 Polyketide/Macrolactones

Another group of antibiotics called as polyketide contains alternating carbonyl groups and methylene groups or is derived from precursors which contain such alternating groups. The sediments of South China Sea were the source for actinobacterial strain SCSIO 01,127 that produced two lobophorin analogues, namely, lobophorins E and F. Studies also revealed that both compounds showed antibacterial activities against *B. thuringiensis*, *Enterococcus faecalis*, and *S. aureus*. It was established that lobophorin F demonstrated improved antibacterial activities against *S. aureus* and *E. faecalis*. The antimicrobial features were enhanced due to the nonexistence of hydroxyl group on C-32 (Niu et al., 2011).

Abyssomicin C, a spirotetronate polyketide derived from *Verrucosispora*, had the ability to limit the folic acid precursor para-aminobenzoic acid. This resulted in causing some kind of mutation in DNA synthesis which in due course caused cell impairment and obstructed the growth of *S. aureus* resistant to methicillin (Riedlinger et al., 2004).

A peptide isolated from epidermal mucus of *Myxine glutinosa L.* (hagfish) called myxinidin showed antibiotic antibacterial action toward both fish and human pathogens. The amino acids in peptide are responsible for antimicrobic actions toward a group of microbes encompassing Gram-positive and Gram-negative groups (Cantisani et al., 2014). Another new antimicrobial metabolite known as 7-O-methyl-50-hydroxy-30-heptenoateemacrolactin was produced with the association of bacteria *B. subtilis* and the seaweed *Anthophycus longifolius*, having a polyketide backbone. An inhibitory zone of 18 mm diameter and 16 mm diameter was disclosed against *Aeromonas hydrophila* and *V. parahaemolyticus*, respectively, by 7-O-methyl-5^0-hydroxy-3^0-heptenoateemacrolactin based on the agar diffusion method. The metabolite being lipophilic penetrated through the bacterial membrane and was successful in exhibiting its bactericidal abilities (Chakraborty et al., 2014).

A polyketide of aromatic origin called as Compound 4 was separated from *Streptomyces* sp. JRG-04 isolated from the sediments of a mangrove habitat. This compound was a structural analogue of aromatic benzoisochromanequinone polyketide antibiotic that possessed activities against varied bacteria. Further, it was potent against Gram-positive and Gram-negative bacteria and caused decease of *S. aureus* cells (methicillin resistant) by disturbing the cell membrane. Govindarajan et al. (2014) also detected the cytotoxic nature of the novel compound in cardiomyoblasts (H9C2) cell lines.

From the sediment of East China Sea, marine *Bacillus* sp. isolated befitted to be a rich source of macrolactin S and possessed 5 oxygenated methines, 5 methylenes, 12 olefinicmethines, a methyl, and a lactone carbonyl carbon. The compound isolated showed germicidal action on *E. coli*, *S. aureus*, and *B. subtilis*. From a mass culture broth of low salinity of marine *Bacillus* sp., macrolactins X, Y, and Z

were isolated which showed antimicrobial action on *Bacillus subtilis, Escherichia coli*, and *Saccharomyces cerevisiae* (Mondol and others, 2013).

Lingdomycin and ascosetin, polyketides derived from *Lindgomycetaceae* fungal strains, possessed discrete domains constituting a tetramic acid and bicyclic hydrocarbon, assorted through a carbonyl bridging displayed pronounced inhibitory activities against *B. subtilis* and *Staphylococcus epidermidis*. Furthermore, a strong antibiotic ability was observed against methicillin-resistant *S. aureus*. In a freshwater environment, *Lindgomycetaceae* members were extracted from underwater parts of plant material and decaying wood (Wu et al., 2015a). A study conducted on two oxaphenalenone dimers talaromycesones A and B, derived from marine fungus *Talaromyces* sp. strain, was reported to demonstrate strong antibacterial activities *S. epidermidis* and methicillin-resistant *S. aureus* (Wu et al., 2015b).

The compounds, penicillstressol, isopenicillstressol, and 0Z-isocitreoviridinol derived from *Penicillium* sp. BB1122 (marine sediment) of polyketide class, have demonstrated remarkable resistance against pathogen, MRSA (Auckloo et al., 2017). Grincamycin L and angucycline derivatives derived from deep-sea sediment have reported to show pronounced antimicrobial effect against *E. faecium* CCARM 5203, *E. coli* CCARM 1009, MDR *E. faecalis* CCARM 5172, *S. aureus* CCARM 3090, and *S. typhimurium* CCARM 8250 (Yang *et al*., 2020).

The polyketide buanmycin and buanquinone collected from marine *Streptomyces* of tidal mudflat in Buan Republic of Korea exhibited potent cytotoxicity against gastric carcinoma cells (SNU-638) and colorectal carcinoma cells (HCT-116). Further, the pathogenic *Salmonella enterica*, a Gram-negative bacterium, was inhibited by the same compound. According to Moon et al. (2015), buanmycin showed control of sortase A, considered as a source for detection of antimicrobials. *Bacillus amyloliquefaciens* MTCC12713 derived from *Kappaphycus alvarezii*, an intertidal macroalga, showed antibacterial activities against multidrug-resistant bacteria. Twenty-one membered macrocyclic lactones, identified as difficidin analogues, presented bactericidal activities against MRSA, VR *E. faecalis*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa* (Chakraborty et al., 2021).

3.3 Anthraquinone Class

Anthraquinone, an aromatic compound group with a 9,10-dioxoanthracene core, has numerous antimicrobial in vitro, and in vivo activities of natural and synthetic anthraquinones have been reported by Malmir et al. (2017). Nevertheless, there has been limited research related to the structural-functional relationship of these compounds, and they exhibit structural diversity and variations in chemical composition which have demonstrated potential therapeutic uses as antiviral, antibacterial, antifungal, anti-inflammatory, antioxidant, and cytotoxic agents (Malmir et al., 2013). Studies by Li and Chen (1986) revealed the capability of cosmomycin B produced by *Streptomyces cyaneofuscatus* M-27 to impede Gram-positive bacteria and obstruct in vitro DNA synthesis of P388 of leukemia cell.

Marine Australian *Streptomyces* sp. (CMB-M0150) served as the source of novel compounds, aranciamycin, aranciamycin A, and aranciamycins I and J. Structurally, on sugar moiety, these compounds lack an amino group making it dissimilar from other anthracycline compounds isolated from microorganisms that prevented the growth of *M. bovis* BCG in vitro and *B. subtilis* strains. The isolated compounds exhibited low cytotoxicity, and hence this paves the way for a scope of carrying out more research in the future especially for the development in the medical field (Khalil et al., 2015).

Marine *Pseudomonas stutzeri* isolated from the Ribbonfish (*Desmodema* spp.) became the source of a new compound, namely, Zafrin. The compound is chemically *4b-methyl-5,6,7,8-tetrahydro1 phenanthrenone* and is a stable uncharged metabolite which is hydrophobic as well as lipophilic. This compound displayed antibacterial activities against *Salmonella typhi* and *S. aureus*. The major benefit of Zafrin when related to other antibiotics like ampicillin, tetracycline, and vancomycin is that it was more destructive on the cytoplasmic membrane of *B. subtilis* (Uzair et al., 2008).

The bioactive compounds AMA11, AMA12, and AMA21, AMA11 CE 6 (quinoxaline-2-carboxamide), AMA11 CE 7 (3-nitro-1,2-benzenedicarboxylic acid and quinoxaline-2-carboxamide) derived from *Streptomyces* spp. of the mangrove sediment belonged to the quinone class have shown potential microbial activity against the drug-resistant microbe MRSA SK1 (Sangkanu et al., 2017). The extraction of C-glycosylated benz[α]anthraquinone derivatives, urdamycinone G, urdamycinone E, and dehydroxyaquayamycin, was carried out from a saltwater strain *Streptomycetes* sp. BCC45596. The biosynthetic precursor urdamycin E was isolated post recultivation and exhibited anti-*Plasmodium falciparum* K1 strain and anti-*Mycobacterium tuberculosis* activity (Supong et al., 2012).

3.4 Polybrominated Biphenyl Class

A $3,3^1,5,5^1$-tetra-bromo-$2,2^1$-biphenyldiol composite formed MC21-A was isolated from marine bacteria, *Pseudomonas*, *Alteromonas*, and *Phenolica* sp., which demonstrated the ability to penetrate bacterial cell membranes, resulting in cellular mortality. It has been reported that no cellular toxicity against human normal fibroblast Vero cells and rat pheochromocytoma was observed up to a concentration 50 mg/ml (Isnansetyo & Kamei, 2003).

Dysidea granulosa, the marine sponge, turned out to be the source of polybrominated diphenyl ethers, 2-(2′,4′-dibromophenoxy)-3,5-dibromophenol, 2-(2′,4′-dibromophenoxy)-3,4,5-tribromophenol and 2-(2′,4′-dibromophenoxy)-4,6-dibromophenol which exhibited broad-spectrum in vitro antimicrobial activity toward MRSA, MSSA *E. coli*, and *Salmonella*. A very strong broad-spectrum antibacterial activity was displayed by 2-(2′, 4″-dibromophenoxy)-3,5-dibromophenol than other two compounds. According to Sun et al. (2015), 2-(2′,4′-dibromophenoxy)-3, 5-dibromophenol perhaps BE employed as a possible principal germicidal molecule against *E. coli*, MRSA, and *Salmonella* for the development of drug. A chief active

antimicrobial compound identified from the methanol extract of *Phyllospongia papyracea*, a marine sponge of the *Dictyoceratida* order, was identified to be 2-(3′,5-′-dibromo-2′-methoxyphenoxy)-3, 5-dibromophenol that was found to be extremely active against *Bacillus subtilis, Staphylococcus aureus, Campylobacter jejuni, Pseudomonas aeruginosa, Streptococcus pneumoniae*, and *Listeria* (Sun et al., 2017).

3.5 Terpenoid Class

Terpenoids are derivatives of terpenes which encompasses 5-carbon isoprene units. The addition/removal of functional groups converts terpenes to terpenoids, and the antimicrobial activity of these compounds is determined from their functional group (Mahizan et al., 2019). Terretonin G, a sesterterpenoid from fungus *Aspergillus* sp., was reported to demonstrate potential antibiotic activity against *S. aureus*, *B. subtilis* PC1219, and *Micrococcus luteus* ATCC9341 (Fukuda et al., 2014).

Priancinins A and B are remarkable compounds isolated from the red sea sponge, *Prinos* sp. The two compounds have similar chemical characteristic composed of 6-memseral ring cyclic peroxide. These metabolites display antibacterial activity against beta-hemolytic *Streptococcus*. It has also been confirmed that the mode of action of these metabolites is several times more efficient than tetracycline (Sokoloff et al., 1982).

Penicillium adametzioides AS-53, marine sponge-derived fungus, triggered the identification of novel bisthiodiketopiperazine spin-offs, adametizines A and B, whereas two different acorane sesquiterpenes, adametacorenols A and B, were picked up from a rice solid culture medium. Adametizines A displayed lethality against brine shrimp (*Artemia salina*) and inhibitory activities against *Aeromonas hydrophila*, *Staphylococcus aureus*, *Vibrio harveyi*, *V. parahaemolyticus*, and *Gaeumannomyces graminis*. Chlorination at C-7 of bisthiodiketopiperazines increased lethality and antimicrobial activity in the brine shrimp (Liu et al., 2015). Sea cucumber contains triterpene saponins, and a non-sulfated triterpene glycoside, 21 lanostane, was isolated from the body walls of *Bohadschia cousteau*. The isolated compounds showed good antifungal activity against *Candida albicans* (Elbandy et al., 2014).

A pentacyclic cytochalasin, diaporthalasin 1 sourced from *Diaporthaceae* sp. (PSU-SP2/4), a marine-based fungal organism, demonstrated significant antagonistic characters toward *Staphylococcus aureus* and MRSA (Khamthong and others, 2014). Micromonohalimanes A and B, halimane-type diterpenoids, were isolated from a *Micromonospora* sp. Micromonohalimane B displayed antagonistic activity toward MRSA (Zhang et al., 2016). The compounds smenotronic acid, ilimaquinone, and pelorol derived from marine sponge, *Hyrtios erectus*, belong to sesquiterpene and quinone class have shown pronounced inhibition against CR-Dd2 strain of *P. falciparum* (Ju *et al*., 2018).

3.6 Alkaloid Class

Stachyin B, a compound derived from the fungus *Stachybotrys* sp. MF347, was reported as the first spirocyclicdrimane. This chemical compound belongs to alkaloid class, coupled by spirodihydrobenzofuranlactam unit, and spirodihydroisobenzofuran with NeC bond was demonstrated to inhibit *B. subtilis* and numerous other bacteria. The Greenland Seas served as a source of the fungus, *Trichoderma* sp. strain MF10, from which pyridine and trichodin were derived. It has been demonstrated that these chemical compounds posed inhibition against *B. subtilis* and *S. epidermidis* bacteria ascertained by intramolecular cyclization of the compound with a particular pyridine basic backbone consisting of phenyl group (Bugni et al., 2004). *A. brasiliensis*, a marine sponge-derived compound, namely, arenosclerins A, B, and C and haliclonacyclamine E, has been reported to demonstrate antimicrobial action against hospital-acquired antibiotic-resistant bacteria (Torres et al., 2002). Studies by Yu et al. (2014) revealed that five new alkaloids of aaptamine derivatives from sea sponge *Aaptos aaptos* were demonstrated to have antifungal and anti-HIV-1 activities.

Antifungal property was observed against the pathogenic strains of *Cryptococcus* sp. and *Candida albicans* in a new crambescin homologue discovered from the marine sponge *Pseudaxinella reticulata* from the Bahamas (Jamison & Molinski, 2015). The compounds, acremolin C, cyclo-(L-Trp-L-Phe), 4-hydroxyphenyl acetic acid, (7S, 11S)-(+)-12-hydroxysydonic acid, and (7S)-(+)-hydroxysydonic acid derived from *Aspergillus sydowii* SP-1 from marine sediment of Antarctic, have shown potential inhibition against pathogen, MRSA, and MRSE (Li *et al*., 2018). Other compounds such as n-hexadecanoic acid, 3-methylpyridazine, octadecanoic acid, and indazol-4-one derived from *Streptomyces* sp. of the alkaloid class have demonstrated pronounced antimicrobial activity against *A. baumannii*, *E. coli*, *E. faecium* ESBL, *K. pneumoniae*, and *S. aureus* (Al-Dhabi et al., 2019). Clathrodin and oroidin, the marine alkaloids isolated from sponges, *Agelas*, showed antagonistic nature to *Staphylococcus aureus*, *Escherichia coli*, *Enterococcus faecalis*, and *Candida albicans*. Oroidin exhibited encouraging antagonistic activity toward G + ve bacteria (Zidar et al., 2014).

An assessment of the antimicrobial components of the Antarctic microorganism was carried out, and an alkaloid acremolin C (1) was separated from Antarctic fungal isolate, *Aspergillus sydowii* SP-1. Furthermore, other combinations, viz., cyclo-(L-Trp-L-Phe) (2), 4-hydroxyphenylacetic acid (3), (7S)-(+)-hydroxysydonic acid (4), and (7S, 11S)-(+)-12-hydroxysydonic acid (5), have also been recognized. Investigations revealed that compounds 2, 4, and 5 were antagonistic to MRSA and MRSE (Li et al., 2018).

The list of few compounds with broad antimicrobial activity against multidrug-resistant pathogens from the recent studies is depicted in the table (Table 1).

Table 1 Composites with broad-spectrum antagonistic activity toward MDR pathogens

Drug-resistant microbes	Bioactivity of the compound	Class	Source	Reference
S. aureus CCARM 3090, *S. typhimurium* CCARM 8250, *E. faecalis* CCARM 5172, *E. faecium* CCARM 5203MDR, *E. coli* CCARM 1009	Grincamycin L and angucycline derivatives	Polyketides	Deep-sea sediment derived *S. lusitanus*	Yang et al. (2020)
S. aureus WC 25 V 880854, *pneumoniae* ATCC70063, ESBL K., *A. baumannii* MDR 4414, *E. coli* (ESBL 4345)	n-Hexadecanoic acid 3-methylpyridazine,3a-methyl-6-((4-ethylphenyl, indazol-4-one, octadecanoic acid	Alkaloids	*Streptomyces* sp. from marine sample	Al-Dhabi et al. (2019)
VR *E. faecalis* ATCC 51213, MRSA ATCC 43300	Salinaphthoquinones B and D	Quinone	*Salinispora arenicola* derived from marine sediments	da Silva et al. (2019)
P. aeruginosa ATCC 2774, *A. baumannii* ATCC MRSA ATCC 33591, *E. coli* ATCC 35218, *E. faecalis* ATCC 310682; and *K. pneumoniae* ATCC 700603, 19,606	Monosulfoxide quinomucin and quinomycin A	Cyclic octapeptide	*Streptomyces* sp. obtained from mangrove soil	Lu et al. (2019)
MRSA ATCC NR-46171 and MRSA ATCC-46071	4-Bromophenol and Bis (2-ethylhexyl) phthalate	Derived from bromophenol and phthalate ester	Marine sediment originated from *Nocardiopsis* sp. strain SCA21	Siddharth (2019)

4 Mechanism of Action of Marine Bioactive Compounds (Marine Antibiotics)

Investigations on the precise mechanism of action of marine antibiotics are scant, and brief description on specific explicit targets of marine antibiotics and their associated mode of actions are explained.

A halophilic marine bacterium identified from tunicates isolated from the Pacific Ocean, a phenazine antibiotic LL-14I352a constituting antibacterial mode of action,

was observed. It is noticed that LL-14I352a possesses an amino acid residue which enables its easy transport through the pathogen's cytoplasmic membrane (Singh et al., 1997). Tunicamycin E was extracted from salt water-based *Streptomyces xinghaiensis* SCSIO S15077 along with other composites streptovirudin D2, tunicamycin A, tunicamycin B, tunicamycin X, tunicamycin C, and tunicamycin C3. The South China Sea sediment is the source for all the aforementioned compounds. These compounds displayed antagonistic action toward *Bacillus thuringiensis* BT01 and *B. thuringiensis* W102 and fungal isolates *Candida albicans* ATCC 96901 and *C. albicans* CMCC (F) 98,001 (Zhang et al., 2020).

Another mechanism involves inhibition of bacterial RNA polymerase by salinamide A (SalA), a bicyclic depsipeptide antibiotic hauled out from the jellyfish *Cassiopea xamachana* (Degen et al., 2014). This antibiotic derived from marine *Streptomyces* sp. is composed of seven and two amino acids and nonamino acid residues, respectively. Through conformational changes, SalA allosterically inhibit active-center function of RNA polymerase. Consequently, SalA restricts the addition of nucleotide in the initiation and elongation process during transcription. Significant antibacterial activities were seen against *Haemophilus influenzae* and *Enterobacter cloacae*. Salinamide F, an analogue of depsipeptide with a potential to constrain bacterial RNA polymerase, was isolated from *Streptomyces* sp., strain CNB-091. The fluorescence perceived RNAP-inhibition assay confirmed the inhibitory activity of salinamide F with identical mode of action on bacterial RNA polymerase (Hassan et al., 2015). Recently, quinomycin A, a cyclic octapeptide derived from actinomycete, *Streptomyces* strain B475, showed the mechanism of action of inducing DNA damage SOS response similar to levofloxacin (Lu et al., 2019).

The microbial world is so elaborate where in each species plays a significant role in creating and sensing to differential chemical cues. The progressive approaches developed by each bacterial community, namely, intracellular and intercellular communication, are controlled by quorum sensing (QS). A variety of physiological events determined by the bacteria including virulence, biofilm formation, antibiotic production, and competence including sporulation is regulated by QS.

In the biofilms of *P. aeruginosa,* there exists a leeway of in situ introduction of N-acyl homoserine lactone (AHL)-facilitated QS (Hentzer et al., 2002). The study stated that a halogenated furanone compound obtained from the Australian macroalgae, namely, *Delisea pulchra*, obstructed the communication between the cells of *P. aeruginosa* by shrinking the quorum sensing-regulated gene expression. Thus, it can be inferred that this compound can infiltrate microcolonies and alter cell signaling in biofilm cells.

5 Conclusion

Since many decades, antibiotics acted as the prime drugs which are being administrated against bacterial infections. The phenomenon of antimicrobial resistance has originated with regard to the acquired resistance and evolution of pathogens albeit, as a result antibacterial agent lost their efficacy and supremacy. As a consequence of this, imminent deleterious bacteria have evolved to sustain that became a threat for

the entire globe, and therefore antimicrobial resistance remained as a problematic condition. From several investigations conducted all over the world, the isolation of antibacterial compounds especially with reference to their mode of action along with the biosynthetic pathway has been discarded for a considerable period of time. And hence, there exists a need to formulate ideal antibiotic compounds from marine sector to effectively combat novel and existing ailments. The organisms surviving in the extreme conditions bear the potential to create unique compounds with exemplary bioactivity, ascertained as a result of the diversity of the environmental framework of the ocean, which has to be divulged by advanced and innovative techniques for the purpose to culture and isolate the compound. As a result, marine sphere undoubtedly acts as a wonderful source for the isolation of bioactive compounds despite the limitations in deriving compounds out of them.

References

Ahn, K. S., Sethi, G., Chao, T. H., Neuteboom, S. T., Chaturvedi, M. M., Palladino, M. A., Younes, A., & Aggarwal, B. B. (2007). Salinosporamide A (NPI-0052) potentiates apoptosis, suppresses osteoclastogenesis, and inhibits invasion through down-modulation of NF-κB–regulated gene products. *Blood, the Journal of the American Society of Hematology, 110*(7), 2286–2295.

Al-Dhabi, N. A., Ghilan, A. K. M., Esmail, G. A., Arasu, M. V., Duraipandiyan, V., & Ponmurugan, K. (2019). Bioactivity assessment of the Saudi Arabian Marine Streptomyces sp. Al-Dhabi-90, metabolic profiling and it's in vitro inhibitory property against multidrug resistant and extended-spectrum beta-lactamase clinical bacterial pathogens. *Journal of Infection and Public Health, 12*(4), 549–556.

Aneiros, A., & Garateix, A. (2004). Bioactive peptides from marine sources: Pharmacological properties and isolation procedures. *Journal of Chromatography B, 803*(1), 41–53.

Auckloo, B. N., Pan, C., Akhter, N., Wu, B., Wu, X., & He, S. (2017). Stress-driven discovery of novel cryptic antibiotics from a marine fungus Penicillium sp. BB1122. *Frontiers in Microbiology, 8*, 1450.

Bae, M., Chung, B., Oh, K. B., Shin, J., & Oh, D. C. (2015). Hormaomycins B and C: New antibiotic cyclic depsipeptides from a marine mudflat-derived *Streptomyces* sp. *Marine Drugs, 13*(8), 5187–5200.

Belarbi, E. H., Gomez, A. C., Chisti, Y., Camacho, F. G., & Grima, E. M. (2003). Producing drugs from marine sponges. *Biotechnology Advances, 21*(7), 585–598.

Berdy, J. (2005). Bioactive microbial metabolites. *The Journal of Antibiotics, 58*(1), 1–26.

Bérdy, J. (2012). Thoughts and facts about antibiotics: Where we are now and where we are heading. *The Journal of Antibiotics, 65*(8), 385–395.

Bugni, T. S., Singh, M. P., Chen, L., Arias, D. A., Harper, M. K., Greenstein, M., Maiese, W. M., Concepción, G. P., Mangalindan, G. C., & Ireland, C. M. (2004). Kalihinols from two Acanthella cavernosa sponges: Inhibitors of bacterial folate biosynthesis. *Tetrahedron, 60*(33), 6981–6988.

Cantisani, M., Finamore, E., Mignogna, E., Falanga, A., Nicoletti, G. F., Pedone, C., Morelli, G., Leone, M., Galdiero, M., & Galdiero, S. (2014). Structural insights into and activity analysis of the antimicrobial peptide myxinidin. *Antimicrobial Agents and Chemotherapy, 58*(9), 5280–5290.

Chakraborty, K., Thilakan, B., & Raola, V. K. (2014). Polyketide family of novel antibacterial 7-O-methyl-5′-hydroxy-3′-heptenoate–macrolactin from seaweed-associated *Bacillus subtilis* MTCC 10403. *Journal of Agricultural and Food Chemistry, 62*(50), 12194–12208.

Chakraborty, K., Kizhakkekalam, V. K., Joy, M., & Dhara, S. (2021). Difficidin class of polyketide antibiotics from marine macroalga-associated Bacillus as promising antibacterial agents. *Applied Microbiology and Biotechnology, 105*(16), 6395–6408.

Charan, R. D., Schlingmann, G., Janso, J., Bernan, V., Feng, X., & Carter, G. T. (2004). Diazepinomicin, a new antimicrobial alkaloid from a marine Micromonospora sp. *Journal of Natural Products, 67*(8), 1431–1433.

Cueto, M., Jensen, P. R., Kauffman, C., Fenical, W., Lobkovsky, E., & Clardy, J. (2001). Pestalone, a new antibiotic produced by a marine fungus in response to bacterial challenge. *Journal of Natural Products, 64*(11), 1444–1446.

da Silva, A. B., Silveira, E. R., Wilke, D. V., Ferreira, E. G., Costa-Lotufo, L. V., Torres, M. C. M., ... Pessoa, O. D. L. (2019). Antibacterial Salinaphthoquinones from a strain of the bacterium *Salinispora arenicola* recovered from the marine sediments of St. Peter and St. Paul Archipelago, Brazil. *Journal of Natural Products, 82*(7), 1831–1838.

Degen, D., Feng, Y., Zhang, Y., Ebright, K. Y., Ebright, Y. W., Gigliotti, M., Vahedian-Movehed, H., Mandal, S., Talaue, M., Connell, N., Arnold, E., Fenical, W., & Ebright, R. H. (2014). Antibiotics derived from marine organisms in "Studies in Natural Products Chemistry". Vol. 51, 1e29. https://doi.org/10.1016/B978-0-444-63932-5.00010-3. Accessed September 4, 2022.

Elbandy, M., Rho, J. R., & Afifi, R. (2014). Analysis of saponins as bioactive zoochemicals from the marine functional food sea cucumber *Bohadschia cousteaui*. *European Food Research and Technology, 238*(6), 937–955.

Fenical, W. (1993). Chemical studies of marine bacteria: Developing a new resource. *Chemical Reviews, 93*(5), 1673–1683.

Fukuda, T., Kurihara, Y., Kanamoto, A., & Tomoda, H. (2014). Terretonin G, a new sesterterpenoid antibiotic from marine-derived *Aspergillus* sp. OPMF00272. *The Journal of Antibiotics, 67*(8), 593–595.

Govindarajan, G., Santhi, V. S., & Jebakumar, S. R. D. (2014). Antimicrobial potential of phylogenetically unique actinomycete, *Streptomyces* sp. JRG-04 from marine origin. *Biologicals, 42*(6), 305–311.

Hassan, H. M., Degen, D., Jang, K. H., Ebright, R. H., & Fenical, W. (2015). Salinamide F, new depsipeptide antibiotic and inhibitor of bacterial RNA polymerase from a marine-derived *Streptomyces* sp. *The Journal of Antibiotics, 68*(3), 206–209.

Haste, N. M., Perera, V. R., Maloney, K. N., Tran, D. N., Jensen, P., Fenical, W., Nizet, V., & Hensler, M. E. (2010). Activity of the streptogramin antibiotic etamycin against methicillin-resistant *Staphylococcus aureus*. *The Journal of Antibiotics, 63*(5), 219–224.

Hentzer, M., Riedel, K., Rasmussen, T. B., Heydorn, A., Andersen, J. B., Parsek, M. R., Rice, S. A., Eberl, L., Molin, S., Hoiby, N., Kjelleberg, S., & Givskov, M. (2002). Inhibition of quorum sensing in Pseudomonas aeruginosa biofilm bacteria by a halogenated furanone compound. *Microbiology, 148*, 87–102.

Hu, G. P., Yuan, J., Sun, L., She, Z. G., Wu, J. H., Lan, X. J., Zhu, X., Lin, Y. C., & Chen, S. P. (2011). Statistical research on marine natural products based on data obtained between 1985 and 2008. *Marine Drugs, 9*(4), 514–525.

Isnansetyo, A., & Kamei, Y. (2003). MC21-A, a bactericidal antibiotic produced by a new marine bacterium, *Pseudoalteromonas phenolica* sp. nov. O-BC30T, against methicillin-resistant *Staphylococcus aureus*. *Antimicrobial Agents and Chemotherapy, 47*(2), 480–488.

Jamison, M. T., & Molinski, T. F. (2015). Antipodal crambescin A2 homologues from the marine sponge *Pseudaxinella reticulata*. Antifungal structure–activity relationships. *Journal of Natural Products, 78*(3), 557–561.

Kanagasabapathy, S., Samuthirapandian, R., & Kumaresan, M. (2011). Preliminary studies for a new antibiotic from the marine mollusk *Melo melo* (Lightfoot, 1786). *Asian Pacific Journal of Tropical Medicine, 4*(4), 310–314.

Kang, H. K., Seo, C. H., & Park, Y. (2015). Marine peptides and their anti-infective activities. *Marine Drugs, 13*(1), 618–654.

Khalil, Z. G., Raju, R., Piggott, A. M., Salim, A. A., Blumenthal, A., & Capon, R. J. (2015). Aranciamycins I and J, antimycobacterial anthracyclines from an Australian marine-derived *Streptomyces* sp. *Journal of Natural Products, 78*(4), 949–952.

Kwon, H. C., Kauffman, C. A., Jensen, P. R., & Fenical, W. (2006). Marinomycins A− D, antitumor-antibiotics of a new structure class from a marine actinomycete of the recently discovered genus "Marinispora". *Journal of the American Chemical Society, 128*(5), 1622–1632.

Leal, M. C., Puga, J., Serôdio, J., Gomes, N. C., & Calado, R. (2012). Trends in the discovery of new marine natural products from invertebrates over the last two decades–where and what are we bioprospecting? *PLoS One, 7*(1), e30580.

Li, M., & Chen, Y. L. (1986). Structural studies on rhodilunancins A and B. *The Journal of Antibiotics, 39*(3), 430–436.

Li, W. T., Luo, D., Huang, J. N., Wang, L. L., Zhang, F. G., Xi, T., ... Lu, Y. Y. (2018). Antibacterial constituents from Antarctic fungus, Aspergillus sydowii SP-1. *Natural Product Research, 32*(6), 662–667.

Liao, Z., Wang, X. C., Liu, H. H., Fan, M. H., Sun, J. J., & Shen, W. (2013). Molecular characterization of a novel antimicrobial peptide from Mytilus coruscus. *Fish & Shellfish Immunology, 34*(2), 610–616.

Lincke, T., Behnken, S., Ishida, K., Roth, M., & Hertweck, C. (2010). Closthioamide: an unprecedented polythioamide antibiotic from the strictly anaerobic bacterium Clostridium cellulolyticum. *Angewandte Chemie, 122*(11), 2055–2057.

Liu, Y., Li, X. M., Meng, L. H., Jiang, W. L., Xu, G. M., Huang, C. G., & Wang, B. G. (2015). Bisthiodiketopiperazines and acorane sesquiterpenes produced by the marine-derived fungus Penicillium adametzioides AS-53 on different culture media. *Journal of Natural Products, 78*(6), 1294–1299.

Lu, Q. P., Ye, J. J., Huang, Y. M., Liu, D., Liu, L. F., Dong, K., ... Sun, C. H. (2019). Exploitation of potentially new antibiotics from mangrove actinobacteria in Maowei sea by combination of multiple discovery strategies. *Antibiotics, 8*(4), 236.

Luesch, H., Yoshida, W. Y., Moore, R. E., Paul, V. J., & Corbett, T. H. (2001). Total structure determination of Apratoxin A, a potent novel cytotoxin from the marine Cyanobacterium *Lyngbya majuscula*. *Journal of the American Chemical Society, 123*(23), 5418–5423.

Mahizan, N. A., Yang, S. K., Moo, C. L., Song, A. A. L., Chong, C. M., Chong, C. W., Abushelaibi, A., Lim, S. H. E., & Lai, K. S. (2019). Terpene derivatives as a potential agent against antimicrobial resistance (AMR) pathogens. *Molecules, 24*(14), 2631.

Malmir, M., Serrano, R., & Silva, O. (2013). Anthraquinones as potential antimicrobial agents: A review. Research Institute for Medicines (iMed. ULisboa), Faculty of Pharmacy, Universidade de Lisboa, Av. Professor Gama Pinto, 1649-003.

Malmir, M., Serrano, R., & Silva, O. (2017). Anthraquinones as potential antimicrobial agents-A review. Antimicrobial research: Novel bioknowledge and educational programs. Formatex, 55–61.

Manivasagan, P., Venkatesan, J., Sivakumar, K., & Kim, S. K. (2014). Pharmaceutically active secondary metabolites of marine actinobacteria. *Microbiological Research, 169*(4), 262–278.

Moon, K., Chung, B., Shin, Y., Rheingold, A. L., Moore, C. E., Park, S. J., ... Oh, D. C. (2015). Pentacyclic antibiotics from a tidal mud flat-derived actinomycete. *Journal of Natural Products, 78*(3), 524–529.

Namikoshi, M., Negishi, R., Nagai, H., Dmitrenok, A., & Kobayashi, H. (2003). Three new chlorine containing antibiotics from a marine-derived fungus *Aspergillus ostianus* collected in Pohnpei. *The Journal of Antibiotics, 56*(9), 755–761.

Niu, S., Li, S., Chen, Y., Tian, X., Zhang, H., Zhang, G., Zhang, W., Yang, X., Zhang, S., Ju, J., & Zhang, C. (2011). Lobophorins E and F, new spirotetronate antibiotics from a South China Sea-derived *Streptomyces* sp. SCSIO 01127. *The Journal of Antibiotics, 64*(11), 711–716.

Nweze, J. A., Mbaoji, F. N., Huang, G., Li, Y., Yang, L., Zhang, Y., Huang, S., Pan, L., & Yang, D. (2020). Antibiotics development and the potentials of marine-derived compounds to stem the tide of multidrug-resistant pathogenic bacteria, fungi, and protozoa. *Marine Drugs, 18*(3), 145.

Riedlinger, J., Reicke, A., Zähner, H. A. N. S., Krismer, B., Bull, A. T., Maldonado, L. A., Ward, A. C., Goodfellow, M., Bister, B., Bischoff, D., & SuSSMUTH, R. D. (2004). Abyssomicins, inhibitors of the para-aminobenzoic acid pathway produced by the marine Verrucosispora strain AB-18-032. *The Journal of Antibiotics, 57*(4), 271–279.

Sangkanu, S., Rukachaisirikul, V., Suriyachadkun, C., & Phongpaichit, S. (2017). Evaluation of antibacterial potential of mangrove sediment-derived actinomycetes. *Microbial Pathogenesis, 112*, 303–312.

Sarika, A. R., Lipton, A. P., Aishwarya, M. S., & Dhivya, R. S. (2012). Isolation of a bacteriocin-producing lactococcuslactis and application of its bacteriocin to manage spoilage bacteria in high-value marine fish under different storage temperatures. *Applied Biochemistry and Biotechnology, 167*(5), 1280–1289.

Siddharth, S. (2019). Isolation and characterization of bioactive compounds with antibacterial, antioxidant and enzyme inhibitory activities from marine-derived rare actinobacteria, Nocardiopsis sp. SCA21. *Microbial Pathogenesis, 137*, 103775.

Silva, O. N., Fensterseifer, I. C., Rodrigues, E. A., Holanda, H. H., Novaes, N. R., Cunha, J. P., ... Franco, O. L. (2015). Clavanin A improves outcome of complications from different bacterial infections. *Antimicrobial Agents and Chemotherapy, 59*(3), 1620–1626.

Singh, M. P., Menendez, A. T., Petersen, P. J., Ding, W. D., Maiese, W. M., & Greenstein, M. (1997). Biological and mechanistic activities of phenazine antibiotics produced by culture LL-14I352. *The Journal of Antibiotics, 50*(9), 785–787.

Sokoloff, S., Halevy, S., Usieli, V., Colorni, A., & Sarel, S. (1982). Prianicin A and B, nor-sesterterpenoid peroxide antibiotics from Red Sea sponges. *Experientia, 38*(3), 337–338.

Sun, S., Canning, C. B., Bhargava, K., Sun, X., Zhu, W., Zhou, N., Zhang, Y., & Zhou, K. (2015). Polybrominated diphenyl ethers with potent and broad spectrum antimicrobial activity from the marine sponge Dysidea. *Bioorganic & Medicinal Chemistry Letters, 25*(10), 2181–2183.

Sun, S., Canning, C., Wang, K., Zhu, W., Yang, F., Zhang, Y., & Zhou, K. (2017). Antibacterial activity of 2-(3', 5'-Dibromo-2'-methoxyphenoxy)-3, 5-dibromophenol Isolated from Phyllospongia papyracea. *Natural Product Communications, 12*(4), 1934578X1701200426.

Supong, K., Thawai, C., Suwanborirux, K., Choowong, W., Supothina, S., & Pittayakhajonwut, P. (2012). Antimalarial and antitubercular C-glycosylated benz [α] anthraquinones from the marine-derived Streptomyces sp. BCC45596. *Phytochemistry Letters, 5*(3), 651–656.

Torres, Y. R., Berlinck, R. G., Nascimento, G. G., Fortier, S. C., Pessoa, C., & de Moraes, M. O. (2002). Antibacterial activity against resistant bacteria and cytotoxicity of four alkaloid toxins isolated from the marine sponge *Arenosclerabra siliensis*. *Toxicon, 40*(7), 885–891.

Uzair, B., Ahmed, N., Ahmad, V. U., Mohammad, F. V., & Edwards, D. H. (2008). The isolation, purification and biological activity of a novel antibacterial compound produced by Pseudomonas stutzeri. *FEMS Microbiology Letters, 279*(2), 243–250.

Wei, L., Gao, J., Zhang, S., Wu, S., Xie, Z., Ling, G., Kuang, Y. Q., Yang, Y., Yu, H., & Wang, Y. (2015). Identification and characterization of the first cathelicidin from sea snakes with potent antimicrobial and anti-inflammatory activity and special mechanism. *Journal of Biological Chemistry, 290*(27), 16633–16652.

Wright, R. K. (1981). Urochordata. In N. A. Ratcliffe & A. F. Rowley (Eds.), *Invertebrate blood cells* (Vol. 2).

Wu, B., Ohlendorf, B., Oesker, V., Wiese, J., Malien, S., Schmaljohann, R., & Imhoff, J. F. (2015a). Acetylcholinesterase inhibitors from a marine fungus *Talaromyces* sp. strain LF458. *Marine Biotechnology, 17*(1), 110–119.

Wu, B., Wiese, J., Labes, A., Kramer, A., Schmaljohann, R., & Imhoff, J. F. (2015b). Lindgomycin, an unusual antibiotic polyketide from a marine fungus of the Lindgomycetaceae. *Marine Drugs, 13*(8), 4617–4632.

Yang, L., Hou, L., Li, H., & Li, W. (2020). Antibiotic angucycline derivatives from the deepsea-derived Streptomyces lusitanus. *Natural Product Research, 34*(24), 3444–3450.

Yu, H. B., Yang, F., Sun, F., Li, J., Jiao, W. H., Gan, J. H., ... Lin, H. W. (2014). Aaptamine derivatives with antifungal and anti-HIV-1 activities from the South China Sea sponge Aaptos aaptos. *Marine Drugs, 12*(12), 6003–6013.

Zhang, Y., Adnani, N., Braun, D. R., Ellis, G. A., Barns, K. J., Parker-Nance, S., Guzei, I. A., & Bugni, T. S. (2016). Micromonohalimanes A and B: Antibacterial halimane-type diterpenoids from a marine Micromonospora species. *Journal of Natural Products, 79*(11), 2968–2972.

Zhang, S., Gui, C., Shao, M., Kumar, P. S., Huang, H., & Ju, J. (2020). Antimicrobial tunicamycin derivatives from the deep sea-derived Streptomyces xinghaiensis SCSIO S15077. *Natural Product Research, 34*(11), 1499–1504.

Zidar, N., Montalvão, S., Hodnik, Ž., Nawrot, D. A., Žula, A., Ilaš, J., Kikelj, D., Tammela, P., & Peterlin Mašič, L. (2014). Antimicrobial activity of the marine alkaloids, clathrodin and oroidin, and their synthetic analogues. *Marine Drugs, 12*(2), 940–963.

Prebiotic- and Probiotic-Based Strategies for the Control of Antimicrobial Resistance

Toms C. Joseph, S. Remya, and S. S. Greeshma

Contents

T. C. Joseph (✉) · S. Remya
Microbiology Fermentation and Biotechnology Division, ICAR-Central Institute of Fisheries Technology (ICAR-CIFT), Kochi, Kerala, India
e-mail: Toms.CJoseph@icar.gov.in; remya.s@icar.gov.in

S. S. Greeshma
Microbiology Fermentation and Biotechnology Division, ICAR-Central Institute of Fisheries Technology (ICAR-CIFT), Kochi, Kerala, India

Mumbai Research Centre, ICAR-Central Institute of Fisheries Technology, Mumbai, India
e-mail: greeshma.ambadi@gmail.com

M. P. Mothadaka et al. (eds.), *Handbook on Antimicrobial Resistance*,
https://doi.org/10.1007/978-981-19-9279-7_38

Abstract

Inappropriate antibiotic use for prophylaxis and treatment of diseases leads to antimicrobial resistance with resultant health hazards to humans and animals. Strict regulations have been imposed by many countries for reducing the usage of antibiotics for preventive and therapeutic purposes in animal farming and aquaculture. Probiotics, prebiotics, and synbiotics can be effectively used for control and treatment of diseases in place of antibiotics as prophylactics in cattle farming, including aquaculture. Probiotics are used in human and animal health aimed at improving the digestive health by modulation of gut microbiota, inhibition of colonization of microbial pathogens in the intestines, and increasing the immune status. Prebiotics act by promoting metabolism, the establishment of native intestinal bacteria, and eliminating the pathogenic bacteria, thereby improving health conditions of the host animal. A synbiotic is an amalgam of prebiotics and probiotics, where prebiotic selectively favors growth of probiotic organism(s). The chapter discusses the present uses of probiotics, synbiotics, and prebiotics, along with the limitations. The need for studies to assure the safety of probiotic organisms used is also presented.

Keywords

Probiotic · Prebiotic · Synbiotic · Animal Health · Human Health · Aquatic Animal Health · Antimicrobial resistance

1 Introduction

In recent years, the misappropriate and overemployment of antibiotics drugs sans prescription or otherwise in faunae and in humans has become a critical well-being part of community (Liu, 2020). There was an increase of 65% in the global antibiotic consumption in humans during 2000–2015 and the DDDs (defined daily dose) showed a rise from 21.1 to 34.8 billion and antibiotic consumption increased to 15.7 from 11.3 (39% rise) DDDs per 1000 persons per day (Klein et al., 2018). Worldwide, the antimicrobial application in the animal husbandry sector, which was estimated to be above 90,000 tons in 2017, is predicted to touch more than 100,000 tons by 2030 (Tiseo et al., 2020). Many antimicrobials, which are employed in the feed of animals to treat or avert diseases (Zhao et al., 2020), have revealed resistance of more than 50% (P50). During 2000–2018, this proportion grew from 0.15 to 0.41 and from 0.13 to 0.34 in chickens and pigs, respectively (Van Boeckel et al., 2019).

Worldwide, besides using antibiotics for therapeutic purposes, animal feeds are also often added with low-dose antibiotics for growth enrichment to enhance the efficacy of feed and for increase in weight and betterment of the health of the animal (Cuong et al., 2018). Antibiotics have thus been extensively used in livestock feed primarily to promote development and avert, regulate, and manage diseases in many

countries (Marshall & Levy, 2011). The application of antimicrobial drugs as agents to increase development and/or enhance efficiency of animal feed depends on the country's legal system. Antibiotic residue in eggs, meat, and milk and their widespread presence in the environment are a common consequence of administering drugs in animals for food production. Undiscerning use of antibiotics and their subsequent presence in animal foods have a deleterious impact on the industry and pose a severe threat to the well-being of the environment and humans that includes the increase in antibiotic resistance in microbes (Kumar et al., 2018). Antibiotics for prophylactic purpose directly increase the selection pressure and thereby favor survival and multiplication of Antibiotic Resistant Bacteria (ARB) (Blau et al., 2019). The ARBs can be transferred from animals to humans and vice versa through personnel (Smith et al., 2013), foodstuffs, and environment. The link between AMR and application of antimicrobials has already been proven in many cases. The far-reaching application of fluoroquinolones resulted in the advent of AMR in *Campylobacter* sp. in the food chain (Alfredson & Korolik, 2007). Though AMR is of public health significance worldwide, countries with increased intake of antibiotics display increased rates of resistance (Fair & Tor, 2014). Due to the increased incidence of AMR, governments of several nations and various organizations have begun to put a stop to antibiotic employment in livestock (European Commission, 2003). On the contrary, complete prohibition of application of drugs in livestock, specifically in the poultry sector, may lead to a surge in infectious diseases by *Clostridium perfringens* and *Campylobacter jejuni* (Alfredson & Korolik, 2007).

There is widespread application of antimicrobial drugs in aquaculture, and there is large variation in the antibiotic usage in this field. While the amount of antibiotic usage is 1000 mg per 1000 kg of aquaculture in Norway, it was 0.700 kg per 1000 kg of aquaculture production in Vietnam. In the same study, it was estimated that nearly 500,000–600,000 kg of antibiotics was used in 1994 for shrimp aquaculture (Smith, 2008). In a study, it was found that 67 antibiotics were used in aquaculture in 11 of 15 countries studied, and the most widely used antibiotics were oxytetracycline, sulfadiazine, and florfenicol (Lulijwa et al., 2020). Assessment of the current rate of antimicrobial use in aquaculture across the world is challenging since distribution and registration methods of antimicrobials in each country are dissimilar. Besides, the quantity of antibiotics and supplementary inputs employed in aquaculture differs between the nations and regions (Burridge et al., 2010). As in the case of livestock, the risk of AMR is high in aquaculture with an increased prophylactic use of antimicrobials owing to selective pressure, making the medication less effective (Watts et al., 2017). Thus, AMR has been confirmed in aquaculture (Elmahdi et al., 2016). The microbes that are resistant to antimicrobials and the genes that are responsible for resistance therein get transmitted from aquatic animals or environment to terrestrial animal and to human, and the reversal of the process leads to hostile consequences on aquatic ecosystems and human and animal health (Santos & Ramos, 2018). Intensive farming promotes insensitive use of antibiotics, leading to residues of antimicrobials in aquatic products (Chen et al., 2020). Around 75% of the antibiotics applied to fish are wasted due to defecation in the surrounding waters (Burridge et al., 2010). The advancement in intensive aquaculture practices has

resulted in several bacterial diseases, causing enhanced application of antimicrobial drugs (Defoirdt et al., 2011). Antibiotic contamination in aquatic environments reduces the diversity of bacteria, including taxa that are responsible for initial fecundity and carbon cycling (Eckert et al., 2019). The pathogens acquire genes of antibiotic resistance possibly from the environmental resistome, resulting in long-term health consequences (Bengtsson-Palme et al., 2018). The unrestricted antibiotic use has caused pollution in the environment, and transmission and selection of ARB and vicissitudes in the microbial ecosystems (Bungau et al., 2021).

The presence of AMR genes was found in untreated sewage, and genes encoding resistance to beta-lactams, aminoglycosides, macrolides, sulfonamides, and tetracyclines were found to be the most prevalent in bacterial resistome from 79 locations in 60 countries (Hendriksen et al., 2019). The problems of growing resistance to infection are similar in animal hospitals and human hospitals. Many alternatives to antibiotics were developed in the management of bacterial infections and in containment of AMR (Ghosh et al., 2019).

2 Prebiotics, Probiotics, and Synbiotics

The use of probiotics is gaining much acceptance in the medical field and animal husbandry, including aquaculture (Collins & Gibson, 1999; Munoz-Atienzal et al., 2013; Téllez et al., 2015; Varankovich et al., 2015). Various agents, like prebiotics, synbiotics, and probiotics, are advocated to substitute antibiotics in poultry production. Use of probiotics is well defined as the use of beneficial organisms to achieve the preferred effect either to prevent the disease or improve the overall well-being of the organism (Collins & Gibson, 1999). The effectiveness of probiotics basically lies in the microbial ecology that gets established and opposes the entry or multiplication of pathogens in the body. Probiotics aid in decreasing the advancement of antibiotic unresponsiveness in microbes by reducing the application of antibiotics (Collins & Gibson, 1999; Munoz-Atienzal et al., 2013; Varankovich et al., 2015). With the growing issues of AMR, application of probiotics as replacement to drugs can prevent infectious diseases and promote the growth/development of animals (Munoz-Atienzal et al., 2013; Téllez et al., 2015).

The structural function of gut microbiome can be greatly influenced by probiotics since they subdue the growth of other microbes by generating antimicrobial agents and compete with binding sites, other microbial organisms, and intestinal mucosal receptors (Abd El-Moneim et al., 2020). The usage of probiotics may help reduce the development of antibiotic-resistant strains due to the intensive application of antibiotics (Munoz-Atienzal et al., 2013; Varankovich et al., 2015). Since the microorganisms share common surroundings in the gastrointestinal tract, threat exists for the possible transmission of antibiotic resistance from probiotics to pathogenic microbes or vice versa. When improperly cooked, probiotic-fed animals for human consumption can also be a potential source of antibiotic resistance transmission for the human gut microflora (Imperial & Ibana, 2016).

Immune modulation, organic acid production, reduction in intestinal pH, and stimulating host immune systems are the plausible modes of action of probiotics for defying bacterial pathogens (Abd El-Moneim et al., 2020). Additionally, probiotics decrease the transmission of pathogens to the intestinal mucosa by retaining immune tolerance and strengthening the integrity of the intestine (Lee & Bak, 2011). A variety of techniques and tools are used, including traditional methods, metagenomic sequence profiling, and experiments of in vivo, to reconnoiter the action of probiotics on structure, purpose, and diversity of gastrointestinal microflora.

Prebiotics, when administered as a dietary supplement, are resilient to digestion by enzymes. They are not absorbed as such and provide health benefits to the host by promoting development, absorption, and formation of the innate gastrointestinal tract (GIT) bacteria and eliminating the pathogenic bacteria (Ricke, 2015). Though only carbohydrate-rich compounds like non-starch polysaccharides and inedible oligosaccharides were earlier regarded as prebiotic agents, nowadays prebiotics include a variety of oligosaccharides of varying lengths of carbon chain and even polyunsaturated fatty acids and polyphenols. The concept has eventually grown into a "substrate used selectively for health-promoting microorganisms" (Gibson et al., 2017; Ricke, 2018). Prebiotics, though not directly, offer the host metals required for metabolic functions and micronutrients due to their efficiency to promote bacterial growth.

Synbiotic is a blend of probiotics and prebiotics, and these have been established for their ability to prevent different infections in farm animals and humans. The synbiotics are more useful than probiotics or prebiotics separately owing to the concurrent characteristics of pre- and probiotics (LeBlanc et al., 2014).

3 Probiotics in Human Health

Currently, probiotic bacteria are added in several foodstuffs that are supposed to endorse the well-being of humans (Songisepp et al., 2012). Probiotic use has been proven to modulate gastrointestinal microflora in children subsequent to the usage of antibiotics (Collado et al., 2012). A significant number of studies revealed that probiotic employment is effective in the regulation and containment of intestinal ailments (Dodoo et al., 2017), management of gastrointestinal cancers (Rai et al., 2021), averting allergies (Dudek-Wicher et al., 2020), obesity, protection of cardiovascular system (Dudek-Wicher et al., 2020), tumor (Mao et al., 2020), etc (Table 1).

Adults with *Clostridium difficile*-associated diarrhea, a disease linked with continued use of antibiotics that destroys normal flora, can be effectively controlled with the administration of probiotics, including fecal transplantation (Gupta et al., 2021; Varankovich et al., 2015). The probiotic Lactic acid bacterial strains were able to improve digestion, absorption, and nutrient uptake in livestock, finfish, and human beings (Téllez et al., 2015; Varankovich et al., 2015). The bacterium *Helicobacter pylori,* which is responsible for gastritis and peptic ulcers, could be suppressed or in some cases totally eliminated with the treatment of probiotics (Varankovich et al., 2015; Liu et al., 2020a). Further, it was observed that side effects of treatment for the

Table 1 Beneficial effects of probiotics in human beings, livestock, and aquatic organisms

Host	Type of organism	Species	Benefits	Reference
Human beings	*Bacteria*	*Lactobacillus* sp. and *Bifidobacterium* sp.	Protect against *Helicobacter pylori*	Canducci et al., 2002
		Enterococcus faecium SF68®	Cures severe diarrhea	Greuter et al., 2020
		Bifidobacterium sp.	Treatment for infectious diarrhea, inflammatory bowel disease, increase immune response in host	Alessandri et al., 2019
		B. longum BB536	Reduces respiratory illness in children and modulates growth of *Faecalibacterium* (beneficial gut microbiota)	Lau et al., 2018
		Saccharomyces boulardii	Reduces diarrhea, inflammatory bowel disease, and ulcerative colitis	Guslandi et al., 2000; McFarland, 2007; Dinleyici et al., 2012; Shan et al., 2013; Sivananthan & Petersen, 2018
		Lactobacillus acidophilus	Prevent urinary tract infection and vesicourethral reflux	Lee et al., 2007
		L. crispatus CTV-05	Prevents vaginal infections and UTI	Stapleton et al., 2011
		Bifidobacterium bifidum, B. lactis, Lactobacillus rhamnosus, and *L. acidophilus*	Control UTI in children	Sadeghi-Bojd et al., 2020
	Yeast	*Torulaspora delbrueckii, Yarrowia lipolytica, Debaryomyces hansenii, Kluyveromyces lactis, K. marxianus,* and *K. lodderae*	Antipathogenic effect	Saber et al., 2017; Homayouni-Rad et al., 2020;
Poultry	Bacteria	*Bacillus subtilis*	Improves the production and quality of eggs, increase in brainstem neurotransmitters, 5-HT levels, as well as a decrease in the	La Ragione & Woodward, 2003; Ribeiro et al., 2014; Yan et al., 2018

(continued)

Table 1 (continued)

Host	Type of organism	Species	Benefits	Reference
			hypothalamus dopamine and norepinephrine, protects from *C. perfringens* and *S.* Enteritidis	
		Rhodobacter capsulatus	Improves health of hen and egg quality during end of egg laying and reduce *Salmonella* infection	Lokhande et al., 2013; Oh et al., 2017
		Bacillus licheniformis	Enhances immune system and acts as hormone regulator, improves egg production and feed intake, restores impaired villi structure in heat-stressed chickens and low serum levels of IL-1 and 6, etc.	Wang et al., 2017a; Deng et al., 2012
		E. faecalis UGRA10	Improves immune system, gut microbiota, and hormones and metabolism, increases the antibody levels against pathogens, alters intestinal tract, reduces *Salmonella* infection	Waters et al., 2005; Franz et al., 2011; Han et al., 2013; Luo et al., 2013; Park et al., 2016; Peralta-Sánchez et al., 2019; Zhao et al., 2013; Zheng et al., 2016
		Lactic acid bacteria	Provides protection against *E. coli* O157: H7, *L. monocytogenes* and *S. enterica*	Carey et al., 2008; Maragkoudakis et al., 2009
		B. bifidum, *B. animalis,* *B. longum,* and *B. infantis*	Improves body weight, immune response status, and decreases intestinal coliform count	Abd El-Moneim et al., 2019
		B. bifidum and *Bacillus toyonensis*	Improves growth, meat quality, and reduces cecal coliforms	Abou-Kassem et al., 2021

(continued)

Table 1 (continued)

Host	Type of organism	Species	Benefits	Reference
		L. johnsonii (strain BS15)	Improves FCR, weight gain, reduced expression of IFN-γ and IL10, restores damaged ileal villi, and reduces NE	Wang et al., 2017b
		Bacillus coagulans	Protection from NE caused by *C. perfringens*	Al-Baadani et al., 2018
		C. butyricum (strain MIYAIRI 588)	Reduces NE infection	Takahashi et al., 2018
		Butyricicoccus pullicaecorum LMG 24109	Reduced NE and occurrence of *E. coli* and *Campylobacter* spp.	Eeckhaut et al., 2016
		E. faecium	Reduce NE infection	Wu et al., 2019
		Lactobacillus salivarius SMXD51	Reduce intestinal Campylobacter load	Saint-Cyr et al., 2017
		L. reuteri CSF8	Reduce intestinal Campylobacter load	Nothaft et al., 2017
		L. reuteri KUB-AC5	Eliminates *S. enterica* serovar Enteritidis	Nakphaichit et al., 2019
		L. plantarum LTC-113	Protects from *Salmonella* Typhimurium	Wang et al., 2018
		C. butyricum HJCB998	Protects from *Salmonella*	Yang et al., 2012
		Propionibacterium freudenreichii B3523	Protects from *Salmonella*	Nair & Kollanoor Johny, 2017
		E. faecium (type NCIMB 10415	Controls colonization of *E. coli* and *Shigella*	Beirão et al., 2018
		E. coli Nissle 1917 expressing Microcin J25	Offer protection against Salmonella infections	Forkus et al., 2017
	Yeast + bacteria	*Saccharomyces cerevisiae* and *L. fermentum*	Elevates expression of TLR-2 and TLR-4 mRNA in the chicken foregut	(Bai et al., 2013).
Domestic animals	Bacteria	*L. plantarum*	Treatment of mastitis in cows	Andrews et al., 2019; Derakhshani et al., 2018; Lima et al., 2017
		L. reuteri, L. rhamnosus, and *P. acidilactici*	Treatment for metritis in cows	Genís et al., 2018
		L. buchneri (DSM type 32,407)	Increase conception in healthy cows	Peter et al., 2018

(continued)

Table 1 (continued)

Host	Type of organism	Species	Benefits	Reference
		Lactobacillus spp. in ewes	Improves the fertility and health status	Quereda et al., 2020
		Lactobacillus spp. and *Paenibacillus polymyxa*	Treatment of clinical bovine respiratory disease (BRD)	Amat et al., 2020
		E. faecalis CECT7121	Activate immune response	Díaz et al., 2018
		Lactobacillus animalis (type NP-51) and *Actinobacterium dietzia*	Treatment for Johne's disease in cattle	Click, 2011
		Lactobacillus spp.	Control diarrhea in pigs due to *E. coli*	Hou et al., 2015; Zhao & Kim, 2015
		B. subtilis DSM 5750 and *B. licheniformis* DSM 5749	Improves immunity against pathogenic bacteria and promote beneficial gut microbiota in pigs	Zhang et al., 2017
		C. butyricum TO-A, *E. faecalis* T-110 and *Bacillus mesentericus* TO-A	Improved immunity to piglets	Inatomi et al., 2017
		L. johnsonii L531 and *L. rhamnosus*	Control Salmonella infections in pigs and piglets	Zhang et al., 2018; He et al., 2019
		Lactobacillus spp.	Control pathogens, viz., *Campylobacter, S.* Enteritidis, *S.* Typhimurium, *S.* Dublin, *E. coli* O157: H7	Saint-Cyr et al., 2016; Lin et al., 2020
		Ruminococcus, Nocardioidaceae, Brevibacterium, Streptococcus, and *Soilbacillus*	Control EHEC in animals	Chopyk et al., 2016
		P. freudenreichii and *L. acidophilus*	Control *E. coli* strain O157 in sheep and cattle	Wisener et al., 2015
	Yeast + bacteria	*S. cerevisiae* and *B. licheniformis*	Offer protection against diarrhea in pigs	Pan et al., 2017
	Yeast	*Candida tropicalis*	Able to control diarrhea in calves	Bi et al., 2017

(continued)

Table 1 (continued)

Host	Type of organism	Species	Benefits	Reference
Feline and canine	Bacteria	*E. faecium* SF68	Promotes immunity in dogs and puppies	Benyacoub et al., 2003
		L. rhamnosus strain	Reduces atopic dermatitis and allergies in dogs	Marsella, 2009
		L. plantarum P-8, L. casei, and *Bifidobacterium animalis* subsp. *lactis V9*	Reduce the levels of opportunistic pathogens and improves beneficial microflora in dogs	Xu et al., 2019
		E. faecium SF68	Reduces diarrhea in cats and dogs	Bybee et al., 2011
		L. acidophilus (NCC2766 and NCC2628) and *L. johnsonii* (NCC2667)	Reduce diarrhea and promotes immunity in dogs	Sauter et al., 2006
		VSL # 3 (a mixture of bacteria belonging to *Lactobacillus, Bifidobacterium,* and *Streptococcus* spp.)	Treatment of IBD in dogs	Rossi et al., 2014
		Lactobacillus murinus LpP2	Management of diarrhea associated with canine distemper	Delucchi et al., 2017
		B. toyonensis	Increase immunity in canine	Franz et al., 2020
Aquaculture	Bacteria	*B. fusiformis*	Increase survival and speeded up metamorphosis of larvae of *Litopenaeus vannamei* and *Penaeus monodon*	Guo et al., 2009
		L. fermentum URLP18	Improves colonization, feed utilization, growth, intestinal microbiota, innate immune response, and protection from *A. hydrophila*	Krishnaveni et al., 2021
		L. plantarum VSG3	Increases growth, immunity, and protection from *A. hydrophila* in rohu	Giri et al., 2013
		Lactobacillus rhamnosus	Improves physiological stress	Dawood et al., 2017

(continued)

Table 1 (continued)

Host	Type of organism	Species	Benefits	Reference
			response, immune responses, and enhanced resistance against stress of low salinity in Red sea bream (*Pagrus major*)	
		Pediococcus pentosaceus	Enhanced feed utilization, growth, number of intestinal microbes, activity of digestive enzymes, and improved health of *L. vannamei* shrimp	Adel et al., 2017
		B. cereus and *P. acidilactici*	Decreased ammonia, nitrate, and biochemical oxygen demand in the pond water rearing white leg shrimp compared to the control group	Khademzade et al., 2020
		Lactobacillus	Protects juvenile hybrid tilapia against *A. hydrophila*	Liu et al., 2013
		E. faecalis W24 and *L. lactis* L19	Increases resistance	Kong et al., 2020
		L. lactis and *B. subtilis*	Enhance the immune response, disease resistance against *A. hydrophila*	Won et al., 2020
		L. plantarum	Protection against *Vibrio alginolyticus* in white leg shrimp and enhanced humoral and cellular immune responses such as SOD activity, PO activity, and proPO and PE mRNA transcription levels	Chiu et al., 2007
		Psychrobacter maritimus	Increase growth performance in Nile tilapia	Makled et al., 2020
		B. subtilis BT23	Protection against *Vibrio harveyi* in *P. monodon*	Vaseeharan & Ramasamy, 2003

(continued)

Table 1 (continued)

Host	Type of organism	Species	Benefits	Reference
		B. licheniformis	Superoxide dismutase and phenoloxidase and decreased *Vibrio* sp. in the intestine of *L. vannamei*	Li et al., 2007
		Bacillus spp.	Antiviral activity against WSS in shrimp	Li et al., 2009
		B. pumilus SE5	Protection against pathogens in *L. vannamei*	Zhang et al., 2020
		Bacillus NP5	Reduces ammonia levels, improves growth and immune status of African catfish	Putra et al., 2020
		Sanolife PRO-F	Reduces ammonia levels, improves growth and immunity of Nile tilapia	Elsabagh et al., 2018
		B. coagulans	Improves survival rates and digestive enzyme in *L. vannamei* larvae	Zhou et al., 2009
		A. faecalis Y311 and *B. cereus* NY5	Reduces phosphorus in culture water and increases the relative abundance of beneficial microbiota of Nile tilapia	Wang et al., 2020
	Bacteria + yeast	*L. lactis, B. subtilis,* and *S. cerevisiae*	Increases dietary performance and growth *in L. rohita* fingerlings	Mohapatra et al., 2012

removal of *H. pylori* were managed with supplementation of probiotics containing *Lactobacillus* sp. and *Bifidobacterium* sp. strains (Canducci et al., 2002).

The use of *Enterococcus faecium* reduced the time taken for recovery due to severe diarrhea significantly (Benyacoub et al., 2003; Greuter et al., 2020). Although Enterococci have probiotic potential, they can be the reason for nosocomial infections and have also been identified as resistant to vancomycin. Hence, the probiotic application in the form of Enterococci has become a contentious issue. Unlike Lactobacillus, the Enterococcus genus species is known to be opportunistic and occasionally are etiological agents for other human infections, such as infectious

endocarditis and bacteremia (Rao et al., 2021; Sanders et al., 2010; Varankovich et al., 2015). In curing acute diarrhea and inhibition of antibiotic-associated diarrhea, double-blind trials, two each of randomized-controlled and open-label studies, were conducted with *E. faecium* SF68® in juvenile and adult patients, concluding that SF68 showed an excellent safety profile with overall acceptance (Greuter et al., 2020).

Bifidobacteria application was found to thwart or diminish contagious diarrhea and alleviate inflammatory bowel disease (IBD) indicators. *B. longum* BB536 was able to reduce respiratory illnesses in children and modulated gut microbiota, with significant enhancement in the quantity of beneficial bacteria, *Faecalibacterium* (Lau et al., 2018). There have been reports on the regulation of immune response in host by Bifidobacteria. *Saccharomyces boulardii* has been found to be efficient in reducing the duration of diarrhea, irrespective of its origin (Shan et al., 2013). The recurrence of IBD, as well as mild ulcerative colitis conditions, was prevented and treated by employing *S. boulardii* (Guslandi et al., 2000). Yeasts belonging to *Torulaspora delbrueckii, Yarrowia lipolytica, Debaryomyces hansenii, Kluyveromyces marxianus, K. lactis*, and *K. lodderae* showed strong antipathogenic effects (Homayouni-Rad et al., 2020; Saber et al., 2017).

The *short chain fatty acids* (SCFAs), namely, acetic, butyric, lactic, and propionic acids, produced by probiotic bacteria are responsible for the prevention of infections associated with *serovar of Salmonella enterica* Typhimurium and *C. difficile* (Tejero-Sariñena et al., 2013). Several probiotics produce various types of antimicrobial composites, viz. acetaldehydes, bacteriocins, diacetyl, ethanol, hydrogen peroxide, organic acids, and peptides. The mode of action of these organic acids is decreasing the pH (Kareem et al., 2014). Probiotics not only produce bioactive compounds that are anti-pathogenic and directly target the bacteria but also activate defense pathways of hosts that act against the pathogens by triggering the mechanism involved in cationic antimicrobial peptide production in various types of cells that include "*Paneth*" in intestinal, epithelial and small intestinal crypts (Figueroa-González et al., 2011). Probiotic bacteria present in commercial probiotic products inhibit bacteria that were pathogenic to human beings (*S.* Typhimurium, *E. coli*, *L. monocytogenes* etc.).

Unusual microflora in the vagina can result in the increased prevalence of urinary tract infection (UTI). Any imbalance in the microbial composition in vagina will result in bacterial vaginosis and UTI. The supplementation of probiotics can help in balancing of *Lactobacillus* sp. and can result in relieving the symptoms (Waigankar & Patel, 2011). A commercial probiotic containing *Lactobacillus acidophilus* with a daily dose of 1.0×10^8 CFU/g was given two times a day to children affected with UTI and vesicourethral reflux and proved to be capable of preventing recurrence of UTI (Lee et al., 2007). Vaginal suppositories harboring *L. crispatus* CTV-05 reduced to a large extent the prevalence of UTI in women (Stapleton et al., 2011). Probiotic assortment containing *Bifidobacterium bifidum, B. lactis, Lactobacillus rhamnosus,* and *L. acidophilus* remarkably brought down the recurrence of UTI in children compared with placebo (Sadeghi-Bojd et al., 2020).

4 Prebiotic and Human Health

Prebiotic oligosaccharides are generally used as a dietary supplement for their beneficial effects on gut microbiota. Galacto-oligosaccharides (GOS) caused a 5–10 times increase in Bifidobacteria in humans, and the result was dose-dependent (Azcarate-Peril et al., 2017; Liu et al., 2017). GOS-treated Caco-2 cells upregulated genes that control the process of digestion, steroids and fatty acids metabolism, antibacterial proteins, and transmembrane-based transport of solutes and amino acids (Lafontaine et al., 2020). The supplementation of extremely purified GOS was linked to an increased relative abundance of *B. longum, B. adolescentis, B. breve, B. catenulatum,* and *B. dentium* (Azcarate-Peril et al., 2017). Supplementation with inulin led to a rise in the amount of *Bifidobacterium* and *Faecalibacterium* in a group of obese women (Dewulf et al., 2013). Prebiotics like laminaran and ulvan, which are derived from marine polysaccharides, have been selectively used by *Bifidobacteria*, *Lactobacilli,* and *Bacteroides* (Seong et al., 2019). The intake of chicory inulin increased the numbers of *Bifidobacterium* sp. and *Anaerostipes hadrus* (Baxter et al., 2019). Intake of chicory fructans is reportedly linked with high proportions of *B. adolescentis, B. bifidum*, *B. longum,* and *F. prausnitzii* (Li et al., 2018).

5 Synbiotics and Human Health

Synbiotics denote synergistic mixtures of prebiotics and probiotics, where the prebiotic selectively favors the probiotic organism(s) (Pandey et al., 2015). It was revealed that the consortia of *Clostridia*, which are strict anaerobes and spore formers, prevent the colonization of pathogens like *Salmonella* in neonatal mice (Kim et al., 2017). Commercially available synbiotic mixture (OMNi-BiOTiC®) comprising prebiotics that include corn starch, fructooligosaccharides, maltodextrin, and inulin, and a mixture of probiotic bacterial strains, belonging to *Bifidobacterium, Lactobacillus,* and *Lactococcus* sp., elevated mucosal microbial diversity, colonic $CD4^+$ T cells, fecal acetate, and butyrate levels. The synbiotic was also able to alleviate the IBS indicators (Moser et al., 2019). A synbiotic formulation, containing prebiotic combination of fructooligosaccharides (FOS), GOS, and xylo-oligosaccharides (XOS), and a combination of five spore-forming *Bacillus* strains, impacted in vitro microbial action and composition of gastrointestinal tract of humans. The synbiotic caused a rise in the microbial diversity of the distal colon and improvement in the levels of *Bacillaceae* in the ascending colon with an augmented production of acetate, butyrate, and propionate (Duysburgh et al., 2019). Soymilk incorporated with XOS as prebiotic and fermented with *Weissella cibaria* FB069 exhibited substantial antiproliferative action in Caco-2 and HCT116 cells sans any toxic impact on cells and could hinder signal stream of "*MD2/TLR4/MyD88/NF-κB*" in HCT116 cells (Le et al., 2020).

6 Probiotics in Poultry Production

The probiotic supplementation in poultry diet has increased over the years. Many probiotics employed in poultry production are bacteria, which occur in the birds alimentary tract and have interesting attributes like inhibitors of pathogenic bacteria and as regulators of signals of intestinal cells. Different strains of bacteria have been examined, and their impacts on the performance of birds, egg production, immunity development, improved digestion and absorption, and change in the gut microbiome have been checked. For example, administartion of *B. subtilis* was able to improve the production and quality of eggs in poultry (Ribeiro et al., 2014). *Rhodobacter capsulatus* was found to improve health of hen and quality of eggs during the laying period (Lokhande et al., 2013) and reduced the contamination of *Salmonella* (Oh et al., 2017) while *Bacillus licheniformis* was able to enhance the immune system and act as a controller of hormone (Wang et al., 2017a).

Similarly, supplementation of the broiler feed with *B. subtilis* was concomitant to upsurge of brainstem neurotransmitters, 5-HT levels, and also decline in dopamine and norepinephrine in the hypothalamus (Yan et al., 2018). Dietary supplementation with *B. licheniformis* showed an enhancement in the production of eggs and feed consumption, and restored the impaired villi structure in heat-stressed chickens, which also resulted in low serum levels of TNF-α, IL-1,6 and corticosterone (Deng et al., 2012).

In broilers, serum immunoglobulin levels increased after supplementation of probiotics in the diet and fostered changes in phagocytosis capacity and the immune cell numbers (Beirão et al., 2018; Zhang et al., 2012). The administration of *E. faecalis* UGRA10 in poultry has shown some benefits since *Enterococci* are a part of normal microflora in warm-blooded animals and intermingle with the structure of resistance (Franz et al., 2011), gut microbiota (Han et al., 2013; Park et al., 2016; Peralta-Sánchez et al., 2019), hormones and metabolic rate (Zhao et al., 2013). The metabolism rate was significantly enhanced when *E. faecium* was employed as a probiotic (Zheng et al., 2016), causing increased antibody levels against pathogens, or producing alterations in the intestinal tract (Luo et al., 2013). In egg-laying hens, a combination of probiotics had a positive influence on the quality of eggs, performance, and immunity response (Zhang et al., 2012). Gastrointestinal microbial communities are important for nutrition and performance of the host (Sohail et al., 2015). Enterococcus used as a probiotic in chickens resulted in the modification of microbiota in the feces (Han et al., 2013) and particularly reduced the population of *Salmonella* (Waters et al., 2005).

The ability of probiotics, especially of LAB, in providing protection against hazardous microbial pathogens in chickens is well documented (Maragkoudakis et al., 2009). *In* ovo inoculation of *B. animalis*, *B. bifidum*, *B. infantis,* and *B. longum* resulted in enhanced body mass, improvement in immune response and antioxidant status, enhancement of serum super dismutase and immunoglobulins, increase in the counts of LAB and *Bifidobacteria,* and decline of total coliforms

and bacterial counts in broilers (Abd El-Moneim et al., 2019). Incorporation of *B. bifidum* and *Bacillus toyonensis* in the feed regimen of Japanese quail enhanced growth and resulted in better quality of meat, and also reduced the cecal coliforms and *E. coli* (Abou-Kassem et al., 2021).

Lactobacillus supplementation in the diet was able to enhance coccidiosis-induced intestinal immunity by modulation of intraepithelial lymphocytes (Wang et al., 2021). *S. cerevisiae* and *Lactobacillus fermentum* probiotic-fed chicken had elevated TLR-2 elucidation and higher proportions of CD3+, CD4+, and CD8+ T-lymphocytes in the foregut of fowls (Bai et al., 2013). Decrease in the use of prophylactic antimicrobials has resulted in the reappearance of necrotic enteritis (NE), resulting in production loss, which in turn has resulted in enhanced applications of probiotics (Caly et al., 2015). NE can be caused by *C. perfringens* and results in lacerations in the liver and small intestine of chicken. This condition at times leads to fatality in chickens. *Bacillus coagulans* fed to chickens with NE resulted in improved growth performance, had lower lesions on the gut, and reduced *C. perfringens* counts in the cecum and liver (Al-Baadani et al., 2018). The replacement of microflora of ilea and ceca from fowls infected in the past can aid in safeguarding infected fowls, repairing intestinal ulcers, and improving the efficacy of feed (Keerqin et al., 2017). Incorporation of strain BS15 of *Lactobacillus johnsonii* lessened the effects caused by NE, with improved FCR, weight gain, reduced expression of IL10 and IFN-γ, and restoration of the damaged ileal villi (Wang et al., 2017b). A butyrate-producing *C. butyricum MIYAIRI 588* strain reduced the severity and occurrence of lesions of NE broilers (Takahashi et al., 2018). Administration of *Butyricicoccus pullicaecorum* LMG 24109 resulted in the reduction of the occurrence of *E. coli* and *Campylobacter* spp., the necrotic lesions in broilers with NE and also resulted in lowering the FCR (Eeckhaut et al., 2016). Application of probiotic *E. faecium* NCIMB 11181 led to the amelioration of NE induced intestinal lesion body, weight loss, intestinal cell apoptosis, and improved the composition of intestinal bacteria (Wu et al., 2019).

The use of probiotics reduced the Campylobacter levels in poultry. *Lactobacillus salivarius* SMXD51 was able to lessen cecal loads of Campylobacter by 2.8-log in 35 days (Saint-Cyr et al., 2017). Observations of microbial communities indicated that reduced levels of *Lactobacilli* in ceca of chicken led to increased levels of *Campylobacter* sp. (Sakaridis et al., 2018). Probiotic *L. reuteri* CSF8, along with oral vaccine of recombinant *E. coli* that expresses N-glycan of *Campylobacter jejuni,* caused a reduction in colonization of *Campylobacter* in specific pathogen-free chickens, possibly due to the strengthening of immune system (Nothaft et al., 2017). Extensive research has shown that probiotics can be successfully used for controlling colonization of *Campylobacter* in chicken.

Probiotic bacteria compete naturally, and as a result, they try to remove pathogenic bacteria by competitive exclusion (CE), bacterial interference, or antagonism (Fuller, 1989). Administration of *B. subtilis* as a pre-dose to broilers had lower levels of *C. perfringens* and *S.* Enteritidis compared to chicks, which were not provided the pre-dose. After the administration of *B. subtilis* probiotic to broilers, the bacteria can remain for 36 days in the bird's intestines and show high resistance to the pathogen

E. coli O78: K80 (La Ragione et al., 2001). *L. reuteri* KUB-AC5 eliminated *S. enterica* serovar Enteritidis from the cecum and ileum in poultry. In the same study, high doses of the probiotic bacteria (10^7 cfu) augmented Lactobacilli and suppressed enterobacteriaceea in ceca (Nakphaichit et al., 2019). The strain *Lactobacillus plantarum* LTC-113 could protect the chicks from *S.* Typhimurium dysbiosis, which is possible due to the upsurge in the TJ (claudin-2/5, ZO-1) assertion as well as reduction of cytokines (IL-1β;6) and microbial colonization in GIT and liver (Wang et al., 2018). Application of *C. butyricum* HJCB998 in broilers significantly reduced the levels of *Salmonella* spp. in ceca (Yang et al., 2012). Feeding *Propionibacterium freudenreichii* B3523 of milk origin to turkeys resulted in decreased colonization of *S. enterica* serovar Heidelberg and also brought decline in cecal colonization and distribution to the liver (Nair & Kollanoor Johny, 2017). *E. faecium* (type NCIMB 10415), when combined with live-attenuated *S.* Enteritidis, improved the production of Salmonella-specific IgA, which also decreased *E. coli* and *Shigella* and enhanced *Anaerotruncus, Blautia,* and *Lactobacillus* in layer birds (Beirão et al., 2018). A probiotic culture based on *Lactobacillus* was able to significantly decrease *S.* Enteritidis in neonatal broiler chicks. Probiotic use in turkeys has been shown to provide additional resistance to *Salmonella* spp. infection (Téllez et al., 2015). Moreover, there are reports on the reduction of idiopathic diarrhea using probiotics in commercial turkey (Téllez et al., 2015). A probiotic that was genetically engineered to express and secrete antimicrobial peptide; Microcin J25, the *E. coli* Nissle 1917, was able to eliminate Salmonella from the ceca of turkeys than the treatment groups (Forkus et al., 2017).

7 Prebiotics in Poultry Production

Prebiotics transform the intestinal microbiota to a favorable condition to the host and to have overall helpful impact not only on the intestinal tract, which is seen in the positive development of production parameters, viz. body mass improvement, feed transformation proportion, egg production, and mortality (Li et al., 2008). In addition to improvement in productivity and health, prebiotics were efficient in diminishing the levels of bacteria, viz. *Campylobacter* sp., *Salmonella* sp., *E. coli,* and *C. perfringens*, which are important in both poultry production and public health (Kim et al., 2011, 2019). These positive impacts as well as the low risk of adverse side effects on the host make prebiotics a good option to lower antibiotic use in poultry production, consequently contributing to reducing AMR (Yadav et al., 2016). Moreover, the prebiotics are inexpensive and simpler to produce on a larger scale compared to probiotics.

Probiotic bacteria such as *Bifidobacterium* spp. and *Lactobacillus* spp. has beneficial impacts on gastrointestinal functioning and, as a result, positively influence the host health. These advantageous bacteria are present at high levels in chicken fed with prebiotic-supplemented foods. They utilise and metabolize prebiotics judiciously by supporting their multiplication (Teng & Kim, 2018). This will result in the production of SCFA, reduction of intestinal pH, improved metabolism by

enhanced activity of gastral enzyme, vitamin production, and lesser levels of triglycerides, lowered levels of cholesterol, and also improved function of immune system. Incorporation of FOS to diet remarkably improved the quantity of *Lactobacillus* and *Bifidobacterium* in the small intestine of chickens and reduced the levels of *E. coli* in relation to the control group. The addition of 0.05% MOS and 0.25% FOS in the feed increased the diversity of *Lactobacillus* and reduced the numbers of *E. coli* and *C. perfringens* in the ileum of poultry birds (Kim et al., 2011). Also, incorporation with various amounts of inulin in the diet significantly decreased the count of coliform bacteria in laying hens with increasing concentration of this prebiotic (Shang et al., 2010). It was observed that GOS administered *in ovo* had higher *Bifidobacterium* communities in duodenum, jejunum, ileum, and cecum, whereas *Lactobacillus* levels were higher in all the four sections in control groups (Slawinska et al., 2019). The possible mechanism by which ingestion of prebiotics provides resistance to pathogens is the selective growth of *Bifidobacterium* and *Lactobacilli* in the GI tract that leads to the upsurge in SCFA, particularly acetate, propionate, butyrate, and lactate throughout the main fermentation process in ceca (Ricke, 2018), which decreases the expression of invasive gene in *Salmonella* at low levels. Prebiotics, particularly MOS, are effective in decreasing colonization of pathogen by direct contact with the lectins in pathogen, preventing their intestinal epithelial cell attachment and therefore preventing colonization in the GIT (Oyofo et al., 1989).

The beneficial effects of administration of prebiotic in poultry include better digestion of crude protein, fat, dry matter, and energy. These effects are due to a rise in the useful microorganisms like *Lactobacillus*, alterations in the intestinal architecture, and enhanced intestinal health, resulting in morphological changes in the gut in turn resulting in improved nutrient transport system and increased nutrient absorption (Wang et al., 2005a). Experiments revealed that supplementation of prebiotics improved the inorganic absorption, especially that of Ca, Cu, Fe, and Zn (Chen & Chen, 2004; Raveschot et al., 2020). Prebiotics application results in the augmented production of secreted IgA in the intestine, prevents bacterial attachment and entry into the lumen, increases the creation of mucus, and hinders swelling (Kim et al., 2011; Yadav et al., 2016). Prebiotics prevent pathogen colonization, modulate the microbiome, regulate the production of antibodies and cytokines, and reduce the production of pathogen-associated molecular patterns (PAMPs) by pathogenic bacteria (Teng & Kim, 2018). Addition of prebiotics to chicken feed resulted in reduced intestinal pH and a rise in *Bifidobacterium* and *Lactobacillus* counts, causing increased levels of fatty acids (Ziggers, 2000). Feed supplementation with fructans led to a rise in *Lactobacillus* and a decrease in potential pathogenic bacteria belonging to *Salmonella* and *Campylobacter* in broiler chicken intestine. Administration of cell wall derived MOS of *S. cerevisiae* led to an increase in Lactobacilli in ceca and reduced Campylobacter and *E. coli* numbers (Baurhoo et al., 2009). The administration of isomalto-oligosaccharides (IMO) caused a dramatic decline in the concentration of *S.* Typhimurium and a rise in the population of Bifidobacterium genus bacteria. Another experiment showed that "*refined functional arbohydrates (RFC)*" consisting of D-mannose, β-glucan, and MOS and β-glucan sourced from

S. cerevisiae cell wall was able to inhibit adhesion of *C. jejuni and Salmonellae* to LMH epithelial cell line of chicken *in vitro* (Froebel et al., 2020).

8 Synbiotics in Poultry Production

Synbiotic preparation comprising of *Lactobacillus* spp., *S. cerevisiae,* and inulin (prebiotic) led to a rise in the beneficial microbes of intestines, *Lactobacillus* spp. and *Bifidobacterium* spp., and a drop in the number of pathogenic microbes. Moreover, symbiotic resulted in an increase in lactic acid and SCFA with decreased levels of BCFA (Śliżewska et al., 2020). Broiler chicks fed symbiotic-containing prebiotic incorporated with fructo-oligosaccharides, chicory, sea algal-based phycophytic substances, cell wall fragments extracted from more than 60 different microbial preparations, inulin, and *E. faecium* improved the feed efficiency, reduced the feed conversion ratio, had increased *Bifidobacterium,* and reduced *E. coli* in cecum in relation to antibiotic-fed birds (Tayeri et al., 2018). Broilers, which were fed with commercial probiotic containing multiple species, viz. *Bifidobacterium animalis*, *L. reuteri, Pediococcus acidilactici,* and *E. faecium,* as well as fructo-oligosaccharides, exhibited reduction in heat stress compared with those fed with control diet (Mohammed et al., 2018). Incorporation of synbiotics in varied stages in diet decreased *E. coli*, total coliform counts, and enhanced LAB population in broiler chicken (Dibaji et al., 2014). Incorporation of "*galactooligosaccharides (GOS)*" in feedstuff improved the proportion of development and feed transformation and of broilers with increased levels of *L. johnsonii*, which was linked to the performance of birds and reduced levels of *Lactobacillus crispatus*. In the same study, assessment of innate immune response highlighted an increase in the expression of ileal and cecal interleukin-17A (Richards et al., 2020). Salmonella-infected turkeys fed with synbiotic product containing *Lactobacillus* spp. and lactose had beneficial effects on the conversion of feed and gain in weight (Vicente et al., 2007). FOS and *B. subtilis* administration in poultry improved moderate growth and better rates of feed conversion along with lowered diarrhea and mortality compared to aureomycin-treated birds (Li et al., 2008).

9 Probiotics in Domestic Animals

Infection of the mammary gland is the foremost reason for the use of antibiotics in dairy animals. In addition, the increased incidence of AMR in mastitis-causing microorganisms has increased attention to the application of probiotics for deterrence and cure of such diseases (Rainard & Foucras, 2018). Probiotic strains of *Lactobacillus* may cause inflammatory responses when inoculated into the mammary gland (MG) (Assis et al., 2015). Research has demonstrated that MG that had been affected with mastitis harbor very low-diversity microbial flora (Andrews et al., 2019; Lima et al., 2017), with low counts of *Lactobacilli* in GIT (Ma et al., 2018). *L. plantarum*-based teat infectant lowered somatic cell count, reduced the counts of

microbes associated with mastitis, and improved the microbiota of teat (Yu et al., 2017). In general, prospects of employing probiotics by intramammary application for the control of mastitis seem unlikely (Rainard & Foucras, 2018). Strategies to overcome the barriers to the usage of intramammary probiotic organisms may include the use of purified antibacterial compounds produced by probiotics.

Bovine vagina contains a range of bacterial flora, including opportunistic bacteria (Bicalho et al., 2017), which can infect uterine tissues during stress and parturition. In a recent work, it was revealed that *Lactobacillus* spp. was a part of uterine microflora (Gärtner et al., 2015). The probiotics application suggested an alternative strategy to reduce the incidence or improve recovery from metritis by modulating the vaginal microbiome. The incidence of metritis was reduced after the administration of *L. reuteri, L. rhamnosus,* and *Propionibacterium acidilactici* by intravaginal route prior to calving (Genís et al., 2018). Some LABs were able to moderate *E. coli*-associated infections and endometrial epithelial cell inflammation, while others diminished the cytokine expression (IL-1β,8), the pro-inflammatory kind (Genís et al., 2016). The interuterine application of *L. buchneri* (DSM type 32407) in Holstein cows enhanced conception of healthy cows, especially those with endometritis, and diminished the endometrial expression of cytokines; IL-1β and IL-8 (Peter et al., 2018). The fertility and health status in ewes improved with intravaginal lactobacilli applications (Quereda et al., 2020).

Clinical bovine respiratory disease (BRD) results in nasopharyngeal microbiota dysbiosis, which changes drastically during the transportation of animals (Timsit et al., 2016). Probiotics can improve the respiratory health and thwart the transmission of opportunistic infections by different microbes. Some of the probiotics belonging to *Lactobacillus* spp. and *Paenibacillus polymyxa* were inhibitory *in vitro* toward *Mannheimia haemolytica*, one of the causative agents of BRD, and eradicate it from epithelial cells of bronchi (Amat et al., 2020). Mice fed with E. faecalis CECT7121 and vaccinated against *M. haemolytica* and *P. multocida* has been found to activate humoral response through high levels of antibody titers and IFN-γ (Díaz et al., 2018).

The causative agent of Johne's disease, *"Mycobacterium avium* subsp. *paratuberculosis* (MAP)," was inhibited from adhering epithelial cells of cattle by *S. cerevisiae* and its cell wall components (Li et al., 2016). Supplementation of *Lactobacillus animalis* (type NP-51) reduced MAP levels in mice (Karunasena et al., 2014). Remarkably, Actinobacterium Dietzia inhibits MAP *in vitro* and cattle administered with Dietzia shed lower numbers of MAP in the feces (Click, 2011).

In a recent study, calves fed with LAB-fermented milk have lesser diarrheal diseases and lower death rates (Maldonado et al., 2018); and in another study, *Candida tropicalis* application showed that the intensity of diarrhea due to *E. coli* can be diminished (Bi et al., 2017). Similarly, strains of *Lactobacillus* spp. lessened *E. coli* load and condensed diarrheal scores of weaning pigs (Zhao & Kim, 2015). There have been reports on the antidiarrheal benefits of many probiotics in pigs (Dubreuil, 2017). Feeding of probiotic "*B. subtilis* DSM 5750 and *B. licheniformis* DSM 5749" augmented mucin-2 expression and ileal goblet cell counts, as well as population of *Turicibacter*, *Lactobacillus,* and *Clostridium* while regulating the growth of *Eubacterium eligens, Bacteroides uniformis*, *Sporobacter,* and

Acetanaerobacterium (Zhang et al., 2017). Weaned pigs challenged with ETEC after the administration of a probiotic containing *S. cerevisiae* and *B. licheniformis* resulted in improved sIgA levels in the jejunal and ileal mucosa, feed efficiency, average daily gains, with reduced incidence of diarrhea comparable to those administered with antimicrobials (Pan et al., 2017). Sows immunized for "porcine epidemic diarrhea virus (PEDV)," when administered with a commercial multistrain probiotic containing "*E. faecalis* T-110, *C. butyricum* TO-A, and *Bacillus mesentericus* TO-A," transported antibodies specific to PEDV to piglets through colostrum efficiently than those that were given probiotics (Inatomi et al., 2017).

Administration of *L. johnsonii* L531 reduced the load of *S. enterica* serovar Infantis in the jejunum and colon of weaned piglets along with an increased elimination of pathogen (He et al., 2019). Application of *L. rhamnosus* limited autophagy caused by *Salmonella* Infantis and slowed down the liver transplantation of the pathogen (Zhang et al., 2018).

Feeding of probiotics leads to a 2-log cfu reduction in the levels of *Campylobacter* in animals that can translate to a 30-fold reduction in infections to humans. *Lactobacillus* spp. are able to demonstrate powerful activity against Campylobacter *in vitro* (Saint-Cyr et al., 2016). In the processing of cattle, hides contaminated with EHEC contribute significantly to the contamination of carcasses in slaughter. Recent microbiome studies observed that the increase in EHEC (serogroups O; 26, 45, 103, 111, 121, 145, and 157) was associated with lesser bacterial diversity on cattle hides before harvest and absence of EHEC is often associated with populations of *Ruminococcus, Nocardioidaceae, Brevibacterium, Streptococcus,* and *Soilbacillus* (Chopyk et al., 2016). There is the possibility of transmission of EHEC from cattle to humans by contamination of food products by fecal matter. The probiotics diminish the prevalence of *E. coli* O157: H7 in both sheep and cattle and humans too (Téllez et al., 2015). The application of *P. freudenreichii* and *L. acidophilus* to steers reduced occurrence of *E. coli* O157 on the hide and in the feces (Wisener et al., 2015). LAB isolated from cattle inhibited the growth of several pathogens originating from foods, which include *E. coli* O157:H7, *S.* Enteritidis, S. Dublin, etc. (Lin et al., 2020).

10 Prebiotics in Domestic Animals

The spray-dried bovine serum in combination with FOS alleviated the severity and incidence of enteric disease in calves (Quigley et al., 2002). Calf fed with MOS resulted in improved consumption, conversion ratio of feed, improvement in mean mass of body, and decrease in fecal coliform count and severity of scours compared to the control group (Ghosh & Mehla, 2012). Prebiotic-containing alfalfa meal, wheat middings, calcium carbonate, yeasts, and extract of *Yucca schidigera* fed to beef cattle could control the presence of shigatoxigenic *E. coli,* indicating that diet can play a major role in reducing the incidence of *E. coli* in cattle (Grispoldi et al., 2017). MOS administered to preweaned Holstein heifers had improved body weight gain and reduction in avirulent and virulent *E. coli* compared with control calves (Lucey et al., 2021). Administration of transgalactosylated oligosaccharides (TOS)

increased the concentration of Lactobacilli and Bifidobacterium in pigs (Smiricky-Tjardes et al., 2003). A combination of GOS in swines resulted in increased Bifidobacterium and acetate levels, with an associated decline in intestinal hydrogen ions in relation to the control group. In addition, the amalgamation of GOS constrained the connection of Enterotoxigenic *E. coli* and *S.* Typhimurium on cells of HT29. The supplementation of ß-galactomannan-oligosaccharides in the feed condensed the incidence of *Salmonella*, seropositivity, and shedding in swines (Andrés-Barranco et al., 2015). Prebiotics like lactulose regulate intestinal microbiota by promoting selective growth of bacteria that are beneficial, particularly LAB (Guerra-Ordaz et al., 2014). Casein glycomacropeptides, soluble substances found in locust bean (*Ceratonia siliqua*), wheat (*Triticum aestivum*) bran, and guar gum (*Cyamopsis tetragonoloba*) (González-Ortiz et al., 2014), galacto-oligosaccharides (GOS) (Shoaf et al., 2006), and chito-oligosaccharides (COS) (Quintero-Villegas et al., 2013), are antiadhesives and prevent the attachment of EPEC or ETEC to HEP human 2 or porcine ileal mucus IPEC-J2 cells in *in vitro* experiments. Low butyrate, an SCFA produced as an end product of metabolism concentration, increases the gene expression associated with virulence required for EHEC cell adhesion (Vogt et al., 2015). Feeding of mannan-oligosaccharides (MOS) extracts of brewer's yeast (dried) enhanced serum immunoglobulin G (IgG) levels in *E. coli* K88-challenged pigs.

11 Synbiotics in Domestic Animals

The addition of MOS and *Streptococcus faecium* in the dairy calf diet improved fecal consistency and reduced fecal calf scours. Calves that were fed symbiotic-incorporated milk with probiotic and *S. cerevisiae* cell wall polysaccharides decreased the pathogenic *E. coli* in calf feces and increased the weight gain. Feeding of synbiotic containing an amalgamation of pulp of *Agave fourcroydes* Lem. and a commercial preparation containing *Lactobacillus salivarius* in calves reduced diarrheal incidence and improved weight and mean daily improvement in mass (Rondón et al., 2019). A combination of *Lactobacilli paracasei* with FOS increased the counts of aerobic and anaerobic microbes in the GI tract of piglets, as well as resulted in increased number of bacteria belonging to *Lactobacilli* and *Bifidobacterium*. Along with these, the levels of *Clostridium* sp., *E. coli,* and Enterobacteriaceae declined in pig stools (Nemcová et al., 1999). A synbiotic product containing bacteria, yeast, fungus, and MOS, when fed to weaning piglets, led to improved nutrient digestion, lessened the emission of harmful gases and prevented infections due to bacteria during weaning, and substituted the application of antibiotics (Lee et al., 2009). Piglet-fed diets with 0.5% synbiotic resulted in higher daily weight gain and reduction in coliforms in the colon, while enhanced levels of Bifidobacteria were observed in the colon and ileum (Shim et al., 2005). Diet containing synbiotic with a probiotic and anaerobic microflora and MOS, when fed to early weaning pigs, led to improved metabolism and reduction in toxic gases and enteropathogenic bacteria (Lee et al., 2009). Diet supplemented with synbiotics containing MOS when fed to

growing pigs showed higher crude protein digestibility and dry matter, reduced fecal amine and ammonia gas emissions, and enhanced fecal acetate production with low fecal *E. coli* population (Chu et al., 2011).

12 Probiotics in Feline and Canine Nutrition

Incorporation of *E. faecium* SF68 (5×10^8 CFU/d) in dog food, from weaning to one-year-old puppies, improved the immunity at both systemic and mucosal levels (Benyacoub et al., 2003). *L. rhamnosus* strain reduced immunological symptoms of atopic dermatitis and reduced allergen-induced IgE in the initial half year of life in genetically predisposed dogs (Marsella, 2009). Probiotic-encompassed *L. plantarum*, *L. casei,* and *B. animalis* subsp. *lactis* diminished the levels of opportunistic pathogens and enhanced the abundance of supportive microorganisms, namely, *Lactobacilli* and *Butyricicocci,* in canines (Xu et al., 2019).

The incidence of diarrheal diseases is high among cats and dogs in animal shelters, and an evaluation of the effects of administration of *E. faecium* SF68 at 2.1×10^9 CFU/g in sheltered dogs and cats revealed reduced diarrhea episodes in probiotic-fed cats. However, probiotics did not exhibit any effect on diarrhea in dogs (Bybee et al., 2011). Probiotics with addition of *L. acidophilus* (NCC2766 and NCC2628) and *L. johnsonii* (NCC2667) in dietary-responsive diarrhea dogs resulted in lessening of Enterobacters and enhanced levels of Lactobacilli in the fecal matter and beneficial intestinal cytokine patterns leading to clinical development (Sauter et al., 2006). In another study, "*NSS (non-specific dietary sensitivity)*" dogs administered with *L. acidophilus* strain DSM13241 exhibited better fecal, dry matter, consistency, and frequency in defecation in relation to the control canines. In addition, the fecal concentration of Bifidobacters and Lactobacilli amplified while *Escherichia* spp. and *C. perfringens* numbers diminished in the probiotic-fed group.

Canines with IBD "(*inflammatory bowel disease*)" harbor different kinds of microbiota in their small intestines to that of the healthy canines. The duodenum in the inflammatory condition of IBD dogs revealed enhanced levels of *Enterobacteria* and *Clostridia*, limited *Bacteroides,* and was free of *Spirochaetes*. The shielding influence of probiotic VSL # 3 (a blend of bacteria belonging to *Lactobacillus, Bifidobacterium,* and *Streptococcus* spp.) significantly reduced clinical and history scores and had reduced infiltration of CD3+ T cells in IBD-impacted canines (Rossi et al., 2014). Administration of a commercial probiotic containing strains of LAB improved the levels of *Lactobacillus* spp. with increased expression of TJP *"(tight junction protein)"* with clinical remission in canines (White et al., 2017). The microbiota of the vagina in healthy bitches is mainly colonized by LAB. Canine vaginal LAB was found to have antibacterial activity and is therefore a potential probiotic (Delucchi et al., 2008) that can be used to compact urinary tract infection in dogs. Administration of *Lactobacillus murinus* LpP2 was useful in the management of diarrhea associated with canine distemper (Delucchi et al., 2017). Puppies administered with *B. toyonensis* and immunized against canine parvovirus

improved the immune response with enhanced expression of IL-4, 17 and IFN-γ (Franz et al., 2020).

13 Prebiotics in Canine and Feline Nutrition

Incorporation of prebiotics into feline feed can positively affect microbial populations. Dogs on a chicory-supplemented diet, composed mainly of inulin, led to reduction in *C. perfringens* and increased levels of *Bifidobacteria* in the stool (Zentek et al., 2003). The inclusion of polydextrose in dog food has led to a decrease in fecal pH and indole while increasing the total fecal amount of SCFA, acetate, and propionate concentrations. In addition, the levels of *Lactobacilli*, *Bifidobacteria,* and *E. coli* in feces remained unaffected while *C. perfringens* levels dropped (Beloshapka et al., 2013). Administration of a commercial prebiotic containing β-glucans and MOS from *S. cerevisiae* to dogs resulted in increased levels of propionate-producing bacteria belonging to Porphyromonadaceae and Prevotellaceae and decrease in potential opportunistic pathogens belonging to Enterobacteriaceae and Fusobacteriaceae (Van den Abbeele et al., 2020). Dogs fed with a prebiotic blend and GOS showed improved immunity through increase in oxidative burst and the concentration of polymorphonuclear cells (Rentas et al., 2020). Cat feed supplemented with FOS had reduced *E. coli* while bifidobacterial concentrations increased. The presence of pectin in the feline diet increased *Lactobacilli* and *C. perfringens* concentration (Barry et al., 2010, 2012). The addition of cell walls of yeast in the food of healthy cats altered the stool bacterial composition, increasing helpful bacteria such as *Lactobacilli*, while lessening dangerous microbes; *C. perfringens, pathogenic E. coli,* etc. (Santos et al., 2018). Prebiotic incorporation in the pet diet may equally benefit intestinal health and gut microbiota and probably protect the animals from noninvasive diseases.

14 Synbiotics in Feline and Canine Nutrition

A combination of *B. bifidum* and GOS modulated canine fecal microbiota (Ogué-Bon et al. 2010). Synbiotics were found to reduce the levels of ammonia and biogenic amines, "BCFA (branched chain fatty acids)," indoles, and phenolic compounds (Swanson et al., 2002). Synbiotic-containing *Bacillus coagulans*, *L. acidophilus,* and *E. faecium* SF68, along with FOS and MOS, resulted in a significant rise in Lactobacillus, increased butyrate concentration, and decreased diarrheal occurrence in canines (Gagné et al., 2013). Supplementation of a combination of FOS and acacia and *E. faecium* NCIMB 10415 E1707 in healthy dogs in an animal shelter led to substantial reduction in diarrheal incidence (Rose et al., 2017). A synbiotic containing multispecies probiotics belonging to the genus Streptococcus and Enterococcus with two prebiotics fed to cats with chronic diarrhea improved character of stool and decreased the fecal score. Cats that received synbiotics after clindamycin administration had decreased vomiting and hyporexia. In addition, synbiotic

administration resulted in clinical benefits that persisted for at least 6 weeks after discontinuing (Stokes et al., 2017). Synbiotics containing seven different probiotic species and a mixture of arabinogalactans and fructooligosaccharides administered in cats and dogs led to an increase in *Streptococcus* spp. and *Enterococcus* (Garcia-Mazcorro et al., 2011). Synbiotics containing *Cucumis sativus* extract and *Lactobacillus acidophilus* C8.1 and *Lactobacillus paracasei* JCM8130 led to enhanced levels of *Bifidobacterium,* SCFA, and *Lactobacilli* in canines (Belà et al., 2019).

15 Probiotics in Aquaculture

While the embryonic development of terrestrial farm animals takes place inside the amnion, the aquatic animals at an early stage in their life cycle release their larvae into the water. Despite the fact that in the absence of well-developed digestive tract and immune system the exposure of larvae to pathogenic microbes is unavoidable, this in turn affects the GIT at very early stages.

In addition, opportunistic pathogens associated with the aquatic animals are the leading causes of death and severe financial losses. Therefore, during the larval stages, application of probiotics is required for proper development of the native gut microbiota. The supplementation of *Bacillus toyoi* spores as a probiotic to the yellowtail feed was reported in 1986. Aquaculture probiotics are obtained from varied sources, including host and non host sources. The probiotics derived from host are often isolated from mucus, gills, digestive tract, and skin, implying that they are a part of the microbiota as well as the water of rearing (Lazado et al., 2015).

Daily supplementation of *B. fusiformis* enhanced the rate of survival and accelerated the transformation of larvae of *Litopenaeus vannamei* and *Penaeus monodon* (Guo et al., 2009). *L. rohita* fingerlings administered with probiotics have shown augmented dietary performance and growth compared to control (Mohapatra et al., 2012). Incorporation of *L. fermentum* URLP18 into *C. carpio* diet resulted in effective colonization, better feed consumption, growth performance, altered intestinal microbiota, enhanced innate immune response, and invulnerability to *A. hydrophila* (Krishnaveni et al., 2021). Dietary supplementation of *L. plantarum* VSG3 to rohu for 2 months brought in positive changes in immune parameters, growth, and reduced suscptibility of rohu against disease by *A. hydrophila* (Giri et al., 2013). Diets incorporated with *Lactobacillus rhamnosus* in red sea bream improved physiological stress response, immune responses, and had improved resistance toward stress related to low-salinity conditions (Dawood et al., 2017). Intake of *Pediococcus pentosaceus* probiotic enhanced feed utilization, growth, quantity of intestinal microbes, activity of enzymes related to digestion, and improved health of *L. vannamei* shrimp. The probiotics also resulted in improved immunological response, such as lysozyme activity, total hemocyte counts, increased the *Bacillus* sp. count, and increased resistance to the *V. anguillarum* infections in relation to the control group (Adel et al., 2017). *B. cereus* and *P. acidilactici* decreased ammonia, nitrate, and biochemical oxygen demand in the pond water rearing white leg shrimp compared to the control group. Treating white

leg shrimp with *B. cereus* resulted in higher weight gain, survival ratio, and increased non specific immune reaction, viz. total protein, hemocyte count, and lysozyme activity in relation to the control group (Khademzade et al., 2020). Highly adhesive *Lactobacillus* when incorporated in feed of juvenile hybrid tilapia, protected against infection by *A. hydrophila* (Liu et al., 2013). Addition of *L. lactis* L19 and *E. faecalis* W24 isolated from the GIT of snakehead fish (*Channa argus*) enhanced the humoral immunity, growth performance, upregulated the expression of genes associated with immunity, IL-6, 10, TNF-α, IL-1β, IFN-γ, HSP70,90 and TGF-β, in the head, spleen, gill, kidney, intestine, and liver, and improved resistance against infection of *A. veronii* (Kong et al., 2020). Dietary incorporation of *L. lactis* and *B. subtilis* improved the immune response, disease resistance, growth performance, and intestinal morphology and higher cumulative survival on *A. hydrophila* challenge in Nile tilapia in relation to the control group (Won et al., 2020). White leg shrimp, when fed diet incorporated with *L. plantarum,* protected against *Vibrio alginolyticus* infection and enhanced humoral and cellular immune responses including levels of SOD, PO, and proPO and PE mRNA transcription (Chiu et al., 2007). The *Psychrobacter maritimus*, a marine psychrotrophic bacterium, significantly increased the growth shown in fingerlings of Nile tilapia (Makled et al., 2020).

Numerous studies have explored ways by which Bacillus as probiotics can regulate the microbiota and provide resistance to diseases. *B. subtilis* BT23, with activity against *Vibrio* sp. under *in vitro* conditions, showed 90% reduction in mortality when challenged with *Vibrio harveyi* in *P. monodon* (Vaseeharan & Ramasamy, 2003). Decline in juvenile mortality was observed in *L. vannamei* when *B. subtilis*-containing feed was given 28 days prior to challenge with *V. harveyi*. The reduction in infection can be attributed to competitive mode of exclusion of the pathogen (Balcazar & Rojas-Luna, 2007).

Li et al. (2007) observed that *B. licheniformis* when administered to *L. vannamei* resulted in increased activity of superoxide dismutase and phenoloxidase and decreased *Vibrio* sp. in the intestine. Studies revealed that Bacillus application resulted in the stimulation of immune system of *L. vannamei*, which has also proved helpful in enhancing shrimp growth and survival (Zokaeifar et al., 2012). Furthermore, certain Bacillus spp.-based probiotics demonstrated antiviral effects against WSS infection in shrimp; however, the mode of action is yet to be known (Li et al., 2009). *B. pumilus* SE5 of shrimp origin, when used as probiotic, increased feed utilization, improved intestinal health and immunity of shrimp, and relieved the complicacies associated with high intake of soyabean meal in shrimp, *L. vannamei* (Zhang et al., 2020).

Incorporation of probiotic *Bacillus* NP5 in rearing water reduced the ammonia levels, improved the growth, and immune status of African catfish infected with *Aeromonas hydrophila* (Putra et al., 2020). Application of a commercial sold probiotic, Sanolife PRO-F, in the feed had useful effects on resistance, growth, gut health, and stress responses, and lowered the ammonia concentration in water of rearing of farmed Nile tilapia (Elsabagh et al., 2018). Yucca extract with yeast as a water additive improved the quality of pond water by reducing ammonia levels, resulting in the reduction in oxidative stress and improved the growth in Nile tilapia.

Further, lesser malondialdehyde value and higher levels of catalase glutathione peroxidase and superoxide dismutase were detected in Nile tilapia (Abdel-Tawwab et al., 2021). Administration of a commercial probiotic in *L. vannamei* ponds increased the levels of beneficial bacterial flora, reduced phosphorus and nitrogen levels in the rearing water, and improved the production of shrimp. When some aquacultured ponds were subjected to probiotic application, it resulted in enhanced levels of ammonifying bacteria, protein-mineralizing bacteria and *Bacillus* sp. counts, and diminished levels of Vibrio counts compared with untreated ponds. The incorporation of probiotics reduced dissolved total inorganic nitrogen, reactive phosphorus, and COD demand in the rearing waters and augmented the DO levels (Wang et al., 2005b). Supplementation of *B. coagulans* in water could improve survival rates and increase the digestive enzyme production in *L. vannamei* larvae (Zhou et al., 2009). The use of *A. faecalis* Y311 and *B. cereus* NY5 as water probiotics lowered phosphorus level in aquaculture water, resulting in an upsurge in the relative abundance of helpful microflora in Nile tilapia. The activities of superoxide dismutase in the skin and alkaline phosphatase in the gill and intestine amplified in *B. cereus* and *A. faecalis*-fed ponds, respectively (Wang et al., 2020).

16 Prebiotics in Aquaculture

Knowledge about the application of prebiotics in aquaculture farming has considerably improved in the recent years. Incorporation of grape seed extract in the feed of rainbow trout upregulated immune gene expression such as complement component 3, lysozyme, *TNF-α,* and *IFN-γ* in different mucosal tissues, and the skin mucus of the fish had bactericidal activity against *Yersinia ruckeri* (Mousavi et al., 2021). Administration of autolyzed *S. cerevisiae* induced IL-8 secretion while cell crushed resulted in the secretion of TNFα in Atlantic salmon (Hansen et al., 2021). Application of a mixture of labiatae plant oils and garlic and galactomannan oligosaccharides led to a reduction in Vibrionales and coliforms bacteria, which includes several pathogenic bacteria of fish, and increased in the butyrate producer taxa in gut microbiota in European sea bass (Rimoldi et al., 2020). The pressure related to crowding was mitigated in Nile tilapia fed with β-glucan amalgamated feed, which enhanced their development, stress tolerance, intestinal morphometry, and immunity. Further, TNF-α, INF-γ, and IL-1β gene transcription increased; however, HSP70 gene transcription decreased (Dawood et al., 2020). Dietary supplementation of seaweed (Enteromorpha) polysaccharides (EPS) resulted in enhanced gain in weight, final weight, and precise proportion of growth in banana shrimp, *F. merguiensis*. EPS also increased the levels of alkaline phosphatase, glutathione peroxidase glutathione S-transferase, lysozyme, phenoloxidase, and superoxide dismutase activities in hemolymph. There was an increase in the *Firmicutes* at phylum level while levels of *Vibrio* at genus level reduced (Liu et al., 2020b). Incorporation of diet of common carp fingerlings with guava resulted in significant upregulation in expression of IL1b and IL-8 (Hoseinifar et al., 2019). Corncob-

derived xylooligosaccharide improved the disease resistance, shown in growth and immune response of inborn kind in Nile tilapia fingerlings (Van Doan et al., 2018).

17 Synbiotics in Aquaculture

The investigations on the employment of prebiotics in fish and shellfish cultivation are scant. Feeds augmented with *Enterococcus faecalis*, mannan oligosaccharides, and polyhydroxybutyrate provided to young rainbow trout resulted in considerable enhancement in weight gain (Rodriguez-Estrada et al., 2009). Dietary applications of fructo- and mannan oligosaccharide and *B. clausii*, alone or in combination, exhibited enhanced efficacy of feed, digestive enzyme activity, nutrient deposition, growth, lipid metabolism, and nonspecific immunity in Japanese flounder (Ye et al., 2011). Pacific white shrimp fed with diet containing commercial synbiotic consisting of *S. cerevisiae*, *B. subtilis*, mannan oligosaccharide, and β-glucan led to higher acid phosphatase, alkaline phosphatase, levels of catalase, lysozyme, total superoxide dismutase, and significantly lower serum malondialdehyde levels with increased levels of *Lactococcus* and decreased abundance of *Vibrio* in the gut. It was also found that synbiotic-fed shrimp had reduced cumulative mortality after *V. parahaemolyticus* challenge (Yao et al., 2021). A commercial synbiotic product containing *B. subtilis,* yeast, and mannan oligosaccharides to the basal diet and fed to large mouth bass resulted in lessened feed conversion ratio and increased gain in weight, serum lysozyme, and intestinal protease activity compared with control (Yang et al., 2020).

A combination of *Wickerhamomyces anomalus* yeast with *B. subtilis ATCC 6633* augmented the performance and development of Catla *(Catla catla)* juveniles (Gupta et al., 2020). Similarly, in case of Japanese eels, *Anguilla japonica,* a mixture of *B. licheniformis* with mannan oligosaccharide and *B. subtilis* with fructooligosaccharide mixed feed, amplified growth rate precisely and so was weight gain. Besides, there was an enhanced expression of immune-related genes, immunoglobulin M, and heat shock protein 70 and an improvement in immune response against *Aeromonas hydrophila* infection in fish fed with synbiotic feeds (Park et al., 2020).

18 Safe Use of Probiotics

The transfer and spread of ARGs is a natural problem that gave rise to AMR in microbial pathogens that is aggravated by the overemployment of drugs in veterinary, human medicine, and in livestock farming. The consumption of antibiotics can be substituted with probiotics to regulate infections of animals and humans that can decrease the selective suppression of antibiotic-sensitive microorganisms in the environment and ultimately leading to reduction in the rapid emergence of resistance to drugs. However, the probiotic bacteria themselves can contribute to the transmission of ARGs. Therefore, even though probiotics are currently considered

safe, implementation of appropriate regulation is important for their use in human and animal settings all over the world to effectively reduce the involvement of probiotic strains in the blowout of ARGs in natural environment.

Microbes used as probiotics are found to carry mechanisms that govern antibiotic resistance (Gueimonde et al., 2013; Varankovich et al., 2015). Before a bacterium is considered for use as a probiotic, it is highly imperative to examine it for incidence of genes coding for antimicrobial resistance. The antibiotic resistance of a probiotic bacteria can be either inherent or attained (Pradhan et al., 2020; Sanders et al., 2010). Two statutes are currently accepted in assessing the safety of potential strains of probiotics: "*Generally Recognized as Safe (GRAS)"* by the USFDA (2017) and "*Qualified Presumption of Safety (QPS)*" by EFSA. GRAS is generally applicable to microbes and components that are derived from microbes employed in food products while QPS is applied to a biological agent, viz. bacteria, fungi, or viruses, which are purposely added to various stages in the food chain. The *Lactobacillus* sp. in some commercial probiotics are found to carry antibiotic-resistance genes that are transferable (Biloni et al., 2013). Genetic transfer of vancomycin-resistant plasmid-derived gene was demonstrated between *L. acidophilus* and *Enterococcus faecium* probiotic strains in the GIT of rats establishing transmission of antibiotic resistance genes between probiotic strains (Mater et al., 2008). In another study, an *E. faecium* isolated from animal could transfer vancomycin-unresponsiveness gene, *vanA,* to a strain of *E. faecium* susceptible to vancomycin of human origin in GIT of human volunteers (Lester et al., 2006). Food and commercially available probiotic supplements sometimes contain lactic acid bacteria with antimicrobial resistance genes in plasmids and transposons. Despite being considered as GRAS, the findings of the study point to the threat of spread of antimicrobial genes through lactic acid bacterial probiotic strains. This is particularly true in countries that do not have the regulations and guidelines in place for biosafety testing of probiotics (Chang et al., 2009).

AMR transmission can occur within the water environment when high bacterial numbers that carry AMR genes are added daily to the aquaculture system. This can cause substantial shifts in the existing bacterial community, resulting in the accumulation of AMR bacteria within the aquaculture system. AMR genes that are carried on the mobile genetic elements such as transposons and plasmids may act as reservoir of transmission of antimicrobial genes to pathogens in the gastrointestinal tract, leading to a concern on saftey (Munoz-Atienzal et al., 2013). Hence, it is highly imperative to test the probiotic strains for the presence of AMR genes while considering whether the bacteria will be used as probiotics. Risk exists for the transmission of antimicrobial genes from probiotic to pathogenic microbes, and vice versa, given the assigned location of bacteria in the GIT (Munoz-Atienzal et al., 2013). Munoz-Atienzal et al. (2013) reported the existence of many ARGs in LAB from animals of marine origin intended to be used as probiotics in aquaculture. The addition of antimicrobials and live bacteria to aquaculture systems in large amounts that may have AMR genes has serious long-term effects in the natural aquatic environment. Mere presence of antibiotic-resistant genes in probiotic strains is not harmful. However, probiotic resistance genes are likely to be transmitted further to microbes of GIT and pathogens sharing intestinal environment, potentially

leading to clinical infections that are unresponsive to antibiotic therapy (Broaders et al., 2013; Imperial & Ibana, 2016).

LAB, viz. *Lactobacillus, Lactococcus, Bifidobacterium,* and *Streptococcus,* are intrinsically resistant to streptomycin, kanamycin, and gentamicin while *Bifidobacterium* sp. have inherently unresponsive to aminoglycosides, ciprofloxacin, mupirocin, nalidixic acid and streptomycin (Varankovich et al., 2015; Wei et al., 2012). The genes that code for resistance to lincosamides, macrolides, tetracycline, and streptogramin B are harbored in transposons of *Bifidobacterium* sp. (Gueimonde et al., 2013).

19 Conclusion

There are noteworthy studies that revealed the useful probiotic influence on human and animal health, especially in improving the immune response, regulation of gastrointestinal disorders, and pathogen protection. In addition, probiotics confer increased production capacity in food animals. Prebiotic in diets also has a positive effect on improvement in immune system, intestinal health, and production. Prophylactic health products, namely, Pre-Pro and synbiotics, are usually regarded safe, reduces the use of antibiotics and have no adverse effects on the environment. The consumption of probiotics has increased recently owing to apprehensions about the spread of antimicrobial resistance among pathogens. Probiotics are considered to have the possibility to replace the employment of antibiotics in clinical settings of human and animals. However, risks associated with transfer of AMR genes from probiotic strains to natural flora including pathogens present in the gut or natural environment cannot be ruled out. Hence, before commercial application, mandatory screening of all strains of probiotics for incidence of ARGs has to be done. In summary, probiotics, prebiotics, and synbiotics show great promise in replacing antibiotics in industrial and therapeutic applications.

References

Abd El-Moneim, A. M. E., Elbaz, A. M., Khidr, R. E. S., & Badri, F. B. (2019). Effect of in ovo inoculation of Bifidobacterium spp. on growth performance, thyroid activity, ileum histomorphometry, and microbial enumeration of broilers. *Probiotics and Antimicrobial Proteins, 12*(3), 1–10.

Abd El-Moneim, A. M. E., Selim, D. A., Basuony, H. A., Sabic, E. M., Saleh, A. A., & Ebeid, T. A. (2020). Effect of dietary supplementation of Bacillus subtilis spores on growth performance, oxidative status, and digestive enzyme activities in Japanese quail birds. *Tropical Animal Health and Production, 52*(2), 671–680.

Abdel-Tawwab, M., Mounes, H. A., Shady, S. H., & Ahmed, K. M. (2021). Effects of yucca, Yucca schidigera, extract and/or yeast, Saccharomyces cerevisiae, as water additives on growth, biochemical, and antioxidants/oxidant biomarkers of Nile tilapia, Oreochromis niloticus. *Aquaculture, 533*, 736122.

Abou-Kassem, D. E., Elsadek, M. F., Abdel-Moneim, A. E., Mahgoub, S. A., Elaraby, G. M., Taha, A. E., Elshafie, M. M., Alkhawtani, D. M., Abd El-Hack, M. E., & Ashour, E. A. (2021).

Growth, carcass characteristics, meat quality, and microbial aspects of growing quail fed diets enriched with two different types of probiotics (*Bacillus toyonensis* and *Bifidobacterium bifidum*). *Poultry Science, 100*(1), 84–93.

Adel, M., Yeganeh, S., Dawood, M. A. O., Safari, R., & Radhakrishnan, S. (2017). Effects of *Pediococcus pentosaceus* supplementation on growth performance, intestinal microflora and disease resistance of white shrimp, *Litopenaeus vannamei*. *Aquaculture Nutrition, 23*(6), 1401–1409.

Al-Baadani, H. H., Abudabos, A. M., Al-Mufarrej, S. I., Al-Baadani, A. A., & Alhidary, I. A. (2018). Dietary supplementation of *Bacillus subtilis*, *Saccharomyces cerevisiae* and their symbiotic effect on serum biochemical parameters in broilers challenged with *Clostridium perfringens*. *Journal of Applied Animal Research, 46*(1), 1064–1072.

Alessandri, G., Ossiprandi, M. C., MacSharry, J., Van Sinderen, D., & Ventura, M. (2019). Bifidobacterial dialogue with its human host and consequent modulation of the immune system. *Frontiers in Immunology, 10*, 2348.

Alfredson, D. A., & Korolik, V. (2007). Antibiotic resistance and resistance mechanisms in *Campylobacter jejuni* and *Campylobacter coli*. *FEMS Microbiology Letters, 277*, 123–132.

Amat, S., Alexander, T. W., Holman, D. B., Schwinghamer, T., & Timsit, E. (2020). Intranasal bacterial therapeutics reduce colonization by the respiratory pathogen Mannheimia haemolytica in dairy calves. *Msystems, 5*(2), e00629-19.

Andrés-Barranco, S., Vico, J. P., Grilló, M. J., & Mainar-Jaime, R. C. (2015). Reduction of subclinical *Salmonella* infection in fattening pigs after dietary supplementation with a ß-galactomannan oligosaccharide. *Journal of Applied Microbiology, 118*, 284–294.

Andrews, T., Neher, D. A., Weicht, T. R., & Barlow, J. W. (2019). Mammary microbiome of lactating organic dairy cows varies by time, tissue site, and infection status. *PLoS One, 14*(11), e0225001.

Assis, B. S., Germon, P., Silva, A. M., Even, S., Nicoli, J. R., & Loir, Y. L. (2015). *Lactococcus lactis* v7 inhibits the cell invasion of bovine mammary epithelial cells by *Echerichia coli* and *Staphylococcus aureus*. *Beneficial Microbes, 6*(6), 879–886.

Azcarate-Peril, M. A., Ritter, A. J., Savaiano, D., Monteagudo-Mera, A., Anderson, C., Magness, S. T., & Klaenhammer, T. R. (2017). Impact of short-chain galactooligosaccharides on the gut microbiome of lactose-intolerant individuals. *Proceedings of the National Academy of Sciences of the USA, 114*, E367–E375. https://doi.org/10.1073/pnas.1606722113

Bai, S. P., Wu, A. M., Ding, X. M., Lei, Y., Bai, J., Zhang, K. Y., & Chio, J. S. (2013). Effects of probiotic-supplemented diets on growth performance and intestinal immune characteristics of broiler chickens. *Poultry Science, 92*(3), 663–670.

Balcazar, J. L., & Rojas-Luna, T. (2007). Inhibitory activity of probiotic *Bacillus subtilis* UTM126 against Vibrio species confers protection against vibriosis in juvenile shrimp (*Litopenaeus vannamei*). *Current Microbiology, 55*, 409–412.

Barry, K. A., Wojcicki, B. J., Middelbos, I. S., Vester, B. M., Swanson, K. S., & Fahey, G. C., Jr. (2010). Dietary cellulose, fructooligosaccharides, and pectin modify fecal protein catabolites and microbial populations in adult cats. *Journal of Animal Science, 88*(9), 2978–2987.

Barry, K. A., Middelbos, I. S., Vester Boler, B. M., Dowd, S. E., Suchodolski, J. S., Henrissat, B., Coutinho, P. M., White, B. A., Fahey, G. C., Jr., & Swanson, K. S. (2012). Effects of dietary fiber on the feline gastrointestinal metagenome. *Journal of Proteome Research, 11*(12), 5924–5933.

Baurhoo, B., Goldflus, F., & Zhao, X. (2009). Purified cell wall of Saccharomyces cerevisiae increases protection against intestinal pathogens in broiler chickens. *International Journal of Poultry Science, 8*(2), 133–137.

Baxter, N. T., Schmidt, A. W., Venkataraman, A., Kim, K. S., Waldron, C., & Schmidt, T. M. (2019). Dynamics of human gut microbiota and short-chain fatty acids in response to dietary interventions with three fermentable fibers. *MBio, 10*(1), e02566-18.

Beirão, B. C., Ingberman, M., Fávaro, C., Jr., Mesa, D., Bittencourt, L. C., Fascina, V. B., & Caron, L. F. (2018). Effect of an Enterococcus faecium probiotic on specific IgA following live Salmonella Enteritidis vaccination of layer chickens. *Avian Pathology, 47*(3), 325–333.

Belà, B., Coman, M. M., Verdenelli, M. C., Bianchi, C., Pignataro, G., Fiorini, D., & Silvi, S. (2019). In vitro fermentation of Cucumis sativus fructus extract by canine gut microbiota in combination with two probiotic strains. *Journal of Functional Foods, 63*, 103585.

Beloshapka, A. N., Dowd, S. E., Suchodolski, J. S., Steiner, J. M., Duclos, L., & Swanson, K. S. (2013). Fecal microbial communities of healthy adult dogs fed raw meat-based diets with or without inulin or yeast cell wall extracts as assessed by 454 pyrosequencing. *FEMS Microbiology Ecology, 84*(3), 532–541.

Bengtsson-Palme, J., Kristiansson, E., & Larsson, D. J. (2018). Environmental factors influencing the development and spread of antibiotic resistance. *FEMS Microbiology Reviews, 42*(1), fux053.

Benyacoub, J., Czarnecki-Maulden, G. L., Cavadini, C., Sauthier, T., Anderson, R. E., Schiffrin, E. J., & von der Weid, T. (2003). Supplementation of food with *Enterococcus faecium* (SF68) stimulates immune functions in young dogs. *The Journal of Nutrition, 133*(4), 1158–1162.

Bi, Y., Yang, C., Diao, Q., & Tu, Y. (2017). Effects of dietary supplementation with two alternatives to antibiotics on intestinal microbiota of preweaned calves challenged with Escherichia coli K99. *Scientific Reports, 7*(1), 1–12.

Bicalho, M. L. S., Santin, T., Rodrigues, M. X., Marques, C. E., Lima, S. F., & Bicalho, R. C. (2017). Dynamics of the microbiota found in the vaginas of dairy cows during the transition period: Associations with uterine diseases and reproductive outcome. *Journal of Dairy Science, 100*(4), 3043–3058.

Biloni, A., Quintana, C., Menconi, A., Kallapura, G., Latorre, J., Pixley, C., Layton, S., Dalmagro, M., Hernandez-Velasco, X., & Wolfenden, A. (2013). Evaluation of effects of EarlyBird associated with FloraMax-B11 on Salmonella Enteritidis, intestinal morphology, and performance of broiler chickens. *Poultry Science, 92*(9), 2337–2346.

Blau, K., Samuel, J., Su, J.-Q., Zhu, Y.-G., Smalla, K., & Jechalke, S. (2019). Manure and doxycycline affect the bacterial community and its resistome in lettuce rhizosphere and bulk soil. *Frontiers in Microbiology, 10*, 725. https://doi.org/10.3389/fmicb.2019.00725

Broaders, E., Gahan, C. G., & Marchesi, J. R. (2013). Mobile genetic elements of the human gastrointestinal tract: Potential for spread of antibiotic resistance genes. *Gut Microbes, 4*, 271–280. https://doi.org/10.4161/gmic.24627

Bungau, S., Behl, T., Aleya, L., Bourgeade, P., Aloui-Sossé, B., Purza, A. L., Abid, A., & Samuel, A. D. (2021). Expatiating the impact of anthropogenic aspects and climatic factors on long-term soil monitoring and management. *Environmental Science and Pollution Research, 28*(24), 30528–30550.

Burridge, L., Weis, J. S., Cabello, F., Pizarro, J., & Bostick, K. (2010). Chemical use in salmon aquaculture: A review of current practices and possible environmental effects. *Aquaculture, 306*(1–4), 7–23.

Bybee, S. N., Scorza, A. V., & Lappin, M. R. (2011). Effect of the probiotic *Enterococcus faecium* SF68 on presence of diarrhea in cats and dogs housed in an animal shelter. *Journal of Veterinary Internal Medicine, 25*(4), 856–860.

Caly, D. L., D'Inca, R., Auclair, E., & Drider, D. (2015). Alternatives to antibiotics to prevent necrotic enteritis in broiler chickens: A microbiologist's perspective. *Frontiers in Microbiology, 6*, 1336.

Canducci, F., Cremonini, F., Armuzzi, A., Di Caro, S., Gabrielli, M., Santarelli, L., Nista, E., Lupascu, A., De Martini, D., & Gasbarrini, A. (2002). Probiotics and *Helicobacter pylori* eradication. *Digestive and Liver Disease, 34*, S81–S83.

Carey, C. M., Kostrzynska, M., Ojha, S., & Thompson, S. (2008). The effect of probiotics and organic acids on Shiga-toxin 2 gene expression in enterohemorrhagic Escherichia coli O157: H7. *Journal of Microbiological Methods, 73*(2), 125–132.

Chang, L. I. U., Zhang, Z. Y., Ke, D. O. N. G., Jian-Ping, Y. U. A. N., & Xiao-Kui, G. U. O. (2009). Antibiotic resistance of probiotic strains of lactic acid bacteria isolated from marketed foods and drugs. *Biomedical and Environmental Sciences, 22*(5), 401–412.

Chen, Y. C., & Chen, T. C. (2004). Mineral utilization in layers as influenced by dietary oligofructose and inulin. *International Journal of Poultry Science, 3*(7), 442–445.

Chen, J., Sun, R., Pan, C., Sun, Y., Mai, B., & Li, Q. X. (2020). Antibiotics and food safety in aquaculture. *Journal of Agricultural and Food Chemistry, 68*(43), 11908–11919.

Chiu, C. H., Guu, Y. K., Liu, C. H., Pan, T. M., & Cheng, W. (2007). Immune responses and gene expression in white shrimp, *Litopenaeus vannamei*, induced by Lactobacillus plantarum. *Fish & Shellfish Immunology, 23*(2), 364–377.

Chopyk, J., Moore, R. M., DiSpirito, Z., Stromberg, Z. R., Lewis, G. L., Renter, D. G., Cernicchiaro, N., Moxley, R. A., & Wommack, K. E. (2016). Presence of pathogenic Escherichia coli is correlated with bacterial community diversity and composition on pre-harvest cattle hides. *Microbiome, 4*(1), 1–11.

Chu, G. M., Lee, S. J., Jeong, H. S., & Lee, S. S. (2011). Efficacy of probiotics from anaerobic microflora with prebiotics on growth performance and noxious gas emission in growing pigs. *Animal Science Journal, 82*(2), 282–229.

Click, R. E. (2011). Successful treatment of asymptomatic or clinically terminal bovine Mycobacterium avium subspecies paratuberculosis infection (Johne's disease) with the bacterium Dietzia used as a probiotic alone or in combination with dexamethasone: Adaption to chronic human diarrheal diseases. *Virulence, 2*(2), 131–143.

Collado, M. C., Bäuerl, C., & Pérez-Martínez, G. (2012). Defining microbiota for developing new probiotics. *Microbial Ecology in Health and Disease, 23*(1), 18579.

Collins, M. D., & Gibson, G. R. (1999). Probiotics, prebiotics, and synbiotics: Approaches for modulating the microbial ecology of the gut. *The American Journal of Clinical Nutrition, 69*(5), 1052s–1057s.

Cuong, N., Padungtod, P., Thwaites, G., & Carrique Mas, J. (2018). Antimicrobial usage in animal production: A review of the literature with a focus on low- and middle-income countries. *Antibiotics (Basel), 7*(3), 75.

Dawood, M. A., Koshio, S., Ishikawa, M., El-Sabagh, M., Yokoyama, S., Wang, W. L., Yukun, Z., & Olivier, A. (2017). Physiological response, blood chemistry profile and mucus secretion of red sea bream (*Pagrus major*) fed diets supplemented with *Lactobacillus rhamnosus* under low salinity stress. *Fish Physiology and Biochemistry, 43*(1), 179–192.

Dawood, M. A., Metwally, A. E. S., El-Sharawy, M. E., Atta, A. M., Elbialy, Z. I., Abdel-Latif, H. M., & Paray, B. A. (2020). The role of β-glucan in the growth, intestinal morphometry, and immune-related gene and heat shock protein expressions of Nile tilapia (*Oreochromis niloticus*) under different stocking densities. *Aquaculture, 523*, 735205.

Dec, M., Nowaczek, A., Urban-Chmiel, R., Stępień-Pyśniak, D., & Wernicki, A. (2018). Probiotic potential of Lactobacillus isolates of chicken origin with anti-Campylobacter activity. *Journal of Veterinary Medical Science, 80*, 1195, 18-0092.

Defoirdt, T., Sorgeloos, P., & Bossier, P. (2011). Alternatives to antibiotics for the control of bacterial disease in aquaculture. *Current Opinion in Microbiology, 14*(3), 251–258.

Delucchi, L., Fraga, M., Perelmuter, K., Cidade, E., & Zunino, P. (2008). Vaginal lactic acid bacteria in healthy and ill bitches and evaluation of in vitro probiotic activity of selected isolates. *The Canadian Veterinary Journal, 49*(10), 991.

Delucchi, L., Fraga, M., & Zunino, P. (2017). Effect of the probiotic Lactobacillus murinus LbP2 on clinical parameters of dogs with distemper-associated diarrhea. *Canadian Journal of Veterinary Research, 81*(2), 118–121.

Deng, W., Dong, X. F., Tong, J. M., & Zhang, Q. (2012). The probiotic *Bacillus licheniformis* ameliorates heat stress-induced impairment of egg production, gut morphology, and intestinal mucosal immunity in laying hens. *Poultry Science, 91*(3), 575–582.

Derakhshani, H., Fehr, K. B., Sepehri, S., Francoz, D., De Buck, J., Barkema, H. W., Plaizier, J. C., & Khafipour, E. (2018). Invited review: Microbiota of the bovine udder: Contributing factors and potential implications for udder health and mastitis susceptibility. *Journal of Dairy Science, 101*(12), 10605–10625.

Dewulf, E. M., Cani, P. D., Claus, S. P., Fuentes, S., Puylaert, P. G., Neyrinck, A. M., Bindels, L. B., de Vos, W. M., Gibson, G. R., Thissen, J. P., & Delzenne, N. M. (2013). Insight into the prebiotic concept: Lessons from an exploratory, double blind intervention study with inulin-type fructans in obese women. *Gut, 62*(8), 1112–1121.

Díaz, A. M., Almozni, B., Molina, M. A., Sparo, M. D., Manghi, M. A., Canellada, A. M., & Castro, M. S. (2018). Potentiation of the humoral immune response elicited by a commercial vaccine against bovine respiratory disease by Enterococcus faecalis CECT7121. *Beneficial Microbes, 9*(4), 553–562.

Dibaji, S. M., Seidavi, A., Asadpour, L., & da Silva, F. M. (2014). Effect of a synbiotic on the intestinal microflora of chickens. *Journal of Applied Poultry Research, 23*(1), 1–6.

Dinleyici, E. C., Eren, M., Ozen, M., Yargic, Z. A., & Vandenplas, Y. (2012). Effectiveness and safety of Saccharomyces boulardii for acute infectious diarrhea. *Expert Opinion on Biological Therapy, 12*(4), 395–410.

Dodoo, C. C., Wang, J., Basit, A. W., Stapleton, P., & Gaisford, S. (2017). Targeted delivery of probiotics to enhance gastrointestinal stability and intestinal colonisation. *International Journal of Pharmaceutics, 530*(1–2), 224–229.

Dubreuil, J. D. (2017). Enterotoxigenic Escherichia coli and probiotics in swine: What the bleep do we know? *Bioscience of Microbiota, Food and Health, 36*, 75, 16-030.

Dudek-Wicher, R., Junka, A., Paleczny, J., & Bartoszewicz, M. (2020). Clinical trials of probiotic strains in selected disease entities. *International Journal of Microbiology, 2020*, 8854119.

Duysburgh, C., Van den Abbeele, P., Krishnan, K., Bayne, T. F., & Marzorati, M. (2019). A synbiotic concept containing spore-forming bacillus strains and a prebiotic fiber blend consistently enhanced metabolic activity by modulation of the gut microbiome in vitro. *International Journal of Pharmaceutics: X, 1*, 100021.

Eckert, E. M., Quero, G. M., Di Cesare, A., Manfredini, G., Mapelli, F., Borin, S., Fontaneto, D., Luna, G. M., & Corno, G. (2019). Antibiotic disturbance affects aquatic microbial community composition and food web interactions but not community resilience. *Molecular Ecology, 28*(5), 1170–1182.

Eeckhaut, V., Wang, J., Van Parys, A., Haesebrouck, F., Joossens, M., Falony, G., Raes, J., Ducatelle, R., & Van Immerseel, F. (2016). The probiotic Butyricicoccus pullicaecorum reduces feed conversion and protects from potentially harmful intestinal microorganisms and necrotic enteritis in broilers. *Frontiers in Microbiology, 7*, 1416.

Elmahdi, S., DaSilva, L. V., & Parveen, S. (2016). Antibiotic resistance of Vibrio parahaemolyticus and Vibrio vulnificus in various countries: A review. *Food Microbiology, 57*, 128–134.

Elsabagh, M., Mohamed, R., Moustafa, E. M., Hamza, A., Farrag, F., Decamp, O., Dawood, M. A., & Eltholth, M. (2018). Assessing the impact of *Bacillus* strains mixture probiotic on water quality, growth performance, blood profile and intestinal morphology of Nile tilapia, *Oreochromis niloticus*. *Aquaculture Nutrition, 24*(6), 1613–1622.

European Commission. (2003) Off J EU 18.10 L268.

Fair, R. J., & Tor, Y. (2014). Antibiotics and bacterial resistance in the 21st century. *Perspectives in Medicinal Chemistry, 6*, 25, PMC-S14459.

Figueroa-González, I., Cruz-Guerrero, A., & Quijano, G. (2011). The benefits of probiotics on human health. *Journal of Microbial and Biochemical Technology, 1*, 1948–5948.

Forkus, B., Ritter, S., Vlysidis, M., Geldart, K., & Kaznessis, Y. N. (2017). Antimicrobial probiotics reduce Salmonella enterica in Turkey gastrointestinal tracts. *Scientific Reports, 7*(1), 1–9.

Franz, C. M., Huch, M., Abriouel, H., Holzapfel, W., & Gálvez, A. (2011). Enterococci as probiotics and their implications in food safety. *International Journal of Food Microbiology, 151*(2), 125–140.

Franz, H. C., Conrad, N. L., Santos, F. D., Gonçalves, V. S., Fonseca, R. N., Brasil, C. L., Hübner, S. D. O., & Leite, F. P. (2020). Immunomodulatory effect of Bacillus toyonensis BCT-7112 supplementation in puppies vaccinated against canine parvovirus. *Pesquisa Veterinaria Brasileira, 40*(11), 898–902.

Froebel, L. K., Froebel, L. E., & Duong, T. (2020). Refined functional carbohydrates reduce adhesion of Salmonella and Campylobacter to poultry epithelial cells in vitro. *Poultry Science, 99*(12), 7027–7034.

Fuller, R. (1989). Probiotics in man and animals. *The Journal of Applied Bacteriology, 66*, 365–378.

Gagné, J. W., Wakshlag, J. J., Simpson, K. W., Dowd, S. E., Latchman, S., Brown, D. A., Brown, K., Swanson, K. S., & Fahey, G. C. (2013). Effects of a synbiotic on fecal quality, short-chain fatty acid concentrations, and the microbiome of healthy sled dogs. *BMC Veterinary Research, 9*(1), 1–10.

Garcia-Mazcorro, J. F., Lanerie, D. J., Dowd, S. E., Paddock, C. G., Grützner, N., Steiner, J. M., Ivanek, R., & Suchodolski, J. S. (2011). Effect of a multi-species synbiotic formulation on fecal bacterial microbiota of healthy cats and dogs as evaluated by pyrosequencing. *FEMS Microbiology Ecology, 78*(3), 542–554.

Gärtner, M. A., Bondzio, A., Braun, N., Jung, M., Einspanier, R., & Gabler, C. (2015). Detection and characterisation of Lactobacillus spp. in the bovine uterus and their influence on bovine endometrial epithelial cells in vitro. *PLoS One, 10*(3), e0119793.

Genís, S., Bach, À., Fàbregas, F., & Arís, A. (2016). Potential of lactic acid bacteria at regulating Escherichia coli infection and inflammation of bovine endometrium. *Theriogenology, 85*(4), 625–637.

Genís, S., Cerri, R. L., Bach, À., Silper, B. F., Baylão, M., Denis-Robichaud, J., & Arís, A. (2018). Pre-calving intravaginal administration of lactic acid bacteria reduces metritis prevalence and regulates blood neutrophil gene expression after calving in dairy cattle. *Frontiers in Veterinary Science, 5*, 135.

Ghosh, S., & Mehla, R. K. (2012). Influence of dietary supplementation of prebiotics (mannanoligosaccharide) on the performance of crossbred calves. *Tropical Animal Health and Production, 44*(3), 617–622.

Ghosh, C., Sarkar, P., Issa, R., & Haldar, J. (2019). Alternatives to conventional antibiotics in the era of antimicrobial resistance. *Trends in Microbiology, 27*(4), 323–338.

Gibson, G. R., Hutkins, R., Sanders, M. E., Prescott, S. L., Reimer, R. A., Salminen, S. J., Scott, K., Stanton, C., Swanson, K. S., Cani, P. D., & Verbeke, K. (2017). Expert consensus document: The International Scientific Association for Probiotics and Prebiotics (ISAPP) consensus statement on the definition and scope of prebiotics. *Nature reviews Gastroenterology & hepatology, 14*(8), 491.

Giri, S. S., Sukumaran, V., & Oviya, M. (2013). Potential probiotic Lactobacillus plantarum VSG3 improves the growth, immunity, and disease resistance of tropical freshwater fish, Labeo rohita. *Fish & Shellfish Immunology, 34*(2), 660–666.

González-Ortiz, G., Pérez, J. F., Hermes, R. G., Molist, F., Jiménez-Díaz, R., & Martín-Orúe, S. M. (2014). Screening the ability of natural feed ingredients to interfere with the adherence of enterotoxigenic *Escherichia coli* (ETEC) K88 to the porcine intestinal mucus. *British Journal of Nutrition, 111*(4), 633–642.

Greuter, T., Michel, M. C., Thomann, D., Weigmann, H., & Vavricka, S. R. (2020). Randomized, placebo-controlled, double-blind and open-label studies in the treatment and prevention of acute diarrhea with Enterococcus faecium SF68. *Frontiers in Medicine, 7*, 276.

Grispoldi, L., Bertero, F., Franceschini, S., Mastrosimone, F., Sechi, P., Iulietto, M. F., Ceccarelli, M., & Cenci-Goga, B. T. (2017). Prevalence and characterisation of shigatoxigenic *Escherichia coli* isolated from beef cattle fed with prebiotics. *Italian journal of food safety, 6*(4), 6958.

Gueimonde, M., Sanchez, B., de Los Reyes-Gavilán, C. G., & Margolles, A. (2013). Antibiotic resistance in probiotic bacteria. *Frontiers in Microbiology, 4*, 202. https://doi.org/10.3389/fmicb.2013.00202

Guerra-Ordaz, A. A., González-Ortiz, G., La Ragione, R. M., Woodward, M. J., Collins, J. W., Pérez, J. F., & Martín-Orúe, S. M. (2014). Lactulose and *Lactobacillus plantarum*, a potential complementary synbiotic to control postweaning colibacillosis in piglets. *Applied and Environmental Microbiology, 80*, 4879–4886.

Guo, J. J., Liu, K. F., Cheng, S. H., Chang, C. I., Lay, J. J., Hsu, Y. O., Yang, J. Y., & Chen, T. I. (2009). Selection of probiotic bacteria for use in shrimp larviculture. *Aquaculture Research, 40*(5), 609–618.

Gupta, S., Bhathena, Z. P., Kumar, S., Nuzaiba, P. M., Srivastava, P. P., Gupta, S., & Jadhao, S. B. (2020). Comparative efficacy of mannan-oligosaccharides from two yeast species fed alone or in

combination with probiotic *Bacillus subtilis* ATCC 6633 to Catla (*Catla catla*) juveniles. *Aquaculture International, 28*(2), 691–710.

Gupta, S., Zhu, J., McCarty, T. R., Pruce, J., Kassam, Z., Kelly, C., Fischer, M., & Allegretti, J. R. (2021). Cost-effectiveness analysis of sequential fecal microbiota transplantation for fulminant *Clostridioides difficile* infection. *Journal of Gastroenterology and Hepatology*. https://doi.org/10.1111/jgh.15483

Guslandi, M., Mezzi, G., Sorghi, M., & Testoni, P. A. (2000). Saccharomyces boulardii in maintenance treatment of Crohn's disease. *Digestive Diseases and Sciences, 45*(7), 1462–1464.

Han, D., Walsh, M. C., Cejas, P. J., Dang, N. N., Kim, Y. F., Kim, J., Charrier-Hisamuddin, L., Chau, L., Zhang, Q., Bittinger, K., & Bushman, F. D. (2013). Dendritic cell expression of the signaling molecule TRAF6 is critical for gut microbiota-dependent immune tolerance. *Immunity, 38*(6), 1211–1222.

Hansen, J. Ø., Lagos, L., Lei, P., Reveco-Urzua, F. E., Morales-Lange, B., Hansen, L. D., Schiavone, M., Mydland, L. T., Arntzen, M. Ø., Mercado, L., & Benicio, R. T. (2021). Down-stream processing of baker's yeast (*Saccharomyces cerevisiae*)–Effect on nutrient digestibility and immune response in Atlantic salmon (*Salmo salar*). *Aquaculture, 530*, 735707.

He, T., Zhu, Y. H., Yu, J., Xia, B., Liu, X., Yang, G. Y., et al. (2019). *Lactobacillus johnsonii* L531 reduces pathogen load and helps maintain short-chain fatty acid levels in the intestines of pigs challenged with Salmonella enterica Infantis. *Veterinary Microbiology, 230*, 187–194. https://doi.org/10.1016/j.vetmic.2019.02.003

Hendriksen, R. S., Munk, P., Njage, P., Van Bunnik, B., McNally, L., Lukjancenko, O., Röder, T., Nieuwenhuijse, D., Pedersen, S. K., Kjeldgaard, J., & Kaas, R. S. (2019). Global monitoring of antimicrobial resistance based on metagenomics analyses of urban sewage. *Nature Communications, 10*(1), 1–12.

Homayouni-Rad, A., Azizi, A., Oroojzadeh, P., & Pourjafar, H. (2020). Kluyveromyces marxianus as a probiotic yeast: A mini-review. *Current Nutrition & Food Science, 16*(8), 1163–1169.

Hoseinifar, S. H., Sohrabi, A., Paknejad, H., Jafari, V., Paolucci, M., & Van Doan, H. (2019). Enrichment of common carp (*Cyprinus carpio*) fingerlings diet with Psidium guajava: The effects on cutaneous mucosal and serum immune parameters and immune related genes expression. *Fish & Shellfish Immunology, 86*, 688–694.

Hou, C., Zeng, X., Yang, F., Liu, H., & Qiao, S. (2015). Study and use of the probiotic Lactobacillus reuteri in pigs: A review. *Journal of Animal Science and Biotechnology, 6*(1), 1–8.

Imperial, I. C., & Ibana, J. A. (2016). Addressing the antibiotic resistance problem with probiotics: Reducing the risk of its double-edged sword effect. *Frontiers in Microbiology, 7*, 1983. https://doi.org/10.3389/fmicb.2016.01983

Inatomi, T., Amatatsu, M., Romero-Pérez, G. A., Inoue, R., & Tsukahara, T. (2017). Dietary probiotic compound improves reproductive performance of porcine epidemic diarrhea virus-infected sows reared in a Japanese commercial swine farm under vaccine control condition. *Frontiers in Immunology, 8*, 1877.

Kareem, K. Y., Ling, F. H., Chwen, L. T., Foong, O. M., & Asmara, S. A. (2014). Inhibitory activity of postbiotic produced by strains of *Lactobacillus plantarum* using reconstituted media supplemented with inulin. *Gut pathogens, 6*(1), 1–7.

Karunasena, E., McMahon, K. W., Chang, D., & Brashears, M. M. (2014). Host responses to the pathogen Mycobacterium avium subsp. paratuberculosis and beneficial microbes exhibit host sex specificity. *Applied and Environmental Microbiology, 80*(15), 4481–4490.

Keerqin, C., Morgan, N. K., Wu, S. B., Swick, R. A., & Choct, M. (2017). Dietary inclusion of arabinoxylo-oligosaccharides in response to broilers challenged with subclinical necrotic enteritis. *British Poultry Science, 58*(4), 418–424.

Khademzade, O., Zakeri, M., Haghi, M., & Mousavi, S. M. (2020). The effects of water additive Bacillus cereus and Pediococcus acidilactici on water quality, growth performances, economic benefits, immunohematology and bacterial flora of whiteleg shrimp (Penaeus vannamei Boone, 1931) reared in earthen ponds. *Aquaculture Research, 51*(5), 1759–1770.

Kim, G. B., Seo, Y. M., Kim, C. H., & Paik, I. K. (2011). Effect of dietary prebiotic supplementation on the performance, intestinal microflora, and immune response of broilers. *Poultry Science, 90*(1), 75–82.

Kim, Y. G., Sakamoto, K., Seo, S. U., Pickard, J. M., Gillilland, M. G., Pudlo, N. A., Hoostal, M., Li, X., Wang, T. D., Feehley, T., & Stefka, A. T. (2017). Neonatal acquisition of Clostridia species protects against colonization by bacterial pathogens. *Science, 356*(6335), 315–319.

Kim, S., Jang, M. J., Kim, S. Y., Yang, Y., Pavlidis, H. O., & Ricke, S. C. (2019). Potential for prebiotics as feed additives to limit foodborne Campylobacter establishment in the poultry gastrointestinal tract. *Frontiers in Microbiology, 10*, 91.

Klein, E. Y., Van Boeckel, T. P., Martinez, E. M., Pant, S., Gandra, S., Levin, S. A., Goossens, H., & Laxminarayan, R. (2018). Global increase and geographic convergence in antibiotic consumption between 2000 and 2015. *Proceedings of the National Academy of Sciences, 115*(15), E3463–E3470.

Kong, Y., Gao, C., Du, X., Zhao, J., Li, M., Shan, X., & Wang, G. (2020). Effects of single or conjoint administration of lactic acid bacteria as potential probiotics on growth, immune response and disease resistance of snakehead fish (*Channa argus*). *Fish and shellfish immunology, 102*, 412–421.

Krishnaveni, G., Vignesh, S., Vidhyalakshmi, N., Vijay, V., & Ramesh, U. (2021). Effects of dietary supplementation of Lactobacillus fermentum URLP18 on growth, innate immunity and survival against Aeromonas hydrophila ATCC 7966 challenge in freshwater fish Cyprinus carpio (common carp). *Aquaculture Research, 52*(3), 1160–1176.

Kumar, A., Patyal, A., & Panda, A. K. (2018). Sub-therapeutic use of antibiotics in animal feed and their potential impact on environmental and human health: A comprehensive review. *Journal of Animal Feed Science and Technology, 6*(15), 25.

La Ragione, R. M., & Woodward, M. J. (2003). Competitive exclusion by Bacillus subtilis spores of Salmonella enterica serotype Enteritidis and Clostridium perfringens in young chickens. *Veterinary Microbiology, 94*(3), 245–256.

La Ragione, R. M., Casula, G., Cutting, S. M., & Woodward, M. J. (2001). Bacillus subtilis spores competitively exclude Escherichia coli O78: K80 in poultry. *Veterinary Microbiology, 79*(2), 133–142.

Lafontaine, G. M. F., Fish, N. M., & Connerton, I. F. (2020). In vitro evaluation of the effects of commercial prebiotic GOS and FOS products on human colonic Caco–2 cells. *Nutrients, 12*(5), 1281.

Lau, A. Y., Yanagisawa, N., Hor, Y. Y., Lew, L. C., Ong, J. S., Chuah, L. O., Lee, Y. Y., Choi, S. B., Rashid, F., Wahid, N., & Sugahara, H. (2018). *Bifidobacterium longum* BB536 alleviated upper respiratory illnesses and modulated gut microbiota profiles in Malaysian pre-school children. *Beneficial Microbes, 9*(1), 61–70.

Lazado, C. C., Caipang, C. M. A., & Estante, E. G. (2015). Prospects of host-associated microorganisms in fish and penaeids as probiotics with immunomodulatory functions. *Fish & Shellfish Immunology, 45*(1), 2–12.

Le, B., Ngoc, A. P. T., & Yang, S. H. (2020). Synbiotic fermented soymilk with Weissella cibaria FB069 and xylooligosaccharides prevents proliferation in human colon cancer cells. *Journal of Applied Microbiology, 128*(5), 1486–1496.

LeBlanc, J. G., de Moreno de LeBlanc, A., de Souza Oliveira, R. P., & Todorov, S. D. (2014). Use of Synbiotics (probiotics and prebiotics) to improve the safety of foods. In *Practical food safety: Contemporary issues and future directions*; Bhat, R., Gomez-Lopez, V.M., Eds.; (pp. 497–531) Wiley: Chichester, UK.

Lee, B. J., & Bak, Y. T. (2011). Irritable bowel syndrome, gut microbiota and probiotics. *Journal of Neurogastroenterology and Motility, 17*(3), 252.

Lee, S. J., Shim, Y. H., Cho, S. J., & Lee, J. W. (2007). Probiotics prophylaxis in children with persistent primary vesicoureteral reflux. *Pediatric Nephrology, 22*(9), 1315–1320.

Lee, D. Y., Seo, Y. S., Rayamajhi, N., Kang, M. L., Lee, S. I., & Yoo, H. S. (2009). Isolation, characterization, and evaluation of wild isolates of *Lactobacillus reuteri* from pig feces. *Journal of Microbiology, 47*(6), 663–672.

Lester, C. H., Frimodt-Møller, N., Sørensen, T. L., Monnet, D. L., & Hammerum, A. M. (2006). In vivo transfer of the vanA resistance gene from an Enterococcus faecium isolate of animal origin to an E. faecium isolate of human origin in the intestines of human volunteers. *Antimicrobial Agents and Chemotherapy, 50*(2), 596–599.

Li, X. J., Piao, X. S., Kim, S. W., Liu, P., Wang, L., Shen, Y. B., Jung, S. C., & Lee, H. S. (2007). Effects of chito-oligosaccharide supplementation on performance, nutrient digestibility, and serum composition in broiler chickens. *Poultry Science, 86*(6), 1107–1114.

Li, X., Qiang, L., & Xu, C. (2008). Effects of supplementation of fructooligosaccharide and/or Bacillus subtilis to diets on performance and on intestinal microflora in broilers. *Archives Animal Breeding, 51*(1), 64–70.

Li, J., Tan, B., & Mai, K. (2009). Dietary probiotic Bacillus OJ and isomaltooligosaccharides influence the intestine microbial populations, immune responses and resistance to white spot syndrome virus in shrimp (*Litopenaeus vannamei*). *Aquaculture, 291*(1–2), 35–40.

Li, L., Katani, R., Schilling, M., & Kapur, V. (2016). Molecular epidemiology of Mycobacterium avium subsp. paratuberculosis on dairy farms. *Annual Review of Animal Biosciences, 4*, 155–176.

Li, J., Zhang, X., Cao, L., Ji, J., & Gao, J. (2018). Three inulin-type fructans from *Codonopsis pilosula* (Franch.) Nannf. roots and their prebiotic activity on *Bifidobacterium longum*. *Molecules, 23*(12), 3123.

Lima, S. F., Teixeira, A. G., Lima, F. S., Ganda, E. K., Higgins, C. H., Oikonomou, G., & Bicalho, R. C. (2017). The bovine colostrum microbiome and its association with clinical mastitis. *Journal of Dairy Science, 100*(4), 3031–3042.

Lin, W. C., Ptak, C. P., Chang, C. Y., Ian, M. K., Chia, M. Y., Chen, T. H., & Kuo, C. J. (2020). Autochthonous lactic acid bacteria isolated from dairy cow feces exhibiting promising probiotic properties and in vitro antibacterial activity against foodborne pathogens in cattle. *Frontiers in Veterinary Science, 7*, 239.

Liu, J. (2020). Tackling the global non-prescription use of antibiotics. *The Lancet Infectious Diseases, 20*(2), 169–170.

Liu, W., Ren, P., He, S., Xu, L., Yang, Y., Gu, Z., & Zhou, Z. (2013). Comparison of adhesive gut bacteria composition, immunity, and disease resistance in juvenile hybrid tilapia fed two different Lactobacillus strains. *Fish and Shellfish Immunology, 35*(1), 54–62.

Liu, F., Li, P., Chen, M., Luo, Y., Prabhakar, M., Zheng, H., He, Y., Qi, Q., Long, H., Zhang, Y., & Sheng, H. (2017). Fructooligosaccharide (FOS) and galactooligosaccharide (GOS) increase Bifidobacterium but reduce butyrate producing bacteria with adverse glycemic metabolism in healthy young population. *Scientific Reports, 7*(1), 1–12.

Liu, A., Wang, Y., Song, Y., & Du, Y. (2020a). Treatment with compound Lactobacillus acidophilus followed by a tetracycline-and furazolidone-containing quadruple regimen as a rescue therapy for Helicobacter pylori infection. *Saudi Journal of Gastroenterology: Official Journal of the Saudi Gastroenterology Association, 26*(2), 78.

Liu, W. C., Zhou, S. H., Balasubramanian, B., Zeng, F. Y., Sun, C. B., & Pang, H. Y. (2020b). Dietary seaweed (Enteromorpha) polysaccharides improves growth performance involved in regulation of immune responses, intestinal morphology and microbial community in banana shrimp Fenneropenaeus merguiensis. *Fish and Shellfish Immunology, 104*, 202–212.

Lokhande, A., Ingale, S. L., Lee, S. H., Kim, J. S., Lohakare, J. D., Chae, B. J., & Kwon, I. K. (2013). The effects of Rhodobacter capsulatus KCTC-2583 on cholesterol metabolism, egg production and quality parameters during the late laying periods in hens. *Asian-Australasian Journal of Animal Sciences, 26*(6), 831–837.

Lucey, P. M., Lean, I. J., Aly, S. S., Golder, H. M., Block, E., Thompson, J. S., & Rossow, H. A. (2021). Effects of mannan-oligosaccharide and Bacillus subtilis supplementation to preweaning Holstein dairy heifers on body weight gain, diarrhea, and shedding of fecal pathogens. *Journal of Dairy Science, 104*(4), 4290–4302.

Lulijwa, R., Rupia, E. J., & Alfaro, A. C. (2020). Antibiotic use in aquaculture, policies and regulation, health and environmental risks: A review of the top 15 major producers. *Reviews in Aquaculture, 12*(2), 640–663.

Luo, J., Zheng, A., Meng, K., Chang, W., Bai, Y., & Li, K. (2013). Proteome changes in the intestinal mucosa of broiler (Gallus gallus) activated by probiotic Enterococcus faecium. *J Proteome, 91*, 226–241.

Ma, C., Sun, Z., Zeng, B., Huang, S., Zhao, J., Zhang, Y., Su, X., Xu, J., Wei, H., & Zhang, H. (2018). Cow-to-mouse fecal transplantations suggest intestinal microbiome as one cause of mastitis. *Microbiome, 6*(1), 1–17.

Makled, S. O., Hamdan, A. M., & El-Sayed, A. F. M. (2020). Growth promotion and immune stimulation in nile tilapia, *Oreochromis niloticus*, fingerlings following dietary administration of a novel marine probiotic, *Psychrobacter maritimus* S. *Probiotics and Antimicrobial Proteins, 12*(2), 365–374.

Maldonado, N. C., Chiaraviglio, J., Bru, E., De Chazal, L., Santos, V., & Nader-Macías, M. E. F. (2018). Effect of milk fermented with lactic acid bacteria on diarrheal incidence, growth performance and microbiological and blood profiles of newborn dairy calves. *Probiotics and Antimicrobial Proteins, 10*(4), 668–676.

Mao, J., Zhang, S. Z., Du, P., Cheng, Z. B., Hu, H., & Wang, S. Y. (2020). Probiotics can boost the antitumor immunity of CD8+ T cells in BALB/c mice and patients with colorectal carcinoma. *Journal of Immunology Research, 2020*, 4092472.

Maragkoudakis, P. A., Mountzouris, K. C., Psyrras, D., Cremonese, S., Fischer, J., Cantor, M. D., & Tsakalidou, E. (2009). Functional properties of novel protective lactic acid bacteria and application in raw chicken meat against *Listeria monocytogenes* and *Salmonella enteritidis*. *International Journal of Food Microbiology, 130*(3), 219–226.

Marsella, R. (2009). Evaluation of *Lactobacillus rhamnosus* strain GG for the prevention of atopic dermatitis in dogs. *American Journal of Veterinary Research, 70*(6), 735–740.

Marshall, B. M., & Levy, S. B. (2011). Food animals and antimicrobials: Impacts on human health. *Clinical Microbiology Reviews, 24*(4), 718–733.

Mater, D. D. G., Langella, P., Corthier, G., & Flores, M. J. (2008). A probiotic Lactobacillus strain can acquire vancomycin resistance during digestive transit in mice. *Journal of Molecular Microbiology and Biotechnology, 14*, 123–127. https://doi.org/10.1159/000106091

McFarland L. V. (2007) Meta-analysis of probiotics for the prevention of traveler's diarrhea. *Travel Medicine and Infectious Disease, 5*, 97–105.

Mohammed, A. A., Jacobs, J. A., Murugesan, G. R., & Cheng, H. W. (2018). Effect of dietary synbiotic supplement on behavioral patterns and growth performance of broiler chickens reared under heat stress. *Poultry Science, 97*(4), 1101–1108.

Mohapatra, S., Chakraborty, T., Prusty, A., Das, P., Paniprasad, K., & Mohanta, K. (2012). Use of different microbial probiotics in the diet of rohu, *Labeo rohita* fingerlings: Effects on growth, nutrient digestibility and retention, digestive enzyme activities and intestinal microflora. *Aquaculture Nutrition, 18*(1), 1–11.

Moser, A. M., Spindelboeck, W., Halwachs, B., Strohmaier, H., Kump, P., Gorkiewicz, G., & Högenauer, C. (2019). Effects of an oral synbiotic on the gastrointestinal immune system and microbiota in patients with diarrhea-predominant irritable bowel syndrome. *European Journal of Nutrition, 58*(7), 2767–2778.

Mousavi, S., Sheikhzadeh, N., Hamidian, G., Mardani, K., Oushani, A. K., Firouzamandi, M., Esteban, M. Á., & Shohreh, P. (2021). Changes in rainbow trout (Oncorhynchus mykiss) growth and mucosal immune parameters after dietary administration of grape (*Vitis vinifera*) seed extract. *Fish Physiology and Biochemistry, 47*(2), 547–563.

Munoz-Atienzal, E., Gomez-Sala, B., Araujo, C., Campanerol, C., Del Campo, R., Hernandez, P. E., Herranz, C., & Cintas, L. M. (2013). Antibiotic susceptibility and virulence factors of lactic acid bacteria of aquatic origin intended for use as probiotics in aquaculture. *BMC Microbiology, 13*(1), 15.

Nair, D. V., & Kollanoor Johny, A. (2017). Food grade pimenta leaf essential oil reduces the attachment of Salmonella enterica Heidelberg (2011 ground Turkey outbreak isolate) on to Turkey skin. *Frontiers in Microbiology, 8*, 2328.

Nakphaichit, M., Sobanbua, S., Siemuang, S., Vongsangnak, W., Nakayama, J., & Nitisinprasert, S. (2019). Protective effect of Lactobacillus reuteri KUB-AC5 against *Salmonella* Enteritidis challenge in chickens. *Beneficial Microbes, 10*(1), 43–54.

Nemcová, R., Bomba, A., Gancarčíková, S., Herich, R., & Guba, P., (1999). Study of the effect of Lactobacillus paracasei and fructooligosaccharides on the faecal microflora in weanling piglets. *Berliner und Munchener Tierarztliche Wochenschrift, 112*, 225–228.

Nothaft, H., Perez-Muñoz, M. E., Gouveia, G. J., Duar, R. M., Wanford, J. J., Lango-Scholey, L., Panagos, C. G., Srithayakumar, V., Plastow, G. S., Coros, C., & Bayliss, C. D. (2017). Coadministration of the Campylobacter jejuni N-glycan-based vaccine with probiotics improves vaccine performance in broiler chickens. *Applied and Environmental Microbiology, 83*(23), e01523-17.

Ogué-Bon, E., Khoo, C., McCartney, A. L., Gibson, G. R., & Rastall, R. A. (2010). *In vitro* effects of synbiotic fermentation on the canine faecal microbiota. *FEMS Microbiology Ecology, 73*(3), 587–600.

Oh, J. K., Pajarillo, E. A. B., Chae, J. P., Kim, I. H., & Kang, D. K. (2017). Protective effects of Bacillus subtilis against Salmonella infection in the microbiome of Hy-Line Brown layers. *Asian-Australasian Journal of Animal Sciences, 30*(9), 1332.

Oyofo, B. A., DeLoach, J. R., Corrier, D. E., Norman, J. O., Ziprin, R. L., & Mollenhauer, H. H. (1989). Prevention of Salmonella typhimurium colonization of broilers with D-mannose. *Poultry Science, 68*(10), 1357–1360.

Pan, L., Zhao, P. F., Ma, X. K., Shang, Q. H., Xu, Y. T., Long, S. F., Wu, Y., Yuan, F. M., & Piao, X. S. (2017). Probiotic supplementation protects weaned pigs against enterotoxigenic Escherichia coli K88 challenge and improves performance similar to antibiotics. *Journal of Animal Science, 95*(6), 2627–2639.

Pandey, K. R., Naik, S. R., & Vakil, B. V. (2015). Probiotics, prebiotics and synbiotics-a review. *Journal of Food Science and Technology, 52*(12), 7577–7587.

Park, J. W., Jeong, J. S., Lee, S. I., & Kim, I. H. (2016). Effect of dietary supplementation with a probiotic (*Enterococcus faecium*) on production performance, excreta microflora, ammonia emission, and nutrient utilization in ISA brown laying hens. *Poultry Science, 95*, 2829–2835. https://doi.org/10.3382/ps/pew241

Park, Y., Kim, H., Won, S., Hamidoghli, A., Hasan, M. T., Kong, I. S., & Bai, S. C. (2020). Effects of two dietary probiotics (*Bacillus subtilis* or *licheniformis*) with two prebiotics (mannan or fructo oligosaccharide) in Japanese eel, *Anguilla japonica*. *Aquaculture Nutrition, 26*(2), 316–327.

Peralta-Sánchez, J. M., Martín-Platero, A. M., Ariza-Romero, J. J., Rabelo-Ruiz, M., Zurita-González, M. J., Baños, A., Rodríguez-Ruano, S. M., Maqueda, M., Valdivia, E., & Martínez-Bueno, M. (2019). Egg production in poultry farming is improved by probiotic bacteria. *Frontiers in Microbiology, 10*, 1042.

Peter, S., Gärtner, M. A., Michel, G., Ibrahim, M., Klopfleisch, R., Lübke-Becker, A., Jung, M., Einspanier, R., & Gabler, C. (2018). Influence of intrauterine administration of Lactobacillus buchneri on reproductive performance and pro-inflammatory endometrial mRNA expression of cows with subclinical endometritis. *Scientific Reports, 8*(1), 1–13.

Pradhan, D., Mallappa, R. H., & Grover, S. (2020). Comprehensive approaches for assessing the safety of probiotic bacteria. *Food Control, 108*, 106872.

Putra, A. N., bayu Syamsunarno, M., Ningrum, W., Jumyanah, J., & Mustahal, M. (2020). Effect of the administration of probiotic Bacillus NP5 in the rearing media on water quality, growth, and disease resistance of African catfish (*Clarias gariepinus*). *Biodiversitas Journal of Biological Diversity, 21*(6), 2566–2575. https://doi.org/10.13057/biodiv/d210629

Quereda, J. J., García-Roselló, E., Barba, M., Mocé, M. L., Gomis, J., Jiménez-Trigos, E., Bataller, E., Martínez-Boví, R., García-Muñoz, Á., & Gómez-Martín, Á. (2020). Use of probiotics in intravaginal sponges in sheep: A pilot study. *Animals, 10*(4), 719.

Quigley III, J. D., Kost, C. J., & Wolfe, T. A. (2002). Effects of spray-dried animal plasma in milk replacers or additives containing serum and oligosaccharides on growth and health of calves. *Journal of Dairy Science, 85*(2), 413–421.

Quintero-Villegas, M. I., Aam, B. B., Rupnow, J., Sørlie, M., Eijsink, V. G., & Hutkins, R. W. (2013). Adherence inhibition of enteropathogenic Escherichia coli by chitooligosaccharides

with specific degrees of acetylation and polymerization. *Journal of Agricultural and Food Chemistry, 61*(11), 2748–2754.

Rai, N., Singh, A. K., Keshri, P. K., Barik, S., Kamble, S. C., Singh, S. K., Kumar, R., Mishra, P., Kotiya, D., & Gautam, V. (2021). Probiotics for management of gastrointestinal cancers. In *Probiotic research in therapeutics* (pp. 191–209). Springer.

Rainard, P., & Foucras, G. (2018). A critical appraisal of probiotics for mastitis control. *Frontiers in Veterinary Science, 5*, 251.

Rao, C., Dhawan, B., Vishnubhatla, S., Kapil, A., Das, B., & Sood, S. (2021). Clinical and molecular epidemiology of vancomycin-resistant Enterococcus faecium bacteremia from an Indian tertiary hospital. *European Journal of Clinical Microbiology and Infectious Diseases, 40*(2), 303–314.

Raveschot, C., Coutte, F., Frémont, M., Vaeremans, M., Dugersuren, J., Demberel, S., Drider, D., Dhulster, P., Flahaut, C., & Cudennec, B. (2020). Probiotic Lactobacillus strains from Mongolia improve calcium transport and uptake by intestinal cells in vitro. *Food Research International, 133*, 109201.

Rentas, M. F., Pedreira, R. S., Perini, M. P., Risolia, L. W., Zafalon, R. V. A., Alvarenga, I. C., Vendramini, T. H. A., Balieiro, J. C. C., Pontieri, C. F. F., & Brunetto, M. A. (2020). Galactoligosaccharide and a prebiotic blend improve colonic health and immunity of adult dogs. *PLoS One, 15*(8), e0238006.

Ribeiro, V., Jr., Albino, L. F. T., Rostagno, H. S., Barreto, S. L. T., Hannas, M. I., Harrington, D., De Araujo, F. A., Ferreira, H. C., Jr., & Ferreira, M. A. (2014). Effects of the dietary supplementation of Bacillus subtilis levels on performance, egg quality and excreta moisture of layers. *Animal Feed Science and Technology, 195*, 142–146.

Richards, P. J., Lafontaine, G. M. F., Connerton, P. L., Liang, L., Asiani, K., Fish, N. M., & Connerton, I. F. (2020). Galacto-oligosaccharides modulate the juvenile gut microbiome and innate immunity to improve broiler chicken performance. *Msystems, 5*(1), e00827-19.

Ricke, S. C. (2015). Potential of fructooligosaccharide prebiotics in alternative and nonconventional poultry production systems. *Poultry Science, 94*(6), 1411–1418.

Ricke, S. C. (2018). Focus: Nutrition and food science: Impact of prebiotics on poultry production and food safety. *The Yale Journal of Biology and Medicine, 91*(2), 151.

Rimoldi, S., Torrecillas, S., Montero, D., Gini, E., Makol, A., Valdenegro, V. V., Izquierdo, M., & Terova, G. (2020). Assessment of dietary supplementation with galactomannan oligosaccharides and phytogenics on gut microbiota of European sea bass (*Dicentrarchus labrax*) fed low fishmeal and fish oil based diet. *PLoS One, 15*(4), e0231494.

Rodriguez-Estrada, U., Satoh, S., Haga, Y., Fushimi, H., & Sweetman, J. (2009). Effects of single and combined supplementation of Enterococcus faecalis, mannan oligosaccharide and polyhydroxybutyrate acid on growth performance and immune response of rainbow trout *Oncorhynchus mykiss*. *Aquaculture Science, 57*(4), 609–617.

Rondón, A., del Valle, A., Milián, G., Arteaga, F., Rodríguez, M., Valdivia, A., & Martínez, M. (2019). Production of a synbiotic biopreparation (mixture of Agave fourcroydes Lem. Pulp and PROBIOLACTIL®) for use in calves. *Ağrı, 25*(2), 1–8.

Rose, L., Rose, J., Gosling, S., & Holmes, M. (2017). Efficacy of a probiotic-prebiotic supplement on incidence of diarrhea in a dog shelter: A randomized, double-blind, placebo-controlled trial. *Journal of Veterinary Internal Medicine, 31*(2), 377–382.

Rossi, G., Pengo, G., Caldin, M., Piccionello, A. P., Steiner, J. M., Cohen, N. D., Jergens, A. E., & Suchodolski, J. S. (2014). Comparison of microbiological, histological, and immunomodulatory parameters in response to treatment with either combination therapy with prednisone and metronidazole or probiotic VSL# 3 strains in dogs with idiopathic inflammatory bowel disease. *PLoS One, 9*(4), e94699.

Saber, A., Alipour, B., Faghfoori, Z., & Khosroushahi, A. Y. (2017). Secretion metabolites of dairy Kluyveromyces marxianus AS41 isolated as probiotic, induces apoptosis in different human cancer cell lines and exhibit anti-pathogenic effects. *Journal of Functional Foods, 34*, 408–421.

Sadeghi-Bojd, S., Naghshizadian, R., Mazaheri, M., Ghane Sharbaf, F., & Assadi, F. (2020). Efficacy of probiotic prophylaxis after the first febrile urinary tract infection in children with normal urinary tracts. *Journal of the Pediatric Infectious Diseases Society, 9*(3), 305–310.

Saint-Cyr, M. J., Guyard-Nicodème, M., Messaoudi, S., Chemaly, M., Cappelier, J.-M., Dousset, X., & Haddad, N. (2016). Recent advances in screening of anti-Campylobacter activity in probiotics for use in poultry. *Frontiers in Microbiology, 7*, 553.

Saint-Cyr, M. J., Haddad, N., Taminiau, B., Poezevara, T., Quesne, S., Amelot, M., Daube, G., Chemaly, M., Dousset, X., & Guyard-Nicodème, M. (2017). Use of the potential probiotic strain lactobacillus salivarius SMXD51 to control Campylobacter jejuni in broilers. *International Journal of Food Microbiology, 247*, 9–17.

Sakaridis, I., Ellis, R. J., Cawthraw, S. A., Van Vliet, A. H., Stekel, D. J., Penell, J., Chambers, M., La Ragione, R. M., & Cook, A. J. (2018). Investigating the association between the caecal microbiomes of broilers and *Campylobacter* burden. *Frontiers in Microbiology, 9*, 927.

Sanders, M. E., Akkermans, L. M., Haller, D., Hammerman, C., Heimbach, J. T., Hörmannsperger, G., & Huys, G. (2010). Safety assessment of probiotics for human use. *Gut Microbes, 1*(3), 164–185.

Santos, L., & Ramos, F. (2018). Antimicrobial resistance in aquaculture: Current knowledge and alternatives to tackle the problem. *International Journal of Antimicrobial Agents, 52*(2), 135–143.

Santos, J. P. F., Aquino, A. A., Glória, M. B. A., Avila-Campos, M. J., Oba, P. M., Santos, K. D. M., Vendramini, T. H. A., Carciofi, A. C., Junior, A. R., & Brunetto, M. A. (2018). Effects of dietary yeast cell wall on faecal bacteria and fermentation products in adult cats. *Journal of Animal Physiology and Animal Nutrition, 102*(4), 1091–1101.

Sauter, S. N., Benyacoub, J., Allenspach, K., Gaschen, F., Ontsouka, E., Reuteler, G., Cavadini, C., Knorr, R., & Blum, J. W. (2006). Effects of probiotic bacteria in dogs with food responsive diarrhoea treated with an elimination diet. *Journal of Animal Physiology and Animal Nutrition, 90*(7–8), 269–277.

Seong, H., Bae, J. H., Seo, J. S., Kim, S. A., Kim, T. J., & Han, N. S. (2019). Comparative analysis of prebiotic effects of seaweed polysaccharides laminaran, porphyran, and ulvan using in vitro human fecal fermentation. *Journal of Functional Foods, 57*, 408–416.

Shan, L. S., Hou, P., Wang, Z. J., Liu, F. R., Chen, N., Shu, L. H., Zhang, H., Han, X. H., Han, X. X., Cai, X. X., & Shang, Y. X. (2013). Prevention and treatment of diarrhoea with Saccharomyces boulardii in children with acute lower respiratory tract infections. *Beneficial Microbes, 4*(4), 329–334.

Shang, H. M., Hu, T. M., Lu, Y. J., & Wu, H. X. (2010). Effects of inulin on performance, egg quality, gut microflora and serum and yolk cholesterol in laying hens. *British Poultry Science, 51*(6), 791–796.

Shim, S. B., Verstegen, M. W. A., Kim, I. H., Kwon, O. S., & Verdonk, J. M. A. J. (2005). Effects of feeding antibiotic-free creep feed supplemented with oligofructose, probiotics or synbiotics to suckling piglets increases the preweaning weight gain and composition of intestinal microbiota. *Archives of Animal Nutrition, 59*(6), 419–427.

Shoaf, K., Mulvey, G. L., Armstrong, G. D., & Hutkins, R. W. (2006). Prebiotic galactooligosaccharides reduce adherence of enteropathogenic *Escherichia coli* to tissue culture cells. *Infection and Immunity, 74*(12), 6920–6928.

Sivananthan, K., & Petersen, A. M. (2018). Review of Saccharomyces boulardii as a treatment option in IBD. *Immunopharmacology and Immunotoxicology, 40*(6), 465–475.

Slawinska, A., Dunislawska, A., Plowiec, A., Radomska, M., Lachmanska, J., Siwek, M., Tavaniello, S., & Maiorano, G. (2019). Modulation of microbial communities and mucosal gene expression in chicken intestines after galactooligosaccharides delivery In Ovo. *PLoS One, 14*(2), e0212318.

Śliżewska, K., Markowiak-Kopeć, P., Żbikowski, A., & Szeleszczuk, P. (2020). The effect of synbiotic preparations on the intestinal microbiota and her metabolism in broiler chickens. *Scientific Reports, 10*(1), 1–13.

Smiricky-Tjardes, M. R., Grieshop, C. M., Flickinger, E. A., Bauer, L. L., & Fahey, G. C., Jr. (2003). Dietary galactooligosaccharides affect ileal and total-tract nutrient digestibility, ileal and fecal bacterial concentrations, and ileal fermentative characteristics of growing pigs. *Journal of Animal Science, 81*(10), 2535–2545.

Smith, P. (2008). Antimicrobial resistance in aquaculture. *Revue scientifique et technique (International Office of Epizootics), 27*(1), 243–264.

Smith, T. C., Gebreyes, W. A., Abley, M. J., Harper, A. L., Forshey, B. M., Male, M. J., Martin, H. W., Molla, B. Z., Sreevatsan, S., Thakur, S., & Thiruvengadam, M. (2013). Methicillin-resistant Staphylococcus aureus in pigs and farm workers on conventional and antibiotic-free swine farms in the USA. *PLoS One, 8*(5), e63704.

Sohail, M. U., Hume, M. E., Byrd, J. A., Nisbet, D. J., Shabbir, M. Z., Ijaz, A., & Rehman, H. (2015). Molecular analysis of the caecal and tracheal microbiome of heat-stressed broilers supplemented with prebiotic and probiotic. *Avian Pathology, 44*(2), 67–74.

Songisepp, E., Hütt, P., Rätsep, M., Shkut, E., Kõljalg, S., Truusalu, K., Stsepetova, J., Smidt, I., Kolk, H., Zagura, M., & Mikelsaar, M. (2012). Safety of a probiotic cheese containing Lactobacillus plantarum Tensia according to a variety of health indices in different age groups. *Journal of Dairy Science, 95*(10), 5495–5509.

Stapleton, A. E., Au-Yeung, M., Hooton, T. M., Fredricks, D. N., Roberts, P. L., Czaja, C. A., Yarova-Yarovaya, Y., Fiedler, T., Cox, M., & Stamm, W. E. (2011). Randomized, placebo-controlled phase 2 trial of a Lactobacillus crispatus probiotic given intravaginally for prevention of recurrent urinary tract infection. *Clinical Infectious Diseases, 52*(10), 1212–1217.

Stokes, J. E., Price, J. M., & Whittemore, J. C. (2017). Randomized, controlled, crossover trial of prevention of clindamycin-induced gastrointestinal signs using a synbiotic in healthy research cats. *Journal of Veterinary Internal Medicine, 31*(5), 1406–1413.

Swanson, K. S., Grieshop, C. M., Flickinger, E. A., Bauer, L. L., Chow, J., Wolf, B. W., Garleb, K. A., & Fahey Jr, G. C. (2002). Fructooligosaccharides and Lactobacillus acidophilus modify gut microbial populations, total tract nutrient digestibilities and fecal protein catabolite concentrations in healthy adult dogs. *The Journal of Nutrition, 132*(12), 3721–3731.

Takahashi, M., McCartney, E., Knox, A., Francesch, M., Oka, K., Wada, K., Ideno, M., Uno, K., Kozłowski, K., Jankowski, J., & Kamiya, S. (2018). Effects of the butyric acid-producing strain Clostridium butyricum MIYAIRI 588 on broiler and piglet zootechnical performance and prevention of necrotic enteritis. *Animal Science Journal, 89*(6), 895–905.

Tayeri, V., Seidavi, A., Asadpour, L., & Phillips, C. J. (2018). A comparison of the effects of antibiotics, probiotics, synbiotics and prebiotics on the performance and carcass characteristics of broilers. *Veterinary Research Communications, 42*(3), 195–207.

Tejero-Sariñena, S., Barlow, J., Costabile, A., Gibson, G. R., & Rowland, I. (2013). Antipathogenic activity of probiotics against Salmonella Typhimurium and *Clostridium difficile* in anaerobic batch culture systems: Is it due to synergies in probiotic mixtures or the specificity of single strains? *Anaerobe, 24*, 60–65.

Téllez, G., Lauková, A., Latorre, J. D., Hernandez-Velasco, X., Hargis, B. M., & Callaway, T. (2015). Food-producing animals and their health in relation to human health. *Microbial Ecology in Health and Disease, 26*, 25876. https://doi.org/10.3402/mehd.v26.25876.

Teng, P. Y., & Kim, W. K. (2018). Roles of prebiotics in intestinal ecosystem of broilers. *Frontiers in veterinary science, 5*, 245.

Timsit, E., Workentine, M., Schryvers, A. B., Holman, D. B., van der Meer, F., & Alexander, T. W. (2016). Evolution of the nasopharyngeal microbiota of beef cattle from weaning to 40 days after arrival at a feedlot. *Veterinary Microbiology, 187*, 75–81.

Tiseo, K., Huber, L., Gilbert, M., Robinson, T. P., & Van Boeckel, T. P. (2020). Global trends in antimicrobial use in food animals from 2017 to 2030. *Antibiotics, 9*(12), 918.

USFDA. GRAS notices. 2017. http://www.accessdata.fda.gov/scripts/fdcc/?set=GRASNotices. Accessed: 10 March 2022.

Van Boeckel, T. P., Pires, J., Silvester, R., Zhao, C., Song, J., Criscuolo, N. G., Gilbert, M., Bonhoeffer, S., & Laxminarayan, R. (2019). Global trends in antimicrobial resistance in animals in low-and middle-income countries. *Science, 365*(6459), eaaw1944.

Van den Abbeele, P., Duysburgh, C., Rakebrandt, M., & Marzorati, M. (2020). Dried yeast cell walls high in beta-glucan and mannan-oligosaccharides positively affect microbial composition and activity in the canine gastrointestinal tract in vitro. *Journal of Animal Science, 98*(6), skaa173.

Van Doan, H., Hoseinifar, S. H., Faggio, C., Chitmanat, C., Mai, N. T., Jaturasitha, S., & Ringø, E. (2018). Effects of corncob derived xylooligosaccharide on innate immune response, disease resistance, and growth performance in Nile tilapia (*Oreochromis niloticus*) fingerlings. *Aquaculture, 495*, 786–793.

Varankovich, N. V., Nickerson, M. T., & Korber, D. R. (2015). Probiotic-based strategies for therapeutic and prophylactic use against multiple gastrointestinal diseases. *Frontiers in Microbiology, 6*, 685.

Vaseeharan, B. A. R. P., & Ramasamy, P. (2003). Control of pathogenic Vibrio spp. by Bacillus subtilis BT23, a possible probiotic treatment for black tiger shrimp *Penaeus monodon*. *Letters in Applied Microbiology, 36*(2), 83–87.

Vicente, J., Wolfenden, A., Torres-Rodriguez, A., Higgins, S., Tellez, G. & Hargis, B. (2007). Effect of a Lactobacillus species-based probiotic and dietary lactose prebiotic on turkey poult performance with or without Salmonella enteritidis challenge. *Journal of Applied Poultry Research, 16*(3), 361–364.

Vogt, S. L., Peña-Díaz, J., & Finlay, B. B. (2015). Chemical communication in the gut: Effects of microbiota-generated metabolites on gastrointestinal bacterial pathogens. *Anaerobe, 34*, 106–115.

Waigankar, S. S., & Patel, V. (2011). Role of probiotics in urogenital healthcare. *Journal of Mid-Life Health, 2*(1), 5–10.

Wang, X. W., Lin, X., Zhang, L., Du, Y. G., Bai, X. F., & Shu, S. G. (2005a). Effect of oligochitosan on broiler performance, small intestine structure and muscle mineral element concentration. *Journal of the Chinese Cereals and Oils Association, 20*, 83–88.

Wang, Y. B., Xu, Z. R., & Xia, M. S. (2005b). The effectiveness of commercial probiotics in northern white shrimp Penaeus vannamei ponds. *Fisheries Science, 71*(5), 1036–1041.

Wang, Y., Du, W., Lei, K., Wang, B., Wang, Y., Zhou, Y., & Li, W. (2017a). Effects of dietary Bacillus licheniformis on gut physical barrier, immunity, and reproductive hormones of laying hens. *Probiotics and Antimicrobial Proteins, 9*(3), 292–299.

Wang, H., Ni, X., Qing, X., Zeng, D., Luo, M., Liu, L., Li, G., Pan, K., & Jing, B. (2017b). Live probiotic *Lactobacillus johnsonii* BS15 promotes growth performance and lowers fat deposition by improving lipid metabolism, intestinal development, and gut microflora in broilers. *Frontiers in Microbiology, 8*, 1073.

Wang, L., Li, L., Lv, Y., Chen, Q., Feng, J., & Zhao, X. (2018). *Lactobacillus plantarum* restores intestinal permeability disrupted by salmonella infection in newly-hatched chicks. *Scientific Reports, 8*(1), 1–10.

Wang, M., Yi, M., Lu, M., Gao, F., Liu, Z., Huang, Q., Li, Q., & Zhu, D. (2020). Effects of probiotics Bacillus cereus NY5 and Alcaligenes faecalis Y311 used as water additives on the microbiota and immune enzyme activities in three mucosal tissues in Nile tilapia *Oreochromis niloticus* reared in outdoor tanks. *Aquaculture Reports, 17*, 100309.

Wang, Y., Lv, X., Li, X., Zhao, J., Zhang, K., Hao, X., Liu, K., & Liu, H. (2021). Protective effect of *Lactobacillus plantarum* P8 on growth performance, intestinal health, and microbiota in Eimeria-infected broilers. *Frontiers in Microbiology, 12*, 705758.

Waters, S. M., Murphy, R. A., & Power, R. F. (2005). Assessment of the effects of Nurmi-type cultures and a defined probiotic preparation on a salmonella typhimurium 29E challenge in vivo. *Journal of Food Protection, 68*(6), 1222–1227.

Watts, J. E., Schreier, H. J., Lanska, L., & Hale, M. S. (2017). The rising tide of antimicrobial resistance in aquaculture: Sources, sinks and solutions. *Marine Drugs, 15*(6), 158.

Wei, Y. X., Zhang, Z. Y., Liu, C., Malakar, P. K., & Guo, X. K. (2012). Safety assessment of Bifidobacterium longum JDM301 based on complete genome sequences. *World Journal of Gastroenterology: WJG, 18*(5), 479.

White, R., Atherly, T., Guard, B., Rossi, G., Wang, C., Mosher, C., Webb, C., Hill, S., Ackermann, M., Sciabarra, P., & Allenspach, K. (2017). Randomized, controlled trial evaluating the effect of multi-strain probiotic on the mucosal microbiota in canine idiopathic inflammatory bowel disease. *Gut Microbes, 8*(5), 451–466.

Wisener, L. V., Sargeant, J. M., O'connor, A. M., Faires, M. C., & Glass-Kaastra, S. K. (2015). The use of direct-fed microbials to reduce shedding of Escherichia coli O157 in beef cattle: A systematic review and meta-analysis. *Zoonoses and Public Health, 62*(2), 75–89.

Won, S., Hamidoghli, A., Choi, W., Park, Y., Jang, W. J., Kong, I. S., & Bai, S. C. (2020). Effects of *Bacillus subtilis* WB60 and *Lactococcus lactis* on growth, immune responses, histology and gene expression in Nile Tilapia, Oreochromis niloticus. *Microorganisms, 8*(1), 67.

Wu, Y., Zhen, W., Geng, Y., Wang, Z., & Guo, Y. (2019). Pretreatment with probiotic Enterococcus faecium NCIMB 11181 ameliorates necrotic enteritis-induced intestinal barrier injury in broiler chickens. *Scientific Reports, 9*(1), 1–17.

Xu, H., Zhao, F., Hou, Q., Huang, W., Liu, Y., Zhang, H., & Sun, Z. (2019). Metagenomic analysis revealed beneficial effects of probiotics in improving the composition and function of the gut microbiota in dogs with diarrhoea. *Food & Function, 10*(5), 2618–2629.

Yadav, A., Kolluri, G., Gopi, M., Karthik, K., Malik, Y., & Dhama, K. (2016). Exploring alternatives to antibiotics as health promoting agents in poultry – a review. *Journal of Experimental Biology and Agricultural Sciences, 4*(3), 68–383.

Yan, F. F., Wang, W. C., & Cheng, H. W. (2018). *Bacillus subtilis* based probiotic improved bone mass and altered brain serotoninergic and dopaminergic systems in broiler chickens. *Journal of Functional Foods, 49*, 501–509.

Yang, C. M., Cao, G. T., Ferket, P. R., Liu, T. T., Zhou, L., Zhang, L., Xiao, Y. P., & Chen, A. G. (2012). Effects of probiotic, *Clostridium butyricum*, on growth performance, immune function, and cecal microflora in broiler chickens. *Poultry Science, 91*(9), 2121–2129.

Yang, P., Yang, W., He, M., Li, X., & Leng, X. J. (2020). Dietary synbiotics improved the growth, feed utilization and intestinal structure of largemouth bass (*Micropterus salmoides*) juvenile. *Aquaculture Nutrition, 26*(2), 590–600.

Yao, W., Li, X., Zhang, C., Wang, J., Cai, Y., & Leng, X. (2021). Effects of dietary synbiotics supplementation methods on growth, intestinal health, non-specific immunity and disease resistance of Pacific white shrimp*, Litopenaeus vannamei*. *Fish & Shellfish Immunology, 112*, 46–55.

Ye, J. D., Wang, K., Li, F. D., & Sun, Y. Z. (2011). Single or combined effects of fructo-and mannan oligosaccharide supplements and *Bacillus clausii* on the growth, feed utilization, body composition, digestive enzyme activity, innate immune response and lipid metabolism of the Japanese flounder Paralichthys olivaceus. *Aquaculture Nutrition, 17*(4), e902–e911.

Yu, J., Ren, Y., Xi, X., Huang, W., & Zhang, H. (2017). A novel lactobacilli-based teat disinfectant for improving bacterial communities in the milks of cow teats with subclinical mastitis. *Frontiers in Microbiology, 8*, 1782.

Zentek, J., Marquart, B., Pietrzak, T., Ballevre, O., & Rochat, F. (2003). Dietary effects on bifidobacteria and Clostridium perfringens in the canine intestinal tract. *Journal of Animal Physiology and Animal Nutrition, 87*(11–12), 397–407.

Zhang, J. L., Xie, Q. M., Ji, J., Yang, W. H., Wu, Y. B., Li, C., Ma, J. Y., & Bi, Y. Z. (2012). Different combinations of probiotics improve the production performance, egg quality, and immune response of layer hens. *Poultry Science, 91*(11), 2755–2760.

Zhang, W., Zhu, Y. H., Zhou, D., Wu, Q., Song, D., Dicksved, J., & Wang, J. F. (2017). Oral administration of a select mixture of Bacillus probiotics affects the gut microbiota and goblet cell function following *Escherichia coli*challenge in newly weaned pigs of genotype MUC4 that are supposed to be enterotoxigenic E. coli f4ab/ac receptor negative. *Applied and Environmental Microbiology, 83*(3), e02747-16.

Zhang, W., Zhu, Y. H., Yang, G. Y., Liu, X., Xia, B., Hu, X., Su, J. H., & Wang, J. F. (2018). *Lactobacillus rhamnosus* GG affects microbiota and suppresses autophagy in the intestines of pigs challenged with Salmonella Infantis. *Frontiers in Microbiology, 8*, 2705.

Zhang, J. J., Yang, H. L., Yan, Y. Y., Zhang, C. X., Ye, J. D., & Sun, Y. Z. (2020). Effects of fish origin probiotics on growth performance, immune response and intestinal health of shrimp (*Litopenaeus vannamei*) fed diets with fish meal partially replaced by soybean meal. *Aquaculture Nutrition, 26*(4), 1255–1265.

Zhao, P. Y., & Kim, I. H. (2015). Effect of direct-fed microbial on growth performance, nutrient digestibility, fecal noxious gas emission, fecal microbial flora and diarrhea score in weanling pigs. *Animal Feed Science and Technology, 200*, 86–92.

Zhao, X., Guo, Y., Guo, S., & Tan, J. (2013). Effects of Clostridium butyricum and Enterococcus faecium on growth performance, lipid metabolism, and cecal microbiota of broiler chickens. *Applied Microbiology and Biotechnology, 97*(14), 6477–6488.

Zhao, Y., Yang, Q. E., Zhou, X., Wang, F., Muurinen, J., Virta, M., Brandt, K., & Zhu, Y. (2020). Antibiotic resistome in the livestock and aquaculture industries: Status and solutions. *Critical Reviews in Environmental Science and Technology, 51*(19), 1–38.

Zheng, A., Luo, J., Meng, K., Li, J., Bryden, W. L., Chang, W., Zhang, S., Wang, L. X. N., Liu, G., & Yao, B. (2016). Probiotic (Enterococcus faecium) induced responses of the hepatic proteome improves metabolic efficiency of broiler chickens (Gallus gallus). *BMC Genomics, 17*(1), 1–12.

Zhou, X. X., Wang, Y. B., & Li, W. F. (2009). Effect of probiotic on larvae shrimp (*Penaeus vannamei*) based on water quality, survival rate and digestive enzyme activities. *Aquaculture, 287*(3–4), 349–353.

Ziggers, D. (2000) Tos, a new prebiotic derived from whey. *Animalence Feed Sci Technology, 5*, 34–36.

Zokaeifar, H., Balcázar, J. L., Saad, C. R., Kamarudin, M. S., Sijam, K., Arshad, A., & Nejat, N. (2012). Effects of *Bacillus subtilis* on the growth performance, digestive enzymes, immune gene expression and disease resistance of white shrimp, *Litopenaeus vannamei*. *Fish and Shellfish Immunoly, 33*(4), 683–689.

Antimicrobial Resistance Through Food: Role of Food Safety Management System

T. V. Sankar

Contents

Abstract

Antimicrobials are biologically active ingredients of manmade or natural origin that eliminate or hinder the development of microorganisms which includes antibacterial, antiviral, antifungal, and antiprotozoal substances (EC, 2017). The progress of resistance to such drugs poses a complex and challenging complications affecting the lives of human beings. The bacterial resistance towards antibiotics can spread through direct contact of humans with food animals and livestock, by means of food chain, or through wastewater from farms and hospitals. The use of antibacterial or antibiotics is inevitable in the present-day world to protect the food animals in their growth phase. But their frequent use endangers the life of consumers subsequently, needing careful attention to mitigate the issue. The first and major requirement is the formulation of a policy involving the stakeholders concerned, with the employment of substances that possess antimicrobial properties, both in food faunas and people, and its implementation. Besides, education, capacity building, and research on antibacterial resistance including surveillance need to be followed for fresh insight into the

T. V. Sankar (✉)
ICAR-Central Institute of Fisheries Technology, Cochin, Kerala, India
e-mail: sankar.tv@icar.gov.in

M. P. Mothadaka et al. (eds.), *Handbook on Antimicrobial Resistance*,
https://doi.org/10.1007/978-981-19-9279-7_39

issue. The spread of AMR through food is another dimension to the problem, and appropriate mitigation measures to comprehend the problem are the need of the hour. The measures to tackle the biological hazards should be addressed sans second thought along the food value sequence. The doctrines associated with hazard analysis critical control point (HACCP) and prerequisite programs (PRPs) need to be treated with all seriousness to ensure that the antibacterial-resistant organisms do not contaminate the food and distress the consumers. In the light of the open trade in the world, this assumes more significance.

Keywords

Antibacterial resistance · Contamination · Food safety · Value chain · Mitigation

1 Introduction

Antibiotics or antibacterial agents have been considered vital substances in dealing with ailments during the preceding era but eventually turned out to be dangerous prescriptions due to the advent and transmission of microbes with resistance to antimicrobial agents, challenging the medical profession (Meyer et al., 2010; Palmer & Kishony, 2013). As already shown, an antibacterial substance is any substance which at a very significantly lower amount destroys or inhibits the development of bacteria causing an impact on the host. The antimicrobial resistance (AMR) denotes the capability of an organism to develop resistance progressively to an bactericidal compound to which they were earlier vulnerable, with the risk of spreading to others (WHO, 2015; EC, 2017).

AMR occurs in bacteria when they acquire resistant gene or their genes mutate to resistant genes enabling them to endure the presence of antimicrobial agents (WHO, 2015). The direct antibacterial usage in human and in food animals facilitates the spread of AMR, and their overuse or misuse accelerates its occurrence (Nelson et al., 2019). The prophylactic employment of antimicrobial agents to promote feed efficiency and also for weight gain supports the incidence of AMR in the foodstuff settings. The incidence of AMR has grown from an issue to a challenge in recent times, and any minor illness becomes life-threatening. The overuse of antibacterial compounds led to the selection of AMR pathogens, and its dispersal into the ecosystem at sub-lethal concentrations sustains its manifestation. The equally alarming aspect is the excessive use of antimicrobial agents in the sector of animals than in humans (CDC, 2013; WHO, 2014) and hence the potential possibility of transmission of zoonoses of resistance. The AMR evolution is reported to occur through vertical gene transfer into the progeny cells followed by intra- and inter-microbial gene transfer by horizontal means (Founou et al., 2016). For instance, the occurrence of methicillin-resistant *Staphylococcus aureus* (MRSA) and extended-spectrum β-lactamase (ESBL)-producing *Enterobacteriaceae* is an example of the incidence of ARB (antibiotic-resistant bacteria) and ARG (antibiotic-resistant genes) originated from animal source, along the food chain.

The AMR indicates the emergence of an imbalance between the nonjudicial usage and the adverse reaction to antibiotics. The AMR is a serious threat to human life, and the effect of AMR with regard to disease and economic loss is difficult to estimate. Some of the common contagions are increasingly problematic and at times difficult for treatment. The ailments associated with AMR bacteria especially of multidrug-resistant kind are important contributing factors inducing sickness and demise of patients with severe health issues (Prestinaci et al., 2015). The advent of antibiotic-resistant strains of certain pathogens like methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant *Enterococcus* (VRE), multidrug-resistant *Pseudomonas aeruginosa*, imipenem-resistant *Acinetobacter baumannii* (IRAB), and *Escherichia coli* and *Klebsiella pneumoniae* that are resistant to cephalosporins of the third generation poses grave dangers to public health (Rossolini et al., 2007; Spellberg et al., 2008; Meyer et al., 2010). The global spread of extended-spectrum β-lactamase (ESBL)-producing pathogens and MRSA and their prevalence in numerous sick bays is another important concern of AMR (Cantas et al., 2013). The separation of Carbapenem or Fluoroquinolone resistant non-*Enterobacteriaceae* microbes, viz., *A. baumannii* and *P. aeruginosa* (Brown et al., 1998; Cantas et al., 2013), resistant foodborne microbes, namely, *Salmonella enterica* and *E. coli* (Fischer et al., 2012, 2013), are going to be a foremost hazard to community well-being in future. The fact that no new antibiotics are being developed in recent times and the quick emergence of resistant pathogens make the situation worst. The present trends in employment of antibiotics in disease treatment leading to the advancement of multidrug-resistant (MDR) bacteria add further stress on the need for drugs employing novel molecular mechanisms (Magiorakos et al., 2012).

2 AMR Status

Food is essential for sustaining human life, and when gets contaminated either directly from the environment or due to spoilage, it is likely to impact the consumers. The presence of chemical contaminants and microbial pathogens makes food unsuitable for human consumption and may occur at any point along the value chain if sufficient care is not taken. The incidence of AMR bacteria during the production to distribution is identified as a major risk not only to the personnel involved in the production process but more importantly to the consumers, who get the penalty for ingesting the food available for distribution. The incidence of AMR bacteria or antibiotic resistant genes is equally riskier as a food safety concern and needs stringent care along the value chain as well.

It is reported that the food animal and livestock production sector used about 63,151 tonnes of antibacterial substances globally in 2010 and is expected to enhance by 67% by 2030 (Laxminarayan et al., 2015). The alarming situation is not the resistance by a single species of bacteria to a particular antimicrobial but to more than one class of the chemicals to different species coexisting in the ecosystem. This complicates the food chain and thus furtherance of the problem (Cohen, 1992).

According to the information available from the CDC, *Campylobacter* spp., *Salmonella* spp., and *Shigella* toxin-producing *E. coli* were reported to be the major agents of foodborne illnesses leading to diseases in severe cases (Founou et al., 2018). These mainly contribute to morbidity and mortality in places where AMR is not given enough importance. The absence of hygiene regime and poor handling practices add to the incidence and food safety in a gigantic way (WHO, 2011; Tack et al., 2019).

The first case of AMR reported was the resistance to penicillin in 1948 and the development of resistance to one or more than one antibiotics by bacteria considered to be natural phenomena (Barber & Rozwadowska-Dowzenko, 1948; Cantas et al., 2013). It is very tantalizing to note that the employment of antibiotics is directly proportional to the development of resistance. The SMART (Study for Monitoring of Antimicrobial Resistance Trends) studies revealed that the AMR development was highest in the Asia-Pacific regions followed by America, Africa, and Europe (Lee et al., 2013). The incidence of ESBL has been a major concern in treatments against *Enterobacteriaceae* (Pitout & Laupland, 2008; Lee et al., 2009). Similar is the apprehension on the development of resistance to carbapenem by strains of *Enterobacteriaceae*.

AMR is a multifaceted, intricate, burning worldwide problematic condition and is a growing apprehension. It is known that the non-judicious and inappropriate use of antibiotics is a persistent issue in sensitive sectors like food, leading to the emergence of AMR. The specific practice of using suitable antibiotic(s) is important in lessening the effect of AMR on the population at large. In order to combat the matters associated with AMR, the Antimicrobial Stewardship Programs (ASP) was outlined with guidelines by the societies, namely, ISDA (Infection Diseases Society of America) and SHEA (Society of Healthcare Epidemiology) of America (Dellit et al., 2007; Owens, 2008). Studies on the development of MRSA due to fluoroquinolone use (Madaras-Kelly et al., 2006), VRE (enterococci resistant to vancomycin) (Harbarth et al., 2002), CRE (cephalosporin-resistant *Enterobacteriaceae)* (Calil et al., 2001), and carbapenem-resistant *Acinetobacter*, *Pseudomonas*, and *Enterobacteriaceae* (Go et al., 1994; Rahal et al., 2002) demonstrate the link between the antibacterial use and emergence of AMR. There is an opposite association among nalidixic acid concentrations with the development of AMR-resistant strains in *Enterobacteriaceae* on exposure to the lower concentrations of the drug signifying the importance of MIC (minimum inhibitory concentration) and MPC (mutant prevention concentration) (Stamey, 1976; Caron & Mousa, 2010). It is also interesting to note that not only the susceptible flora but also the coexisting flora as well are affected by antibiotic treatment as shown by the lowering of the concentration of some Gram-negative bacteria by vancomycin, besides its actual action on Gram-positive bacteria (Robinson & Young, 2010). It is a great concern that necessitates immediate attention with thorough understanding of microbial virulence, chemistry, and epidemiology of the drug. It is also imperative to use the drug at an optimal level to avoid overuse leading to the development of AMR.

More often than not, the food chains are responsible for transmission of pathogenic organisms. The spread of *Campylobacter difficile* resistant to drugs and

non-typhoid salmonella, ESBLs, and MRSA are serious threats (CDC, 2013). It is also a concern that at least 15% of the diseases emerging, of late, are associated with the food chain, probably with the sourcing of raw materials or the ingredients (Parmley et al., 2012). The MDR bacteria are important source of food safety apprehensions. Among many organisms, Gram-negative bacteria from animal sources causing gastroenteritis, *Salmonella* that are transmitted through fecal contamination (Silva et al., 2014) and the G-negative curved bacteria *Campylobacter jejuni*, cause diarrhea, fever, etc., through undercooked or improperly cooked chicken and unpasteurized milk (CDC, 2013). The Gram-positive organism *Clostridium difficile*, which causes serious healthcare-associated infections at the community level, is also an emerging concern. The symptoms of infection, such as pain of the abdomen, diarrhea, pyrexia, etc., are related to food manufacture and animal husbandry. The Gram-positive bacteria *Staphylococcus aureus* shares the usual skin and nasal bacterial flora of persons. The development of methicillin-resistant *S. aureus*, again, is related to healthcare-associated infection. Its ability to produce specific toxins leading to intoxication-related poisoning of foods and the syndrome of toxic shock is well documented (WHO, 2014). The ESBL-producing *Enterobacteriaceae* also exude apprehensions in the present-day environment. Even the most common *E. coli* has shown to develop resistance which is a public issue. More recently the AMR to the antibiotic colistin is creating a major alarm across the globe as the application of the antibiotic is noticed in food production systems in several countries (Anna George, 2018).

3 Antibiotics as Veterinary Medicine

The employment of antibiotics at the levels of food production leads to matters of safety such as antibiotic residue presence and the occurrence of ARB (antibiotic-resistant bacteria) or ARG (antibiotic-resistant genes) in food animals and food products, while the inadequacy of shipping and stowage services, together with the absence of practices of hygiene, contributes in its spread (Founou et al., 2021).

The demand for food across the globe upsurges due to population explosion and hence the preference for food animal, particularly fish. The requirement is met by increased production, intensive aquaculture, and animal husbandry activities which invariably use excess antibiotics to limit the health issues of the animal concerned while promoting growth. The data available indicate that about 70% of the drugs are used for prophylactic purpose (Roura et al., 1992; Silbergeld et al., 2008) and for growth promotion (Anderson et al., 2003; Roe & Pillai, 2003; Anthony et al., 2001; Cabello, 2006). The long-time supplementation of a drug in small doses through feed, particularly in aquaculture, facilitates the enrichment of resistant microbial populations (Gullberg et al., 2011; Alexander et al., 2011). Related information are existing on the incidence of ESBL-producing and carbapenemase-positive *Enterobacteriaceae* strains, MRSA, and plasmid-mediated quinolone resistance in food animals and their products (Cuny et al., 2010; Horton et al., 2011; Nordmann et al., 2011; Fischer et al., 2012, 2013). Incidence of multiple drug-resistant (MDR)

bacteria in fish aquaculture farms due to the increased use of antibiotics is also reported (Cabello, 2006; Colquhoun et al., 2007; Shah et al., 2012; Cantas et al., 2013). The use of antibiotics in aquaculture is essential in managing the insusceptible structure of the animal affected by the intensive culture practices and similarly to prevent the infection leading to mass mortality. At the same time, its presence in the animal promotes AMR in the consumers leading to safety concerns.

4 Possible Solutions

Bacteria are present everywhere, and occurrence in the food chain is almost certain. The resistant genes can be transmitted, like any other food safety hazard, along the food chain, through unscientific handling practices or by cross-contamination. The processes which facilitate the incidence of microbiological hazards are likely to introduce AMR bacteria into the food. The problem of AMR starts from the farm, either in aquaculture or animal husbandry. The most ideal solution to minimize the dependency on antibiotics is by adopting the best management practices. Literature shows that 73% of antibiotics sold globally are consumed by animals raised for food (Van Boeckel et al., 2017) and the prolonged application of antibiotics promotes the selection of resistance genes and facilitates their horizontal transfer. Some of the solutions suggested for farm-level management in animal husbandry are based on innovative management of Bio-secured systems, namely, "all-in-all-out" structures; use of antibiotic-free agents of growth; the improved utility of supplementary animal well-being measures, viz., bacteriophages, disinfectants, and serums (vaccines); vector control; upgraded testing to facilitate appropriate drug selection; reduced dependence on antibiotics for the initial stages of growth; and better waste management practices (Robinson et al., 2016), together with capacity building activities for farmers in AMR mitigation measures. This can be adopted effectively for fisheries as well, in addition to the management of antibacterial usage through good aquaculture practices. Also, measures are to be taken upstream of the food groups in order to reduce the emergence of AMR. The sensible and harmonious application of agents under the guidance of skilled personnel in livestock farming, aquaculture and human medicine would have to be taken seriously. Though absence of chemical, microbiological, and physical hazards are significant in food from food safety point of view, the microbial hazards are important in the sense that they can be controlled during production or along the food chain by appropriately following the recommended food safety protocols.

The possible entry of antimicrobial agents into the human or animal body may be deliberate or accidental – deliberate in the sense given to animal or human as part of disease treatment or prevention and accidental as a result of sharing the common ecosystem. In either case, the ultimate prey is the consumer. Food, obviously the common element across the living system, is a potential commodity facilitating the transmission of bacteria, specifically AMR bacteria, irrespective of whether it is plant or based on animal. The food commodity gets infected at the farm or at the

slaughterhouse (animal) and gets carried forward. In the case of noninfected raw material, the AMR bacteria can come from the infected food handler, processor, or even contaminated water used for processing and further along the food chain, if preventive action is not taken to control them. The increased risk is from the food animal as the exposure risks are more due to the reasons explained above.

The five keys proposed by the WHO for controlling biological threats, viz., "choose safe raw materials," "keep hands and utensils clean," "separate raw and cooked food," "cook thoroughly," and "keep food at a safe temperature," provide the basic requirement to tackle biological dangers in a food production system. Food contamination facilitates the spread of the microbe from a single source, and sensitizing the people involved in the food production system, both livestock and fisheries, is critical, and a tenacious food safety management system can minimize the transmission of AMR organisms which is imperative. The absolute management could be difficult, but the implementation of good practices with special reference to the use of antimicrobials at all stages from production to consumption following good hygiene practices (GHP) needs to be strictly adhered to make it a system.

5 Management of AMR in the Food System

Managing hazards in a food system is a very complex proposition. Addressing antibacterial resistance along the food system requires the active involvement and cooperation of many different sectors connected with the production, processing, and transportation of not only the raw materials but also the intermediate products and other ingredients as well. In this line, the most important requirement is the judicial application of antibiotics on food animals by adopting better health programs and hygiene practices.

Microbial resistance to antibiotics impacts the application of the drug for human health, and the benefits of the drug cannot be realized. A similar situation appears in the livestock sector as well, and the issues of food protection and well-being as envisaged in the SDG (Sustainable Development Goals) of the United Nations and addressing the food security, animal welfare, and ultimately the social and economic development cannot be addressed. Exposure to food animals grimly impacts the food chain and ultimately the stakeholders rather directly leading to serious health issues. The global trade of animals-based food and seafood across the borders as well as international travel of the stakeholders contributes to the spread of AMR length and breadth making it a universal concern (Founou et al., 2016).

Minimizing the application of drugs and chemicals on food animals is the most needed solution to ensure the food-related spread of AMR among the population. The main concern is its release into the ecosystem, particularly into the water bodies, which becomes a source for the advent and spread of AMR in the farm−/pond-to-plate continuum (Berendonk et al., 2015; Martinez, 2009).

Pathogens by themselves are a threat to consumers, and if the pathogens are resistant to antimicrobials, they pose a greater threat due to increased health risk

and potential of treatment failures. The AMR through food clearly indicates the usage of antimicrobials in food animals or more severely the contamination of places of animal rearing and in either case is an impending risk factor (FAO, 2011).

The most important requirement for control of AMR in food systems is the prudent use and understanding clearly the significance of non-judicious usage. The fact remains that the possibility of resistance development is imminent in cases where extensive use is practiced for immediate benefit. The USDA antibacterial resistance action plan suggested a plan of action encompassing investigation, deterrence and control, enquiry, and growth and clearly indicated that responsible use of antibacterial substances is not reduced usage but judicious use and understanding of the consequences (USDA, 2014). The WHO has released Global Principles for the AMR control in food animals (WHO, 2000) and WOAH documents related to AMR, together with Strategies for the Responsible and Judicious Use, as well as the methodology for risk analysis, monitoring, surveillance, and laboratory methodologies, signifying its importance (OIE, 2015). Most of these documents emphasize actions to prevent disease occurrence, nonantibiotic mitigation measures if disease occurs and in imminent cases, and the use of less harmful antibiotics. The AMR emerging as a public health threat is a global concern leading to a two-dimensional impact, viz., increasing morbidity and mortality in the developing nations and financial burden for treatments among the developed countries (Harbarth et al., 2015). The "Tripartite Alliance" between WHO, FAO, and WOAH initiated a GAP (Global Action Plan) on AMR in 2015, to address the concern with five major objectives, viz., creating awareness of AMR, strengthening knowledge, reducing infectious disease, optimizing rational use of antibiotics, and making a framework for combined deterrence and control measure of antibacterial resistance in the food stratification (WHO, 2015).

A proactive action is initiated by world countries in this regard. Norway has considerably reduced antibiotic usage, the EU has excluded the use of antibiotics in the promotion of growth, and the USA has brought out legislation for controlled termination of utilization of antibiotics for prophylactic purposes and also making stringent labelling provisions (Founou et al., 2016). In developing countries, there is a situation of concern due to the improper implementation of measures on AMR. Equally concerning is the fact that most of the developing countries are global exporters of meat and fish, and there is a potential threat of spreading AMR through the hierarchy of foods (Fernandes et al., 2016; Holmes et al., 2016), a problem that needs urgent mitigation.

Basically, the biological hazards are pathogenic microorganisms, such as bacteria (e.g., cholera,), viruses (e.g., hepatitis A or B), or parasites (*Trichinella*), the presence of which (infection) or the toxins produced by them (intoxication) cause illness in human beings. In the context of AMR, the concern is several folds larger due to difficult control measures. As far as food safety is concerned, a robust strategy is required, from production to table in line with a scientific understanding. Some can be managed in the production process itself or at the receiving sites

following simple hygiene processes. Certain pathogens require strategic steps and could be removed or reduced to manageable limits during the processing, such as controlling pathogens in milk by pasteurization, controlling microorganisms by chilling or freezing fish, etc. Time and temperature management are the most important requirements to control the biological hazards in the product development along with sanitation and hygiene. Hospital-related infections also contribute to the incidence of AMR globally, and improving water, sanitation, and hygiene (WASH) and infection prevention and control (IPC) are crucial to its control (Holmes et al., 2016).

Any management system which reduces or removes the introduction of hazard should eliminate the possibility of transmission of AMR and a foolproof safety management system at all levels is an absolute requirement. Similarly, the process of cooking removes bacteria, and the management of critical control point (CCP) is absolutely required for maintaining an AMR-free food chain. A robust food safety management system (FSMS) is often set up to minimize the incidence of threats, which has three fundamental programs, namely, good manufacturing practices (GMPs), prerequisite programs (PRPs), and the HACCP system which leads to foods that are free from threats for the consumers. In this case, the actual control starts at the farm level with GMP, and the PRP and HACCP take over when the produce is ready for product development and movement along the value chain. Moreover, testing at the receiving stage, the removal of hazards following appropriate measures during the subsequent handling stages is also equally important. The sanitation and hygiene in the production facility and the personnel involved in the process are absolute requirements. A strict hygiene regime needs to be considered starting from the design of the production facility, equipment, development of a product traceability system, and the implementation of prerequisite programs including good manufacturing practices (GMPs) and good hygiene practices (GHP). The development of an integrated framework has already been initiated globally to tackle the issue of food-related hazards. In controlling AMR hazards, the protocol for food safety is to be strictly adhered to, besides taking extra effort to unconditionally remove factors such as biofilms, etc. that favor multiplication of bacteria and development of AMR bacteria.

While addressing the seven principles of HACCP (Fig. 1), the potential hazard needs to be identified taking into consideration the raw materials, ingredients used, the overall production process, the end user or the consumer requirements, and the possible limitations.

The hazard analysis forms an important step in the risk assessment where the potential hazard is analyzed for its severity and frequency of incidence in order to get information on the severity index and the possible point where it is addressed in the production process. The food production system controls the incidence of biological hazards as most of them follow FSMS protocol following the principles of HACCP (Tables 1 and 2).

The critical control point (CCP) and critical limit (CL) are to be defined for the hazard identified with a possible action plan for its control. The facilities with

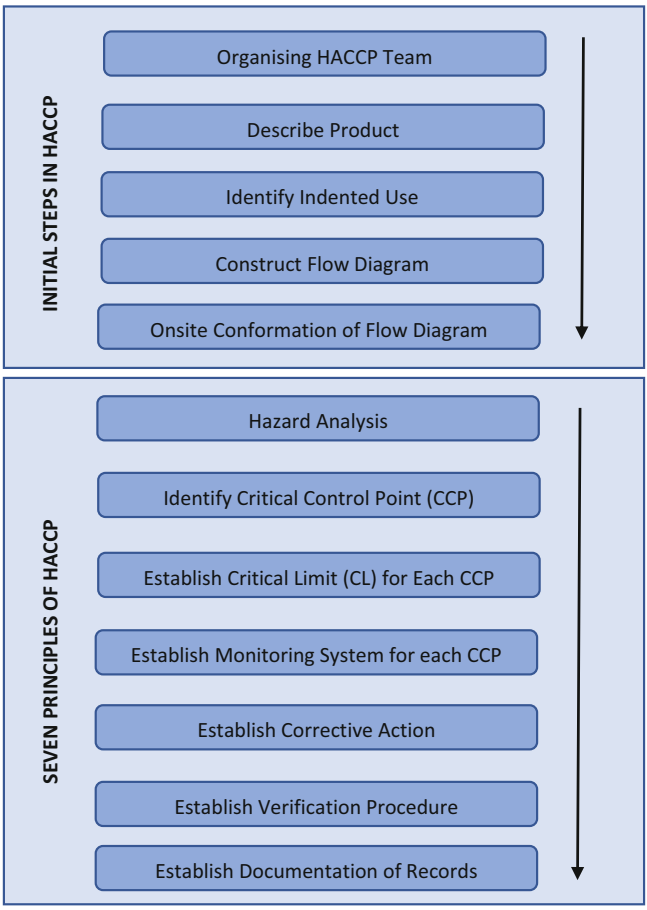


Fig. 1 HACCP steps and principles

suboptimum sanitary conditions or where PRPs are not given importance or the development of biofilms poses a problem to control mechanisms (Kovac, 2019). The biofilms are in fact the areas where surviving microbes position themselves and create havoc, and the physical barriers provide protection against cleaning and the use of sanitizers (Galié et al., 2018), which need to be avoided using appropriate cleaning protocols.

6 Conclusion

The judicious application of antibacterial agents in the food production process including the raw materials by following scientific protocols provides the basic requirement for the possible control of AMR. Capacity building of stakeholders,

Table 1 Prerequisite program (PRP) for control of biological hazard

Stage	Control measure	Action
Raw material receiving	Temperature control	Adhering to the specifications and sanitation and hygiene
Storage of RM	Temperature control Prevent cross contamination	Maintain support system Separate inappropriate food components
Processing/ product preparation	Prevent contamination – handling/personal hygiene Prevent cross contamination (Raw/cooked products) Prevent biofilm formation	Maintain personal hygiene Temperature control Planning protocol to suit the production process Proper cleaning and disinfection Cleaning instruments, etc. to remove residual food and biofilm with appropriate protocol
Packaging	Separating raw/cooked product Prevent cross contamination	Temperature control Proper cleaning and disinfection Cleaning instruments, etc. to remove residual food and biofilm with appropriate protocol
Transportation	Prevent contamination – handling/personal hygiene Prevent cross contamination (Raw/cooked products) Prevent biofilm formation	Maintain personal hygiene Temperature control Proper cleaning and disinfection Cleaning instruments, etc. to remove residual food and biofilm with appropriate protocol
Retailing	Prevent contamination – handling/personal hygiene Prevent cross contamination (Raw/cooked products)	Maintain personal hygiene Temperature control Proper cleaning and disinfection
Waste management	Cross contamination	Waste management

Table 2 HACCP for biological hazard in a food production system

Stage	Control measure	Critical limit	Action
Raw material	Temperature control	Fresh <4 °C Frozen -18 °C $\pm$ 2 °C	Maintain support system
Cooked product	Temperature control	Check cooking parameters	Maintain support system
Raw packaged products	Temperature/humidity control; control of packaging material	<30 °C/$<60\%$ RH	Maintain support system

development of tenacious FSMS, incorporating the principles of prerequisite program, and HACCP protocol suitable for the candidate food system are the cornerstone in controlling the incidence of AMR through the food production system, which otherwise may lead to serious health consequences.

References

Alexander, T. W., Yanke, J. L., Reuter, T., Topp, E., Read, R. R., Selinger, B. L., & McAllister, T. A. (2011). Longitudinal characterization of antimicrobial resistance genes in feces shed from cattle fed different sub-therapeutic antibiotics. *BMC Microbiology, 11*, 1–12. https://doi.org/10.1186/1471-2180-11-19

Anderson, A. D., Nelson, J. M., Rossiter, S., & Angulo, F. J. (2003). Public health consequences of use of antimicrobial agents in food animals in the United States. *Microbial Drug Resistance, 9*, 373–379.

Anthony, F., Acar, J., Franklin, A., Gupta, R., Nicholls, T., Tamura, Y., Thompson, S., Threlfall, E. J., Vose, D., van Vuuren, M., & White, D. G. (2001). Antimicrobial resistance: Responsible and prudent use of antimicrobial agents in veterinary medicine. *Reviews in Science and Technology, 20*, 829–839.

Barber, M., & Rozwadowska-Dowzenko, M. (1948). Infection by penicillin-resistant staphylococci. *Lancet, 2*, 641–644.

Berendonk, T. U., Manaia, C. M., Merlin, C., Fatta-Kassinos, D., Cytryn, E., Walsh, F., Bürgmann, H., Sorum, H., Norström, M., Pons, M., Kreuzinger, N., Huovinen, P., Stefani, S., Schwartz, T., Kisand, V., Baquero, F., & Luis Martinez, J. (2015). Tackling antibiotic resistance: The environmental framework. *Nature Reviews. Microbiology, 13*, 310–317.

Brown, S., Bantar, C., Young, H. K., & Amyes, S. G. (1998). Limitation of *Acinetobacter baumannii* treatment by plasmid-mediated carbapenemaseARI-2. *Lancet, 351*, 186–187.

Cabello, F. C. (2006). Heavy use of prophylactic antibiotics in aquaculture: A growing problem for human and animal health and for the environment. *Environmental Microbiology, 8*, 1137–1144.

Calil, R., Marba, S. T., von Nowakonski, A., & Tresoldi, A. T. (2001). Reduction in colonization and nosocomial infection by multi-resistant bacteria in a neonatal unit after institution of educational measures and restriction in the use of cephalosporins. *American Journal of Infection Control, 29*, 133–138.

Cantas, L., Shah, S. Q., Cavaco, L. M., Manaia, C. M., Walsh, F., Popowska, M., Garelick, H., Burgmann, H., & Sorum, H. (2013). A brief multi-disciplinary review on antimicrobial resistance in medicine and its linkage to the global environmental microbiota. *Frontiers in Microbiology, 4*, 96. https://doi.org/10.3389/fmicb.2013.00096

Caron, W. P., & Mousa, S. A. (2010). Prevention strategies for antimicrobial resistance: A systematic review of the literature. *Infection and Drug Resistance, 3*, 25–33.

CDC. (2013). *Antibiotic resistance threats in the United States*. *US Department of Health and Human Services*. http://www.cdc.gov/drugresistance/pdf/ar-threats-2013-508.pdf

Cohen, M. L. (1992). Epidemiology of drug resistance: Implications for a post—Antimicrobial era. *Science, 257*, 1050–1055.

Colquhoun, D. J., Aarflot, L., & andMelvold, C.F. (2007). gyrA and parC mutations and associated quinolone resistance in *Vibrio anguillarum* serotype O2b strains isolated from farmed Atlantic cod (*Gadus morhua*) in Norway. *Antimicrobial Agents and Chemotherapy, 51*, 2597–2599.

Cuny, C., Friedrich, A., Kozytska, S., Layer, F., Nubel, U., Ohlsen, K., Strommenger, B., Walther, B., Wieler, L., & Witte, W. (2010). Emergence of methicillin-resistant *Staphylococcus aureus* (MRSA) in different animal species. *International Journal of Medical Microbiology, 300*, 109–117.

Dellit, T. H., Owens, R. C., McGowan, J. E., Jr., Gerding, D. N., Weinstein, R. A., Burke, J. P., Huskins, W. C., Paterson, D. L., Fishman, N. O., Carpenter, C. F., Brennan, P. J., Billeter, M., & Hooton, T. M. (2007). Infectious Diseases Society of America and the Society for Healthcare Epidemiology of America guidelines for developing an institutional program to enhance antimicrobial stewardship. *Clinical Infectious Diseases, 44*(2), 159–177. https://doi.org/10.1086/510393. Epub 2006 Dec 13.

EC. (2017). *A European One Health Action Plan Against Antimicrobial Resistance (AMR)*. European Commission. Online: https://ec.europa.eu/health/sites/default/files/antimicrobialresistance/docs/amr_2017_action-plan.pdf (Accessed on 25 April 2019).

FAO. (2011). *Guidelines for risk analysis of foodborne antimicrobial resistance CAC/GL 77–2011* (pp. 1–29). Codex Alimentarius Commission/Guidelines.

Fernandes, M. R., Moura, Q., Sartori, L., Silva, K. C., Cunba, M. P., Esposito, F., et al. (2016). Silent dissemination of colistin-resistant Escherichia coli in South America could contribute to the global spread of the mcr-1 gene. *Euro Surveillance, 21*, 30214. https://doi.org/10.2807/1560-7917.ES.2016.21.17.30214

Fischer, J., Rodriguez, I., Schmoger, S., Friese, A., Roesler, U., Helmuth, R., & Guerra, B. (2012). *Escherichia coli* producing VIM-1 carbapenemase isolated in a pig farm. *Journal of Antimicrobial Chemotherapy, 67*(7), 1793–1795.

Fischer, J., Rodriguez, I., Schmoger, S., Friese, A., Roesler, U., Helmuth, R., & Guerra, B. (2013). *Salmonella enterica* subsp. enterica producing VIM-1 carbapenemase isolated from livestock farms. *Journal of Antimicrobial Chemotherapy, 68*(2), 478–480.

Founou, L. L., Founou, R. C., & Essack, S. Y. (2016). Antibiotic resistance in the food chain: A developing country-perspective. *Frontiers in Microbiology, 7*, 1881. https://doi.org/10.3389/fmicb.2016.01881

Founou, L. L., Amoako, D. G., Founou, R. C., & Essack, S. Y. (2018). Antibiotic resistance in food animals in Africa: A systematic review and meta-analysis. *Microbial Drug Resistance, 24*(5), 648–665.

Founou, L. L., Founou, R. C., & Essack, S. T. (2021). Antimicrobial resistance in the farm-to-plate continuum: More than a food safety issue. *Future Science OA, 7*, FSO692. https://doi.org/10.2144/fsoa-2020-0189

Galie, S., García-Gutiérrez, C., Miguélez, E. M., Villar, C. J., & Lombo, F. (2018). Biofilms in the food industry: Health aspects and control methods. *Frontiers in Microbiology, 9*, 898. https://doi.org/10.3389/fmicb.2018.00898

George, A. (2018). Editorial commentary: Antimicrobial resistance, trade, food safety and security. *One Health, 5*, 6–8.

Go, E. S., Urban, C., Burns, J., Kreiswirth, B., Eisner, W., Mariano, N., Mosinka-Snipas, K., & Rahal, J. J. (1994). Clinical and molecular epidemiology of Acinetobacter infections sensitive only to polymyxin B and sulbactam. *Lancet, 344*, 1329–1332.

Gullberg, E., Cao, S., Berg, O. G., Ilback, C., Sandegren, L., Hughes, D., & Andersson, D. I. (2011). Selection of resistant bacteria at very low antibiotic concentrations. *PLoS Pathogens, 7*, e1002158. https://doi.org/10.1371/journal.ppat.1002158

Harbarth, S., Cosgrove, S., & Carmeli, Y. (2002). Effects of antibiotics on nosocomial epidemiology of vancomycin-resistant enterococci. *Antimicrobial Agents and Chemotherapy, 46*, 1619–1628.

Harbarth, S., Balkhy, H. H., Goossens, H., Jarlier, V., Kluytmans, J., Laxminarayan, R., et al. (2015). Antimicrobial resistance: One world, one fight! *Antimicrobial Resistance and Infection Control, 4*, 49. https://doi.org/10.1186/s13756-015-0091-2

Holmes, A. H., Moore, L. S., Sundsfjord, A., Steinbakk, M., Regmi, S., Karley, A., et al. (2016). Understanding the mechanisms and drivers of antimicrobial resistance. *Lancet, 387*, 176–187. https://doi.org/10.1016/S0140-6736(15)00473-0

Horton, R. A., Randall, L. P., Snary, E. L., Cockrem, H., Lotz, S., Wearing, H., Duncan, D., Rabie, A., McLaren, I., La Watson, E., Ragione, R. M., & Coldham, N. G. (2011). Fecal carriage and shedding density of CTX-M extended-spectrum β-lactamase-producing *Escherichia coli*in cattle, chickens, and pigs: Implications for environmental contamination and food production. *Applied and Environmental Microbiology, 77*(11), 3715–3719. https://doi.org/10.1128/AEM.02831-10. Epub 2011 Apr 8.

Kovac, J. (2019). Precision food safety: A paradigm shift in detection and control of food-borne pathogens. *MSystems, 4*, e00164–e00119. https://doi.org/10.1128/mSystems.00164-19

Laxminarayan, R., Van Boeckel, T., & Teillant, A. (2015). *The economic costs of withdrawing antimicrobial growth promoters from the livestock sector*. OECD Food, Agriculture and Fisheries Papers, No. 78, OECD Publishing, Paris. https://doi.org/10.1787/5js64kst5wvl-en

Lee, J. H., Jeong, S. H., Cha, S. S., & Lee, S. H. (2009). New disturbing trend in antimicrobial resistance of gram-negative pathogens. *PLoS Pathogens, 5*, e1000221. https://doi.org/10.1371/journal.ppat.1000221

Lee, C.-R., Cho, I. H., Jeong, B. C., & Lee, S. H. (2013). Review strategies to minimize antibiotic resistance. *International Journal of Environmental Research and Public Health, 10*, 4274–4305. https://doi.org/10.3390/ijerph10094274

Madaras-Kelly, K. J., Remington, R. E., Lewis, P. G., & Stevens, D. L. (2006). Evaluation of an intervention designed to decrease the rate of nosocomial methicillin-resistant *Staphylococcus aureus* infection by encouraging decreased fluoroquinolone use. *Infection Control and Hospital Epidemiology, 27*, 155–169.

Magiorakos, A. P., Srinivasan, A., Carey, R. B., Carmeli, Y., Falagas, M. E., Giske, C. G., Harbarth, S., Hindler, J. F., Kahlmeter, G., Olsson-Liljequist, B., Paterson, D. L., Rice, L. B., Stelling, J., Struelens, M. J., Vatopoulos, A., Weber, J. T., & Monnet, D. L. (2012). Multidrug-resistant, extensively drug-resistant and pan drug resistant bacteria: An international expert proposal for interim standard definitions for acquired resistance. *Clinical Microbiology and Infection, 18*, 268–281. https://doi.org/10.1111/j.1469-0691.2011.03570.x

Martinez, J. L. (2009). Environmental pollution by antibiotics and by antibiotic resistance determinants. *Environmental Pollution, 157*, 2893–2902.

Meyer, E., Schwab, F., Schroeren-Boersch, B., & Gastmeier, P. (2010). Dramatic increase of third-generation cephalosporin-resistant *E. coli* in German intensive care units: Secular trends in antibiotic drug use and bacterial resistance, 2001 to 2008. *Critical Care, 14*, R113. https://doi.org/10.1186/cc9062

Nelson, D. W., Moore, J. E., & Rao, J. R. (2019). Antimicrobial resistance (AMR): Significance to food quality and safety. *Food Quality and Safety, 3*, 15–22. https://doi.org/10.1093/fqsafe/fyz003

Nordmann, P., Poirel, L., Toleman, M. A., & Walsh, T. R. (2011). Does broad-spectrum β-lactam resistance due to NDM-1 herald the end of the antibiotic era for treatment of infections caused by gram-negative bacteria? *Journal of Antimicrobial Chemotherapy, 66*, 689–692.

OIE. (2015). *OIE standards, guidelines and resolution on antimicrobial resistance and the use of antimicrobial agents* (pp. 1–66). World Organisation for Animal Health.

Owens, R. C., Jr. (2008). Antimicrobial stewardship: Concepts and strategies in the 21st century. *Diagnostic Microbiology and Infections Diseases, 61*, 110–128.

Palmer, A. C., & Kishony, R. (2013). Understanding, predicting and manipulating the genotypic evolution of antibiotic resistance. *Nature Reviews Genetics, 14*, 243–248.

Parmley, J., Leung, Z., Leger, D., Finley, R., Irwin, R., Pintar, K., Pollari, F., Reid-Smith, R., Waltner-Toews, D., Karmali, M., & Engelhardt, R. (2012). One health and food safety—The Canadian experience: A holistic approach toward enteric bacterial pathogens and antimicrobial resistance surveillance. In E. R. Choffnes, D. A. Relman, L. Olsen, R. Hutton, & A. Mack (Eds.), *Improving food safety through a one health approach* (pp. 176–188). National Academy Press.

Pitout, J. D., & Laupland, K. B. (2008). Extended-spectrum β-lactamase-producing Enterobacteriaceae: An emerging public-health concern. *Lancet Infectious Diseases, 8*, 159–166.

Prestinaci, F., Pezzotti, P., & Pantosti, A. (2015). Antimicrobial resistance: A global multifaceted phenomenon. *Pathogens and Global Health, 109*(7), 303–318.

Rahal, J. J., Urban, C., & Segal-Maurer, S. (2002). Nosocomial antibiotic resistance in multiple gram-negative species: Experience at one hospital with squeezing the resistance balloon at multiple sites. *Clinical Infectious Diseases, 34*(4), 499–503.

Robinson, C. J., & Young, V. B. (2010). Antibiotic administration alters the community structure of the gastrointestinal micobiota. *Gut Microbes, 1*, 279–284.

Robinson, T. P., Bu, D. P., Carrique-Mas, J., Fèvre, E. M., Gilbert, M., Grace, D., Hay, S. I., Jiwakanon, J., Kakkar, M., Kariuki, S., Laxminarayan, R., Lubroth, J., Magnusson, U., Thi Ngoc, P., Van Boeckel, T. P., & Woolhouse, M. E. J. (2016). Opinion paper: Antibiotic

resistance: Mitigation opportunities in livestock sector development. *Animal, 11*, 1–3., The Animal Consortium. https://doi.org/10.1017/S1751731116001828

Roe, M. T., & Pillai, S. D. (2003). Monitoring and identifying antibiotic resistance mechanisms in bacteria. *Poultry Science, 82*, 622–626.

Rossolini, G. M., Mantengoli, E., Docquier, J. D., Musmanno, R. A., & Coratza, G. (2007). Epidemiology of infections caused by multi-resistant gram-negatives: ESBLs, MBLs, pan-resistant strains. *The New Microbiologica: Official Journal of the Italian Society for Medical Virology (SIVIM), 30*(3), 332–339.

Roura, E., Homedes, J., & Klasing, K. C. (1992). Prevention of immunologic stress contributes to the growth-permitting ability of dietary antibiotics in chicks. *Journal of Nutrition, 122*, 2383–2390.

Shah, S. Q., Colquhoun, D. J., Nikuli, H. L., & Sorum, H. (2012). Prevalence of antibiotic resistance genes in the bacterial flora of integrated fish farming environments of Pakistan and Tanzania. *Environmental Science & Technology, 46*, 8672–8679.

Silbergeld, E. K., Graham, J., & Price, L. B. (2008). Industrial food animal production, antimicrobial resistance, and human health. *Annual Review of Public Health, 29*, 151–169.

Silva, C., Calva, E., & Maloy, S. (2014). One health and food-borne disease: Salmonella transmission between humans, animals, and plants. In R. M. Atlas & S. Maloy (Eds.), *One health: People, animals, and the environment* (pp. 137–148). American Society for Microbiology.

Spellberg, B., Guidos, R., Gilbert, D., Bradley, J., Boucher, H. W., Scheld, W. M., Bartlett, J. G., Edwards, J., Jr., & Infectious Diseases Society of America. (2008). The epidemic of antibiotic-resistant infections: A call to action for the medical community from the Infectious Diseases Society of America'. *Clinical Infectious Diseases, 46*, 155–164.

Stamey, T. A. (1976). Resistance to nalidixic acid: A misconception due to underdosage. *Journal of American Microbiological Association, 236*(16), 1857–1860. https://doi.org/10.1001/jama.1976.03270170023019

Tack, D. M., Marder, E. P., & Griffin, P. M. (2019). Preliminary incidence and trends of infections with pathogens transmitted commonly through food — Foodborne Diseases Active Surveillance Network, 10 U.S. Sites, 2015–2018. *MMWR. Morbidity and Mortality Weekly Report, 68*, 369–373.

USDA. (2014). *Antimicrobial resistance ACTION PLAN*. United States Department of Agriculture, 26p.

Van Boeckel, T. P., Glennon, E. E., Chen, D., Gilbert, M., Robinson, T. P., Grenfell, B. T., Levin, S. A., Bonhoeffer, S., & Laxminarayan, R. (2017). Reducing antimicrobial use in food animals. *Science, 357*(6358), 1350–1352.

WHO. (2000). WHO global principles for the containment of antimicrobial resistance in animals intended for food, Report of a WHO Consultation with the participation of the Food and Agriculture Organization of the United Nations and the Office International des Epizooties, Geneva, Switzerland 5–9 June 2000, 27p.

WHO. (2011). *World Health Organization. Tackling antibiotic resistance from a food safety perspective in Europe*. WHO, Europe.

WHO. (2014). *Antimicrobial resistance: Global report on surveillance*. World Health Organization.

WHO. (2015). *World Health Organization. Global action plan on antimicrobial resistance*. Online https://apps.who.int/iris/bitstream/handle/10665/193736/9789241509763_eng.pdf?sequence=1%26isAllowed=y (Accessed on 15 May 2017).

The Role of Vaccines in Combating Antimicrobial Resistance

Nagendra R. Hegde

Contents

Abstract

Besides sanitation, vaccines and antimicrobials (AMs) have been central to improvements in public health. However, antimicrobial resistance (AMR) appears to have been hastened by the introduction of antibiotics and other AMs. AMR has now been recognized as one of the major paradoxes, where unrestricted use is at odds with dire requirements, necessitating the global community to act at all levels through inter-sectoral collaboration in order to identify and implement concerted action. Among the many facets of managing AMR is the use of vaccines. Available evidence indicates that vaccines against bacterial pathogens have immense benefit in controlling the disease and reducing the individual- as well as population-level load/carriage of AMR pathogens. In addition, vaccines against certain viral diseases can reduce secondary bacterial infections and consequently the prescription and use of antibiotics. The combined benefit of these and other effects are a reduction in the levels of AMR. However,

N. R. Hegde (✉)
National Institute of Animal Biotechnology, Hyderabad, India
e-mail: hegde@niab.org.in

M. P. Mothadaka et al. (eds.), *Handbook on Antimicrobial Resistance*,
https://doi.org/10.1007/978-981-19-9279-7_40

there are still obstacles in the form of pathogen variation (serotypes and strains which may already exist or arise new), lack of clarity and understanding on how many pathogens we should target (since AMR can be horizontally transferred), a fraction of the populace being against any vaccination, and need for implementation of a holistic approach to place a value on the impact. Ultimately, vaccines will have to be used prudently along with rational use of antibiotics, and other methods to mitigate AMR, rather than advocating any one approach alone to combat AMR.

Keywords

AMR · Vaccines · Bacterial pathogens · Antibiotics

Abbreviations

ABR	Antibiotic resistance
AIDS	Acquired immunodeficiency syndrome
AM	Antimicrobial
AMR	Antimicrobial resistance
AMU	Antimicrobial use
AOM	Acute otitis media
BHV-1	Bovine herpesvirus 1
BRD	Bovine respiratory disease
DALYs	Disability-adjusted life years
ESKAPE (pathogens)	*Enterococcus faecium*, *S. aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, *Enterobacter* spp.
GBS	Group B *Streptococcus*
Hib	*Haemophilus influenzae* type b
IB	Infectious bronchitis
IPD	Invasive pneumococcal disease
MDR	Multidrug resistance/resistant
Mtb	*Mycobacterium tuberculosis*
ND	Newcastle disease
NP	Nasopharynx/nasopharyngeal
NS	Non-susceptible
NVT	Non-vaccine type
PCV	Pneumococcal conjugate vaccine
PRRS	Porcine reproductive and respiratory syndrome
PD	Pneumococcal disease
PPV	Pneumococcal polysaccharide vaccine
R	Resistance
VT	Vaccine type

1 Introduction

1.1 Role of Vaccines in Infectious Diseases

The control of infections and diseases caused by bacteria, viruses, fungi, protozoa, and parasites has been a chief focus of public health interventions for more than a century. The fact that chronic and metabolic diseases have only recently been recognized in alarming proportions is a testament to the fact that infectious diseases were the major contributing factors to healthcare before lifestyle-related diseases were even considered as serious threats.

Three major contributions in healthcare intervention, namely, cleanliness and sanitation, vaccines, and antibiotics, have had tremendous impact on preventing infections. However, the expanse to which these interventions, either individually or combinatorially, have had socioeconomic impact beyond just reducing morbidity or mortality is only beginning to be understood in recent years. These have implications for rationalizing health services, both in the hospitals and in the community, and to work towards a One Health approach at the interphase of humans, animals, pathogens, and the environment.

The practice of vaccination has existed since the tenth century in one form or another but gathered a greater importance during the early and late eighteenth century. Further developments during the late nineteenth century, followed by the birth of immunology, led to immunization being adopted as one of the cornerstones of public health interventions. Vaccination is probably the sole intervention capable of eradicating a disease and combating global threats due to infectious diseases. Vaccines have played a major role in the eradication of small pox in humans and rinderpest in animals, not to mention that regional control has been achieved for more than a dozen other human diseases, including major bacterial (diphtheria, *Haemophilus influenzae* type b-associated disease, pertussis, tetanus, typhoid) and viral (hepatitis B, measles, mumps, polio, rabies, rotavirus gastroenteritis, rubella, yellow fever) diseases and a few animal diseases such as foot-and-mouth disease and peste des petits ruminants (Buczkowski et al. 2014; Greenwood 2014; Plotkin et al. 2017; Rappuoli et al. 2011). In addition, the last two to three decades have witnessed a geometric rise in the number of vaccines being developed and deployed.

Vaccines were originally developed to save lives. In the last few years however, various other factors such as the burden and consequences (complications, sequelae) of infectious diseases, shift in demographics to urban population, aging, emerging and re-emerging diseases, increased morbidity and hospitalization, advocacy, larger understanding of the economic impact, and the capacity for manufacturing and distribution of vaccines have all driven the widespread use of vaccines. However, in recent times, the overall ramifications of vaccination beyond simply preventing ill health is being better appreciated by factoring in proxy outcomes such as disability or quality adjusted life years, besides averting less conspicuous disease outcomes, hospital visits or hospitalizations, medical expenses or care costs, etc. (Thomas et al. 2019; Walker et al. 2010). A number of reports and reviews have revealed that similar to sanitation, vaccines provide huge imperceptible benefits to public health.

1.2 Antimicrobials and Antimicrobial Resistance (AMR)

Bacteria have existed for millions, possibly billions, of years. Bacteria and certain other microorganisms are not only pathogens or simple commensals, but have a deeper association and interaction with animals and humans, influencing health and disease in multiple ways.

Most bacteria are extracellular organisms and are hence amenable to treatment with drugs which do not need to enter host cells. Any compound which can either kill or stop the growth of a microbe can be classified under the broad definition of an antimicrobial (AM) and may include compounds against bacteria, viruses, protozoa, or fungi. However, in a narrower context, AMs typically refer to antibiotics and antibacterial agents.

Antibiotics have played a key role in combatting life-threatening bacterial infections, saving lives, improving health and enhancing wealth. The tryst of human medicine with antibiotics started with the discovery of penicillin by Alexander Fleming in 1928, although anti-infective effects of antiseptics, disinfectants, and various plant and animal products as well as fungi have been documented earlier (Gould 2016). Since then, a number of antibiotics belonging to various groups, classified mainly on chemical structure, have been discovered and chemically synthesized. In addition, AMs include nonantibiotic chemotherapeutic agents such as sulfonamides and pyrimidine antifolates. AMs target certain biochemical processes, resulting in stasis or lysis of bacterial cells, through the inhibition of the synthesis or assembly of cell wall (penicillins and cephalosporins), disruption of the cell membrane (polymyxins, lipopeptides), and inhibition of protein synthesis (macrolides, lincosamides, tetracyclines, glycylcyclines, oxazolidinones), enzymes essential for bacterial genome replication (rifamycins, quinolones), or folate synthesis (sulfonamides) (Davies and Davies 2010). A majority of the marketed antibiotics over the years were developed in the first 25–30 years, followed by a gap of about 30 years of lean period, until a handful of new classes have been discovered recently.

In most cases, the deployment of AMs has been followed almost immediately by observations of resistance to the antibiotic introduced. Antimicrobial resistance (AMR) can be intrinsic or acquired. Intrinsic resistance may be due to impermeability of the bacterial membrane to a particular substance, absence of drug targets in the bacterial cell, or other properties. On the other hand, acquired resistance is typically due to mutation(s) which are stably transferred over generations through vertical transfer from mother to daughter cells or through horizontal transfer of extrachromosomal elements between bacteria (Alekshun and Levy 2007; Blair et al. 2015; Chernova et al. 2021; Christaki et al. 2020); the resultant AMR is probably a consequence of an evolutionary process of survival of the fittest on a continuous basis. Such mutations have predated antibiotic discovery (although not necessarily the existence of AM properties of other biotic species), as evidenced by the observation that genes encoding resistance to tetracycline and glycopeptide antibiotics were detected in plant and animal permafrost samples dating back 30,000 years ago (D'Costa et al. 2011). Whether the resistance is intrinsic or acquired, the result is a restricted penetration or efflux of the drug, destruction or modification of the

antibiotic, switching or sequestration of the target, or modification of the target site. Some of the acquired resistance can be adaptive, where the organisms may transiently and reversibly modulate gene expression through epigenetic modifications, following specific environmental cues, including the use of AMs (Fernandez et al. 2011; Lee 2019; Motta et al. 2015; zur Wiesch et al. 2011). In any case, evolution of AMR is a major impediment for controlling bacterial diseases.

Antimicrobials are widely used indiscriminately (Morgan et al. 2011; Ocan et al. 2015), and antimicrobial use (AMU) is thought to be a major contributing factor for the emergence of, and increase in, AMR. A strong correlation between AMU and subsequent development of AMR has been noted at the level of hospital wards, communities, populations, and countries (Davies and Davies 2010; Bronzwaer et al. 2002; van de Sande-Bruinsma et al. 2008). Based on survey of prescriptions about two decades ago, each person in the USA was estimated to consume AM for 22% of the year (Gums 2002). Besides inappropriate consumption in humans, the use of AMs in agriculture (e.g., use of streptomycin) and animal husbandry (e.g., for growth promotion) and the contamination of groundwater with residues from active pharmaceutical ingredients (ECDC/EFSA/EMA 2017; Goossens 2009) all add to the emergence and sustenance of AMR. Enormous amounts of AMs are used in livestock and poultry production, besides also being used during the production of fruits and vegetables (McManus et al. 2002). AMU in animal production is projected to further increase geometrically (Van Boeckel et al. 2019). Not too long ago, about two-thirds of antibiotic usage in veterinary medicine was for nontherapeutic and growth promotion purposes (Anderson et al. 2003; Anthony et al. 2001; Cabello 2006), where the use of subtherapeutic doses is frequent. Incidentally, the use of low concentrations of AMs has been correlated with emergence of AMR (Alexander et al. 2011; Gullberg et al. 2011). As more than a third of the pathogens are shared between humans and animals, both of whom share overlapping environments, the situation could lead to potential seamless transmission of AMR across species and national borders.

Over the years, the frequency of AMR strains has increased. In fact, nosocomial infections with multidrug-resistant (MDR) bacteria are one of the top ten leading causes of mortality worldwide (Arias and Murray 2009; Vincent et al. 2009). It is estimated that currently 700,000 die as a consequence of AMR, with varying rates of infections and fatalities in different regions of the world (Anon 2016); by 2050, annual fatalities could reach 7 million globally, with a projected consolidated cost of USD 100 trillion if the problem is left untackled (Anon 2016; Naylor et al. 2018). A recently published study on the burden of AMR in terms of deaths and disability-adjusted life years (DALYs) associated with and attributable to 23 bacterial pathogens and 88 pathogen-drug combinations in 204 countries and territories in 2019 estimated that (a) 4.95 million deaths were associated with AMR, with 72% of them being accounted for by 6 pathogens and 30% being accounted for by lower respiratory infections, (b) 25.65% of the deaths associated with AMR were attributable to bacterial AMR, (c) 8%–14% of the deaths were attributable to specific pathogen-drug combinations, and (d) deaths were higher in sub-Saharan Africa (Antimicrobial Resistance Consortium 2022). Antibiotic use, including overuse, abuse, and misuse,

thus has adverse health and economic impacts. The rise in AMR has increased the morbidity and mortality frequencies and rates, length of hospital stays, cost of care, the use of increasingly costly AMs which have serious side effects, and therapeutic failures, as well as cost of care for impending medical conditions such as cancer therapy, organ transplantation, and major surgeries, thus escalating the overall healthcare costs (Gums 2002; Cassini et al. 2019; Cosgrove and Carmeli 2003; Friedman et al. 2016). The economic burden of AMR could set the world GDP back by 2%–3.5% by 2050 (KPMG LLP 2014; Taylor et al. 2014). There is therefore an urgent need to tackle AMR, through a variety of approaches, one of which is the discovery, development, and deployment of vaccines.

2 Evidence for the Role of Vaccines in Combating AMR

2.1 Vaccines for Diseases Caused by *Haemophilus influenzae*

Haemophilus influenzae is an opportunistic pathogen which causes secondary bacterial diseases such as epiglottitis, pneumonia, bacteremia, meningitis, and in some cases bone and joint infections, mostly in infants and young children. Of the six types, which are differentiated based on the capsular polysaccharide, type b (Hib) is the most pervasive. An effective Hib vaccine was developed and introduced in the 1980s and used in immunization programs since the early 1990s for children below the age of five years, initially as a monovalent vaccine but now available in combination with vaccines against diphtheria, pertussis, tetanus, and hepatitis B. While the first-generation Hib vaccines contained only the polysaccharide, the second-generation vaccines contain the polysaccharide conjugated to a protein (Barbour 1996). The vaccine has been successful in reducing bacterial colonization, disease incidence, and purchase of antimicrobials for outpatients (Gounder and Hennessy 2014; Murphy et al. 1993; Palmu et al. 2014). Some replacement in serotype of circulating strains has been observed, with not much change in antibiotic resistance over time (Lipsitch 1999; Reilly et al. 2022; Whittaker et al. 2017), making the Hib vaccine one of the success stories in prevention of infectious diseases. Although data is available on the AMR of *H. influenzae* (Tristram et al. 2007), not sufficient data is available to appreciate the contribution of Hib vaccine in the reduction of AMR, albeit it cannot be ignored.

2.2 Vaccines for Diseases Caused by *Streptococcus pneumoniae*

Pneumococcus (*Streptococcus pneumoniae*) is the poster child of evidence for the role of vaccines in combating AMR. It is the most common cause of bacterial infection in children under 3 years of age, presenting as multiple clinical manifestations such as sinusitis, pneumonia, otitis, meningitis, empyema, and bacteremia, with a high rate of morbidity and mortality in young children. An estimated 15 million cases of invasive pneumococcal disease (IPD) and 1 million deaths

(amounting to ~11% of all-cause mortality) occur in children less than 5 years of age, mostly in developing countries (Wahl et al. 2018). Pneumococcal meningitis is equally fatal, with high rates of neurological sequelae or hearing loss (Schuchat et al. 1997). Following the introduction of the Hib vaccine, *S. pneumoniae* emerged as the most common cause of meningitis.

The organism *S. pneumoniae* colonizes the nasopharynx during the first 2 years of life and can be carried as a commensal by persons of all ages. The bacterium exists as about 100 serotypes, which are differentiable based on the composition of the capsular polysaccharide. Even though there is a wide spatial, temporal, demographic, and socioeconomic variation in the incidence, carriage, serotype distribution, and AMR, only a limited number of serotypes appear to be associated with the disease, especially in pediatric subjects (Hackel et al. 2013; Hausdorff 2002; Hausdorff et al. 2000; Jauneikaite et al. 2012; Linares et al. 2010; Lynch 3rd and Zhanel 2010; Sniadack et al. 1995; Song et al. 2012). Nasopharyngeal (NP) carriage of pneumococcus reflects the serotypes circulating and causing disease in a community (Bogaert et al. 2004a, b; Gray et al. 1980). *S. pneumoniae* is carried in proportions higher than 10% in children younger than 5 years, asymptomatically by children in childcare centers and schools and their parents, and is a major reason for transmission among communities (Bogaert et al. 2004a, b; Leiberman et al. 1999; O'Brien et al. 2009). The proportion of pneumococcal isolates with AMR as well as rates of respiratory and systemic disease is reflected by their NP carriage, which increases from infancy to toddlerhood, especially among those attending childcare (Bogaert et al. 2004a; Leiberman et al. 1999; Simell et al. 2012).

Resistance of pneumococci to antibiotics was first reported in the mid-1960s. The greatest source of resistance is children, as they carry the bacteria more often, for longer periods than adults, are more likely to be in crowded areas and in contact, and have a greater likelihood for the use of antibiotics (Bogaert et al. 2004a; Leiberman et al. 1999; Simell et al. 2012). Dramatic increase in non-susceptibility to penicillin, the historical drug of choice against pneumococcus, was observed all over the world, with a parallel dramatic increase in the proportion of MDR strains (Butler et al. 1998; Fenoll et al. 2009; Hoban et al. 2001; Low 2005; Song et al. 2004). The pattern of AMR varies among age of the carriers, pneumococcal serotypes, and geographic locations and is influenced by the levels of crowding in schools as well as prescription and use of antibiotics, even within the same serotypes (Bronzwaer et al. 2002; van de Sande-Bruinsma et al. 2008; Goossens 2009; Fenoll et al. 2009; Diekema et al. 2000; Farrell et al. 2007; Goossens et al. 2005; Song et al. 1999; Van Eldere et al. 2007). A high proportion of resistance to penicillin and other antibiotics was observed in several Asian countries, particularly in the Orient, with progressive increase over several years since the early 1980s (Song et al. 1999; Jaiswal et al. 2014; Kim et al. 2012). A higher rate of resistance has been shown to be associated with increased hospitalizations, poor clinical outcomes, and additional costs (Naylor et al. 2018; Friedman et al. 2016; Reynolds et al. 2014). However, efforts to decrease AMU have shown conflicting outcomes with no change as well as positive effects (Fenoll et al. 2009; Cohen et al. 2006; Hicks et al. 2011; Katsarolis et al. 2009).

Pneumococcal vaccine development was based on epidemiological data of the prevalence of the serotypes as well as their AMR phenotype (Hausdorff 2002; Feikin and Klugman 2002). Early vaccines, which were the whole-cell inactivated type and introduced in 1911, were followed by the second-generation vaccines containing polysaccharides from multiple serotypes. Pneumococcal polysaccharide vaccines (PPVs) were available for several years, and a composition containing polysaccharides from 23 serotypes (PPV23) was introduced in 1983. PPV23 reduced IPD in adults and the general population, more so in low-income than high-income countries (Moberley et al. 2013). However, these vaccines elicit short-term antibody responses without much immunological memory and could not be administered to young children. Later, protein-conjugated polysaccharides were developed as vaccines. Five, seven, ten, thirteen, or twenty-one serotypes have been targeted to develop PCV4 (pneumococcal conjugate vaccine 4-valent), PCV5, PCV7, PCV10, PCV13, or PCV21, respectively. PCVs have been available commercially for more than two decades and have been used in children in more than 120 countries and in adults in more than 100 countries. The serotypes covered or not covered by vaccine are typically designated as vaccine-type (VT) and non-vaccine-type (NVT) strains, respectively.

Several studies showed that the PCVs reduced (a) pneumonia and invasive disease overall as well as that associated with antibiotic-non-susceptible strains; (b) hospitalization and complications due to all-cause pneumonia; (c) prevalence of AMR and MDR pneumococci among vaccines as well as their unvaccinated contacts, including adults; (d) NP carriage of VT serotypes and associated AMR phenotype; (e) healthcare utilization, including visits to clinics and hospitals, and use of emergency service; (f) overall morbidity and mortality; and (g) antibiotic prescription and use, beyond that already reduced following Hib vaccination (Linares et al. 2010; Azzari and Resti 2008; Black et al. 2001; Bonten et al. 2015; Cohen et al. 2017; Dagan and Klugman 2008; Grabenstein and Weber 2014; Hammitt et al. 2006; Hampton et al. 2012; Harboe et al. 2010a; Klugman and Black 2018; Leibovitz 2008; O'Brien et al. 2003; Reinert 2004; Shah and Ratner 2006; Tin Tin Htar et al. 2013; Weil-Olivier et al. 2012; Weinberger et al. 2011; Wilby and Werry 2012). Vaccination has been observed to provide collateral benefit to unvaccinated children, adults, and the elderly, even without a mandated national immunization program, owing to a herd effect and a consequent decreased transmission among the population (Davis et al. 2013; Harboe et al. 2010b; Kellner et al. 2005; McBean et al. 2005; O'Brien and Dagan 2003; Whitney et al. 2003). On the other hand, the proportion and types of resistance have been observed to be higher in individuals who have been partially vaccinated or where follow-up doses have been delayed (Ozdemir et al. 2014).

Population-level change in serotype distribution, commonly referred to as serotype replacement, that is, decrease in VT and a parallel increase in NVT, has been commonly observed following vaccination, with a rebound in the proportion of overall carriage as well as non-susceptibility in healthy populations, owing to increase in frequency of NVT strains, and an associated variable invasiveness, and sometimes reduced vaccine efficacy (Song et al. 2012; Dagan and Klugman 2008;

Harboe et al. 2010a; Leibovitz 2008; Weinberger et al. 2011; Whitney et al. 2003; Cohen 2009; Dagan 2009; Hanquet et al. 2010; Kaur et al. 2016; Ortqvist et al. 2005; Spratt and Greenwood 2000; Tin Tin Htar et al. 2015). Since carriage is highest in young children and the most resistant strains belong to VT, the most remarkable effect of vaccination was typically observed with VT in young children; a concomitant rise in carriage of NVT was also observed (Song et al. 2012; Low 2005; Harboe et al. 2010a; Ginsburg and Klugman 2017). The reduction in VT carriage has been proposed to be due to elimination of susceptible strains and expansion of pre-existing NVT strains and prevention of new acquisitions rather than termination of existing carriage episodes or change in proportion of individuals with bacterial colonization (Beall et al. 2006; Hanage 2007; Pillai et al. 2009). However, nonconformities such as no clear effect on NVT, reduction in non-susceptibility of VT to certain antibiotics, or no increase in NVT have been observed in a few cases (Davies et al. 2008; Link-Gelles et al. 2013; Moore et al. 2004; Pelton et al. 2004; Tomczyk et al. 2016; Whitney et al. 2000), possibly due to the increase in NVT or other inexplicable, dynamic changes in the proportion of carriage of bacteria at the host population level.

Data show that the use of 23-valent PPVs, the non-coverage of serotypes by PCV7, and the subsequent coverage by PCV10 and PCV13 were effective in further reducing infection, disease, dissemination, hospitalization, and mortality as well as ABR types and NP carriage, particularly of VT strains, although no change in resistance to some antibiotics or NP carriage and no effect or increased disease owing to resistant, often NVT, strains have been reported (Kim et al. 2012, 2016; Dagan and Klugman 2008; Kaur et al. 2016; Tin Tin Htar et al. 2015, 2019; Andrews et al. 2014; Savulescu et al. 2017; Stanek et al. 2016).

One of the most common childhood diseases is acute otitis media (AOM), which is typically due to a viral infection, followed by a secondary bacterial infection. *S. pyogenes* was the most common culprit in the early twentieth century, but *S. pneumoniae* and *Haemophilus influenzae* now make up 80% of the cases (Ruuskanen and Heikkinen 1994; Yatsyshina et al. 2016). Immunization with the Hib vaccine precipitously reduced invasive disease as well as AOM (Reilly et al. 2022; Whittaker et al. 2017), through a highly significant herd effect by reducing bacterial colonization and carriage in vaccinated and unvaccinated individuals (Barbour 1996; Peltola et al. 1999). There was a reduction in the number of AOM episodes as well as drug-resistance strains and AMR, with a speculated reduction in filling prescriptions and outpatient antibiotic purchases (Peltola et al. 1999; Adam et al. 2010; Eythorsson et al. 2018a, b). As far as *S. pneumoniae* is concerned, again, there is a strong association between NP carriage and serotype distribution of AMR strains in AOM (Lynch 3rd and Zhanel 2010; Dagan 2000; Dagan et al. 2000; Kempf et al. 2015). PCV7 and PCV13 have been shown to be efficacious against all-cause AOM, reducing episodes, particularly relating to VT serotypes, as well as decreasing tympanostomies and visits to the emergency department, consequently resulting in reduced use of antibiotics and decline in AMR, although episodes due to NVT were reported (Tin Tin Htar et al. 2019; Casey and Pichichero 2004; Cohen et al. 2015; Dagan 2003; Marom et al. 2017; Palmu et al. 2018; Poehling et al. 2007; Taylor et al.

2012). As expected, a parallel increase in NVTs and *H. influenzae* f has been observed (Casey et al. 2010; Dupont et al. 2010; Martin et al. 2014; Ozawa et al. 2015; Shea et al. 2011; Stamboulidis et al. 2011). Like with IPD, carriage remained similar, but the proportion of AOM decreased, possibly due to decreased burden or a lower potential of certain VTs and NVTs.

Overall, PCVs have been very impactful in reducing vaccine as well as all-type IPD, AOM, and meningitis in vaccine-eligible and vaccine-ineligible children, with a decline in associated hospitalization rates and mortality, a decrease in adult infections, and a reduction in AM prescription, purchase, and use. NP carriage, which can be a surrogate measure of the effectiveness of vaccines in the community, and AMR are also reduced for VT, whereas overall carriage is not affected. Modelling and other studies have shown that PCVs could ultimately lead to a balance between susceptible and resistant bacteria, as well as an increase in fitness through other means (Albarracin Orio et al. 2011; Andersson and Hughes 2010; Lehtinen et al. 2017; Lenski 1998; Maher et al. 2012; Obolski et al. 2018; Rozen et al. 2007; Trzcinski et al. 2006). Emergence of NVT and other virulence determinants may erode vaccine impact over time, and vaccine formulations may need to address the changing epidemiology and newly emerging serotypes, particularly those that are resistant to currently used antibiotics (Klugman and Black 2018). It is, however, difficult to exactly pinpoint if serotype switch or emergence of NVT is a consequence exclusively of vaccination; other factors such as spatiotemporal distribution of serotypes, natural evolution of bacteria, independent selection pressure as a result of AMU, etc. could also contribute to serotype switch (Andam and Hanage 2015; Croucher et al. 2014; de Celles et al. 2015).

In terms of antibiotic prescriptions and consumption, overall reductions have been observed as a consequence of PCVs in several cases (Dagan and Klugman 2008; Wilby and Werry 2012; Cohen 2009; Kaltoft and Zeuthen 2000; Lee et al. 2014), although reports to the contrary also exist (Sa-Leao et al. 2009). Specifically, PCV13 has been estimated to have avoided 11.4 million days of ABU per year in children under 5 years of age (Kingwell 2018) and to have decreased antibiotic usage by about 47% (Makri 2019). For AOM, as only a fraction of the cases require antibiotic treatment, stricter guidelines have been shown to decrease AMU (American Academy of Pediatrics Subcommittee on Management of Acute Otitis Media 2004; Sabuncu et al. 2009). However, efforts to decrease AMU have resulted in conflicting outcomes with positive, negative, or no effect observed in different parts of the world (Fenoll et al. 2009; Cohen et al. 2006; Hicks et al. 2011; Katsarolis et al. 2009; Dagan et al. 2009; Nilsson and Laurell 2006; van Gils et al. 2009; Veenhoven et al. 2003).

A major problem with *S. pneumoniae* has been the existence of close to 100 serotypes and hence the requirement for multivalency of the vaccine. Using few serotypes in the vaccine does not negate capsular-type switch or subsequent increase on NVTs. On the other hand, it is impractical to include antigens for all the serotypes (Bagnoli et al. 2011). One way is to target the major types that are present in particular geographical locations, although this requires extensive and continuous epidemiological data and surveillance. The other way is to target conserved proteins

as antigens, an approach which is gaining much traction (Bagnoli et al. 2011; Buchy et al. 2020; Malley and Anderson 2012). Alternatively, the most resistant strains could be targeted for vaccine development, driving the emergence of susceptible strains, which can then be taken care of by the use of antibiotics (de Celles et al. 2015; Buchy et al. 2020; Tekle et al. 2012). However, modelling studies show that even a combination of vaccine and therapy may not eliminate both disease and pathogen colonization (Tekle et al. 2012).

2.3 Vaccines for Typhoid

Typhoid, caused by related serovars belonging to *Salmonella enterica* subspecies *enterica*, is a mostly waterborne disease. It is a serious burden to low- and middle-income countries, particularly those in Asia and sub-Saharan Africa. A higher mortality due to typhoid is associated with resistance to multiple antibiotics (Antillon et al. 2017a, b; Bhutta 1996; Pieters et al. 2018). Although typhoid accounts for only a minority of febrile illnesses (Marks et al. 2017; Ochiai et al. 2008), heterogeneity in geographic, temporal, and disease manifestation poses challenges for timely diagnosis (Andrews et al. 2019). Overlapping symptoms with other diseases, especially viral diseases, and a lack of appropriate diagnosis, particularly owing to poor sensitivity of tests, lead to patients being treated empirically and frequently without confirmatory diagnosis (Andrews et al. 2018; D'Acremont et al. 2014; John et al. 2016; Mayxay et al. 2013; Mogasale et al. 2016). Based on conservative estimates, it has been projected that over 50 million cases of fever are being treated as suspected cases of typhoid (Andrews et al. 2019). Data from Asia show that for every true case of typhoid, 3–25 false cases are treated as typhoid (Marks et al. 2017; Ochiai et al. 2008; Andrews et al. 2018, 2019; John et al. 2016), leading to unnecessary use as well as overuse of antibiotics. It has been postulated that suspected typhoid may be one of the major drivers of AMU in the Indian subcontinent (Andrews et al. 2019; Laxminarayan et al. 2016). AMU against unconfirmed but suspected cases of typhoid not only doesn't have a clinical benefit but also could disrupt gut microbiome and increase AMR (Andrews et al. 2019).

The first-generation typhoid vaccines contained Vi capsular polysaccharide, but similar to the polysaccharide vaccines of *S. pneumoniae*, these had a moderate efficacy, poor immunological memory, and short duration of protection and could not be administered to young children, resulting in poor uptake in endemic settings. The second-generation typhoid vaccines, which consist of polysaccharides conjugated with proteins, are quite effective in reducing cases as well as averting mortality, but efficacy and effectiveness are variable depending on the disease burden, including the level of chronic carriage, the latter being inversely proportional to effectiveness of typhoid vaccines (Antillon et al. 2017a; Ochiai et al. 2008; Cook et al. 2008; Lo et al. 2018; Steele et al. 2016).

Not a lot of data is available on the effect of typhoid vaccine on AMR. Typhoid vaccines may reduce AMU, with a possible reduction in selection pressure as well as threat of AMR infections (Andrews et al. 2019). Modelling studies predict a decline

in incidence of infection and AMR cases as well as cases averted, with increasing vaccination coverage, although rebound infection rates as well as proportion of AMR cases are expected within a short period of 4–5 years (Kaufhold et al. 2019). The proportion of cases is predicted to be most influenced by the relative infectiousness or fitness of AMR strains, the transmission rate, the rate of emergence of resistance, the fraction of patients who are symptomatic and treated, the recovery rate from infection, the fraction of patients that become chronic carriers, and the consequent infectiousness of the strains, whereas vaccine efficacy and duration of immunity conferred by the vaccine may have no impact (Kaufhold et al. 2019; Pitzer et al. 2014). Vaccination is also expected to reduce the proportion of susceptible strains (Kaufhold et al. 2019). It must be noted that there is an increase in non-typhoidal *Salmonella* infections, with a substantial contribution of their transmission from animals to humans (Nair et al. 2018).

3 Other Bacterial Diseases

A few other bacterial vaccines are in use in humans. One of them is meningococcal vaccine, which targets the invasive meningococcal disease caused by *Neisseria meningitidis* (Rouphael and Stephens 2012; Soumahoro et al. 2021). The disease is a serious risk to infants and young children, with high fatalities as well as post-recovery sequelae (Pace and Pollard 2012). Penicillin was the drug of choice for treatment of meningococcal infections for a long period of time, but high levels of resistance have necessitated the use of higher antibiotics (Acevedo et al. 2019; Lin et al. 2021; Rostamian et al. 2022). However, despite a low incidence of the disease, resistance of the organism to β-lactamase and antibiotics such as fluoroquinolones has raised an alarm (Aye et al. 2020; Bai et al. 2019). Both polysaccharide and polysaccharide conjugate vaccines have been used to prevent meningococcal disease. According to the capsular polysaccharide, *N. meningitidis* can be classified into 12 serogroups, of which 6 (A, B, C, W, X, Y) are responsible for the major burden of disease globally (Bai et al. 2019). Although monovalent vaccines were initially used, emergence of other serotypes necessitated the development of multivalent vaccines containing antigens for up to four serotypes (A, C, W, Y), which have been shown to be effective in various settings (Bai et al. 2019; McMillan et al. 2022). Not much data is available on the effect of meningococcal vaccines in reducing AMR burden; any such studies must take into consideration the cost of the recommended treatment regimen (cephalosporins and fluoroquinolones), as well as the unintended consequences of increasing AMR in commensal and other pathogenic organisms.

Another bacterial disease is gonorrhea, the sexually transmitted disease (mainly cervicitis or urethritis but also pelvic inflammatory disease) caused by *Neisseria gonorrhoeae*, afflicting close to 90 million adults worldwide (Unemo et al. 2019). The disease has existed for a long time, and treatment have evolved from penicillin to ciprofloxacin to other antibiotics, but progressively resistant strains have emerged over time (Mlynarczyk-Bonikowska et al. 2020; Suay-Garcia and Perez-Gracia 2017). There is no vaccine available specifically for gonorrhea as of now. However,

meningococcal vaccine has recently been found to reduce rates of gonorrhea as much as 30% (Azze 2019; Paynter et al. 2019). Modelling studies have indicated that even with modest efficacy, gonorrhea vaccines could have a significant impact on AMU (Craig et al. 2015).

Pertussis (whooping cough), caused by *Bordetella pertussis*, is one of the leading ten causes of childhood mortality and is considered as a re-emerging disease (Stefanelli 2019). It is a severe respiratory disease, especially of children. Trimethoprim-sulfamethoxazole and macrolides have been commonly used to treat pertussis (Cimolai 2021; von Konig 2005), but erythromycin-resistant strains have been on the rise and causing epidemics in various places (Yao et al. 2020). Vaccines have been available against pertussis but provide short-term protection (Wilkinson et al. 2021). On the other hand, studies on reduction in AMR due to pertussis vaccine are scanty.

Group B streptococcus (GBS), *Streptococcus agalactiae*, is responsible for a significant fraction of life-threatening invasive bacterial infections in newborns globally (Jansen and Anderson 2018; Seale et al. 2017). Neonatal sepsis, bacteremia, and meningitis as well as osteoarthritis and pneumonia have been associated with GBS. The organism is a common inhabitant of the genital and gastrointestinal tracts and causes infections perinatally (Melin and Efstratiou 2013). Owing to variations in the capsular polysaccharide, GBS exists as ten serotypes, whose proportions vary temporally and spatially as well as in disease manifestations (Melin and Efstratiou 2013; Bianchi-Jassir et al. 2020). As far as AMR is concerned, GBS is typically susceptible to penicillin as well as most β-lactams, but increasing resistance to macrolides and lincosamide has been observed (Melin and Efstratiou 2013). Intrapartum administration with β-lactam antibiotics is recommended based either on perceived risk or antenatal screening for colonization with GBS; however, this is not without drawbacks, one of the concerns being development of AMR (Melin and Efstratiou 2013). Vaccines against GBS are under late-stage development.

Vaccines for several other pathogens which are responsible for considerable amount of morbidity and AMR-related mortality are on the anvil and at various stages of development. The most important of them is *Staphylococcus aureus*, which is responsible for a large fraction of nosocomial as well as community infections, especially those associated with wounds and surgical sites. Methicillin and vancomycin resistance in *S. aureus* is of grave concern worldwide, and a multitude of efforts are ongoing to develop efficacious vaccines against *S. aureus* infections (Bagnoli et al. 2011, 2012; Schaffer and Lee 2009; Spellberg and Daum 2012). The second important pathogen is *Mycobacterium tuberculosis* (Mtb), which has the highest burden of infected individuals of any single infectious disease, and is responsible for worldwide deaths of about 700,000, among which a third are due to MDR Mtb (Buchy et al. 2020; Raviglione et al. 2012), not to mention the burden of Mtb in immunodeficiency disorders such as acquired immunodeficiency syndrome (AIDS). AMR in the case of Mtb is compounded by the organism undergoing latency and causing chronic infections and the unavailability of acceptable vaccines or lack of wide use of available vaccines (Atkins and Lipsitch 2018). Members of the Family *Enterobacteriaceae*, along with other ESKAPE pathogens (*Enterococcus*

faecium, *S. aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, *Enterobacter* spp.), also display high levels of MDR and are the cause of a high proportion of treatment failures in various clinical conditions (Manrique et al. 2022). Another important pathogen which is carried by healthy adults but asymptomatically colonizes hospital patients and can cause treatment failures is *Clostridium difficile* (Cohen et al. 2010). Other important pathogens include *Aeromonas* and *Shigella*. However, no vaccines are available for any of these pathogens, and the impact of vaccines on AMR due to them can only be examined once the vaccines are deployed. One issue with some of these bacteria is that they are also commensals and that vaccines against pathogenic strains may perturb the balance of beneficial microbiota. In such cases, it may be worthwhile developing vaccines by targeting only the virulence or resistance determinants (defined by the absence of such determinants in commensals) (Lipsitch and Siber 2016).

3.1 Vaccines for Viral Diseases

Viruses are intracellular pathogens and viral infections are not treatable with antibiotics. In addition, there are very few antivirals that are effective in controlling clinical viral diseases. However, some of the most common viral diseases are accompanied by secondary infections, most often with commensal bacteria. This viral-bacterial synergy in disease manifestation and aggravation is directly related to a large proportion of AMU worldwide, especially in developing countries. For example, respiratory syncytial virus and influenza virus infections increased AMU, both appropriately for secondary infections and inappropriately due to incorrect diagnosis in Canada (Kingwell 2018). A third of the AM prescriptions in ambulatory care in the USA have been estimated to be for viral infections (Lipsitch and Siber 2016). In developing countries, factors such as unhygienic conditions (and hence the fear of secondary infection), poor access to diagnostics, a lack of follow-up clinic/hospital visits by patients, weak recording system, patient demands and their fear of hospitalization costs, etc. often force clinicians to prescribe antibiotics for viral infections. It should be noted that vaccination for the primary pathogen (virus) is better than other measures to mitigate secondary bacterial infections (McGarry et al. 2013; Pilishvili and Bennett 2015). However, measuring the effect of antiviral vaccines in reducing AMU and mitigating AMR is difficult as the data is indirect. Some efforts have, however, been made in recent times, specifically in the case of influenza.

Influenza is one of the most common upper as well as lower respiratory tract infections and is frequently associated with secondary infection or co-infections, often due to *S. pneumoniae* (Chertow and Memoli 2013; Kash and Taubenberger 2015). About half of the deaths due to the 2009 pandemic influenza are estimated to have occurred in Asia and Africa, partly due to secondary bacterial infections (Kim et al. 2011; Reinert 2009). Influenza is known to predispose children to AOM, thus increasing AMU (Dbaibo et al. 2020).

As much as half of the outpatient AM prescriptions for upper respiratory tract infections are thought to be inappropriate or irrational in uncomplicated cases of influenza (Kronman et al. 2014; Misurski et al. 2011). This is a direct consequence of the uncertainty in clinical diagnosis, absence of follow-up confirmatory laboratory testing, and the fear of secondary complications (Misurski et al. 2011; Ghazi et al. 2016; Low 2008; Neuzil et al. 2000), not to mention the actual secondary comorbidity and/or complications, which are not uncommon, leading to antibiotic consumption (Low 2008; Kwong et al. 2009). Abuse and misuse of antibiotics in influenza infection have been documented in the USA (Fleming-Dutra et al. 2016; Grijalva et al. 2009).

Influenza vaccine has been shown to reduce the likelihood of secondary bacterial infections, including episodes and incidence of AOM (Norhayati et al. 2017; Principi et al. 2012), resulting in the decrease in inappropriate or appropriate AM prescription and use to treat influenza-associated respiratory disease (Kwong et al. 2009; Fleming-Dutra et al. 2016; Bridges et al. 2000). The mean number of antibiotic courses per person, the proportion of persons receiving antibiotics (including families and community contacts), duration of antibiotic use, antibiotic prescriptions per person-days, and the overall primary as well as secondary use of antibiotics have all been shown to be reduced, although no effect has been noted in a few cases (Kwong et al. 2009; Fleming-Dutra et al. 2016; Bridges et al. 2000; Allsup et al. 2003; Esposito et al. 2003; Hoberman et al. 2003; Jansen et al. 2008; Principi et al. 2003). Vaccine-related reduction in influenza disease appears to also reduce the selection pressure driven by antibiotic treatment (Misurski et al. 2011).

Data on the contribution of other viral vaccines to reduction in AM prescriptions and use, levels of AMR, and evolutionary pressure for selection of resistant bacterial strains are very few. Antibiotics are commonly used to prevent complications resulting from measles (Kabra and Lodha 2013), and measles vaccines can be envisaged to reduce antibiotic use, although this may not be the case in certain instances (Hansen et al. 2017). Reduction in AMR has also been reported with chicken pox (Bozzola et al. 2016), where *S. aureus* and group A streptococci are common secondary infective agents (Lesko et al. 2001; Peterson et al. 1996). Two diseases where bacterial infections and AMR are major issues are respiratory syncytial virus pneumonia and AIDS. However, no effective vaccines are yet available for both of these diseases, but it is certain that any vaccine against them will likely have a great impact on AMU and AMR.

3.2 Vaccines for Diseases of Animals

Modern livestock rearing for food production is intensive in nature, where animals or birds are housed in large numbers and often in densities that are unnatural. These conditions have led to the use of antimicrobials for therapeutic, prophylactic, or metaphylactic purposes. It is estimated that more than half of all AMU used globally is in large-scale, commercial, agricultural, and livestock rearing practices (Anon 2015; Oliver et al. 2011). Growing evidence suggests association between AMU in

livestock and AMR (Tang et al. 2017), which has led to several countries restricting the use of antimicrobials in animal production only for therapeutic purposes (Nunan 2022).

Besides bacterial diseases, viral-bacterial synergy is an important contributor to morbidity and mortality in intensive livestock farming. One such condition is bovine respiratory disease (BRD), where bovine herpesvirus 1 (BHV-1), bovine viral diarrhea virus, bovine parainfluenza virus type 3, and bovine respiratory syncytial virus could be involved individually or in combination. All these viruses can cause immune suppression, followed by secondary bacterial infections with *Mannheimia haemolytica*, *Histophilus somni*, *Pasteurella multocida*, *Moraxella bovis*, and even *Mycobacterium bovis* (Kao et al. 2007; Srikumaran et al. 2007). Other conditions include viral infections followed by contagious bovine/ovine/caprine pleuropneumonia. Similarly, calfhood rota- or coronaviral infections can be complicated by secondary infections with bacteria such as *E. coli* (Hess et al. 1984).

Besides biosecurity measures, vaccines also decrease the burden or severity of disease; in addition, viral vaccines could reduce secondary bacterial infections and consequently AMU in food production animals (Postma et al. 2017; Rojo-Gimeno et al. 2016). Other effects could include herd protection and use of narrower-spectrum antibiotics, thus reducing the development and dissemination of AMR (Hoelzer et al. 2018a). In pigs, up to 80% reduction in consumption of oxytetracycline was observed following vaccination against ileitis caused by *Lawsonia intracellularis*; this was in addition to improvements in clinical and production parameters as well as fewer pigs being treated (Bak and Rathkjen 2009; Peiponen et al. 2018). Reduction in antibiotic consumption has been reported with vaccination against *Actinobacillus pleuropneumoniae* in Denmark (Hoelzer et al. 2018a). Similarly, vaccines against porcine multisystemic wasting syndrome (caused by porcine circovirus-2) and porcine reproductive and respiratory syndrome (PRRS) have been shown in some European countries as well as Canada to reduce bacterial infections, antibiotic consumption, and treatment costs (Mavromatis et al. 1999; Raith et al. 2016). Observations on reduction in prescription and use of antimicrobials have been reported following the introduction of vaccines for infections with *Mycoplasma* (Kruse et al. 2019). However, other studies have failed to show any influence in prescriptions or use of antimicrobials with some pig diseases (Kruse et al. 2019; Kristensen et al. 2014; Postma et al. 2016; Temtem et al. 2016).

Vaccines could reduce the severity of BRD in cattle, including co-infection with *M. haemolytica* (Babiuk et al. 1987; Jericho et al. 1991; Stilwell et al. 2008). Similarly, vaccination against infectious bronchitis (IB) or Newcastle disease (ND) could protect birds against colibacillosis (Cook et al. 1986; Huang and Matsumoto 2000; Matthijs et al. 2005). There are several other bacterial diseases of bovines, swine, and poultry which could potentially reduce AMU. Two important ones in poultry are necrotic enteritis (caused by *Clostridium perfringens*) and coccidiosis (caused by *Eimeria* species), the latter of which predisposes birds to secondary bacterial infections (Hoelzer et al. 2018b). Infestations with ecto- and endoparasites are other conditions which could lead to secondary bacterial infections. However, no data is available on any of these. On the other hand, live vaccines such as those

against PRRS, ND, and IB could also have some immunosuppressive effect, subsequently not being able to dampen disease complicated by *Mycoplasma hyopneumoniae*, *M. gallisepticum*, or *E. coli* (Potter et al. 2008; Thacker et al. 2000). Live vaccines may also transmit to weak or immunocompromised animals, causing breakthrough disease. Furthermore, a host of other factors could reduce vaccine efficacy in animals, leading to ineffectiveness in reducing AMU (Hoelzer et al. 2018a). It may be possible to use antibiotics below therapeutic threshold levels when used in combination with vaccination (Speksnijder et al. 2015), but subtherapeutic doses could also induce and propagate AMR.

No data on AMU or AMR is available with other respiratory viral infections such as avian influenza or infectious bursal disease in poultry and other birds or gastroenteritic viral (calici, reo, and rota) infections in pigs and other animals.

4 Commentary and Perspectives

Antimicrobials have existed for several centuries, but they have been put to clinical application only in the last eight decades. Their properties of non-specificity (broad spectrum), therapeutic effect (they can be used after infection and often after the appearance of clinical symptoms, whereas vaccines can only be used before infection), and rapid action (hours after administration as against vaccines which require several weeks to be effective) are indispensable for human and animal health. Antimicrobials are particularly useful but are used excessively in those parts of the world which have a high burden of infectious diseases and where socioeconomics and a lack of infrastructure for storage and administration of vaccines have resulted in limited immunization coverage.

Antimicrobials are life-saving drugs that we cannot avoid, particularly for common medical interventions such as surgeries, cancer chemotherapy, transplantations, etc. It has been propounded that providing antibiotics universally could prevent close to half a million deaths of children under 5 years of age in more than hundred countries (Laxminarayan et al. 2016). However, the use of antibiotics over several decades has selected resistant bacterial strains, owing to which AMR has become a global public health threat. Antimicrobials not only select bystander reservoir resistance but can also disrupt human microbiome, leading to dysbiosis and affecting general health, nutritional status, and immune function (Kim et al. 2017; Lange et al. 2016; McDonnell et al. 2021). AMR has been responsible for considerable proportion of morbidity and associated mortality, treatment failures, and heightened nosocomial infections, confounding choices for healthcare management, and increasing costs. Deaths due to AMR have been estimated to be at least 23,000 in the USA (CDC 2019), 33,000 in Europe (Cassini et al. 2019), 38,000 in Thailand (Pumart et al. 2012), and 58,000 babies alone in India (Laxminarayan et al. 2013). The numbers in Europe amounted to 5.5% of all the infections (Cassini et al. 2019). Without course correction, AMR is estimated to lead to 10 million deaths by 2050 (Jonas et al. 2017). It has been projected that 10% of all expenditure in developing countries is on AMR-related complications (OECD 2018). Therefore, judicious and

prudent provision and use of antibiotics is the crux of antimicrobial stewardship programs (Ginsburg and Klugman 2017; Makri 2019; Buchy et al. 2020; Lipsitch and Siber 2016; Lee et al. 2013).

Resistance to any new antimicrobial is inevitable and is likely to have already emerged even before the deployment of the antimicrobial for clinical use. Alternatives to antibiotics are therefore a very intense area of research. Vaccines have been the central pillars of eradication of smallpox, near elimination of polio, and a marked decrease in the incidence of measles, mumps, rubella, diphtheria, tetanus, and pertussis. On the other hand, appreciation of vaccines in reducing AMR as a secondary or even a tertiary consequence of disease prevention or reducing pathogen load has received attention only very recently.

Vaccines are prophylactic in nature and, by design, are expected to reduce the incidence and burden of diseases. Besides reducing the disease burden, vaccines against bacterial diseases have several direct or cascading effects, such as (a) preventing infection at individual level even before disease is manifested; (b) reducing the prevalence of infection at population level; (c) inhibition of bacterial colonization; (d) limiting the exposure of bacteria to AM and thereby reducing the possibility of acquiring resistance; (e) reducing the transmission and spread of AMR strains; (f) minimizing patients visiting medical facilities and nosocomial infections; (g) minimizing treatment; (h) reducing antimicrobial prescriptions and therefore use; (i) avoiding indiscriminate use of antibiotics or use of rational, narrower-spectrum antibiotics; (j) providing herd immunity, thus limiting overall infections in the community as well as of high-risk individuals, such as the elderly, cancer patients, and those who are immunocompromised, to whom vaccines are contraindicated; and (k) minimizing the effect of AMs on microbiome (Klugman and Black 2018; Kingwell 2018; Makri 2019; Atkins and Lipsitch 2018; Lipsitch and Siber 2016; Atkins et al. 2018; Bloom et al. 2018; Clift and Salisbury 2017; Fine 1993; Goncalves 2008; Jansen et al. 2018; Kennedy and Read 2017, 2018; Mallory et al. 2018; Mishra et al. 2012; Tagliabue and Rappuoli 2018). Vaccines are also used when the pathogen population is low, with fewer circulating variants, as against the therapeutic application of antibiotics, where the organisms have already reached sufficient numbers to cause disease. In addition, vaccines activate multiple arms of the immune system and in different ways in different individuals and, in many cases, elicit an anamnestic response, protecting for much longer periods, as against drugs, which have a narrow mechanism of action and become ineffective or even obsolete in a very short time. Together, these two features of vaccines greatly lessen the chance of development of resistance or even onward transmission of pathogens (Blair et al. 2015; Kingwell 2018; Barnighausen et al. 2014; Malarski et al. 2019; Plotkin 2010). The development of bacterial resistance is much less of a concern with vaccines than with antibiotics (Kennedy and Read 2018; Malarski et al. 2019).

There are several intangible effects of vaccines on AMR. First of all, vaccines are typically evaluated for their effectiveness in reducing the burden of disease caused by a specific pathogen as well as cost-benefit, particularly where the disease burden is high (Bloom et al. 2017). Secondary or tertiary consequences beyond direct costs

or disability-adjusted life years (DALYs) are not currently part of such evaluation. Second, the costs of AMR emergence, disruption of microbiome, not using the appropriate antimicrobial where one is available, cost of treating side effects of AMs, etc. as a consequence of AMU instead of a vaccine are rarely calculated (Andrews et al. 2019; Atkins and Lipsitch 2018). Third, the influence of vaccines on the AMR in bystander bacteria, which could have been effected by AMU, is difficult to estimate, as there are bound to be large variations in the effect itself. Fourth, the benefits of viral vaccines in reducing the burden of bacterial disease and AMR need to be included as a contributor for overall public health (Lipsitch and Siber 2016), although quantification of the effect in cases of viral-bacterial synergy is difficult since establishing causal relationships and teasing out consequences due to commensal organisms are complex (Atkins and Lipsitch 2018). AMR-related end points therefore need to be built into vaccine clinical trials and post-marketing surveys (Klugman and Black 2018; Kingwell 2018; Bloom et al. 2018). Fifth, it is also important to remember that AMs, especially antibiotics, have effects on multiple bacteria, whereas vaccines target a single pathogen; on the other hand, vaccine (s) against a single pathogen can affect AMU and AMR with multiple pathogens. Sixth, it is also necessary to take into consideration the benefits and implementation costs of antimicrobial stewardship programs, including the investments required on the development and deployment of diagnostics and setting up laboratories as well as developing microbiology expertise (Andrews et al. 2019; Bloom et al. 2018). Seventh, it is possible that vaccinations may drive change in social attitudes, which in turn could burden the health systems. For example, a gonorrhea vaccine may lead to riskier sexual behavior (Bloom et al. 2018). Therefore, such complex scenarios must be considered while making assessments of the contribution of vaccines against AMR. Eighth, vaccines are supposed to prevent disease, which, if occurring frequently, is a continuous source of turnover for the industry where commercial interests frequently override public health interests (Anon 2016; Lipsitch and Siber 2016). The benefits of diverting the resources to vaccine development and manufacturing instead of the same for AMs are also never measured (Kingwell 2018). In this context, it is worthwhile mentioning that the research and development costs for new AMs have skyrocketed as the pipeline has narrowed drastically (Dunais et al. 2011). Ninth, how the use of vaccines to reduce AMU in animal and fish industry will impact human health and overall costs needs a One Health approach which factors in livestock rearing, food production practices, consumption habits, consequent infections and diseases, treatment costs, etc. Finally, a macroeconomy picture in terms of lifetime parameters beyond health, that is, consumption of goods and services for leisure, degree of stability in consumption, budgetary pressures and financial risk protection over life, fiscal balance, poverty, equity, etc., needs to be factored in to evaluate the overall as well as AMR-specific contribution of vaccines in public health (Bloom et al. 2018). In this context, the assessments need to include the primary, secondary, and subsequent effects as well as control between the rich and the poor (Wilby and Werry 2012; Atkins et al. 2018; Bloom et al. 2018; Goncalves 2008; Wang et al. 2016). Ultimately, the mechanisms

to assess the overall contribution of vaccines to health and welfare need to be uniform and universally applied.

However, the factors, limitations, and confounders which could influence the outcome of studies must also be borne in mind. These include the understanding of (a) the differences in clinical guidelines and definitions and consequently clinical practice; (b) the differential application and implementation and actual practices of antimicrobial stewardship programs; (c) influence of health services, micro- and macroeconomy, social behaviors, community pressures, climate, etc. on the needs for and access to antibiotics; (d) the inherent problems in defining targeted populations based on disease diagnosis rather than general population and, on the contrary, the practice of enrolling healthy volunteers in clinical trials; (e) the bias of publications, their differential approaches and influences, variations in inclusion and exclusion criteria, and non-publication of results; (f) the unavailability of information for which the antimicrobials were prescribed; (g) the fact that vaccines have varying efficacies and duration of protection, with the possibility of inducing a carrier status in immunized individuals; (h) the importance of continuous surveillance for pathogen serotypes and other variants; and (i) the variation in considering factors and evaluation criteria from one vaccine to another, from one country, region, or socioeconomic status to another (Cohen 2009; Atkins et al. 2018; Buckley et al. 2019; Tagliabue et al. 2019).

Despite these issues, and the requirement to include multiple metrics in estimating the full value of vaccines, we need to start somewhere, maybe with fewer factors such as assessing the landscape of AM prescribing without or with vaccines. Additional factors can then be added and layered to expand to other scenarios, including envisioning the emergence of re-emergence of pathogens. Similarly, we can begin with individual-, household-, or community-level analysis and expand to national, regional, and international levels. It is also important to parallely ensure policy changes on prioritizing existing vaccines; innovate regulatory pathways; fund research and development of promising or newer vaccine candidates; ensure affordability, access, and market stability and sustainability; better disseminate real-world evidence of the safety, efficacy, and effectiveness of vaccines; and enhance public perception (Buchy et al. 2020; Tagliabue and Rappuoli 2018; Baker et al. 2018; Levy and Marshall 2004; Rappuoli et al. 2017; Sherman et al. 2016; Vetter et al. 2018). Ultimately, AMR cannot be tackled by a single approach, and vaccines are an important tool which complement other tools in mitigating AMR (Kingwell 2018; Atkins et al. 2018; Jansen et al. 2018). And since pathogens do not recognize geographical borders or barriers, the war is a global one (Baker et al. 2018).

Acknowledgments More than one thousand original articles could be cited for statements made in this review. However, due to space constraints, it is impossible to do so, and I apologize to hundreds of authors for not being able to cite their work. The author's own work on AMR was supported through a grant from the Department of Biotechnology, Government of India (No. BT/IN/Indo-UK/AMR/05/NH/2018-19).

References

Acevedo, R., et al. (2019). The Global Meningococcal Initiative meeting on prevention of meningococcal disease worldwide: Epidemiology, surveillance, hypervirulent strains, antibiotic resistance and high-risk populations. *Expert Review of Vaccines,* 18, 15–30.

Adam, H. J., et al. (2010). Changing epidemiology of invasive Haemophilus influenzae in Ontario, Canada: Evidence for herd effects and strain replacement due to Hib vaccination. *Vaccine,* 28, 4073–4078.

Albarracin Orio, A. G., Pinas, G. E., Cortes, P. R., Cian, M. B., & Echenique, J. (2011). Compensatory evolution of pbp mutations restores the fitness cost imposed by beta-lactam resistance in Streptococcus pneumoniae. *PLoS Pathogens,* 7, e1002000.

Alekshun, M. N., & Levy, S. B. (2007). Molecular mechanisms of antibacterial multidrug resistance. *Cell,* 128, 1037–1050.

Alexander, T. W., et al. (2011). Longitudinal characterization of antimicrobial resistance genes in feces shed from cattle fed different subtherapeutic antibiotics. *BMC Microbiology,* 11, 19.

Allsup, S., Gosney, M., Haycox, A., & Regan, M. (2003). Cost-benefit evaluation of routine influenza immunisation in people 65-74 years of age. *Health Technology Assessment,* 7, iii-x, 1-65.

American Academy of Pediatrics Subcommittee on Management of Acute Otitis Media. (2004). Diagnosis and management of acute otitis media. *Pediatrics,* 113, 1451–1465.

Andam, C. P., & Hanage, W. P. (2015). Mechanisms of genome evolution of Streptococcus. *Infection, Genetics and Evolution,* 33, 334–342.

Anderson, A. D., Nelson, J. M., Rossiter, S., & Angulo, F. J. (2003). Public health consequences of use of antimicrobial agents in food animals in the United States. *Microbial Drug Resistance,* 9, 373–379.

Andersson, D. I., & Hughes, D. (2010). Antibiotic resistance and its cost: Is it possible to reverse resistance? *Nature Reviews. Microbiology,* 8, 260–271.

Andrews, J. R., et al. (2018). High rates of enteric fever diagnosis and lower burden of culture-confirmed disease in peri-urban and rural Nepal. *The Journal of Infectious Diseases,* 218, S214–S221.

Andrews, J. R., et al. (2019). Typhoid conjugate vaccines: A new tool in the fight against antimicrobial resistance. *The Lancet Infectious Diseases,* 19, e26–e30.

Andrews, N. J., et al. (2014). Serotype-specific effectiveness and correlates of protection for the 13-valent pneumococcal conjugate vaccine: A postlicensure indirect cohort study. *The Lancet Infectious Diseases,* 14, 839–846.

Anon. (2015). *Antimicrobials in agriculture and the environment: Reducing unnecessary use and waste*. Review on antimicrobial resistance, commissioned by the UK Government, chaired by O'Neill J.

Anon. (2016). Tackling drug-resistant infections globally: Final report and recommendations. Review on antimicrobial resistance, commissioned by the UK Government, chaired by O'Neill J.

Anthony, F., et al. (2001). Antimicrobial resistance: Responsible and prudent use of antimicrobial agents in veterinary medicine. *Revue Scientifique et Technique,* 20, 829–839.

Antillon, M., Bilcke, J., Paltiel, A. D., & Pitzer, V. E. (2017a). Cost-effectiveness analysis of typhoid conjugate vaccines in five endemic low- and middle-income settings. *Vaccine,* 35, 3506–3514.

Antillon, M., et al. (2017b). The burden of typhoid fever in low- and middle-income countries: A meta-regression approach. *PLoS Neglected Tropical Diseases,* 11, e0005376.

Antimicrobial Resistance Consortium. (2022). Global burden of bacterial antimicrobial resistance in 2019: A systematic analysis. *Lancet,* 399, 629.

Arias, C. A., & Murray, B. E. (2009). Antibiotic-resistant bugs in the 21st century – A clinical super-challenge. *The New England Journal of Medicine,* 360, 439–443.

Atkins, K. E., & Lipsitch, M. (2018). Can antibiotic resistance be reduced by vaccinating against respiratory disease? *The Lancet Respiratory Medicine,* 6, 820–821.

Atkins, K. E., et al. (2018). Use of mathematical modelling to assess the impact of vaccines on antibiotic resistance. *The Lancet Infectious Diseases,* 18, e204–e213.

Aye, A. M. M., et al. (2020). Meningococcal disease surveillance in the Asia-Pacific region (2020): The global meningococcal initiative. *The Journal of Infection,* 81, 698–711.

Azzari, C., & Resti, M. (2008). Reduction of carriage and transmission of Streptococcus pneumoniae: The beneficial "side effect" of pneumococcal conjugate vaccine. *Clinical Infectious Diseases,* 47, 997–999.

Azze, R. F. O. (2019). A meningococcal B vaccine induces cross-protection against gonorrhea. *Clinical and Experimental Vaccine Research,* 8, 110–115.

Babiuk, L. A., et al. (1987). Protection of cattle from bovine herpesvirus type I (BHV-1) infection by immunization with individual viral glycoproteins. *Virology,* 159, 57–66.

Bagnoli, F., Bertholet, S., & Grandi, G. (2012). Inferring reasons for the failure of Staphylococcus aureus vaccines in clinical trials. *Frontiers in Cellular and Infection Microbiology,* 2, 16.

Bagnoli, F., et al. (2011). Designing the next generation of vaccines for global public health. *OMICS,* 15, 545–566.

Bai, X., et al. (2019). Prevention and control of meningococcal disease: Updates from the Global Meningococcal Initiative in Eastern Europe. *The Journal of Infection,* 79, 528–541.

Bak, H., & Rathkjen, P. H. (2009). Reduced use of antimicrobials after vaccination of pigs against porcine proliferative enteropathy in a Danish SPF herd. *Acta Veterinaria Scandinavica,* 51, 1.

Baker, S. J., Payne, D. J., Rappuoli, R., & De Gregorio, E. (2018). Technologies to address antimicrobial resistance. *Proceedings of the National Academy of Sciences of the United States of America,* 115, 12887–12895.

Barbour, M. L. (1996). Conjugate vaccines and the carriage of Haemophilus influenzae type b. *Emerging Infectious Diseases,* 2, 176–182.

Barnighausen, T., et al. (2014). Reassessing the value of vaccines. *The Lancet Global Health,* 2, e251–e252.

Beall, B., et al. (2006). Pre- and postvaccination clonal compositions of invasive pneumococcal serotypes for isolates collected in the United States in 1999, 2001, and 2002. *Journal of Clinical Microbiology,* 44, 999–1017.

Bhutta, Z. A. (1996). Impact of age and drug resistance on mortality in typhoid fever. *Archives of Disease in Childhood,* 75, 214–217.

Bianchi-Jassir, F., et al. (2020). Systematic review of Group B Streptococcal capsular types, sequence types and surface proteins as potential vaccine candidates. *Vaccine,* 38, 6682–6694.

Black, S. B., et al. (2001). Postlicensure evaluation of the effectiveness of seven valent pneumococcal conjugate vaccine. *The Pediatric Infectious Disease Journal,* 20, 1105–1107.

Blair, J. M., Webber, M. A., Baylay, A. J., Ogbolu, D. O., & Piddock, L. J. (2015). Molecular mechanisms of antibiotic resistance. *Nature Reviews. Microbiology,* 13, 42–51.

Bloom, D. E., Black, S., Salisbury, D., & Rappuoli, R. (2018). Antimicrobial resistance and the role of vaccines. *Proceedings of the National Academy of Sciences of the United States of America,* 115, 12868–12871.

Bloom, D. E., Brenzel, L., Cadarette, D., & Sullivan, J. (2017). Moving beyond traditional valuation of vaccination: Needs and opportunities. *Vaccine,* 35(Suppl 1), A29–A35.

Bogaert, D., De Groot, R., & Hermans, P. W. (2004a). Streptococcus pneumoniae colonisation: The key to pneumococcal disease. *The Lancet Infectious Diseases,* 4, 144–154.

Bogaert, D., et al. (2004b). Colonisation by Streptococcus pneumoniae and Staphylococcus aureus in healthy children. *Lancet,* 363, 1871–1872.

Bonten, M. J., et al. (2015). Polysaccharide conjugate vaccine against pneumococcal pneumonia in adults. *The New England Journal of Medicine,* 372, 1114–1125.

Bozzola, E., et al. (2016). Varicella skin complications in childhood: A case series and a systematic review of the literature. *International Journal of Molecular Sciences,* 17, 688.

Bridges, C. B., et al. (2000). Effectiveness and cost-benefit of influenza vaccination of healthy working adults: A randomized controlled trial. *JAMA,* 284, 1655–1663.

Bronzwaer, S. L., et al. (2002). A European study on the relationship between antimicrobial use and antimicrobial resistance. *Emerging Infectious Diseases,* 8, 278–282.
Buchy, P., et al. (2020). Impact of vaccines on antimicrobial resistance. *International Journal of Infectious Diseases,* 90, 188–196.
Buckley, B. S., et al. (2019). Impact of vaccination on antibiotic usage: A systematic review and meta-analysis. *Clinical Microbiology and Infection,* 25, 1213–1225.
Buczkowski, H., Muniraju, M., Parida, S., & Banyard, A. C. (2014). Morbillivirus vaccines: Recent successes and future hopes. *Vaccine,* 32, 3155–3161.
Butler, J. C., Dowell, S. F., & Breiman, R. F. (1998). Epidemiology of emerging pneumococcal drug resistance: Implications for treatment and prevention. *Vaccine,* 16, 1693–1697.
Cabello, F. C. (2006). Heavy use of prophylactic antibiotics in aquaculture: A growing problem for human and animal health and for the environment. *Environmental Microbiology,* 8, 1137–1144.
Casey, J. R., Adlowitz, D. G., & Pichichero, M. E. (2010). New patterns in the otopathogens causing acute otitis media six to eight years after introduction of pneumococcal conjugate vaccine. *The Pediatric Infectious Disease Journal,* 29, 304–309.
Casey, J. R., & Pichichero, M. E. (2004). Changes in frequency and pathogens causing acute otitis media in 1995-2003. *The Pediatric Infectious Disease Journal,* 23, 824–828.
Cassini, A., et al. (2019). Attributable deaths and disability-adjusted life-years caused by infections with antibiotic-resistant bacteria in the EU and the European Economic Area in 2015: A population-level modelling analysis. *The Lancet Infectious Diseases,* 19, 56–66.
CDC. (2019). *Antibiotic resistance threats in the United States.* The Centers for Diseases Control and Prevention, United States Department of Health and Human Services, Atlanta, GA, USA.
Chernova, O. A., et al. (2021). Antimicrobial drug resistance mechanisms among Mollicutes. *International Journal of Antimicrobial Agents,* 57, 106253.
Chertow, D. S., & Memoli, M. J. (2013). Bacterial coinfection in influenza: A grand rounds review. *JAMA,* 309, 275–282.
Christaki, E., Marcou, M., & Tofarides, A. (2020). Antimicrobial resistance in bacteria: Mechanisms, evolution, and persistence. *Journal of Molecular Evolution,* 88, 26–40.
Cimolai, N. (2021). Pharmacotherapy for Bordetella pertussis infection. II. A synthesis of clinical sciences. *International Journal of Antimicrobial Agents,* 57, 106257.
Clift, C., & Salisbury, D. M. (2017). Enhancing the role of vaccines in combatting antimicrobial resistance. *Vaccine,* 35, 6591–6593.
Cohen, R. (2009). The need for prudent use of antibiotics and routine use of vaccines. *Clinical Microbiology and Infection,* 15(Suppl 3), 21–23.
Cohen, R., Cohen, J. F., Chalumeau, M., & Levy, C. (2017). Impact of pneumococcal conjugate vaccines for children in high- and non-high-income countries. *Expert Review of Vaccines,* 16, 625–640.
Cohen, R., et al. (2006). Impact of pneumococcal conjugate vaccine and of reduction of antibiotic use on nasopharyngeal carriage of nonsusceptible pneumococci in children with acute otitis media. *The Pediatric Infectious Disease Journal,* 25, 1001–1007.
Cohen, R., et al. (2015). A 13-year survey of pneumococcal nasopharyngeal carriage in children with acute otitis media following PCV7 and PCV13 implementation. *Vaccine,* 33, 5118–5126.
Cohen, S. H., et al. (2010). Clinical practice guidelines for Clostridium difficile infection in adults: 2010 update by the society for healthcare epidemiology of America (SHEA) and the infectious diseases society of America (IDSA). *Infection Control and Hospital Epidemiology,* 31, 431–455.
Cook, J., et al. (2008). The cost-effectiveness of typhoid Vi vaccination programs: Calculations for four urban sites in four Asian countries. *Vaccine,* 26, 6305–6316.
Cook, J. K., Smith, H. W., & Huggins, M. B. (1986). Infectious bronchitis immunity: Its study in chickens experimentally infected with mixtures of infectious bronchitis virus and Escherichia coli. *The Journal of General Virology,* 67(Pt 7), 1427–1434.
Cosgrove, S. E., & Carmeli, Y. (2003). The impact of antimicrobial resistance on health and economic outcomes. *Clinical Infectious Diseases,* 36, 1433–1437.

Craig, A. P., et al. (2015). The potential impact of vaccination on the prevalence of gonorrhea. *Vaccine,* 33, 4520–4525.

Croucher, N. J., et al. (2014). Evidence for soft selective sweeps in the evolution of pneumococcal multidrug resistance and vaccine escape. *Genome Biology and Evolution,* 6, 1589–1602.

D'Acremont, V., et al. (2014). Beyond malaria--causes of fever in outpatient Tanzanian children. *The New England Journal of Medicine,* 370, 809–817.

Dagan, R. (2000). Clinical significance of resistant organisms in otitis media. *The Pediatric Infectious Disease Journal,* 19, 378–382.

Dagan, R. (2003). Antibiotic resistance and the potential impact of pneumococcal conjugate vaccines. *Communicable Diseases Intelligence,* 27(Suppl), S135–S143.

Dagan, R. (2009). Serotype replacement in perspective. *Vaccine,* 27(Suppl 3), C22–C24.

Dagan, R., Givon-Lavi, N., Leibovitz, E., Greenberg, D., & Porat, N. (2009). Introduction and proliferation of multidrug-resistant Streptococcus pneumoniae serotype 19A clones that cause acute otitis media in an unvaccinated population. *The Journal of Infectious Diseases,* 199, 776–785.

Dagan, R., & Klugman, K. P. (2008). Impact of conjugate pneumococcal vaccines on antibiotic resistance. *The Lancet Infectious Diseases,* 8, 785–795.

Dagan, R., Leibovitz, E., Leiberman, A., & Yagupsky, P. (2000). Clinical significance of antibiotic resistance in acute otitis media and implication of antibiotic treatment on carriage and spread of resistant organisms. *The Pediatric Infectious Disease Journal,* 19, S57–S65.

Davies, J., & Davies, D. (2010). Origins and evolution of antibiotic resistance. *Microbiology and Molecular Biology Reviews,* 74, 417–433.

Davies, T. A., et al. (2008). Effects of the 7-valent pneumococcal conjugate vaccine on U.S. levofloxacin-resistant Streptococcus pneumoniae. *Microbial Drug Resistance,* 14, 187–196.

Davis, S. M., Deloria-Knoll, M., Kassa, H. T., & O'Brien, K. L. (2013). Impact of pneumococcal conjugate vaccines on nasopharyngeal carriage and invasive disease among unvaccinated people: Review of evidence on indirect effects. *Vaccine,* 32, 133–145.

Dbaibo, G., et al. (2020). Quadrivalent influenza vaccine prevents illness and reduces healthcare utilization across diverse geographic regions during five influenza seasons: A randomized clinical trial. *The Pediatric Infectious Disease Journal,* 39, e1–e10.

D'Costa, V. M., et al. (2011). Antibiotic resistance is ancient. *Nature,* 477, 457–461.

de Celles, M. D., et al. (2015). Interaction of vaccination and reduction of antibiotic use drives unexpected increase of pneumococcal meningitis. *Scientific Reports,* 5, 11293.

Diekema, D. J., Brueggemann, A. B., & Doern, G. V. (2000). Antimicrobial-drug use and changes in resistance in Streptococcus pneumoniae. *Emerging Infectious Diseases,* 6, 552–556.

Dunais, B., et al. (2011). Antibiotic prescriptions in French day-care centres: 1999-2008. *Archives of Disease in Childhood,* 96, 1033–1037.

Dupont, D., et al. (2010). Evolving microbiology of complicated acute otitis media before and after introduction of the pneumococcal conjugate vaccine in France. *Diagnostic Microbiology and Infectious Disease,* 68, 89–92.

ECDC/EFSA/EMA. (2017). ECDC/EFSA/EMA second joint report on the integrated analysis of the consumption of antimicrobial agents and occurrence of antimicrobial resistance in bacteria from humans and food-producing animals: Joint interagency antimicrobial consumption and resistance analysis (JIACRA) report. *EFSA Journal,* 15, e04872.

Esposito, S., et al. (2003). Effectiveness of influenza vaccination of children with recurrent respiratory tract infections in reducing respiratory-related morbidity within the households. *Vaccine,* 21, 3162–3168.

Eythorsson, E., et al. (2018a). Decreased acute otitis media with treatment failure after introduction of the ten-valent pneumococcal haemophilus influenzae protein D conjugate vaccine. *The Pediatric Infectious Disease Journal,* 37, 361–366.

Eythorsson, E., et al. (2018b). Impact of the 10-valent pneumococcal conjugate vaccine on antimicrobial prescriptions in young children: A whole population study. *BMC Infectious Diseases,* 18, 505.

Farrell, D. J., Klugman, K. P., & Pichichero, M. (2007). Increased antimicrobial resistance among nonvaccine serotypes of Streptococcus pneumoniae in the pediatric population after the introduction of 7-valent pneumococcal vaccine in the United States. *The Pediatric Infectious Disease Journal,* 26, 123–128.

Feikin, D. R., & Klugman, K. P. (2002). Historical changes in pneumococcal serogroup distribution: Implications for the era of pneumococcal conjugate vaccines. *Clinical Infectious Diseases,* 35, 547–555.

Fenoll, A., et al. (2009). Temporal trends of invasive Streptococcus pneumoniae serotypes and antimicrobial resistance patterns in Spain from 1979 to 2007. *Journal of Clinical Microbiology,* 47, 1012–1020.

Fernandez, L., Breidenstein, E. B., & Hancock, R. E. (2011). Creeping baselines and adaptive resistance to antibiotics. *Drug Resistance Updates,* 14, 1–21.

Fine, P. E. (1993). Herd immunity: History, theory, practice. *Epidemiologic Reviews,* 15, 265–302.

Fleming-Dutra, K. E., et al. (2016). Prevalence of inappropriate antibiotic prescriptions among US ambulatory care visits, 2010-2011. *JAMA,* 315, 1864–1873.

Friedman, N. D., Temkin, E., & Carmeli, Y. (2016). The negative impact of antibiotic resistance. *Clinical Microbiology and Infection,* 22, 416–422.

Ghazi, I. M., et al. (2016). Antibiotic utilization and opportunities for stewardship among hospitalized patients with influenza respiratory tract infection. *Infection Control and Hospital Epidemiology,* 37, 583–589.

Ginsburg, A. S., & Klugman, K. P. (2017). Vaccination to reduce antimicrobial resistance. *The Lancet Global Health,* 5, e1176–e1177.

Goncalves, G. (2008). Herd immunity: Recent uses in vaccine assessment. *Expert Review of Vaccines,* 7, 1493–1506.

Goossens, H. (2009). Antibiotic consumption and link to resistance. *Clinical Microbiology and Infection,* 15(Suppl 3), 12–15.

Goossens, H., Ferech, M., Stichele, R. V., Elseviers, M., & E. P. Group. (2005). Outpatient antibiotic use in Europe and association with resistance: A cross-national database study. *Lancet,* 365, 579–587.

Gould, K. (2016). Antibiotics: From prehistory to the present day. *The Journal of Antimicrobial Chemotherapy,* 71, 572–575.

Gounder, P. P., & Hennessy, T. W. (2014). The effect of antimicrobial use on nasopharyngeal colonization by Streptococcus pneumoniae and Haemophilus influenzae. *The Pediatric Infectious Disease Journal,* 33, 228.

Grabenstein, J. D., & Weber, D. J. (2014). Pneumococcal serotype diversity among adults in various countries, influenced by pediatric pneumococcal vaccination uptake. *Clinical Infectious Diseases,* 58, 854–864.

Gray, B. M., Converse, G. M., 3rd, & Dillon, H. C., Jr. (1980). Epidemiologic studies of Streptococcus pneumoniae in infants: Acquisition, carriage, and infection during the first 24 months of life. *The Journal of Infectious Diseases,* 142, 923–933.

Greenwood, B. (2014). The contribution of vaccination to global health: Past, present and future. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences,* 369, 20130433.

Grijalva, C. G., Nuorti, J. P., & Griffin, M. R. (2009). Antibiotic prescription rates for acute respiratory tract infections in US ambulatory settings. *JAMA,* 302, 758–766.

Gullberg, E., et al. (2011). Selection of resistant bacteria at very low antibiotic concentrations. *PLoS Pathogens,* 7, e1002158.

Gums, J. G. (2002). Assessing the impact of antimicrobial resistance. *American Journal of Health-System Pharmacy,* 59, S4–S6.

Hackel, M., et al. (2013). Serotype prevalence and antibiotic resistance in Streptococcus pneumoniae clinical isolates among global populations. *Vaccine,* 31, 4881–4887.

Hammitt, L. L., et al. (2006). Indirect effect of conjugate vaccine on adult carriage of Streptococcus pneumoniae: An explanation of trends in invasive pneumococcal disease. *The Journal of Infectious Diseases,* 193, 1487–1494.

Hampton, L. M., et al. (2012). Prevention of antibiotic-nonsusceptible Streptococcus pneumoniae with conjugate vaccines. *The Journal of Infectious Diseases,* 205, 401–411.

Hanage, W. P. (2007). Serotype replacement in invasive pneumococcal disease: Where do we go from here? *The Journal of Infectious Diseases,* 196, 1282–1284.

Hanquet, G., et al. (2010). Pneumococcal serotypes in children in 4 European countries. *Emerging Infectious Diseases,* 16, 1428–1439.

Hansen, N. S., et al. (2017). Effect of early measles vaccine on pneumococcal colonization: A randomized trial from Guinea-Bissau. *PLoS One,* 12, e0177547.

Harboe, Z. B., et al. (2010a). Temporal trends in invasive pneumococcal disease and pneumococcal serotypes over 7 decades. *Clinical Infectious Diseases,* 50, 329–337.

Harboe, Z. B., et al. (2010b). Early effectiveness of heptavalent conjugate pneumococcal vaccination on invasive pneumococcal disease after the introduction in the Danish Childhood Immunization Programme. *Vaccine,* 28, 2642–2647.

Hausdorff, W. P. (2002). Invasive pneumococcal disease in children: Geographic and temporal variations in incidence and serotype distribution. *European Journal of Pediatrics,* 161(Suppl 2), S135–S139.

Hausdorff, W. P., Bryant, J., Paradiso, P. R., & Siber, G. R. (2000). Which pneumococcal serogroups cause the most invasive disease: Implications for conjugate vaccine formulation and use, part I. *Clinical Infectious Diseases,* 30, 100–121.

Hess, R. G., et al. (1984). Synergism in experimental mixed infections of newborn colostrum-deprived calves with bovine rotavirus and enterotoxigenic Escherichia coli (ETEC). *Zentralblatt für Veterinärmedizin. Reihe B,* 31, 585–596.

Hicks, L. A., et al. (2011). Outpatient antibiotic prescribing and nonsusceptible Streptococcus pneumoniae in the United States, 1996-2003. *Clinical Infectious Diseases,* 53, 631–639.

Hoban, D. J., Doern, G. V., Fluit, A. C., Roussel-Delvallez, M., & Jones, R. N. (2001). Worldwide prevalence of antimicrobial resistance in Streptococcus pneumoniae, Haemophilus influenzae, and Moraxella catarrhalis in the SENTRY Antimicrobial Surveillance Program, 1997-1999. *Clinical Infectious Diseases,* 32(Suppl 2), S81–S93.

Hoberman, A., et al. (2003). Effectiveness of inactivated influenza vaccine in preventing acute otitis media in young children: A randomized controlled trial. *JAMA,* 290, 1608–1616.

Hoelzer, K., et al. (2018a). Vaccines as alternatives to antibiotics for food producing animals. Part 1: Challenges and needs. *Veterinary Research,* 49, 64.

Hoelzer, K., et al. (2018b). Vaccines as alternatives to antibiotics for food producing animals. Part 2: New approaches and potential solutions. *Veterinary Research,* 49, 70.

Huang, H. J., & Matsumoto, M. (2000). Nonspecific innate immunity against Escherichia coli infection in chickens induced by vaccine strains of Newcastle disease virus. *Avian Diseases,* 44, 790–796.

Jaiswal, N., et al. (2014). Distribution of serotypes, vaccine coverage, and antimicrobial susceptibility pattern of Streptococcus pneumoniae in children living in SAARC countries: A systematic review. *PLoS One,* 9, e108617.

Jansen, A. G., Sanders, E. A., Hoes, A. W., van Loon, A. M., & Hak, E. (2008). Effects of influenza plus pneumococcal conjugate vaccination versus influenza vaccination alone in preventing respiratory tract infections in children: A randomized, double-blind, placebo-controlled trial. *The Journal of Pediatrics,* 153, 764–770.

Jansen, K. U., & Anderson, A. S. (2018). The role of vaccines in fighting antimicrobial resistance (AMR). *Human Vaccines & Immunotherapeutics,* 14, 2142–2149.

Jansen, K. U., Knirsch, C., & Anderson, A. S. (2018). The role of vaccines in preventing bacterial antimicrobial resistance. *Nature Medicine,* 24, 10–19.

Jauneikaite, E., Jefferies, J. M., Hibberd, M. L., & Clarke, S. C. (2012). Prevalence of Streptococcus pneumoniae serotypes causing invasive and non-invasive disease in South East Asia: A review. *Vaccine,* 30, 3503–3514.

Jericho, K. W., Loewen, K. G., Smithson, S. E., & Kozub, G. C. (1991). Protective effect of inactivated bovine herpesvirus-1 in calves experimentally infected with bovine herpesvirus-1 and Pasteurella haemolytica. *Research in Veterinary Science,* 51, 209–214.

John, J., Van Aart, C. J., & Grassly, N. C. (2016). The burden of typhoid and paratyphoid in India: Systematic review and meta-analysis. *PLoS Neglected Tropical Diseases,* 10, e0004616.

Jonas, O. B., Irwin, A., Berthe, F. C. J., Le Gall F. G., & Marquez, P. V. (2017). *Drug-resistant infections: A threat to our economic future*. Vol. 2 final report, Working paper, The World Bank.

Kabra, S. K., & Lodha, R. (2013). Antibiotics for preventing complications in children with measles. *Cochrane Database of Systematic Reviews*, 2013, CD001477.

Kaltoft, N., & Zeuthen, H. B. (2000). Konradsen, Epidemiology of invasive pneumococcal infections in children aged 0-6 years in Denmark: A 19-year nationwide surveillance study. *Acta Paediatrica. Supplement,* 89, 3–10.

Kao, R. R., et al. (2007). Mycobacterium bovis shedding patterns from experimentally infected calves and the effect of concurrent infection with bovine viral diarrhoea virus. *J R Soc Interface,* 4, 545–551.

Kash, J. C., & Taubenberger, J. K. (2015). The role of viral, host, and secondary bacterial factors in influenza pathogenesis. *The American Journal of Pathology,* 185, 1528–1536.

Katsarolis, I., et al. (2009). Risk factors for nasopharyngeal carriage of drug-resistant Streptococcus pneumoniae: Data from a nation-wide surveillance study in Greece. *BMC Infectious Diseases,* 9, 120.

Kaufhold, S., Yaesoubi, R., & Pitzer, V. E. (2019). Predicting the impact of typhoid conjugate vaccines on antimicrobial resistance. *Clinical Infectious Diseases,* 68, S96–S104.

Kaur, R., Casey, J. R., & Pichichero, M. E. (2016). Emerging Streptococcus pneumoniae strains colonizing the nasopharynx in children after 13-valent pneumococcal conjugate vaccination in comparison to the 7-valent Era, 2006-2015. *The Pediatric Infectious Disease Journal,* 35, 901–906.

Kellner, J. D., Church, D. L., MacDonald, J., Tyrrell, G. J., & Scheifele, D. (2005). Progress in the prevention of pneumococcal infection. *CMAJ,* 173, 1149–1151.

Kempf, M., et al. (2015). Decline in antibiotic resistance and changes in the serotype distribution of Streptococcus pneumoniae isolates from children with acute otitis media; A 2001-2011 survey by the French Pneumococcal Network. *Clinical Microbiology and Infection,* 21, 35–42.

Kennedy, D. A., & Read, A. F. (2017). Why does drug resistance readily evolve but vaccine resistance does not? *Proceedings of the Biological Sciences,* 284, 1.

Kennedy, D. A., & Read, A. F. (2018). Why the evolution of vaccine resistance is less of a concern than the evolution of drug resistance. *Proceedings of the National Academy of Sciences of the United States of America,* 115, 12878–12886.

Kim, K. H., et al. (2011). Nasopharyngeal pneumococcal carriage of children attending day care centers in Korea: Comparison between children immunized with 7-valent pneumococcal conjugate vaccine and non-immunized. *Journal of Korean Medical Science,* 26, 184–190.

Kim, L., McGee, L., Tomczyk, S., & Beall, B. (2016). Biological and epidemiological features of antibiotic-resistant streptococcus pneumoniae in pre- and post-conjugate vaccine eras: A United States perspective. *Clinical Microbiology Reviews,* 29, 525–552.

Kim, S., Covington, A., & Pamer, E. G. (2017). The intestinal microbiota: Antibiotics, colonization resistance, and enteric pathogens. *Immunological Reviews,* 279, 90–105.

Kim, S. H., et al. (2012). Changing trends in antimicrobial resistance and serotypes of Streptococcus pneumoniae isolates in Asian countries: An Asian Network for Surveillance of Resistant Pathogens (ANSORP) study. *Antimicrobial Agents and Chemotherapy,* 56, 1418–1426.

Kingwell, K. (2018). Vaccines take a shot at antimicrobial resistance. *Nature Reviews. Drug Discovery,* 17, 229–231.

Klugman, K. P., & Black, S. (2018). Impact of existing vaccines in reducing antibiotic resistance: Primary and secondary effects. *Proceedings of the National Academy of Sciences of the United States of America,* 115, 12896–12901.

KPMG LLP. (2014). *The global economic impact of anti-microbial resistance*. KPMG LLP.

Kristensen, C. S., Vinther, J., Svensmark, B., & Baekbo, P. (2014). A field evaluation of two vaccines against Mycoplasma hyopneumoniae infection in pigs. *Acta Veterinaria Scandinavica,* 56, 24.

Kronman, M. P., Zhou, C., & Mangione-Smith, R. (2014). Bacterial prevalence and antimicrobial prescribing trends for acute respiratory tract infections. *Pediatrics,* 134, e956–e965.

Kruse, A. B., Kristensen, C. S., Rosenbaum Nielsen, L., & Alban, L. (2019). A register-based study on associations between vaccination, antimicrobial use and productivity in conventional Danish finisher pig herds during 2011 to 2014. *Preventive Veterinary Medicine,* 164, 33–40.

Kwong, J. C., Maaten, S., Upshur, R. E., Patrick, D. M., & Marra, F. (2009). The effect of universal influenza immunization on antibiotic prescriptions: An ecological study. *Clinical Infectious Diseases,* 49, 750–756.

Lange, K., Buerger, M., Stallmach, A., & Bruns, T. (2016). Effects of Antibiotics on Gut Microbiota. *Digestive Diseases,* 34, 260–268.

Laxminarayan, R., et al. (2013). Antibiotic resistance-the need for global solutions. *The Lancet Infectious Diseases,* 13, 1057–1098.

Laxminarayan, R., et al. (2016). Access to effective antimicrobials: A worldwide challenge. *Lancet,* 387, 168–175.

Lee, C. R., Cho, I. H., Jeong, B. C., & Lee, S. H. (2013). Strategies to minimize antibiotic resistance. *International Journal of Environmental Research and Public Health,* 10, 4274–4305.

Lee, G. C., et al. (2014). Outpatient antibiotic prescribing in the United States: 2000 to 2010. *BMC Medicine,* 12, 96.

Lee, J. H. (2019). Perspectives towards antibiotic resistance: From molecules to population. *Journal of Microbiology,* 57, 181–184.

Lehtinen, S., et al. (2017). Evolution of antibiotic resistance is linked to any genetic mechanism affecting bacterial duration of carriage. *Proceedings of the National Academy of Sciences of the United States of America,* 114, 1075–1080.

Leiberman, A., Dagan, R., Leibovitz, E., Yagupsky, P., & Fliss, D. M. (1999). The bacteriology of the nasopharynx in childhood. *International Journal of Pediatric Otorhinolaryngology,* 49(Suppl 1), S151–S153.

Leibovitz, E. (2008). The effect of vaccination on Streptococcus pneumoniae resistance. *Current Infectious Disease Reports,* 10, 182–191.

Lenski, R. E. (1998). Bacterial evolution and the cost of antibiotic resistance. *International Microbiology,* 1, 265–270.

Lesko, S. M., O'Brien, K. L., Schwartz, B., Vezina, R., & Mitchell, A. A. (2001). Invasive group A streptococcal infection and nonsteroidal antiinflammatory drug use among children with primary varicella. *Pediatrics,* 107, 1108–1115.

Levy, S. B., & Marshall, B. (2004). Antibacterial resistance worldwide: Causes, challenges and responses. *Nature Medicine,* 10, S122–S129.

Lin, E. Y., Adamson, P. C., & Klausner, J. D. (2021). Epidemiology, treatments, and vaccine development for antimicrobial-resistant Neisseria gonorrhoeae: Current strategies and future directions. *Drugs,* 81, 1153–1169.

Linares, J., Ardanuy, C., Pallares, R., & Fenoll, A. (2010). Changes in antimicrobial resistance, serotypes and genotypes in Streptococcus pneumoniae over a 30-year period. *Clinical Microbiology and Infection,* 16, 402–410.

Link-Gelles, R., et al. (2013). Geographic and temporal trends in antimicrobial nonsusceptibility in Streptococcus pneumoniae in the post-vaccine era in the United States. *The Journal of Infectious Diseases,* 208, 1266–1273.

Lipsitch, M. (1999). Bacterial vaccines and serotype replacement: Lessons from Haemophilus influenzae and prospects for Streptococcus pneumoniae. *Emerging Infectious Diseases,* 5, 336–345.

Lipsitch, M., & Siber, G. R. (2016). How can vaccines contribute to solving the antimicrobial resistance problem? *MBio,* 7, 607.

Lo, N. C., et al. (2018). Comparison of strategies and incidence thresholds for Vi conjugate vaccines against typhoid fever: A cost-effectiveness modeling study. *The Journal of Infectious Diseases,* 218, S232–S242.

Low, D. (2008). Reducing antibiotic use in influenza: Challenges and rewards. *Clinical Microbiology and Infection,* 14, 298–306.

Low, D. E. (2005). Changing trends in antimicrobial-resistant pneumococci: It's not all bad news. *Clinical Infectious Diseases,* 41(Suppl 4), S228–S233.

Lynch, J. P., 3rd, & Zhanel, G. G. (2010). Streptococcus pneumoniae: Epidemiology and risk factors, evolution of antimicrobial resistance, and impact of vaccines. *Current Opinion in Pulmonary Medicine,* 16, 217–225.

Maher, M. C., et al. (2012). The fitness cost of antibiotic resistance in Streptococcus pneumoniae: Insight from the field. *PLoS One,* 7, e29407.

Makri, A. (2019). Progress lags on vaccines to beat antimicrobial resistance. *Lancet,* 394, 1793–1794.

Malarski, M., et al. (2019). Vaccine impact on antimicrobial resistance to inform Gavi, the vaccine alliance's 2018 vaccine investment strategy: Report from an expert survey. *F1000Res,* 8, 1685.

Malley, R., & Anderson, P. W. (2012). Serotype-independent pneumococcal experimental vaccines that induce cellular as well as humoral immunity. *Proceedings of the National Academy of Sciences of the United States of America,* 109, 3623–3627.

Mallory, M. L., Lindesmith, L. C., & Baric, R. S. (2018). Vaccination-induced herd immunity: Successes and challenges. *The Journal of Allergy and Clinical Immunology,* 142, 64–66.

Manrique, P. D., Lopez, C. A., Gnanakaran, S., Rybenkov, V. V., & Zgurskaya, H. I. (2022). New understanding of multidrug efflux and permeation in antibiotic resistance, persistence, and heteroresistance. *Annals of the New York Academy of Sciences* 1519, 46–62.

Marks, F., et al. (2017). Incidence of invasive salmonella disease in sub-Saharan Africa: A multicentre population-based surveillance study. *The Lancet Global Health,* 5, e310–e323.

Marom, T., et al. (2017). Comparison of first year of life acute otitis media admissions before and after the 13-valent pneumococcal conjugate vaccine. *International Journal of Pediatric Otorhinolaryngology,* 97, 251–256.

Martin, J. M., et al. (2014). Emergence of Streptococcus pneumoniae serogroups 15 and 35 in nasopharyngeal cultures from young children with acute otitis media. *The Pediatric Infectious Disease Journal,* 33, e286–e290.

Matthijs, M. G., van Eck, J. H., de Wit, J. J., Bouma, A., & Stegeman, J. A. (2005). Effect of IBV-H120 vaccination in broilers on colibacillosis susceptibility after infection with a virulent Massachusetts-type IBV strain. *Avian Diseases,* 49, 540–545.

Mavromatis, I., Kritas, S. K., Alexopoulos, C., Tsinas, A., & Kyriakis, S. C. (1999). Field evaluation of a live vaccine against porcine reproductive and respiratory syndrome in fattening pigs. *Zentralblatt für Veterinärmedizin. Reihe B,* 46, 603–612.

Mayxay, M., et al. (2013). Causes of non-malarial fever in Laos: A prospective study. *The Lancet Global Health,* 1, e46–e54.

McBean, A. M., Park, Y. T., Caldwell, D., & Yu, X. (2005). Declining invasive pneumococcal disease in the U.S. elderly. *Vaccine,* 23, 5641–5645.

McDonnell, L., et al. (2021). Association between antibiotics and gut microbiome dysbiosis in children: Systematic review and meta-analysis. *Gut Microbes,* 13, 1–18.

McGarry, L. J., et al. (2013). Impact of 13-valent pneumococcal conjugate vaccine (PCV13) in a pandemic similar to the 2009 H1N1 in the United States. *BMC Infectious Diseases,* 13, 229.

McManus, P. S., Stockwell, V. O., Sundin, G. W., & Jones, A. L. (2002). Antibiotic use in plant agriculture. *Annual Review of Phytopathology,* 40, 443–465.

McMillan, M., Marshall, H. S., & Richmond, P. (2022). 4CMenB vaccine and its role in preventing transmission and inducing herd immunity. *Expert Review of Vaccines,* 21, 103–114.

Melin, P., & Efstratiou, A. (2013). Group B streptococcal epidemiology and vaccine needs in developed countries. *Vaccine,* 31(Suppl 4), D31–D42.

Mishra, R. P., Oviedo-Orta, E., Prachi, P., Rappuoli, R., & Bagnoli, F. (2012). Vaccines and antibiotic resistance. *Current Opinion in Microbiology,* 15, 596–602.

Misurski, D. A., Lipson, D. A., & Changolkar, A. K. (2011). Inappropriate antibiotic prescribing in managed care subjects with influenza. *The American Journal of Managed Care,* 17, 601–608.

Mlynarczyk-Bonikowska, B., Majewska, A., Malejczyk, M., Mlynarczyk, G., & Majewski, S. (2020). Multiresistant Neisseria gonorrhoeae: A new threat in second decade of the XXI century. *Medical Microbiology and Immunology,* 209, 95–108.

Moberley, S., Holden, J., Tatham, D. P., & Andrews, R. M. (2013). Vaccines for preventing pneumococcal infection in adults. *Cochrane Database of Systematic Reviews,* 1, CD000422.

Mogasale, V., Ramani, E., Mogasale, V. V., & Park, J. (2016). What proportion of Salmonella Typhi cases are detected by blood culture? A systematic literature review. *Annals of Clinical Microbiology and Antimicrobials,* 15, 32.

Moore, M. R., et al. (2004). Impact of a conjugate vaccine on community-wide carriage of nonsusceptible Streptococcus pneumoniae in Alaska. *The Journal of Infectious Diseases,* 190, 2031–2038.

Morgan, D. J., Okeke, I. N., Laxminarayan, R., Perencevich, E. N., & Weisenberg, S. (2011). Non-prescription antimicrobial use worldwide: A systematic review. *The Lancet Infectious Diseases,* 11, 692–701.

Motta, S. S., Cluzel, P., & Aldana, M. (2015). Adaptive resistance in bacteria requires epigenetic inheritance, genetic noise, and cost of efflux pumps. *PLoS One,* 10, e0118464.

Murphy, T. V., Pastor, P., Medley, F., Osterholm, M. T., & Granoff, D. M. (1993). Decreased Haemophilus colonization in children vaccinated with Haemophilus influenzae type b conjugate vaccine. *The Journal of Pediatrics,* 122, 517–523.

Nair, D. V. T., Venkitanarayanan, K., & Kollanoor Johny, A. (2018). Antibiotic-resistant salmonella in the food supply and the potential role of antibiotic alternatives for control. *Food,* 7, e00428–e00416.

Naylor, N. R., et al. (2018). Estimating the burden of antimicrobial resistance: A systematic literature review. *Antimicrobial Resistance and Infection Control,* 7, 58.

Neuzil, K. M., Mellen, B. G., Wright, P. F., Mitchel, E. F., Jr., & Griffin, M. R. (2000). The effect of influenza on hospitalizations, outpatient visits, and courses of antibiotics in children. *The New England Journal of Medicine,* 342, 225–231.

Nilsson, P., & Laurell, M. H. (2006). A 10-year follow-up study of penicillin-non-susceptible S. pneumoniae during an intervention programme in Malmo, Sweden. *Scandinavian Journal of Infectious Diseases,* 38, 838–844.

Norhayati, M. N., Ho, J. J., & Azman, M. Y. (2017). Influenza vaccines for preventing acute otitis media in infants and children. *Cochrane Database of Systematic Reviews,* 10, CD010089.

Nunan, C. (2022). *Ending routine farm antibiotic use in Europe. Achieving responsible farm antibiotic use through improving animal health and welfare in pig and poultry production.* European Public Health Alliance, Brussels, Belguim.

O'Brien, K. L., et al. (2009). Burden of disease caused by Streptococcus pneumoniae in children younger than 5 years: Global estimates. *Lancet,* 374, 893–902.

Obolski, U., et al. (2018). Vaccination can drive an increase in frequencies of antibiotic resistance among nonvaccine serotypes of Streptococcus pneumoniae. *Proceedings of the National Academy of Sciences of the United States of America,* 115, 3102–3107.

O'Brien, K. L., & Dagan, R. (2003). The potential indirect effect of conjugate pneumococcal vaccines. *Vaccine,* 21, 1815–1825.

O'Brien, K. L., et al. (2003). Efficacy and safety of seven-valent conjugate pneumococcal vaccine in American Indian children: Group randomised trial. *Lancet,* 362, 355–361.

Ocan, M., et al. (2015). Household antimicrobial self-medication: A systematic review and meta-analysis of the burden, risk factors and outcomes in developing countries. *BMC Public Health,* 15, 742.

Ochiai, R. L., et al. (2008). A study of typhoid fever in five Asian countries: Disease burden and implications for controls. *Bulletin of the World Health Organization,* 86, 260–268.

OECD. (2018). *Stemming the superbug tide: Just a few dollars more*. https://doi.org/10.1787/9789264307599-en

Oliver, S. P., Murinda, S. E., & Jayarao, B. M. (2011). Impact of antibiotic use in adult dairy cows on antimicrobial resistance of veterinary and human pathogens: A comprehensive review. *Foodborne Pathogens and Disease,* 8, 337–355.

Ortqvist, A., Hedlund, J., & Kalin, M. (2005). Streptococcus pneumoniae: Epidemiology, risk factors, and clinical features. *Seminars in Respiratory and Critical Care Medicine,* 26, 563–574.

Ozawa, D., et al. (2015). Impact of the seven-valent pneumococcal conjugate vaccine on acute otitis media in Japanese children: Emergence of serotype 15A multidrug-resistant streptococcus pneumoniae in middle ear fluid isolates. *The Pediatric Infectious Disease Journal,* 34, e217–e221.

Ozdemir, H., et al. (2014). Nasopharyngeal carriage of Streptococcus pneumoniae in healthy Turkish children after the addition of PCV7 to the national vaccine schedule. *European Journal of Pediatrics,* 173, 313–320.

Pace, D., & Pollard, A. J. (2012). Meningococcal disease: Clinical presentation and sequelae. *Vaccine,* 30(Suppl 2), B3–B9.

Palmu, A. A., Rinta-Kokko, H., Nohynek, H., Nuorti, J. P., & Jokinen, J. (2018). Impact of national ten-valent pneumococcal conjugate vaccine program on reducing antimicrobial use and tympanostomy tube placements in Finland. *The Pediatric Infectious Disease Journal,* 37, 97–102.

Palmu, A. A., et al. (2014). Effect of pneumococcal Haemophilus influenzae protein D conjugate vaccine (PHiD-CV10) on outpatient antimicrobial purchases: A double-blind, cluster randomised phase 3-4 trial. *The Lancet Infectious Diseases,* 14, 205–212.

Paynter, J., et al. (2019). Effectiveness of a group B outer membrane vesicle meningococcal vaccine in preventing hospitalization from Gonorrhea in New Zealand: A retrospective cohort study. *Vaccines (Basel),* 7, 5.

Peiponen, K. S., Tirkkonen, B. T., Junnila, J. J. T., & Heinonen, M. L. (2018). Effect of a live attenuated vaccine against Lawsonia intracellularis in weaned and finishing pig settings in Finland. *Acta Veterinaria Scandinavica,* 60, 18.

Peltola, H., et al. (1999). Perspective: A five-country analysis of the impact of four different Haemophilus influenzae type b conjugates and vaccination strategies in Scandinavia. *The Journal of Infectious Diseases,* 179, 223–229.

Pelton, S. I., Loughlin, A. M., & Marchant, C. D. (2004). Seven valent pneumococcal conjugate vaccine immunization in two Boston communities: Changes in serotypes and antimicrobial susceptibility among Streptococcus pneumoniae isolates. *The Pediatric Infectious Disease Journal,* 23, 1015–1022.

Peterson, C. L., et al. (1996). Children hospitalized for varicella: A prevaccine review. *The Journal of Pediatrics,* 129, 529–536.

Pieters, Z., Saad, N. J., Antillon, M., Pitzer, V. E., & Bilcke, J. (2018). Case fatality rate of enteric fever in endemic countries: A systematic review and meta-analysis. *Clinical Infectious Diseases,* 67, 628–638.

Pilishvili, T., & Bennett, N. M. (2015). Pneumococcal disease prevention among adults: Strategies for the use of pneumococcal vaccines. *American Journal of Preventive Medicine,* 49, S383–S390.

Pillai, D. R., et al. (2009). Genome-wide dissection of globally emergent multi-drug resistant serotype 19A Streptococcus pneumoniae. *BMC Genomics,* 10, 642.

Pitzer, V. E., et al. (2014). Predicting the impact of vaccination on the transmission dynamics of typhoid in South Asia: A mathematical modeling study. *PLoS Neglected Tropical Diseases,* 8, e2642.

Plotkin, S. A. (2010). Correlates of protection induced by vaccination. *Clinical and Vaccine Immunology,* 17, 1055–1065.

Plotkin, S. A., Orenstein, W. A., Offit, P. A., & Edwards, K. M. (2017). *Plotkin's vaccines*. (7th edn.), Elsevier B.V.

Poehling, K. A., et al. (2007). Reduction of frequent otitis media and pressure-equalizing tube insertions in children after introduction of pneumococcal conjugate vaccine. *Pediatrics,* 119, 707–715.

Postma, M., Vanderhaeghen, W., Sarrazin, S., Maes, D., & Dewulf, J. (2017). Reducing antimicrobial usage in pig production without jeopardizing production parameters. *Zoonoses and Public Health,* 64, 63–74.

Postma, M., et al. (2016). Evaluation of the relationship between the biosecurity status, production parameters, herd characteristics and antimicrobial usage in farrow-to-finish pig production in four EU countries. *Porcine Health Manag,* 2, 9.

Potter, A., Gerdts, V., & Littel-van den Hurk, S. (2008). Veterinary vaccines: Alternatives to antibiotics? *Animal Health Research Reviews,* 9, 187–199.

Principi, N., Baggi, E., & Esposito, S. (2012). Prevention of acute otitis media using currently available vaccines. *Future Microbiology,* 7, 457–465.

Principi, N., Esposito, S., Marchisio, P., Gasparini, R., & Crovari, P. (2003). Socioeconomic impact of influenza on healthy children and their families. *The Pediatric Infectious Disease Journal,* 22, S207–S210.

Pumart, P., Phoda, T., Thamlikitkul, V., & Riewpaiboon, A. (2012). Health and economic impacts of antimicrobial resistance in Thailand. *Journal of Health Services Research & Policy,* 6, 352–360.

Raith, J., et al. (2016). Influence of porcine circovirus type 2 vaccination on the level of antimicrobial consumption on 65 Austrian pig farms. *The Veterinary Record,* 178, 504.

Rappuoli, R., Bloom, D. E., & Black, S. (2017). Deploy vaccines to fight superbugs. *Nature,* 552, 165–167.

Rappuoli, R., Mandl, C. W., Black, S., & De Gregorio, E. (2011). Vaccines for the twenty-first century society. *Nature Reviews. Immunology,* 11, 865–872.

Raviglione, M., et al. (2012). Scaling up interventions to achieve global tuberculosis control: Progress and new developments. *Lancet,* 379, 1902–1913.

Reilly, A. S., McElligott, M., Mac Dermott Casement, C., & Drew, R. J. (2022). Haemophilus influenzae type f in the post-Haemophilus influenzae type b vaccination era: A systematic review. *Journal of Medical Microbiology,* 71, 001606.

Reinert, R. R. (2004). Pneumococcal conjugate vaccines--a European perspective. *International Journal of Medical Microbiology,* 294, 277–294.

Reinert, R. R. (2009). The antimicrobial resistance profile of Streptococcus pneumoniae. *Clinical Microbiology and Infection,* 15(Suppl 3), 7–11.

Reynolds, C. A., Finkelstein, J. A., Ray, G. T., Moore, M. R., & Huang, S. S. (2014). Attributable healthcare utilization and cost of pneumonia due to drug-resistant streptococcus pneumonia: A cost analysis. *Antimicrobial Resistance and Infection Control,* 3, 16.

Rojo-Gimeno, C., et al. (2016). Farm-economic analysis of reducing antimicrobial use whilst adopting improved management strategies on farrow-to-finish pig farms. *Preventive Veterinary Medicine,* 129, 74–87.

Rostamian, M., et al. (2022). A systematic review and meta-analysis on the antibiotic resistance of Neisseria meningitidis in the last 20 years in the world. *Indian Journal of Medical Microbiology,* 40, 323–329.

Rouphael, N. G., & Stephens, D. S. (2012). Neisseria meningitidis: Biology, microbiology, and epidemiology. *Methods in Molecular Biology,* 799, 1–20.

Rozen, D. E., McGee, L., Levin, B. R., & Klugman, K. P. (2007). Fitness costs of fluoroquinolone resistance in Streptococcus pneumoniae. *Antimicrobial Agents and Chemotherapy,* 51, 412–416.

Ruuskanen, O., & Heikkinen, T. (1994). Otitis media: Etiology and diagnosis. *The Pediatric Infectious Disease Journal,* 13, S23–S26; discussion S50-24.

Sabuncu, E., et al. (2009). Significant reduction of antibiotic use in the community after a nationwide campaign in France, 2002-2007. *PLoS Medicine,* 6, e1000084.

Sa-Leao, R., et al. (2009). Changes in pneumococcal serotypes and antibiotypes carried by vaccinated and unvaccinated day-care centre attendees in Portugal, a country with widespread use of the seven-valent pneumococcal conjugate vaccine. *Clinical Microbiology and Infection,* 15, 1002–1007.

Savulescu, C., et al. (2017). Effect of high-valency pneumococcal conjugate vaccines on invasive pneumococcal disease in children in SpIDnet countries: An observational multicentre study. *The Lancet Respiratory Medicine,* 5, 648–656.

Schaffer, A. C., & Lee, J. C. (2009). Staphylococcal vaccines and immunotherapies. *Infectious Disease Clinics of North America,* 23, 153–171.

Schuchat, A., et al. (1997). Bacterial meningitis in the United States in 1995. Active Surveillance Team. *The New England Journal of Medicine,* 337, 970–976.

Seale, A. C., et al. (2017). Estimates of the burden of group B streptococcal disease worldwide for pregnant women, stillbirths, and children. *Clinical Infectious Diseases,* 65, S200–S219.

Shah, S. S., & Ratner, A. J. (2006). Trends in invasive pneumococcal disease-associated hospitalizations. *Clinical Infectious Diseases,* 42, e1–e5.

Shea, K. M., Weycker, D., Stevenson, A. E., Strutton, D. R., & Pelton, S. I. (2011). Modeling the decline in pneumococcal acute otitis media following the introduction of pneumococcal conjugate vaccines in the US. *Vaccine,* 29, 8042–8048.

Sherman, R. E., et al. (2016). Real-world evidence – What is it and what can it tell us? *The New England Journal of Medicine,* 375, 2293–2297.

Simell, B., et al. (2012). The fundamental link between pneumococcal carriage and disease. *Expert Review of Vaccines,* 11, 841–855.

Sniadack, D. H., et al. (1995). Potential interventions for the prevention of childhood pneumonia: geographic and temporal differences in serotype and serogroup distribution of sterile site pneumococcal isolates from children--implications for vaccine strategies. *The Pediatric Infectious Disease Journal,* 14, 503–510.

Song, J. H., Dagan, R., Klugman, K. P., & Fritzell, B. (2012). The relationship between pneumococcal serotypes and antibiotic resistance. *Vaccine,* 30, 2728–2737.

Song, J. H., et al. (1999). Spread of drug-resistant Streptococcus pneumoniae in Asian countries: Asian Network for Surveillance of Resistant Pathogens (ANSORP) Study. *Clinical Infectious Diseases,* 28, 1206–1211.

Song, J. H., et al. (2004). High prevalence of antimicrobial resistance among clinical Streptococcus pneumoniae isolates in Asia (an ANSORP study). *Antimicrobial Agents and Chemotherapy,* 48, 2101–2107.

Soumahoro, L., Abitbol, V., Vicic, N., Bekkat-Berkani, R., & Safadi, M. A. P. (2021). Meningococcal disease outbreaks: A moving target and a case for routine preventative vaccination. *Infectious Disease and Therapy,* 10, 1949–1988.

Speksnijder, D. C., Jaarsma, D. A., Verheij, T. J., & Wagenaar, J. A. (2015). Attitudes and perceptions of Dutch veterinarians on their role in the reduction of antimicrobial use in farm animals. *Preventive Veterinary Medicine,* 121, 365–373.

Spellberg, B., & Daum, R. (2012). Development of a vaccine against Staphylococcus aureus. *Seminars in Immunopathology,* 34, 335–348.

Spratt, B. G., & Greenwood, B. M. (2000). Prevention of pneumococcal disease by vaccination: Does serotype replacement matter? *Lancet,* 356, 1210–1211.

Srikumaran, S., Kelling, C. L., & Ambagala, A. (2007). Immune evasion by pathogens of bovine respiratory disease complex. *Animal Health Research Reviews,* 8, 215–229.

Stamboulidis, K., et al. (2011). The impact of the heptavalent pneumococcal conjugate vaccine on the epidemiology of acute otitis media complicated by otorrhea. *The Pediatric Infectious Disease Journal,* 30, 551–555.

Stanek, R. J., Norton, N. B., & Mufson, M. A. (2016). A 32-year study of the effect of pneumococcal vaccines on invasive Streptococcus pneumoniae Disease. *The American Journal of the Medical Sciences,* 352, 563–573.

Steele, A. D., Hay Burgess, D. C., Diaz, Z., Carey, M. E., & Zaidi, A. K. (2016). Challenges and opportunities for typhoid fever control: A call for coordinated action. *Clinical Infectious Diseases,* 62(Suppl 1), S4–S8.

Stefanelli, P. (2019). Pertussis: Identification, prevention and control. *Advances in Experimental Medicine and Biology,* 1183, 127–136.

Stilwell, G., Matos, M., Carolino, N., & Lima, M. S. (2008). Effect of a quadrivalent vaccine against respiratory virus on the incidence of respiratory disease in weaned beef calves. *Preventive Veterinary Medicine,* 85, 151–157.

Suay-Garcia, B., & Perez-Gracia, M. T. (2017). Drug-resistant Neisseria gonorrhoeae: Latest developments. *European Journal of Clinical Microbiology & Infectious Diseases,* 36, 1065–1071.

Tagliabue, A., Leite, L. C. C., Leroy, O. Y., & Rappuoli, R. (2019). Editorial: A global perspective on vaccines: priorities, challenges and online information. *Frontiers in Immunology,* 10, 2556.

Tagliabue, A., & Rappuoli, R. (2018). Changing priorities in vaccinology: Antibiotic resistance moving to the top. *Frontiers in Immunology,* 9, 1068.

Tang, K. L., et al. (2017). Restricting the use of antibiotics in food-producing animals and its associations with antibiotic resistance in food-producing animals and human beings: A systematic review and meta-analysis. *Lancet Planet Health,* 1, e316–e327.

Taylor, J., Hafner, M. Yesushalmi, E., Smith, R., Bellasi, J., Vardavas, R., Bienkowska-Gibbs, T., & Rubin, J. (2014). *Estimating the economic costs of antimicrobial resistance: Model and results*. RAND Europe, Cambridge, U.K.

Taylor, S., Marchisio, P., Vergison, A., Hausdorff, W. P., & Haggard, M. (2012). Pneumococcal conjugate vaccines and otitis media. *International Journal of Otolaryngology,* 2012, 764573.

Tekle, Y. I., et al. (2012). Controlling antimicrobial resistance through targeted, vaccine-induced replacement of strains. *PLoS One,* 7, e50688.

Temtem, C., Kruse, A. B., Nielsen, L. R., Pedersen, K. S., & Alban, L. (2016). Comparison of the antimicrobial consumption in weaning pigs in Danish sow herds with different vaccine purchase patterns during 2013. *Porcine Health Manag,* 2, 23.

Thacker, E. L., Thacker, B. J., Young, T. F., & Halbur, P. G. (2000). Effect of vaccination on the potentiation of porcine reproductive and respiratory syndrome virus (PRRSV)-induced pneumonia by Mycoplasma hyopneumoniae. *Vaccine,* 18, 1244–1252.

Thomas, L. F., Bellet, C., & Rushton, J. (2019). Using economic and social data to improve veterinary vaccine development: Learning lessons from human vaccinology. *Vaccine,* 37, 3974–3980.

Tin Tin Htar, M., Christopoulou, D., & Schmitt, H. J. (2015). Pneumococcal serotype evolution in Western Europe. *BMC Infectious Diseases,* 15, 419.

Tin Tin Htar, M., et al. (2013). A review of the impact of pneumococcal polysaccharide conjugate vaccine (7-valent) on pneumococcal meningitis. *Advances in Therapy,* 30, 748–762.

Tin Tin Htar, M., et al. (2019). The impact of routine childhood immunization with higher-valent pneumococcal conjugate vaccines on antimicrobial-resistant pneumococcal diseases and carriage: A systematic literature review. *Expert Review of Vaccines,* 18, 1069–1089.

Tomczyk, S., et al. (2016). Prevention of antibiotic-nonsusceptible invasive pneumococcal disease with the 13-valent pneumococcal conjugate vaccine. *Clinical Infectious Diseases,* 62, 1119–1125.

Tristram, S., Jacobs, M. R., & Appelbaum, P. C. (2007). Antimicrobial resistance in Haemophilus influenzae. *Clinical Microbiology Reviews,* 20, 368–389.

Trzcinski, K., Thompson, C. M., Gilbey, A. M., Dowson, C. G., & Lipsitch, M. (2006). Incremental increase in fitness cost with increased beta-lactam resistance in pneumococci evaluated by competition in an infant rat nasal colonization model. *The Journal of Infectious Diseases,* 193, 1296–1303.

Unemo, M., et al. (2019). Gonorrhoea. *Nature Reviews. Disease Primers,* 5, 79.

Van Boeckel, T. P., et al. (2019). Global trends in antimicrobial resistance in animals in low- and middle-income countries. *Science,* 365, eaaw1944.

van de Sande-Bruinsma, N., et al. (2008). Antimicrobial drug use and resistance in Europe. *Emerging Infectious Diseases,* 14, 1722–1730.

Van Eldere, J., Mera, R. M., Miller, L. A., Poupard, J. A., & Amrine-Madsen, H. (2007). Risk factors for development of multiple-class resistance to Streptococcus pneumoniae Strains in Belgium over a 10-year period: Antimicrobial consumption, population density, and geographic location. *Antimicrobial Agents and Chemotherapy,* 51, 3491–3497.

van Gils, E. J., et al. (2009). Effect of reduced-dose schedules with 7-valent pneumococcal conjugate vaccine on nasopharyngeal pneumococcal carriage in children: A randomized controlled trial. *JAMA,* 302, 159–167.

Veenhoven, R., et al. (2003). Effect of conjugate pneumococcal vaccine followed by polysaccharide pneumococcal vaccine on recurrent acute otitis media: A randomised study. *Lancet,* 361, 2189–2195.

Vetter, V., Denizer, G., Friedland, L. R., Krishnan, J., & Shapiro, M. (2018). Understanding modern-day vaccines: What you need to know. *Annals of Medicine,* 50, 110–120.

Vincent, J. L., et al. (2009). International study of the prevalence and outcomes of infection in intensive care units. *JAMA,* 302, 2323–2329.

von Konig, C. H. (2005). Use of antibiotics in the prevention and treatment of pertussis. *The Pediatric Infectious Disease Journal,* 24, S66–S68.

Wahl, B., et al. (2018). Burden of Streptococcus pneumoniae and Haemophilus influenzae type b disease in children in the era of conjugate vaccines: Global, regional, and national estimates for 2000-15. *The Lancet Global Health,* 6, e744–e757.

Walker, D. G., Hutubessy, R., & Beutels, P. (2010). WHO guide for standardisation of economic evaluations of immunization programmes. *Vaccine,* 28, 2356–2359.

Wang, B., et al. (2016). Effect of influenza vaccination of children on infection rate in hutterite communities: Follow-up study of a randomized trial. *PLoS One,* 11, e0167281.

Weil-Olivier, C., van der Linden, M., de Schutter, I., Dagan, R., & Mantovani, L. (2012). Prevention of pneumococcal diseases in the post-seven valent vaccine era: A European perspective. *BMC Infectious Diseases,* 12, 207.

Weinberger, D. M., Malley, R., & Lipsitch, M. (2011). Serotype replacement in disease after pneumococcal vaccination. *Lancet,* 378, 1962–1973.

Whitney, C. G., et al. (2000). Increasing prevalence of multidrug-resistant Streptococcus pneumoniae in the United States. *The New England Journal of Medicine,* 343, 1917–1924.

Whitney, C. G., et al. (2003). Decline in invasive pneumococcal disease after the introduction of protein-polysaccharide conjugate vaccine. *The New England Journal of Medicine,* 348, 1737–1746.

Whittaker, R., et al. (2017). Epidemiology of invasive haemophilus influenzae disease, Europe, 2007-2014. *Emerging Infectious Diseases,* 23, 396–404.

Wilby, K. J., & Werry, D. (2012). A review of the effect of immunization programs on antimicrobial utilization. *Vaccine,* 30, 6509–6514.

Wilkinson, K., Righolt, C. H., Elliott, L. J., Fanella, S., & Mahmud, S. M. (2021). Pertussis vaccine effectiveness and duration of protection – A systematic review and meta-analysis. *Vaccine,* 39, 3120–3130.

Yao, K., et al. (2020). The epidemic of erythromycin-resistant Bordetella pertussis with limited genome variation associated with pertussis resurgence in China. *Expert Review of Vaccines,* 19, 1093–1099.

Yatsyshina, S., et al. (2016). Detection of respiratory pathogens in pediatric acute otitis media by PCR and comparison of findings in the middle ear and nasopharynx. *Diagnostic Microbiology and Infectious Disease,* 85, 125–130.

zur Wiesch, P. A., Kouyos, R., Engelstadter, J., Regoes, R. R., & Bonhoeffer, S. (2011). Population biological principles of drug-resistance evolution in infectious diseases. *The Lancet Infectious Diseases,* 11, 236–247.

Nanoparticle Approach to Control AMR

Visnuvinayagam Sivam, Anandan Rangasamy, and Pavan Kumar Dara

Contents

Abstract

The chapter describes the antibacterial activity of metal nanoparticles and the possibility to control the antimicrobial-resistant (AMR) pathogens. A vast application of metal nanoparticles is being practiced in engineering fields such as solar

V. Sivam (✉)
Microbiology, Fermentation and Biotechnology Division, ICAR-Central Institute of Fisheries Technology (ICAR-CIFT), Kochi, India

A. Rangasamy · P. K. Dara
Biochemistry and Nutrition Division, ICAR-Central Institute of Fisheries Technology, Kochi, India

M. P. Mothadaka et al. (eds.), *Handbook on Antimicrobial Resistance*,
https://doi.org/10.1007/978-981-19-9279-7_42

panels, gas sensors, and biosensors. But a limited application of nanoparticles is available in controlling AMR. Even though there is a considerable report on the antimicrobial activity of the AMR, the main inference in the application of nanoparticles is toxicity. Controlling pathogen by direct application on food or any environment is not permitted. Various types of metal nanoparticles and their role in the destruction of microbes and the possibility of their application to control the AMR are discussed. The application of nanoparticles is of great interest in the packaging materials for food to control the surface bacterial load, which is getting more attention due to the meager toxic effect. The addition of nanoparticles in the packaging material and cloths is based on the migration level. In addition, the antimicrobial activity can be augmented by techniques, viz., doping, capping, the addition of other nanoparticles, and surface modification with stabilizers and chemicals, producing better antimicrobial activity with a lesser toxicity. From the clinical aspect, since it has a better antioxidant activity, based on the criticality of the patient, the NP can be applied for treatments. In the case of controlling AMR in the environment, a suitable treatment method is to be designed to reduce the toxic level of the nanoparticles.

Keywords

Nanoparticles · Metal Oxide Nanoparticles · AMR · Pathogen · Zinc Oxide Nanoparticles

1 Introduction

Worldwide, antimicrobial resistance (AMR) in microbes is steadily increasing in human and animal healthcare systems because of the frequent application of various antibiotics, which imposed a selective tendency for antimicrobial resistance to emerge. Recent evidence suggests that even some bacteria acquire resistance to new antimicrobial agents (Raffi et al., 2010). International organizations predicted that AMR pathogens will pose a significant risk to human and animal health (Raffi et al., 2010). Recently, there is increasing evidence for the prevalence of multidrug-resistant (MDR) bacteria in food, including the retail fish market (Visnuvinayagam et al., 2015, 2016, 2017, 2019a); most of the water bodies (Vaiyapuri et al., 2021a); industrial effluent releasing area (Sivaraman et al., 2017, 2021); retail poultry market (Murugadas et al., 2015); dry fish markets (Sivarman et al., 2021), all have a high level of MDR bacteria. To combat this growing AMR public health threat, initiatives were taken worldwide. An alternative to antibiotics is the main promising strategy devised for mitigating AMR issues. Several alternatives were tried to control the AMR/pathogenic bacteria, that is, bacteriocins (Neha et al., 2019), bacteriophages (Vaiyapuri et al., 2021b; Karthika et al., 2021; Benala et al., 2021), plant extracts (Viji et al., 2015, 2016, 2017; Annamalai et al., 2018; Parvathy et al., 2018: Murthy et al., 2017: Maqbool et al., 2020), nanoparticles (Visnuvinayagam et al., 2019b,

2021; Dara et al. 2021a, 2021b), electron beam irradiation (Visnuvinayagam et al., 2020; Annamalai et al., 2020), etc. Each method has certain advantages as well as limitations. Nanoparticle-based antimicrobial composites are considered one of the promising alternatives to antibiotics. Among the nanoparticles, metal/metal oxide nanoparticles have lately gained importance as a tool to control MDR bacteria in various sectors.

Silver ions and silver-based compounds have been used as resourceful health additives in the Indian and Chinese Ayurvedic systems of medicine since the year 1000 BCE (Ellis, 2013). During the eighth century, Moyer was the first to mention the use of silver for therapeutic uses (Moyer, 1965). Inorganic metals like copper and silver are used to store the water/food for disinfection. Currencies made up of gold, silver, and copper have played a significant role in reducing the pathogen while handling. But these applications were limited due to their larger size (Suvith & Philip, 2014).

Nanotechnology is a topic of contemporary materials science studies that can be used for a variety of novel applications. The term nanoparticles is specifically defined for any materials with 1–100 nm size, either widthwise or lengthwise (EU, 2009). The International Organization for Standardization (ISO) also states that "any material with any exterior dimension between 1 and 100 nm are considered a Nanoparticles" (Deshmukh et al., 2019). Whenever a material's size decreases, it loses many of its physical properties, particularly compared with bulk materials, that is, they exhibit size-related properties that vary significantly from their bulk materials. In contrast to other materials, NPs have larger systems due to their small scale, because of which they are used in various fields, including optical, electromagnetic nanomaterials, biosensors, nanomedicine, and bionanotechnology.

Nanoparticles made from various metals and their oxides have a greater antimicrobial activity. Metal/metal oxide nanoparticles, that is, Ag, Cu, Au, ZnO, CuO, TiO_2, MgO_2, Fe_2O_3, Al_2O_3, Y_2O_3, and CeO_2, have an antibacterial effect on a variety of microorganisms, including bacteria (both Gram-positive and Gram-negative) and fungus. Even though metallic nanoparticles have potent antimicrobial activity, concerns about their discharge and intake have yet to be addressed in the context of ecological and social safety in detail. For example, releasing the excessive nanosilver into the environment damages the ecosystem, and it may be consumed by humans and animals indirectly (Usman et al., 2013). This chapter discusses a wide range of antimicrobial properties on resistant pathogenic microorganisms in detail. Also, different types of metal nanoparticles and their role in the destruction of microbes and the possibility of their application to control the AMR are also discussed.

2 Silver Nanoparticles (SNPs)

In the 1960s, silver began to be used for treatments as a colloidal material. Silver was initially used as a disinfectant and in the treatment of wounds as well. Then, a huge number of studies were carried out after the advent of the nanotechnology

(Deshmukh et al., 2019). SNP is the most studied nanoparticle (NPs) compared with others because of its superior antibacterial activity; hence, it is being widely used as an antimicrobial agent for biomedical applications such as wound-dressing materials, air/water purification, and coating material in the paint industry (Burdus et al., 2018). In addition, it has been shown to have antiviral activities. While optimizing the SNP for the antimicrobial activity, physical, chemical, and optical properties should be considered for better applications, for example, surface property, the distribution pattern of the different sizes, morphological structure, particle compositions, and dissolution rate and capping agents used. Capping technology on the nanoparticles makes the NPs more stable with a long duration and without agglomeration (Burdus et al., 2018).

SNPs can absorb electromagnetic radiation between 380 and 450 nm. Hence, based on the absorption maxima (λ max), the size of the SNP can be assessed. Here, λ max is lower for the small-sized SNP, for example, λmax for the 7-nm-sized SNP is about 410 nm; likewise, 29-nm and 89-nm-sized SNPs have the λmax of 425 nm and 490 nm, respectively. λmax is not only strictly based on the size of the SNPs but also varies based on the shape of the SNPs. Different shapes such as nanosphere, necklaces, nanobars, nanocubes, nanoprisms, nanostar, bipyramids, nanowires, and nanorods can be produced by changing the pH, temperature, composition, etc. (Tran et al., 2015).

In biomedical applications, especially in cancer imaging, the SNP nanoshell is used to find the location of the cancer cell in the body based on the photothermal effect. It has also been used to detect the aromatic components in vivo multiple molecular imaging (Kang et al., 2013).

2.1 Antibacterial Activities

Nanosilver systems offer interesting advantages that help in using them as antimicrobials. They are highly active against a variety of microbes even at low doses. While testing with a clinically important dental microbe, the bacteriostatic and bactericidal effects of SNP were five times higher than chlorhexidine (Panpaliya et al., 2019). The minimum inhibition concentrations (MICs) for *Streptococcus mutants, Streptococcus oralis, Lactobacillus acidophilus,* and *Lactobacillus fermentum* were 60, 45, 15, and 90 μg/ml, respectively (Panpaliya et al., 2019). Regarding foodborne pathogens, the MIC was still lower than the clinical pathogens. The MICs for *Escherichia coli, Salmonella* sp., and *Klebsiella* sp. were about 7.8, 3.9, and 3.9 μg/mL, respectively. SNPs were found to be active against bacteria such as *Streptomyces albogriseolus, Sporosarcina koreensis*, *Acinetobacter calcoaceticus*, *Brevibacterium casei, Streptacidiphilus durhamensis, Aeromonas* sp., molds and yeasts (*Schizosaccharomyces* sp.), C. *albicans*, *Aspergillus* sp., *Penicillium* sp., *Fusarium* sp., *Trichoderma* sp., *Paracoccus* sp., *Phanerochaete chrysosporium, Synechococcus* sp., *Scytonema* sp., *Tinea versicolor*, and white and brown-rot fungi. SNP-coated cotton, polyester, and nylon textiles had a stronger

antibacterial activity against *Escherichia coli* than *Chromobacterium haemolyticum*, and an even better antibacterial activity against *Bacillus cereus* (Syafiuddin et al., 2020).

The antibacterial activity of the SNP also varied with bacterial type. In general, Gram-negative bacteria are comparatively much susceptible to SNP than Gram-positive bacteria because of the outer higher lipid bilayer and poor cell wall thickness in Gram-negative bacteria. Even though the peptidoglycan layers of both Gram-positive and Gram-negative are negatively charged, the thickness of the peptidoglycan is only 3–4 mm for Gram-positive and 30 mm for Gram-negative bacteria (Abbaszadegan et al., 2015). The addition of other chemicals has been shown to augment the antibacterial action of the SNPs (Dara et al., 2020).

2.2 Mode of Action

Assessing the antibacterial property of SNPs is of utmost prerequisite to enhance the antibacterial activity as well as control its toxicity. The proposed mechanism of action of SNPs is hypothetical, thereby lacking clarity on antibacterial activity (Rashki et al., 2021). The most common mode of action reported is attachment to the bacterial cell membrane, modifying the lipid bilayer and enhancing the permeability of the membrane, which leads to intracellular penetration. After internalization, this SNP triggers the reactive oxygen species (ROS) as well as free radicals, which play a main role in the destruction of the cell organelles in the bacteria. In addition, SNP internalization causes a change in the modulation of the pathway, leading to the death of the bacterial cells. A similar mechanism was reported in the fungus and other eukaryotes such as cell lines.

Another mechanism of action includes damages to the thiol group of enzymes, which are responsible for cellular respiration being impaired, leading to cell death. ROS, H_2O_2, superoxide (O_2-), hydroxy radical (OH-) are highly elevated during the SNP application on the cell. An increase in cellular toxicity due to the SNP internalization causes the apoptosis of the cell (Kim et al., 2011).

2.3 Toxicity of SNPs

SNPs can be used in various sectors; their potential antibacterial toxicity is also considered to protect the environment. Research on toxicity is still in a nascent state, especially in food applications. Silver has a high affinity with most of the compounds present in the environment, especially with anions, for example, sulfides, halides, and phosphates. Similarly, it has an affinity with organic molecules, viz., amines and thiols. Cations such as Ca_{2+} and Mg_{2+} also cause Ag NP aggregation by displacing their citrate ligand shell (Le Ouay & Stellacci, 2015).

Although many research findings recommend the use of SNPs to control foodborne pathogens, international regulations have not permitted any nanomaterials, including SNPs (Visnuvinayagam et al., 2021). The nanoparticle toxicity

has hampered their usage in more refined applications. A toxicity report based on the intro-toxicity experiment of silver nanoparticles in rat hepatocytes revealed that even modest levels of silver nanoparticle exposure lead to oxidative stress and mitochondrial damage. However, it can be applied indirectly for food applications such as SNP-coated packaging films and canes, which control the surface-contaminated food material by antibacterial activity (Imran et al., 2010). The main objective of safety is addressed in the concept of migration potential. The international organization recommends the migration percentage limit for the SNP in the packaging films of surface coating; this is also applicable to textiles. SNP-coated cloths are considered to have better antimicrobial properties. Still, as per the regulation, the migration level has to be checked from the cloth to the skin (Rovira et al., 2017).

3 Zinc Oxide Nanoparticles (ZnO-NP)

In India, zinc has been used for treating health problems through naturopathy (Ayurveda) for 2000 years. According to Ayurvedic books, ZnO is one of the ideal therapies for diabetes. In allopathy, physicians prescribe zinc gluconate zinc salt to the zinc-deficient person as a substitute for zinc (Umrani & Paknikar, 2014).

The USFDA classified zinc oxide (ZnO) as "Generally Accepted As Safe (GRAS)"(21CFR182.8991). Zinc is the most used food additive for the fortification of cereal-based products. The GRAS status is applicable to the bulk ZnO. A misunderstanding of GRAS status to ZnO-NPs for use in food applications exists. But it has been approved for use in the food packaging materials such as packages. Because of this, various applications have been introduced for food can's inner surface containing various meat and vegetable products to preserve color and avoid spoilage of food due to their antimicrobial properties.

ZnO-NPs have been often used in engineering fields since the last few decades with many improved applications. But in case of biological application, it is still in the nascent stage. Considerable research on the antibacterial properties is still required. ZnO is heat-resistant, more selective, and more durable than organic and inorganic materials. The general properties of the NPs, that is, high surface-to-volume ratio, small size (M100 nm), and stronger antimicrobial, are also applicable to the ZnO-NP. Besides, it has a UV absorption rate of between 315 nm and 400 nm (UVA region) and 280 nm and 315 nm (UVB region), which is helpful in antibacterial activity and is used as a cosmetic as a UV protector.

The size of the NPs can be indirectly measured by UV spectra because their absorption range is between 327 and 370 nm. Any absorption peaks that are >370 nm are not considered NPs. Most of the ZnO-NP λmax values are between 350 and 370 (Fig. 1a). Hence, the bulk ZnO particle's absorption peak (λ max) ranges between 380 and 385 nm (Fig. 1b). Chemically, the absorption edge moves to a lower wavelength with a decreasing nanoparticle size. This is called a blue shift; when comparing the λmax value of the bulk materials, the NP wavelength is lower. The change in the λ max value is an indication of the smaller particles compared with others, that is, bulk particles (Gupta et al., 2015). A lower λmax provides the smallest

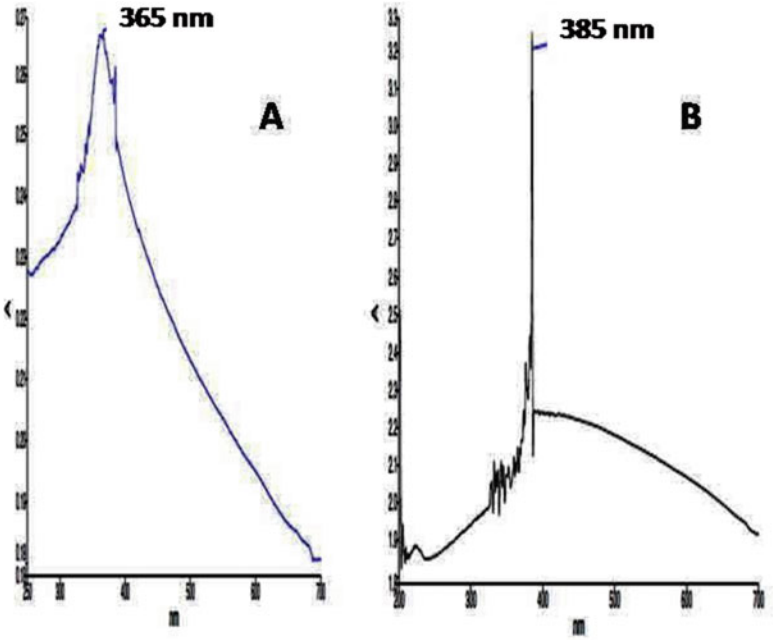


Fig. 1 UV absorption spectra for zinc oxide nanoparticles and bulk particles. (**a**) λmax of ZnO-nanoparticles; (**b**) λmax of ZnO- bulk particles. (Source: Courtesy of the Journal of Environmental Biology, Thrivey Academy, Lucknow, India)

size of the NPs. However, the exact particle size of the ZnO-NP cannot be assessed by UV spectra, and it can be confirmed by scanning electron microscope (SEM) or transmission electron microscope (TEM). In addition, TEM or SEM provides visualization of the shape of NPs. Since the shape also plays a huge role in antibacterial activity, the shape of the ZnO-NPs can be altered by changing the preparation method.

3.1 Synthesis of ZnO-NP

A variety of ZnO nanostructures can be synthesized by modifying the synthesis process, but the chemical method is a good option for controlling the shapes by changing pH, solvent type, temperature, and precursors. The different shapes of the NPs are nanobelts, nanocages, nanocombs, and nanosprings/nanohelixes spirals, flakes drums, stars, polyhedrons, boxes, discs, flowers, and plates. The sharp edges in these may penetrate and cause extensive damage to the bacterial cell membrane (Fig. 2).

Based on the morphology, flakes with sharp edges can penetrate the bacterial surface, leading to bacterial cell wall damage (Visnuvinayagam et al., 2019b, 2021). Different methods of ZnO-NP preparation provide different shapes and sizes of

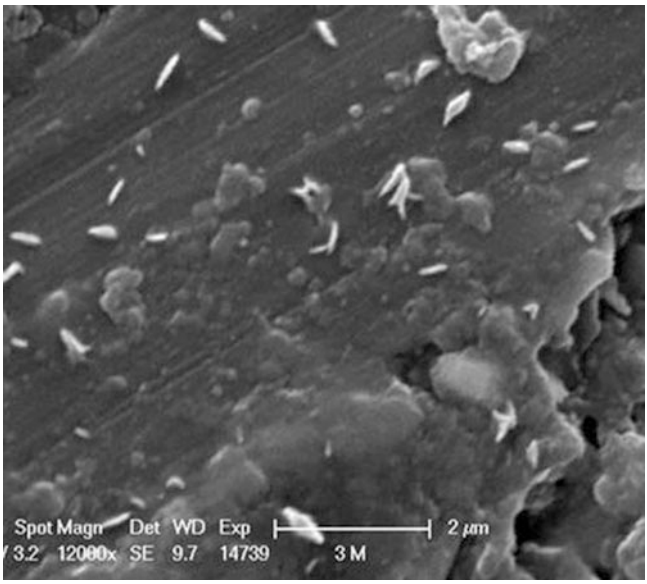


Fig. 2 Zinc oxide nanoparticle in SEM image. (Source: Courtesy of Journal of Environmental Biology, Thrivey Academy, Lucknow, India)

ZnO-NP. According to nanochemistry, the nanosize can be of any dimension. Vabbina et al. (2015) synthesized ZnO-nanoflakes with a thickness of 20 nm in one direction; with the other dimension, it was very long (5 micrometer × 5 micrometer). Similarly, Lee et al. (2002) prepared a zinc oxide nanowire with an average length of 13 nanometer and a typical thickness of 50 nm to produce ZnO-NPs. Only a few reports are available where the nanowire had a width of 80–100 nanometers and length of 300–500 nanometers.

3.2 Antibacterial Activities of ZnO-NP

ZnO-NPs have a wide range of antibacterial activities, that is, they can destroy both Gram-positive and Gram-negative bacteria. Most of the research is focused on major foodborne pathogens such as *E. coli* O157:H7, *Salmonella, L. monocytogenes*, *S. aureus,* and *C. jejuni.* The antimicrobial properties of ZnO-NP at an average size of 30 nm against *Campylobacter jejuni* found that ZnO-NP was strong enough at an MIC value of 0.05 mg. mL^{-1} (Xie et al., 2011). For *Staphylococcus aureus,* the value was 1 mM (Jones et al., 2008). The variation in the size of nanoparticles can be attributed to the variation in the MIC for the same bacterial species, as reported for *S. aureus* (0.3 mg. mL^{-1}) (Visnuvinayagam et al. 2019b). Padmavathy and Vijayaraghavan (2008) synthesized the ZnO-NPs of various sizes and observed that the NP size was inversely related to the antibacterial activity.

Xie et al. (2011) reported that the activity of ZnO nanoparticles on *C. jejuni* was bactericidal, not bacteriostatic. The ability of zinc oxide nanoparticles to destroy

the *C. jejuni* was compared with the ability to inactivate other foodborne bacteria. To inhibit 1–2 of *E. coli* O157:H7 and Salmonella, around 20- to 100-fold greater doses of ZnO-NPs are needed. As a result, the bactericidal activity of ZnO nanoparticles against C. jejuni was extremely effective (Xie et al., 2011).

Most of the literature indicates using MIC values for ZnO-NP; there is limited literature available on MIC and MBC. Only a few reports have compared the ZnO-NPs with others, that is, ZnO-bulk particles (ZnO-BP). Visnuvinayagam et al. (2021) reported extremely low MIC values (better) of *S. aureus* and MRSA, that is, <0.3 mg/mL. The MIC values of bacteria such as *L. monocytogenes, Pseudomonas* species (spoilage bacteria), and H_2S-forming bacteria (food spoilage bacteria) are approximately 1 mg/mL (MIC values). The MIC values of *V. cholerae* and *Salmonella* Typhi are approximately 8 mg/mL. Organisms such as *B. thermosphacta* and *P. aeruginosa* have higher MIC (poor) values of >33 mg/mL. In case of ZnO-BP, the MIC values are 2–40 times higher than the NPs. The MBC values of ZnO-NP are approximately 2–5 times higher than the MIC values of both ZnO-NP and ZnO-BP.

3.3 Mechanism of Action

The exact mechanism of ZnO-NP against bacteria must also be understood to make the best use of them in food products. Even though the process of their antibacterial activity is unknown, few studies have shown that the disruption of cell membrane activity is the fundamental mechanism of antibacterial action (Fig. 3).

Scanning electron microscope (SEM) showed that ZnO-NP caused abnormal cell surfaces and membrane blebbing, as well as a rise in membrane permeability and drastic shift in *C. jejuni* cell morphology, with the treated cells dominating coccoid types but the control cell (untreated cells) was not changed, that is, maintaining the spiral structure. This considerable alteration in chromatin structure was identified not

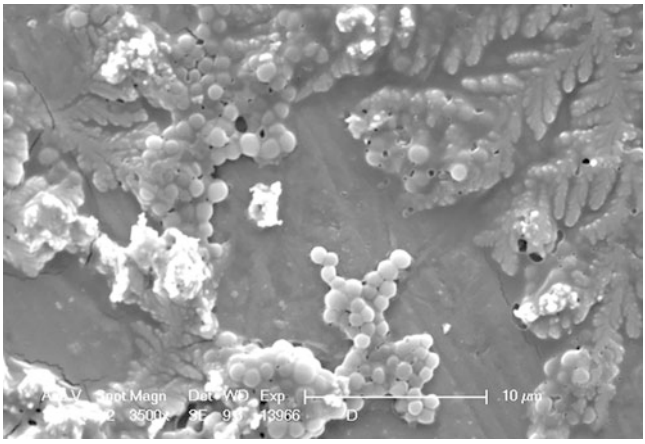


Fig. 3 SEM image of ZnO-NP-treated MRSA. (Source: Courtesy of Journal of Environmental Biology, Thrivey Academy, Lucknow, India)

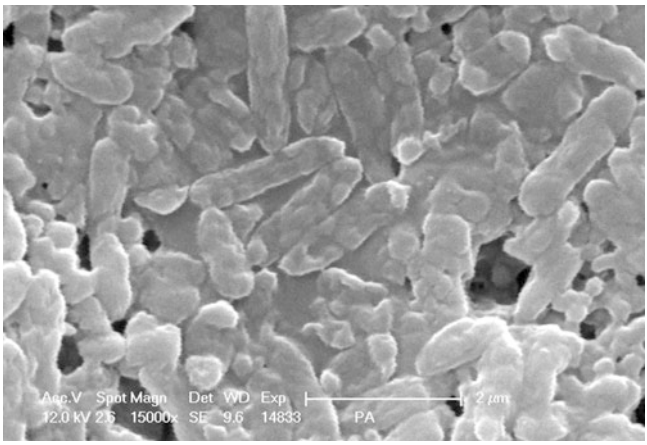


Fig. 4 SEM image of ZnO-NP-treated *Pseudomonas aeruginosa*. (Source: Courtesy of Journal of Environmental Biology, Thrivey Academy, Lucknow, India)

just in *C. jejuni* cells with ZnO-NP but also in another *Campylobacter* sp. and the highly associated genus *Helicobacter* as cells were exposed to multiple circumstances. When *E. coli* (O_{157}:H_7) cells were treated with ZnO-nanoparticles, this induced membrane leakage, which was consistent by transmission electron microscopic and Raman spectroscopic analysis. Changes in the morphology were noticed in MRSA after treating with ZnO-NP, that is, shrinkage, a fusion of cocci, and leakage of the cellular content (Fig. 3). But *Pseudomonas aeruginosa* did not show any damage, which may be why the high MIC and MBC values destroy the bacterial cells (Fig. 4). Based on the above reports, direct interaction of ZnO-NP with cell surface membrane has been observed, which changes the porosity of membranes through which the nanoparticles travel, causing oxidative stress in bacteria, leading to inhibition effects and eventually cell death.

3.4 Limitations of ZnO-NP

ZnO is insoluble in water and agglomerated immediately with water in synthesis. Small-sized nanoparticles are especially likely to be aggregated; hence, nanoparticles of up to 10 nm size are ineffective. This explains the high dispersal of antibacterial activity in small nanoparticles. The synthesis processes are hindered by accumulation, re-precipitation, rearrangement, or nondissolution. Several researchers have considered this problem by using certain additives that do not have significant effects on antibacterial activity. Studies have shown that the ZnO-NP is cytotoxic by damaging the mitochondrial membrane (Deng et al., 2009).

3.5 Enhancement of Antibacterial Activity of the ZnO-NP

Various methods are available to potentiate the antibacterial activity of the ZnO-NP, that is, doping, the addition of other nanoparticles, and surface modification with stabilizers and chemicals.

Doping and implants of external metals on ZnO nanostructures have become topics for researchers to develop functional antibacterial agents. Doping of ZnO-NP with other metals may lead to a higher antibacterial activity. Three bacterial strains were tested to enhance the antibacterial properties of ZnO-NPs. In comparison, ZnO-doped samples showed significant activity toward *S. aureus* than *E. coli* and *P. aeruginosa*. The inhibitions zone was 37% higher than ZnO nanostructures. In medical applications, nanospheres and nanorod doped and undoped ZnOs were synthesized with a wet chemical method and annealed to 600 °C for 2 h. Even though CuO and ZnO are similar to both Gram-positive and -negative bacteria during the exponential stages of bacteria growth, in late and stationary phases, ZnO-NPs were practically inactive while CuO-NP retained significant activity. The growth of *Mycobacterium tuberculosis* strains resistant to antibiotics, but not leading to bacterial death, is inhibited by Ag and ZnO-NP at different ratios.

In contrast to zirconium (IV) oxide (ZrO_2), the nanoparticles of ZrO_2-ZnOs have marked antimicrobial effects, but the antimicrobial effects of ZrO_2-ZnOs do not exceed the ZNO's. But metal oxides are not always combined to produce the synergetic effects. The antimicrobial effect is comparable to that of cadmium oxide (CdO) nanoparticles, particularly for nanoparticles of CdO-ZnO. ZnO nanodoping with Fe ions allows a major antibacterial effect of *P. aeruginosa* against *Escherichia coli.* The bactericidal effect against *E. coli* is more pronounced with TiO_2/ZnO compared with using individually. Combined NPs of Ag/TiO_2/ZnO are more efficient than TiO_2/ZnO. The antimicrobial activities of ZnO nanoparticles *against K. pneumoniae, S. dysenteriae, S. Typhimurium, P. aeruginosa,* and other bacteria are higher than ZnO nanoparticles. ZnO nanoparticles and carbon nanoparticles, particularly the spindle-shaped graph oxide (GO) nanoparticles, can also be used as a method to enhance the efficiency of antimicrobials. The development of Gram-negative (*E. coli, S.* Typhimurium) and Gram-positive (*Bacillus subtilis, Enterococcus faecalis*) bacteria was shown to effectively inhibit nanoparticles of GO-ZnO. The antibacterial effects of GO-ZnO nanoparticles were almost two times the values of ZnO and almost four times the effectiveness of GO nanoparticles.

ZnO nanoparticles coated with modifying agents increase the antibacterial activity. The nanoparticles of gelatin-coated ZnO showed greater growth in Gram-negative bacteria than Gram-positive bacteria. As mentioned above, it is more difficult to overcome antibiotic resistance in Gram-negative bacteria. Chemical surface modification of nanopart with trimethoxy silane (GPTMS, 3-glycidyloxypropyl) and size decreases up to 5 nm will increase nanoparticles' anti-*S. aureus* antimicrobial effects. Polystyrene treatment increased the bacteriostatic impact of ZnO-NPs on *Escherichia coli* and *L. monocytogenes*, thereby preventing *L. monocytogenes* from being bacteriostatic to uncoated ZnO nanoparticles. ZnO's modification of polyethylene or starch nanoparticles also affects

the properties of nanoparticles. The bacteriostatic effects of ZnO-NP on *E. coli* and *S. aureus* were augmented by modification of polyethylene glycol; the effects against Gram-negative bacteria were higher. ZnO nanoparticles have improved cytotoxicity to the cancer cell line (MG-63) through the induction of apoptosis by polyethene. The starch modification allowed the maintenance, compared with modification with polyethylene glycol, of antibacterial properties of ZnO nanoparticles and reduced cytotoxicity. Thioglycerol treatment did not increase bacteriostatic levels, contrary to expectations. The addition of polyvinyl alcohol, polyvinylpyrrolidone, and poly-α, β, l-glutamic acid as stabilizers improved ZnO morphology and ZnO-NP size.

Even though ZnO-NPs are not food grade, they can be used in many food applications, for example, ZnO-NPs can be incorporated into the packaged film for the shelf-life improvement of the food materials. But the European Food Safety Authority (EFSA) recommends the ZnO-NP in food packaging application with the Specific Migration Limit (SML) of 25 mg/kg. The actual migration level observed in the LDPE is 0.05 mg/kg. Since it is within the limit, ZnO-NP can be incorporated into the packaging. Since it is an important mineral for the body, the per-day intake limit for adults is 50 mg/person. The EFSA has now set a limit that is half of the safety level (EFSA, 2015, 2016).

Based on this, it has been observed that the antibacterial activity is due to various reasons, but it may be a sequence of all the reactions, that is, electrostatic communication among ZnO and bacterial cell membranes, which leads to the disruption of cell membrane activity that facilitates the cellular internalization of ZnO nanoparticles. Once the NPs are inside the bacterial cells, they stimulate the production of intercellular reactive oxygen species, that is, H_2O_2, and ultimately the bacterial cell death. Here, a higher membrane damage may be on the Gram-positive bacteria because they have weak LPS. Accordingly, membrane damage will be predominant in the Gram-positive bacteria. In contrast, in Gram-negative bacteria, since the LPS is very strong the possibility of membrane damage on the cell wall is poor or the bacteria may survive; here, the internalization of the ZnO-NP would be prominent and ROS would be a great effect. Hence, all mechanisms vary with the bacterial cell wall and cell LPS.

4 Copper/Copper Oxide Nanoparticles

Copper is one of the important trace elements necessary for humans, its nanoform, that is, the copper nanoparticles (CNP) or copper oxide nanoparticles (CONP) (copper (II) oxide/cupric oxide) have potent industrial applications. It is widely used in gas sensors, superconductors, solar panels, and preservative agents of the fish (Zhang et al., 2015). CNPs are highly sensitive materials; in the presence of air, they will convert into CONP. Similar to the other nanoparticles, the λ max for the CNP is 580 nm. Regarding copper sharing its properties with other costly noble metals, such as silver and gold, its application has increased. Copper is preferred in studies because of its cost; furthermore, copper nanoparticles are said to have antimicrobial activity against a broad range of bacteria and fungi (Wei et al., 2010).

The antibacterial property of CONP is poor compared with CNP, that is, CONP has nearly 2–10 times lesser concentration of CNP, which is enough to completely destroy the bacteria (Ren et al., 2009). Most of the studies were carried out on CONP, and scant literature is available on CNP. The antibacterial activities of the CNP/CONPs have been identified against different types of pathogenic bestial such as infectious organisms like *Staphylococcus aureus,* Bacillus *subtilis, E. coli, Vibrio cholera, Pseudomonas aeruginosa,* and *Syphilis typhus* (Akhavan & Ghaderi, 2010).) The reported MBC values for *MRSA, S. aureus, E. coli,* and *P. aeruginosa* are 1, 0.1–2.5, 0.25, and 5 mg/mL, respectively (Ren et al., 2009). Studies indicate that 100 mg/mL of CONP can prevent complete formation of biofilm on both glass and acrylic denture surfaces (Khan et al., 2013).

Before the advent of the nanotechnology concept, most of the experiments were carried out on micron-sized particles, between 2500 and 10,000 nm. However, the advent of the era of nanoparticles showed more concern about toxicity (Karlsson et al., 2009). Most of the initial toxicity studies are cell line-oriented. In recent years, the production of nanoparticles has gradually increased. Hence, studying the toxic effects of the NP is of paramount importance. It has been reported that CONP has no toxic effects on human cells at the concentration of 100–5000 μg/ml (Alake et al., 2010).

4.1 Mechanism of Action

The killing mechanism of the CONP is similar to the other metal NPs. Initially the CONP attaches to the bacterial cell envelope, then causes severe damage, which leads to the internalization further to the formation of cuprous oxide (Cu_2O). This leads to rapid activation of Fumarase A and iron–sulfur enzyme. Finally, ROS and superoxide are produced from the cells (Meghana, et al., 2015).

5 Titanium Dioxide (TIO_2)

Titanium dioxide (TiO_2) is used in everyday applications, for example, food-coloring agents, drug color agents, paints, cosmetics, ceramics, batteries, solar panels, etc. Worldwide, its production has crossed 4 MMT per year because of its various applications (Ziental et al., 2020). External availability and cheap cost make it useful for many applications. Even though TiO_2 is a GRAS-listed chemical (E171), it is also approved by the European Union (EU). The maximum permitted limit for the TiO_2 as a food-colorings agent in medicines is 1% (Carp et al., 2004). Similar to other NPs, the bulk TiO_2 is only approved by the US FDA and EU, but not the titanium oxide nanoparticles (TONP). Accordingly, the parameters related to the food safety concern on ZnO-NP will also suit the TONP. Most of the biochemical characteristics are similar to ZnO-NP as both have photocatalytic activity, that is, able to absorb the maximum UV. TiO_2 is one of the essential materials for humans and animals. But the latter is responsible for the reduction in the colony-forming unit

(CFU) count. Similarly, a reduction in the *E. coli* count was observed by (Othman et al. 2014). A drop test method was followed on the TiO_2-coated surface and found that the destruction of the *E. coli* was observed (Trapalis et al., 2003). TONP is generally used to destroy pathogens in water. Several factors are responsible for the effects of TONP such as hydroxylation level, level of pH, heat, and the availability of O_2 time. In addition, the effects of TONP can be increased while increasing the intensity of the light (i.e., 180–1660 μE/s m2), and time of actual exposure (Matsunaga et al., 1988), higher dosage of TiO_2 (i.e., 0.1–1.0 g/l), intensity of light, and contact time. The toxicity of all NPs is similar in category, for example, trigger the ROS, superoxide radical, and H_2O_2 (Li et al., 2008).

6 Iron Oxide Nanoparticles (IONPs)

Iron oxide nanoparticles (IONPs) have magnetic and semiconductor properties; therefore, they are widely used from a biomedical aspect (Sangaiya & Jayaprakash, 2018). UV absorption band for the IONP is between 330 and 350 nm. The surface plasmon resonance (SPR), that is, λmax for the positive and negative IONP are 367 and 359 nm, respectively (Arakha et al., 2015). Hajipour et al. (2012) tested 17 pathogens against IONP and found that 5 bacterial species, viz., *S. aureus, Aeromonas hydrophila, E. coli, B. subtilis,* and *B. cereus,* were inhibited by the IONP. Another study revealed that a higher concentration of IONP is needed to inhibit *Bacillus subtilis* and *E. coli* (Arakha et al., 2015). The poor antibacterial activity is due to the tendency of the IONP aggregate at pH 7.4. A suitable biocompatible coating is needed for a better antimicrobial activity. Armijo et al. (2020) reported that polyethylene glycol-capped IONP has shown better antimicrobial activity. Based on the well diffusion analysis, the antibacterial activity has been established for *S. aureus*, *Proteus vulgaris,* and *Pseudomonas aeruginosa* at the concentration of 100 μg/mL.

7 Aluminum Oxide Nanoparticles (AONPs)

Aluminum oxide (Al_2O_3) or alumina is used in energetic systems, especially to replace the lead primers for the preparation of explosives (Mukherjee et al., 2011). Unlike other bulk materials (ZnO, TiO_2, MgO_2), its bulk component is not food grade. Hence, it has limitations in the food application. Aluminum oxide nanoparticles (AONPs) are stable in variable temperatures (Mukherjee et al., 2011). Bala et al. (2011) synthesized the silver–alumina NP composite and found it effective to destroy *Staphylococcus epidermis* and *E. coli*. AONP effectively controls the EPS production, which leads to a greater reduction in biofilm formation (Muzammil et al., 2020). Al_2O_3 NPs can efficiently reduce EPS production by *A. baumannii,* thus affecting biofilm architecture.

8 Magnesium Oxide Nanoparticles (MONPs)

Magnesium oxide (MgO) is recommended to control heartburn and antacid; in addition, it has a bone regeneration capacity. The antibacterial activity of MONPs has been proved by the reduction in colony-forming unit and agar well diffusion assay (Imani & Safaei, 2019). *E. coli* (O_{157}:H_7) and *Salmonella* show greater inhibition; the authors also reported the synergistic activity of the MONP with nisin. The mechanism of action is also similar to the other NPs like ZnO-NP and SNP. Similar to the other NPs, MONPs can also be confirmed by the λmax value of 301 nm (Prasanth et al., 2019). The antibacterial activity of the MONP was also tested with *S. aureus, S. pneumoniae, E. coli,* and *S. typhi* (Jin & He, 2011). MONP was prepared by the green synthesis, and the antibacterial activity was tested using MIC analysis, which reports the MIC values for both *S. aureus* and *E. coli* as 125 μg/mL; the value is 250 mg/mL for *Bacillus* species (Vergheese & Vishal, 2018).

9 Yttrium Oxide Nanoparticles (YONPs)

Yttrium oxide (Y_2O_3) is a metal oxide that releases the highest free energy-releasing value. Because of its potent antioxidant activity, it has attracted more attention in biomedical applications, especially in the field of cancer research (Hosseini et al., 2013). It has been used as a neuroprotective agent in cadmium-induced neurotoxicity in the rats' hippocampus, and the results were promising (Hosseini et al., 2015). It has also been reported that the combined effects of cerium oxide (CeO_2) and yttrium oxide (Y_2O_3) show better results (Schubert et al., 2006). But the antibacterial activity is poorly studied. Only a few studies were carried out on its antimicrobial activity. Kannan and Sundrarajan (2015) studied the antibacterial activity of the YONP and found the MIC value of 14 μg/mL for *S. aureus* and 8 $\mu g.mL^{-1}$ for *E. coli* and *P. aeruginosa.* Even though it has a better antioxidant activity, its toxicity needs to be considered. The concentration and time of exposure-based toxicity were observed in cell lines for the YONP (Selvaraj et al., 2014). Hence, based on the criticality of the patient, the NPs can be used for treatments. It can be also effectively used to reduce oxidative stress in any biological application (Srinivasan et al., 2010).

10 Cerium Oxide Nanoparticles (CEONPs)

Cerium oxide (CeO_2) is known as a superior radical-scavenging compound; it is reduced from Ce4+ to Ce3+, resulting in loss of O_2 molecule (Zhang et al., 2019). It also has the same biological properties as YONP. A combined CEONP and YONP provide better results in most biological applications. Biologists do not give importance to the antibacterial activity of CEONP, which has led to scant availability of literature regarding the antibacterial activity of CEONP. The antibacterial activity against *E. coli* has been observed. Gold NP-coated CEONP shows enhanced activity against *S. aureus, B. subtilis, Salmonella,* and *E. coli* (Chen et al., 2014). The unique

property of CEONP is the migration of the O_2 molecule, that is, reversible conversion (Ce4 + →Ce3 + →Ce4+) on the NP surface, which is called autoregenerative cycle NPs (Tarnuzzer et al., 2005).

11 Conclusions

Based on the above literature, the antibacterial activities of the all-metal/metal oxide nanoparticles are almost similar, though a minor variation was observed. Even though various methods are hypothesized, most are a sequence of reactions. The sequences of reactions are as follows:

1. Electrostatic interaction between ZnO and bacterial cell membranes leads to an interruption in cell membrane activity, which facilitates the cellular internalization of ZnO nanoparticles.
2. After the internalization of the NPs, it stimulates the production of inter-cellular-reactive oxygen species, H_2O_2, and ultimately bacterial cell death. Here, higher membrane damage may be on the Gram-positive bacteria because they have weak LPS. Hence, membrane damage will be predominant in the Gram-positive bacteria. In contrast, in Gram-negative bacteria, the LPS is very strong and the possibility of membrane damage on the cell wall will be poor or the bacteria may survive.
3. Prominence of ROS would be a great effect on the destruction of the bacterial cells.
4. Since all the actions are mostly based on the mechanical action, NPs act on both AMR bacterial and sensitive bacteria in the same manner. Because of this, based on the sensitivity and resistance pattern, its mechanism of action will be unchanged.

The destruction of the bacterial cell takes place based on the mechanical action. Hence, there is no difference between the MIC level for the resistance bacteria and susceptible bacteria. The research carried out on the susceptible bacteria's MIC has not changed much on the resistant bacteria. If the resistance is based on the production of LPS or envelope, it may lead to higher MIC for the resistance.

All metal oxide antibacterial activities can be enhanced by various methods such as doping, combined with other nanoparticles, and surface modification by stabilization to circumvent the aggregation and combined with the chemical.

Clinically none of the metal/metal oxide nanoparticles are directly approved. However, indirectly it is widely practiced, for example, nanocoated materials to reduce the bacterial load and painting with a high antibacterial activity. In biomedical applications, mostly indirect applications of the NPs have been practiced. For instance, they are being used for the scanning and image capturing method that are used indirectly. Similarly, none of the metal oxide nanoparticles are approved for food applications. Most of the researchers claim that copper, manganese, and zinc are food-grade materials that can be used for controlling pathogenic bacteria in the

food. But the US FDA and EU recommended using the bulk form, not the nanoform. Hence, direct application on food is still at the research level. The indirect application of ZnO-NP can be incorporated into packaging film to control the foodborne pathogens of the packaged materials provided with a lesser migration level. Since each packaging material varies with the migration level, proper analysis must be performed while using it for the food packaging. For example, the European Food Safety Authority (EFSA) recommended using the ZnO-NP in food packaging application with the Specific Migration Limit (SML) of 25 mg/kg for ZnO-NP. The actual migration level observed in the LDPE is 0.05 mg/kg (EFSA, 2015, 2016).

Detailed studies were carried out regarding the antibacterial activity of the SNP and ZnO-NP only; other metal nanoparticles are not properly reported. For other NPs, mostly the data are related to the reduction in the colony-forming unit (CFU) or well diffusion only. Furthermore, a clear report is required on the MIC and MBC levels for different bacteria. Additional research inputs are needed for the assessment of MIC and MBC for different NPs with important AMR bacteria. In addition, various doping methods, synergistic methods with different nanoparticles, suitable surface modifiers, and compatible chemicals for the enhancement of the antimicrobial activity of the metal or metal oxide nanoparticles are needed. Finally, the toxicity is also not well studied in most of the NPs. Along with the toxicity, a suitable method to control the NPs' toxicity can be formulated. Proper disposal of the NP guidance is essential for a clean and safe atmosphere.

References

Abbaszadegan, A.. Ghahramani, Y., Gholami, A., Hemmateenejad, B., Dorostkar, S., Nabavizadeh, M., & Sharghi, H. (2015). The effect of charge at the surface of silver nanoparticles on antimicrobial activity against gram-positive and gram-negative bacteria: A preliminary study. Journal of Nanomaterials 720654.

Akhavan, O., & Ghaderi, E. (2010). Cu and CuOnanoparticles immobilized by silica thin films as antibacterial materials and photocatalysts. *Surface and Coatings Technology, 205*(1), 219–223.

Annamalai, J., Lakshmi, N. M., Sivam, V., & Chandragiri Nagaraja Rao, R. (2018). A comparative study on the quality changes of croaker (Johnius dussumieri) fish stored in slurry ice and flake ice. *Journal of Aquatic Food Product Technology, 27*(4), 508–517.

Annamalai, J., Sivam, V., Unnikrishnan, P., Sivasankara, S. K., Pansingh, R. K., Abdul, K. S., Lakshmi, N. M., & Nagarajarao, R. C. (2020). Effect of electron beam irradiation on the biochemical, microbiological and sensory quality of *Litopenaeus vannamei* during chilled storage. *Journal of Food Science and Technology, 57*(6), 2150–2158.

Arakha, M., Saleem, M., Mallick, B. C., & Jha, S. (2015). The effects of interfacial potential on antimicrobial propensity of ZnO nanoparticle. *Scientific Reports, 5*(1), 1–10.

Armijo, L. M., Wawrzyniec, S. J., Kopciuch, M., Brandt, Y. I., Rivera, A. C., Withers, N. J., Cook, N. C., Huber, D. L., Monson, T. C., Hugh, D. C., Smyth, H. D. C., & Osiński, M. (2020). Antibacterial activity of iron oxide, iron nitride, and tobramycin conjugated nanoparticles against *Pseudomonas aeruginosa* biofilms. *Journal of Nanbiotechnology, 18*, 35. https://doi.org/10.1186/s12951-020-0588-6

Bala, T., Armstrong, G., Laffir, F., & Thornton, R. (2011). Titania–silver and alumina–silver composite nanoparticles: Novel, versatile synthesis, reaction mechanism and potential antimicrobial application. *Journal of Colloid and Interface Science, 356*(2), 395–403.

Benala, M., Vaiyapuri, M., Visnuvinayagam, S., George, J. C., Raveendran, K., George, I., Mothadaka, M. P., & Badireddy, M. R. (2021). A revisited two-step microtiter plate assay: Optimization of in vitro multiplicity of infection (MOI) for Coliphage and Vibriophage. *Journal of Virological Methods, 294*, 114177.

Burdusel, A.-C., Gherasim, O., Grumezescu, A. M., Mogoantă, L., Ficai, A., & Andronescu, E. (2018). Biomedical applications of silver nanoparticles: An up-to-date overview. *Nanomaterials, 8*, 681.

Carp, O., Huisman, C. L., & Reller, A. (2004). Photoinduced reactivity of titanium dioxide. *Progress in Solid State Chemistry, 32*(1–2), 33–177.

Chen, B. H., Suresh Babu, K., & Anandkumar, M. (2014). Cytotoxicity and antibacterial activity of gold-supported cerium oxide nanoparticles. *International Journal of Nanomedicine, 9*, 5515.

Dara, P. K., Mahadevan, R., Digita, P. A., Visnuvinayagam, S., Kumar, L. R., Mathew, S., Ravishankar, C. N., & Anandan, R. J. S. A. S. (2020). Synthesis and biochemical characterization of silver nanoparticles grafted chitosan (Chi-Ag-NPs): In vitro studies on antioxidant and antibacterial applications. *SN Applied Sciences, 2*(4), 1–12.

Dara, P. K., Raghavankutty, M., Balaraman, G., Ashraf, P. M., Visnuvinayagam, S., Tejpal, C. S., Mathew, S., Nagarajarao, R. C., & Rangasamy, A. (2021a). Biocompatibility and histopathological evaluation of chitosan nanoparticles grafted fish gelatin bio-nanocomposite membranes in rats. *Iranian Polymer Journal*, 1–12.

Dara, P. K., Raghavankutty, M., Deekonda, K., Vemu, A. K., Sivam, V., Mathew, S., Rangasamy, A., Chandragiri Nagarajarao, R., & Subramanian, S. (2021b). Synthesis of biomaterial-based hydrogels reinforced with cellulose nanocrystals for biomedical applications. *International Journal of Polymer Science*. https://doi.org/10.1155/2021/4865733

Deng, X., Luan, Q., Chen, W., Wang, Y., Wu, M., Zhang, H., & Jiao, Z. (2009). Nanosized zinc oxide particles induce neural stem cell apoptosis. *Nanotechnology, 20*(11), 115101.

Deshmukh, S. P., Patil, S. M., Mullani, S. B., & Delekar, S. D. (2019). Silver nanoparticles as an effective disinfectant: A review. *Materials Science & Engineering, C: Materials for Biological Applications, 97*, 954–965. https://doi.org/10.1016/j.msec.2018.12.102

EFSA. (2015). European Food Safety Authority, Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF), 2015. Scientific opinion on the safety evaluation of the substance zinc oxide, nanoparticles, uncoated and coated with [3-(methacryloxy) propyl] trimethoxysilane, for use in food contact materials. *EFSA Journal, 13*(4), 4063.

EFSA. (2016). Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF), 2016. Safety assessment of the substance zinc oxide, nanoparticles, for use in food contact materials. *EFSA Journal, 14*(3), 4408.

Ellis, R. (2013). *Tiger bone & rhino horn: The destruction of wildlife for traditional Chinese medicine*. Island Press.

EU, European UNION, P. (2009). Regulation (EC) No 1223/2009 of the european parliament and of the council. *Official Journal of the European Union L, 342*, 59.

Gupta, A., Srivastava, P., Bahadur, L., Amalnerkar, D. P., & Chauhan, R. (2015). Comparison of physical and electrochemical properties of ZnO prepared via different surfactant-assisted precipitation routes. *Applied Nanoscience, 5*(7), 787–794.

Hajipour, M. J., Fromm, K. M., Ashkarran, A. A., de Aberasturi, D. J., de Larramendi, I. R., Rojo, T., Serpooshan, V., Parak, W. J., & Mahmoudi, M. (2012). Antibacterial properties of nanoparticles. *Trends in Biotechnology, 30*(10), 499–511.

Hosseini, A., Baeeri, M., Rahimifard, M., Navaei-Nigjeh, M., Mohammadirad, A., Pourkhalili, N., Hassani, S., Kamali, M., & Abdollahi, M. (2013). Antiapoptotic effects of cerium oxide and yttrium oxide nanoparticles in isolated rat pancreatic islets. *Human & Experimental Toxicology, 32*(5), 544–553.

Hosseini, A., Sharifi, A. M., Abdollahi, M., Najafi, R., Baeeri, M., Rayegan, S., Cheshmehnour, J., Hassani, S., Bayrami, Z., & Safa, M. (2015). Cerium and yttrium oxide nanoparticles against lead-induced oxidative stress and apoptosis in rat hippocampus. *Biological Trace Element Research, 164*(1), 80–89.

Imani, M. M., & Safaei, M. (2019). Optimized synthesis of magnesium oxide nanoparticles as bactericidal agents. *Journal of Nanotechnology, 2019*. https://doi.org/10.1155/2019/6063832

Imran, M., Revol-Junelles, A. M., Martyn, A., Tehrany, E. A., Jacquot, M., Linder, M., & Desobry, S. (2010). Active food packaging evolution: Transformation from micro-to nanotechnology. *Critical Reviews in Food Science and Nutrition, 50*(9), 799–821.

Jin, T., & He, Y. (2011). Antibacterial activities of magnesium oxide (MgO) nanoparticles against foodborne pathogens. *Journal of Nanoparticle Research, 13*(12), 6877–6885.

Jones, N., Ray, B., Ranjit, K. T., & Manna, A. C. (2008). Antimicrobial activity of ZnO nanoparticles suspensions on a broad spectrum of microorganisms. *FEMS Microbiology Letters, 279*, 71–76.

Kang, H., Jeong, S., Park, Y., Yim, J., Jun, B. H., Kyeong, S., Yang, J. K., Kim, G., Hong, S., Lee, L. P., & Kim, J. H. (2013). Near-infrared SERS nanoprobes with plasmonic Au/Ag hollow-shell assemblies for in vivo multiplex detection. *Advanced Functional Materials, 23*(30), 3719–3727.

Kannan, S. K., & Sundrarajan, M. (2015). Biosynthesis of Yttrium oxide nanoparticles using Acalypha indica leaf extract. *Bulletin of Materials Science, 38*(4), 945–950.

Karlsson, H. L., Gustafsson, J., Cronholm, P., & Möller, L. (2009). Size-dependent toxicity of metal oxide particles—A comparison between nano-and micrometer size. *Toxicology Letters, 188*(2), 112–118.

Karthika, R., George, I., Murugadas, V., Jeswin, J., Sherin, P. A., Sanjeev, D., Visnuvinayagam, S., Manikanta, B., Prasad, M. M., & Rao, B. M. (2021). Characterization of tailed phages against E. coli using DNA restriction digestion analysis. *Fishery Technology, 58*, 53–58.

Khan, S. T., Ahamed, M., Al-Khedhairy, A., & Musarrat, J. (2013). Biocidal effect of copper and zinc oxide nanoparticles on human oral microbiome and biofilm formation. *Materials Letters, 97*, 67–70.

Kim, J. Y., Lee, C., Love, D. C., Sedlak, D. L., Yoon, J., & Nelson, K. L. (2011). Inactivation of MS2 coliphage by ferrous ion and zero-valent iron nanoparticles. *Environmental Science & Technology, 45*, 6978–6984.

Le Ouay, B., & Stellacci, F. (2015). Antibacterial activity of silver nanoparticles: A surface science insight. *Nano Today, 10*(3), 339–354.

Lee, C. J., Lee, T. J., Lyu, S. C., Zhang, Y., Ruh, H., & Lee, H. J. (2002). Field emission from well-aligned zinc oxide nanowires grown at low temperature. *Applied Physics Letters, 81*(19), 3648–3650.

Li, Q., Mahendra, S., Lyon, D. Y., Brunet, L., Liga, M. V., Li, D., & Alvaez, P. J. J. (2008). Antimicrobial nanomaterials for water disinfection and microbial control: Potential applications and implications. *Water Research, 42*(18), 4591–4602.

Maqbool, H., Visnuvinayagam, S., Zynudheen, A. A., Safeena, M. P., & Sathish Kumar, K. (2020). Antibacterial activity of beetroot peel and whole radish extract by modified well diffusion assay. *International Journal of Current Microbiology and Applied Sciences, 9*(1), 1222–1231.

Matsunaga, T., Tomoda, R., Nakajima, T., Nakamura, N., & Komine, T. (1988). Continuous sterilization system that uses photosemiconductor powders. *Applied and Environmental Microbiology, 54*(6), 1330–1333.

Meghana, S., Kabra, P., Chakraborty, S., & Padmavathy, N. (2015). Understanding the pathway of antibacterial activity of copper oxide nanoparticles. *RSC Advances, 5*(16), 12293–12299.

Moyer, C. A. (1965). A treatment of burns. *Transactions & Studies of the College of Physicians of Philadelphia, 33*(2), 53–103.

Mukherjee, A., Sadiq, I.M., Prathna, T.C., & Chandrasekaran, N. (2011). Antimicrobial activity of aluminium oxide nanoparticles for potential clinical applications. In *Science against microbial pathogens: Communicating current research and technological advances*, Formatex Research, Spain *1*, 245–251.

Murthy, L. N., Phadke, G. G., Mohan, C. O., Chandra, M. V., Annamalai, J., Visnuvinayagam, S., Unnikrishnan, P., & Ravishankar, C. N. (2017). Characterization of spray-dried hydrolyzed proteins from pink perch meat added with Maltodextrin and gum Arabic. *Journal of Aquatic Food Product Technology, 26*(8), 913–928.

Murugadas, V., Visnuvinayagam, S., Purushothaman, V., Prabhakar, T. G., Prabhahar, P., & Ventakaraman, K. (2015). Prevalence of chicken host and non-host adapted Salmonella in retail outlet of Chennai, India. *Asian Journal of Animal and Veterinary Advances, 10*, 885–893.

Muzammil, S., Khurshid, M., Nawaz, I., Siddique, M. H., Zubair, M., Nisar, M. A., Imran, M., & Hayat, S. (2020). Aluminium oxide nanoparticles inhibit EPS production, adhesion and biofilm formation by multidrug-resistant Acinetobacter baumannii. *Biofouling, 36*(4), 492–504.

Neha, V. H., Visnuvinayagam, S., Murthy, L. N., Jeyakumari, A., Sivaraman, G. K., & Prasad, M. M. (2019). Characterization of vibriocin and prospect of co-culture method to overcome the diminishing antibacterial activity. *International Journal of Current Microbiology and Applied Sciences, 8*(10): 671–681.

Osman, A. O., Mahgoub, S. A., & Sitohy, M. Z. (2013). Preservative action of 11S (glycinin) and 7S (β-conglycinin) soy globulin on bovine raw milk stored either at 4 or 25 C. *Journal of dairy research, 80*(2), 174–183.

Othman, N., Babakir-Mina, M., Noori, K., & Rashid, Y. (2014). Pseudomonas aeruginosa infection in burn patients in Sulaimaniyah, Iraq: risk factors and antibiotic resistance rates. *The Journal of Infection in Developing Countries, 8*(11), 1498–1502.

Padmavathy, N., & Vijayaraghavan, R. (2008). Enhanced bioactivity of ZnO nanoparticles – An antimi- crobial study. *Science and Technology of Advanced Materials, 9*, 1–7.

Panpaliya, N. P., Dahake, P. T., Kale, Y. J., Dadpe, M. V., Kendre, S. B., Siddiqi, A. G., & Maggavi, U. R. (2019). In vitro evaluation of antimicrobial property of silver nanoparticles and chlorhexidine against five different oral pathogenic bacteria. *The Saudi Dental Journal, 31*(1), 76–83. https://doi.org/10.1016/j.sdentj.2018.10.004

Parvathy, U., Zynudheen, A. A., Murthy, L.N., Jeyakumari, A., & Visnuvinayagam, S. (2018). Characterization and profilling of protein hydrolysates from white and red meat of tuna (Euthynnus affinis). Peel extracts: Effects on quality changes and shelflife of Indian mackerel. *Fishery Technology, 55*, 248–257.

Prasanth, R., Kumar, S. D., Jayalakshmi, A., Singaravelu, G., Govindaraju, K, & Kumar, V. G., (2019). Green synthesis of magnesium oxide nanoparticles and their antibacterial activity. *Indian Journal of Geo Marine Sciences,* 48, 1210–1215.

Raffi, M., Mehrwan, S., Bhatti, T. M., Akhter, J. I., Hameed, A., Yawar, W., & ul Hasan, M. M. (2010). Investigations into the antibacterial behavior of copper nanoparticles against Escherichia coli. *Annals of Microbiology, 60*(1), 75–80.

Rashki, S., Asgarpour, K., Tarrahimofrad, H., Hashemipour, M., Ebrahimi, M. S., Fathizadeh, H., Khorshidi, A., Khan, H., Marzhoseyni, Z., Salavati-Niasari, M., & Mirzaei, H. (2021). Chitosan-based nanoparticles against bacterial infections. *Carbohydrate Polymers, 251*, 117108.

Ren, G., Hu, D., Cheng, E. W., Vargas-Reus, M. A., Reip, P., & Allaker, R. P. (2009). Characterisation of copper oxide nanoparticles for antimicrobial applications. *International Journal of Antimicrobial Agents, 33*(6), 587–590.

Rovira, J., Nadal, M., Schuhmacher, M., & Domingo, J. L. (2017). Trace elements in skin-contact clothes and migration to artificial sweat: Risk assessment of human dermal exposure. *Textile Research Journal, 87*(6), 726–738.

Sangaiya, P., & Jayaprakash, R. (2018). A review on iron oxide nanoparticles and their biomedical applications. *Journal of Superconductivity and Novel Magnetism, 31*(11), 3397–3413.

Schubert, D., Dargusch, R., Raitano, J., & Chan, S. W. (2006). Cerium and yttrium oxide nanoparticles are neuroprotective. *Biochemical and Biophysical Research Communications, 342*(1), 86–91.

Selvaraj, V., Bodapati, S., Murray, E., Rice, K. M., Winston, N., Shokuhfar, T., Zhao, Y., & Blough, E. (2014). Cytotoxicity and genotoxicity caused by yttrium oxide nanoparticles in HEK293 cells. *International Journal of Nanomedicine, 9*, 1379.

Sivaraman, G. K., Vishnuvinayagam, S., Jha, A. K., Renuka, V., Remya, S., & Vanik, D. (2017). Assessment of microbial quality of fish processing industrial effluent in bar-mouth at Bhidia landing sire, Veraval, Gujarat, India. *Journal of Environmental Biology, 37*, 537–541.

Sivaraman, G. K., Sivam, V., Ganesh, B., Elangovan, R., Vijayan, A., & Mothadaka, M. P. (2021). Whole genome sequence analysis of multi drug resistant community associated methicillin

resistant Staphylococcus aureus from food fish: Detection of clonal lineage ST 28 and its antimicrobial resistance and virulence genes. *Peer Journal, 9*, e11224.

Srinivasan, R., Yogamalar, R., & Bose, A. C. (2010). Structural and optical studies of yttrium oxide nanoparticles synthesized by co-precipitation method. *Materials Research Bulletin, 45*(9), 1165–1170.

Suvith, V. S., & Philip, D. (2014). Catalytic degradation of methylene blue using biosynthesized gold and silver nanoparticles. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy, 118*, 526–532.

Syafiuddin, A., Fulazzaky, M. A., Salmiati, S., Roestamy, M., Fulazzaky, M., Sumeru, K., & Yusop, Z. (2020). Sticky silver nanoparticles and surface coatings of different textile fabrics stabilised by Muntingia calabura leaf extract. *SN Applied Sciences, 2*(4), 1–10.

Tarnuzzer, R. W., Colon, J., Patil, S., & Seal, S. (2005). Vacancy engineered ceria nanostructures for protection from radiation-induced cellular damage. *Nano Letters, 5*(12), 2573–2577.

Tran, H. N., Nghiem, T. H. L., Vu, T. T. D., Chu, V. H., Le, Q. H., Hoang, T. M. N., Nguyen, L. T., Pham, D. M., Tong, K. T., Do, Q. H., Vu, D., Nguyen, T. N., Pham, M. T., Duong, C. N., Tran, T. T., Vu, V. S., Nguyen, T. T., Nguyen, T. B. N., Tran, A. D., Trinh, T. T., & Nguyen, T. T. A. (2015). Optical nanoparticles: Synthesis and biomedical application. *Advances in Natural Sciences: Nanoscience and Nanotechnology, 6*, 023002.

Trapalis, C. C., Keivanidis, P., Kordas, G., Zaharescu, M., Crisan, M., Szatvanyi, A., & Gartner, M. (2003). TiO2 (Fe3+) nanostructured thin films with antibacterial properties. *Thin Solid Films, 433*(1–2), 186–190.

Umrani, R. D., & Paknikar, K. M. (2014). Zinc oxide nanoparticles show antidiabetic activity in streptozotocin-induced Type 1 and 2 diabetic rats. *Nanomedicine, 9*(1), 89–104.

Usman, M. S., El Zowalaty, M. E., Shameli, K., Zainuddin, N., Salama, M., & Ibrahim, N. A. (2013). Synthesis, characterization, and antimicrobial properties of copper nanoparticles. International journal of nanomedicine, 8, 4467.

Vabbina, P. K., Kaushik, A., Pokhrel, N., Bhansali, S., & Pala, N. (2015). Electrochemical cortisol immunosensors based on sonochemically synthesized zinc oxide 1D nanorods and 2D nanoflakes. *Biosensors and Bioelectronics, 63*, 124–130.

Vaiyapuri, M., Raveendran, K., George, I., Gundubilli, D., Sivam, V., Krishnan, S. G., George, J. C., Mothadaka, M. P., Nagarajarao, R. C., & Badireddy, M. R. (2021a). Comparison of single and multi-host enrichment approach for harnessing lytic phages against antimicrobial-resistant E. coli: Repurposing the enrichment step. *Biologia, 76*(3), 1041–1052.

Vaiyapuri, M., Sebastian, A. S., George, I., Variem, S. S., Vasudevan, R. N., George, J. C., Badireddy, M. R., Sivam, V., Peeralil, S., Sanjeev, D., & Thandapani, M. (2021b). Predominance of genetically diverse Esbl Escherichia coli identified in resistance mapping of largest fresh Cum Brackish water of Vembanad Lake, India. *Environmental Science and Pollution Research International, 28*(46), 66206–66222.

Vergheese, M., & Vishal, S. K. (2018). Green synthesis of magnesium oxide nanoparticles using Trigonella foenum-graecum leaf extract and its antibacterial activity. *J Pharmacognosy and Phytochemistry, 7*(3), 1193–1200.

Viji, P., Binsi, P. K., Visnuvinayagam, S., Bindu, J., Ravishankar, C. N., & Gopal, T. K. S. (2015). Efficacy of mint (Mentha arvensis) leaf and citrus (Citrus aurantium) peel extracts as natural preservatives for shelf life extension of chill stored Indian mackerel. *Journal of Food Science and Technology, 52*(10), 6278–6289.

Viji, P., Binsi, P. K., Visnuvinayagam, S., Ravishankar, C. N., Venkateshwarlu, G., & Gopal, T. K. S. (2016). Modified icing system containing mint leaf and citrus peel extracts: Effects on quality changes and shelflife of Indian mackerel. *Indian Journal of Fisheries, 3*(2), 93–101.

Viji, P., Binsi, P. K., Visnuvinayagam, S., Mohan, C. O., Venkateshwarlu, G., & Srinivasa Gopal, T. K. (2017). Lipid oxidation and biochemical quality of Indian mackerel during frozen storage: Effect of previous treatment with plant extracts. *Journal of Food Biochemistry, 41*(1), e12308.

Visnuvinayagam, S., Joseph, T. C., Murugadas, V., Chakrabarti, R., & Lalitha, K. V. (2015). Status on methicillin resistant and multiple drug resistant Staphylococcus aureus in fishes of Cochin and Mumbai coast, India. *Journal of Environmental Biology, 36*(3), 571.

Visnuvinayagam, S., Viji, P., Murthy, L. N., Jeyakumari, A., & Sivaraman, G. K. (2016). Occurrence of faecal indicators in freshwater fishes of Navi Mumbai in retail outlets. *Fishery Technology, 53*, 334–338.

Visnuvinayagam, S., Murthy, L. N., Viji, P., & Sivaraman, G. K. (2017). Study on retail fish markets: Possible occurance and transmission of emergeing pathogen from faecal indicators. *Journal of Environmental Biology, 38*(3), 465–470.

Visnuvinayagam, S. Murthy, L. N., Parvathy, U., Jeyakumari, A., Adiga, T. G., & Sivaraman, G. K. (2019a). Detection of multi drug resistant bacteria in retail fish market water samples of Vashi, Navi Mumbai. *Proceedings of the National Academy of Sciences, India Section B: Biological Sciences*, pp. 1–6.

Visnuvinayagam, S., Murthy, L. N., Jeyakumari, A., Parvathy, U., Anandan, R., Sivaraman, G. K., & Ravishankar, C. N. (2019b). Combined effect of zinc oxide nano particle incorporated chitosan for better antimicrobial activity towards wound healing. *Journal of Environmental Biology, 40*, 691–697.

Visnuvinayagam, S., Murthy, L. N., Parvathy, U., Jeyakumari, A., Rawat, K. P., Khadar, S. A., & Sarma, K. S. S. (2020). Destructive dose determination of electron beam irradiation for pathogenic bacteria in water medium by 96 well plate assay. *Journal of Environmental Biology, 41*(5), 1013–1017.

Visnuvinayagam, S., Murthy, L. N., Parvathy, U., Jeyakumari, A., Sivaraman, G. K., & Karthikeyan, D. (2021). Food grade zinc oxide bulk particle composite can replace the toxic zinc oxide nano composite towards the control of pathogenic and spoilage bacteria. *EMS Microbiology Letters, 368*(1), fnaa210. https://doi.org/10.1093/femsle/fnaa210. PMID: 33320950.

Wei, Y., Chen, S., Kowalczyk, B., Huda, S., Gray, T. P., & Grzybowski, B. A. (2010). Synthesis of stable, low-dispersity copper nanoparticles and nanorods and their antifungal and catalytic properties. *The Journal of Physical Chemistry C, 114*(37), 15612–15616.

Xie, Y., He, Y., Irwin, P. L., Jin, T., & Shi, X. (2011). Antibacterial activity and mechanism of action of zinc oxide nanoparticles against Campylobacter jejuni. *Applied and Environmental Microbiology, 77*(7), 2325–2331.

Zhang, X., Wang, G., Liu, X., Vu, D., Nguyen, T. N., Pham, M. T., Duong, C. N., Tran, T. T., Vu, V. S., Nguyen, T. T., Nguyen, T. B. N., Tran, A. D., Trinh, T. T., & Nguyen, T. T. A. (2015). Different CuO nanostructures: Synthesis, characterization, and applications for glucose sensors. *The Journal of Physical Chemistry. C, Nanomaterials and Interfaces, 112*(43), 16845–16849.

Zhang, M., Zhang, C., Zhai, X., Luo, F., Du, Y., & Yan, C. (2019). Antibacterial mechanism and activity of cerium oxide nanoparticles. *Science China Materials, 62*(11), 1727–1739.

Ziental, D., Czarczynska-Goslinska, B., Mlynarczyk, D. T., Glowacka-Sobotta, A., Stanisz, B., Goslinski, T., & Sobotta, L. (2020). Titanium dioxide nanoparticles: Prospects and applications in medicine. *Nanomaterials, 10*(2), 387.

Phytomolecules as an Alternative Medicine to Combat Antimicrobial Resistance

Palanisamy Sankar, Karunakaran Vijayakaran, and Kalaivanan Ramya

Contents

P. Sankar (✉) · K. Vijayakaran
Department of Veterinary Pharmacology and Toxicology, Veterinary College and Research Institute, Namakkal, Tamil Nadu, India

K. Ramya
Department of Veterinary Microbiology, Veterinary College and Research Institute, Namakkal, Tamil Nadu, India

M. P. Mothadaka et al. (eds.), *Handbook on Antimicrobial Resistance*,
https://doi.org/10.1007/978-981-19-9279-7_43

Abstract

Antimicrobials are natural, semisynthetic, or man-made biological molecules that are effective against infectious microorganisms. They play a crucial part in handling various communicable ailments and contribute the financial viability of productivity in animal sector as an increment which is lower than regular treatment dosages, to enhance the development and transformation of food supply, and to prevent disease. Resistance to bacterial resistance reduced the efficacy of antimicrobials, altering the life-saving model that was created on them. This brings serious public health concern and is considered an apprehension by organizations such as the WHO, FAO, and WOAH. Under these circumstances, the immediate prerequisite is to find alternative antibiotics to prevent and treat microbes. Employment of naturally occurring botanicals is one of the most powerful methods that can be used as an alternative or complementary antibiotic. Conventional therapies use herbal remedies rich in plant-based chemicals, namely, alkaloids, coumarin, essential oils, a collection of regular constituents with capricious phenolic arrangements known as flavonoids, steroids, tannins, and 5-carbon isoprenoids rarely create resistance. The pharmacodynamics of phytochemicals is not well understood but the positive phytochemicals impact is known to their antioxidant and antibacterial features. With the goal of eliminating "superbugs" that are unresponsive to antimicrobials, the development of innovative antimicrobials is very important and the phytochemicals released from the plants can be an attractive source of future, cost-effective, and safe antimicrobial drugs.

Keywords

Antimicrobials · Phytochemicals · Phytomolecules · Plant-derived antibiotics · Plant botanicals

1 Introduction

Antimicrobials are natural, semisynthetic, or synthetic organic molecules active against various infectious microorganisms. They are well recognized as the wonder drugs of the modern world with crucial part for treating of various infectious diseases. The golden age of discovery of new antibiotics is from 1950 to 1970 (Aminov, 2010). The discovery of antimicrobials ensued a tremendous decrease in decease due to diseases (Kapil, 2005; Tiwari et al., 2013). However, decades ago, bacteria that could have been resistant to many antibiotics began to multiply rapidly, leading to rise in disease progression, death, and the cost of health care. Multitudes of factors are responsible for the increase in drug-resistant organisms (Bebell & Muiru, 2014; Chang et al., 2015). The rampant application of antimicrobials both in the sectors of animals and humans contributed significantly to the emergence of drug insusceptibility.

2 Antimicrobial's Usage in Human Beings

The inclination of humans to the antibiotics as prophylactic measures to reduce the disease incidence has amplified significantly in the recent years. The effectiveness of antimicrobials, however, is confined to the development of obduracy to antimicrobials and thus removes the life-saving concept. This major draft is considered to be serious by establishments such as the WHO, FAO, and WOAH confirmed by the entire medical world (WHO, 2017). Despite remarkable advances in modern and scientific medicine, the prospects for antibiotics are diminishing with the manifestation of a number of immunity resistance processes developed by infectious microorganisms that bring greater concern for advanced antibiotics. The significant obduracy modes of action that contribute to the incessant unresponsiveness against antibiotics is through the production of various enzymes, alterations in cell availability, alterations in drug target sites, internal expression of efflux pumps, genetic elements (plasmids, insertion sequences, transposons), and biofilm development that act as a barrier for the antibiotics to act (Dunphy et al., 2019; Hoiby et al., 2010; Ioannidis et al., 2015; Lister et al., 2009; Pang et al., 2019; Reygaert, 2018; Beceiro et al., 2013). Dealing with the snowballing application of antimicrobials, the advent of opportunistic infections, drug-unresponsive strains for the existing medical regimes, makes it difficult to treat them in hospitals and public health sectors.

Increased resistance to antimicrobials resulting from the indiscriminate use of antimicrobials has turned out to be a significant hazard to global human well-being. The status is exacerbated by the ban on drug production since the late 1960s, and the prolonged duration need to test novel antimicrobials prior to legal acceptance by the established order for profit (Spellberg et al., 2004). Increasing mobility and patient movement world over contribute to the increase within and between the nations spread of drug-unresponsive pathogens (Richet et al., 2001).

3 Antibiotics Usage in Animals

Antibiotics are being used in subtherapeutic doses as feed additives added in animal feed that contributes to finances of producing commercial animals by successful growth and modification of feedstuff and preventing disease (Castanon, 2007). In animal husbandry, the in-feed antibiotics application is the generally followed method that results in enhanced modern animal production with apprehensions about the development of antimicrobial resistance, which puts public health in jeopardy (Gadde et al., 2017). There is a probable correlation with application of antibiotics and emergence of resistance to antibiotics among microbes. Additionally, augmented application of antibiotics in the veterinary field further contributed strain on the advent of unresponsiveness to drugs (Silbergeld et al., 2008; Ahmad et al., 2011; Wielinga & Schlundt, 2012).

4 Antimicrobial Resistance: A Public Health Hazard

The observed pervasiveness of unyielding to antimicrobial has now become a foremost hazard of public well-being, with specialists of medicine stern cautioning of a reversion into period of pre-antibiotics (Davies & Davies, 2010); it could be community-associated infections predominantly owed to MRSA, VRE enterococci fashioned by Gram-negative organisms (Kumar & Singh, 2013).

5 Need for Alternatives

Intense enhancement observed in resistance to antimicrobials due to superbugs resulted in decline in antimicrobial drug production, from the later part of the 1960s and the prolonged periods of time necessitated to screen novel antimicrobials prior to the approval by the policy makers for commercial purposes (Spellberg et al., 2004). There is increased public awareness of the problems associated with careless usage. The worldwide blowout of resistance to antimicrobials is asking for the advancement of innovative cures. The document released by WHO of United Nations on priority drugs affirms that "infections caused by antibacterial resistant pathogens are associated with a pharmacological gap that many treatments are already ineffective and many others will soon be ineffective" (Atanasov et al., 2013). However, the pipeline for new antibiotics discovery has been slow. The FDA has permitted only 18 fresh antibiotics during the period 1983–2018. Meanwhile, the swift blowout of drug-immune germs in both health care and community settings has made futility of drugs currently (Li & Webster, 2017). In this scenario, the impending prerequisite is to identify unconventional cures for antibiotics to thwart and treat communicable illnesses. The key methods are usage of material that exists by nature sans support of artificial ingredients, herbs with a prospective employment as substitute or complementary antibiotic. Typical healing programs include traditional folk remedies rich in chemicals, like tannins, flavonoids, terpenoids alkaloids, steroids, coumarin, and essential oils (Atanasov et al., 2013), and that rarely create resistance (Fakhrudin et al., 2014).

6 Mode of Advancement of Immunity to Drugs

The presence of genes of unyielding to antibiotics in microorganism are a natural progression. The gene encoding resistance is produced by the bacteria which act as a self-defense process or usually due to an automatic mutation occurring in the chromosome of bacteria. The frequency of impromptus mutation for antibiotic resistance occurs one in hundred million to one billion cells. Even though, the incidence of mutation is rare phenomenon, the rapid multiplication of pathogenic microbes and the total population of cells found do not take long time to develop immunity against the antibiotics in its milieu (Tiwari & Tiwari, 2011). Once resistance develops, the mutated gene is passed directly on to the bacterial offspring

during the reproduction process. The major accountable reasons to development of antibiotic resistance in microorganisms are as follows:

1. Plasmids
2. Inactivation of antibiotic
3. Target site modification
4. Prevent drug uptake
5. Efflux pumps (EP)
6. Biofilm formation

7 Factors Causing AMR

Microbes are the only living species on earth and contain 50% of all living biomass. There are sharp differences amongst microbes such as contagious and non-contagious, albeit genetic mutations (Ama'bile-Cuevas, 2003) can transform non-pathogenic microorganisms into pathogenic microbes. Antimicrobial agents are applied in treatment of contagious illnesses both in faunae and in humans. Antimicrobials are chemical substances, which are toxic to many forms of microbes. Irregular and intentional use of antibiotics, migration of infected people (Memish et al., 2003), long-term hospitalization, deprivation, and malnourishment are important reasons in evolving antibiotic resistance (Byarugaba, 2004; Vila & Pal, 2010). The practice of including critical antimicrobials in livestock significantly as feed additives is an important reason for the emergence of AMR among zoonotic pathogens through food web in humans across the globe (Memish et al., 2003).

8 Phytochemicals

The ethnobotanical methodology to drug detection has many advantages. The hunt for impressed plant-sourced chemicals and the research experiments on conventional medicine have gained momentum in recent times. Ethnobotany, or the interrelationship between humans and plants, is a field that has also been mentioned as "the science of life" (Tiwari & Tiwari, 2011).

Allen et al. (2014) observed that from the beginning of civilization, human beings were employing the herbals as a cradle of medication. Any plant as whole, one or more of its parts that harbors ingredients that are useful for healing purposes and also for production of valuable drugs as predecessors were given good description as plants of medicine in many ancient treatises of Egypt, India, China, and Mediterranean countries. The natural bioactive components present in plants are called phytochemicals or phytobiotics or phytogenics (Pan et al., 2014). Of the 2,50,000 to 3,50,000 plant species recognized to date, approximately 35,000 are employed worldwide for pharmaceutical needs. The WHO information revealed that every 8 out of 10 African inhabitants and every 4 out of 10 of the Chinese populace bank on phytochemicals for their therapeutic requirements (Prance, 2007). Approximately, 80% of rural people in India use herbal remedies (WHO, 2002). Different

phytochemical compounds that are complex in nature are produced by plants. The derivatives of plant sources such as phenolics, terpenoids, polyphenols, indispensable oils, lectins, polypeptides, and other alkaloids have long established to exhibit antibacterial properties (Sahoo & Manchikanti, 2013).

9 Exploring Botanicals as Alternatives

Traditional Medicine (TM) can offer a luxury of exciting opportunities to fight against drug resistance, as observed by Ahmad and Beg (2001), Narayanan et al. (2011), and Potroz and Cho (2015). Herbs show an array of natural functions and can be well linked to disease control. Combined methods of healthy eating and use of herbal plants can provide powerful tools for controlling many diseases (Shah & Krishnamurthy, 2013). Traditional medicine systems including Ayurveda, Traditional Medicine of China (TCM), Campo, amplifications of Greek system of medicine that resulted in advent of *Unani* system or traditional South India-originated *Siddha* have so far failed to put entry into main stream of medicine for various reasons (Arora et al., 2010; Gupta et al., 2014).

Necessary efforts are being made to create the required mode of operation to adapt traditional therapy to modern medical practice. In countries like China, traditional therapies are already playing an eminent part in the management of contagious ailments (Chen et al., 2013). Plants are source for various biologically active secondary products that are insignificant in their basic body height, but show the potential for plant evolution in adverse abiotic and biotic environmental conditions (Stefanovic & Comic, 2012). The properties of secondary metabolites are well developed during evolution, which can act by disrupting cell targets in herbivores and microbes (Wink et al., 2012) and impacting signals in cells or guard against stress that is of oxidative or UV in nature (Wink, 2008), thus serving as protective mechanism.

10 Plant-Based Chemicals and Their Benefits

The astonishing aspect of plant extracts is that they are chemically complex in nature; they may contain hundreds of different chemical associations in a single extraction solution. Their combined actions will definitely produce an improved outcome (Sahoo & Manchikanti, 2013). Phytochemicals work through a number of mechanisms such as the inactivity of proteins, adhesions, and enzymes in targeted microorganisms. They also block cell signaling pathways (Cech et al., 2012) and disruption of biofilms (Quave et al., 2015). Most herbal remedies work in concerted ways (Quave et al., 2012). The synergistic property of plant extracts provides a distinctive room in the face of emerging antimicrobial resistance that can surpass the concept of single drug treatment for a specific disease by making it more difficult for bacteria to mutate and resist multiple attacks (Hu et al., 2016). Traditional medicine used for centuries has not produced resistance, as it uses the concept of interaction of

plants, which are chemically diverse and complex. The compounds in phytochemicals are natural and are thought to have significant health benefits. It has been proven that phytochemicals can affect gut microbiota and can act as an adjunct in the management of overweight and in treating inflammatory illnesses (Miller & Su, 2011). The microbiota of guts is significant for human body besides contributing to a number of key features related to our health. No wonder, by all possible means, it is rational to conclude that "feed your microbiota and get feed by it."

11 Nutritional Phytochemicals as Modulants of the Gut Microbiota

The availability of plant-sourced chemicals in food and their intake was associated to reducing the hazard of serious long-lasting diseases (Miller & Su, 2011). Foods made with nutraceuticals are opulent cradle of various bioavailable amalgamations, micronutrients, and non-nutrient integrates (Ratra & Gupta, 2015b). These nutraceuticals play more vital role in healthy living of the individual and thus contribute to health and wellness. Apart from the datum, the wide-ranging group of plant-based chemicals such as flavonoids, isoflavones etc. are often referred to as the interdependent group (Srivastava, 2018). Each chemical in each group has different compound properties, is used and absorbed differently, and has different health effects. About 8000 fractions of polyphenols have been found, divided into the four major groups: (1) flavonoids, (2) phenolic acid (curcumin), (3) stilbenoids (resveratrol), and (4) lignane. The antimicrobial properties of phenolic compound derived from tea, wine, olives, and berries were reported by Miller and Su (2011).

12 Flora-Sourced Chemicals as Substitutions to Antimicrobials and Feed Additives

In the present-day scenario, there is an enhanced awareness in decreasing the application of antibiotics in animal farming methods by identifying the substitute to antimicrobials and additives to feed for improving development and health position of farming animals (Panche et al., 2016). Firmicute, Bacteroidetes, Proteobacteria, and Actinobacteria at the levels of 64%, 23%, 8%, and 3%, respectively, are major residents of the microbiota, which are causative agents for the disintegration of food particles and aid assimilation process (Sharma et al., 2018). Carbohydrate-rich foods promote the growth of Firmicute and Proteobacteria, whereas, the unsaturated fatty acids, namely, the carbon chains that have one or more double bonds with a terminal carboxylic group and foods rich in proteins endorse the development of *Bacteriode* spp. and *Actinobacter* spp., in intestinal lumen (De Filippo et al., 2010). Polyphenols are crucial in reducing obesity and rabble-rousing illnesses and attract the interest of researchers and nutritionists (De Filippo et al., 2010). Phenolics present in tea prevent the bacterial growth of various species (Gadde et al., 2017). Catechins of tea mimic the mucin content of ileum, interfere

with the adhesion of bacteria and the subsequent colony (Chiva-Blanch & Badimon, 2017), and also favor the development of *Clostridium coccoides*, *Escherichia coli*, and *Eubacterium rectale* groups and, however, prevent the *Clostridium histolyticum* development. On the other hand, catechins favor growth of useful microorganisms, namely, *Bifidobacterium* spp. and *Lactobacilli*. Attachment of *Lactobacillus* spp. to epithelial cells of guts reduces the action of phenolic compounds and flavonoids (Gadde et al., 2017). Anthocyanin, a group of flavonoids, inhibits the replication of many harmful microbes that include *Bacillus cereus*, *Helicobacter pylori*, *Salmonellae*, and *Staphylococci* (Miller & Su, 2011).

13 Biochemical Sources from Plants as Another Possibility to Antimicrobials in Animal Sector

The phytobiotics or phytogenics are plant-origin usual amalgams that are further incorporated into animal feedstuff to improve efficiency of production (Gadde et al., 2017). Appropriate antibiotic substitutes should mimic effect as AGPs (Antibiotic Growth Promoters), ensure better performance, increase in nutrient availability of animals. Phytochemicals can be used in a variety of ways such as powder, dried, or as fragments, as essential oils, and oleoresin based on the practice employed to obtain the dynamic constituents (Gadde et al., 2017). Among the bioactive amalgams, the important ones are polyphenols that come under plant-sourced chemicals and their conformation and amount differ on various factors (Gadde et al., 2017). Nowadays, phytochemicals are being used as promoters of normal development in the poultry, cattle, and swine industries. Numerous basils and flavors, namely, cinnamon, coriander, garlic, ginger, marjoram, oregano, rosemary, thyme, yarrow etc., are used in the poultry industry as alternative to conventional AGP (Gadde et al., 2017). Unusual effects were reported on the use of phytochemicals due to variations in the conformation, variety, and source of the phytochemicals used and sets of environments (Gadde et al., 2017). The chemical activity of phytochemicals is not well understood but its incorporation into the diet modulates and strengthens the intestinal microbiome and decreases small toxic metabolites in the gut, due to its specific antibacterial effect to different pathogenic bacteria, leading to reduction in immune-mediated stress and intestinal challenge, thus improving animal performance (Kim et al., 2015). The potential useful characteristics of phytochemicals are said to be due to their microbicide and antioxidants also dependent on the composition of active ingredients (Settle et al., 2014).

14 Nutritive Plant-Based Chemicals for Augmenting Inborn Protection in Fowls

Many evidence-based researches point out that health benefit properties of plant-sourced chemicals are associated with the capacity to enhance the host defense mechanisms to most of the illnesses originating from infections (Lillehoj & Lee,

2012; Lillehoj et al., 2011). Immunomodulatory potential of herbs like mustard (*Brassica juncea*), safflower (*Carthamus tinctorius*), and dandelion (*Taraxacum officinale*) was tested in vitro in chicken lymphocytes and macrophages (Lee et al., 2010a). These substances inhibit the growth of tumor cells, stimulate the immune system, and produce antioxidant effects in chickens (Lee et al., 2010a). Cinnamaldehyde, an active component of cinnamon (*Cinnamomum cassia*), stimulated the proliferation of chicken lymphocyte in laboratory conditions and a large phagocytic cells in immobile form in the tissues or as a mobile white blood cell, at sites of infection that are otherwise called the macrophages in spleen resulting in production of nitric oxide at high levels (Lee et al., 2010b). Due to increased awareness about the usage of AGP and its control in poultry production, there is a need for antibiotic-free control strategies to reduce various infectious diseases that were managed with conventional dietary antimicrobials (Gadde et al., 2017). The newly hatched chicken supplemented with a combination of pepper (*Capsicum annuum*), turmeric (*Curcuma longa*), and shiitake mushroom (*Lentinus edodes*) in diet showed upgraded antibodies and serum antibody levels countering profiling with upended levels of serum antibody, and significantly decreased oocysts of feces and infected birds maintained in a normal nourishment or food regime with pepper and mushroom (Lee et al., 2010b).

15 Plant-Originated Nutritive Chemicals for Betterment of Pig Health

In pig production, one of the most problematic and crucial stages is weaning. During this time, pigs are rapidly exposed to a combination of stress factors and predispose to various diseased conditions that affect the survival rate of piglets at an early age (Djeridane et al., 2006). At this weaning stage, providing a combination of phytoconstituents with capsicum oleoresin, cinnamaldehyde, and caracole at 2%, 3%, and 5%, respectively, prolonged the gastric retention time and improved intestinal health by decreasing the total quantity of amount of ileal microbes and enhanced ratio of lactobacilli: enterobacteria (Manzanilla et al., 2004; Nofrarias et al., 2006).

The most plausible reason for mortality in weaned pigs is postweaning diarrhea triggered by *Escherichia coli*. This is also responsible for severe financial losses due to illness, declining functional growth, and medication costs (Fairbrother et al., 2005). Enterotoxigenic *E. coli* causes diarrhea in piglets prior to and after weaning period. In the *E. coli*-infected piglets, the dietary supplementation of capsicum oleoresin, garlicin, and turmeric oleoresin resulted in decreased ilial macrophages and neutrophils, leucocyte, serum TNF-α, and haptoglobin etc. (Liu et al., 2013).

The addition of phytochemicals in pigs reduced DNA damage caused by oxidative stress produced by food to lymphocytes, indicating their potential beneficial effects on the immune system (Frankič et al., 2010). The concept of antioxidant characteristics of phytochemicals concomitant with their biochemical configuration is well documented by Teissedre and Waterhouse (2000).

16 Application of Plant-Sourced Nutritive Chemicals in Ruminants

In grazing animals, there is an interdependent relationship between host and rumen microorganisms where the animal makes available nutrients and the right environment for fermentation, as well as microbes that lower fiber and bind proteins such as energy which is supplied to the host, respectively. Protein depletion is important in providing nitrogen for growth of bacteria in rumen. The excessive production of nitrogen in rumen bacteria leads to enhanced costs in making expenditures and also nitrogen emissions in the environment. Hence, the guidelines for proteolysis, peptidolysis, and deamination needs attention as a target for rumen ripening optimization (Calsamiglia et al., 2006).

Phytonutrients modify the amount of nutrients by altering the utilization of the digestive tract nutrients or other systematic pathways of metabolism. In the study of a mixture of essential oils compounds (MEO) and silage sources of silage, namely, alfalfa silage (AS) vs. corn silage (CS) to assess the usefulness on absorption, fermentation of rumen, microbial populations of rumen, production and composition of milk, the results indicated that effects are minimal (Benchaar et al., 2007). Research into alternative antimicrobial therapies such as animal feed supplements needs to be given emphasis on molecules and quantities that can bring about refined variations in metabolism in microbes and optimize the growth level (Cox et al., 2001). The prolonged supplementation of plant-based biomolecules to ruminants alters the growth rates due to quantitative modifications in the microbial populations in rumen ultimately leading to variations in the fermentation profile. Alteration in ruminal *Prevotella* numbers; a large population of microorganisms implicated in the reduction of amino acids leads to a reduction in protein degradation and ammonia production (Ferme et al., 2004).

Phytonutrients in animal feed of interest are divided into three important groups. They are saponins, Eos, and tannins. In most of the plant-based chemicals, the saponins and sarsaponins are important active compounds, exhibiting antibacterial activity (Wallace et al., 1994) and antiprotozoal activity (Min et al., 2005), which has led to decline in the ammonia nitrogen levels. The tannins are the phenolic compounds divided into water-based and condensed tannins. Condensed tannins can bind and reduce protein and can help in regulating protein consumption by ruminants; however, in huge amounts they can reduce occurrence of ballooning (Min et al., 2003).

Studies of Busquet and others in 2005 and 2006 in laboratory conditions revealed that essential oils like that of garlic decreases the quantity of acetate and branched-chain VFAs. Besides, they help in aggregating the intensities of propionate and butyrate.

The characteristic feature of plant-based chemicals, namely, cinnamaldehyde and eugenol is to lessen and increase the concentrations of acetate and propionate/butyrate in rumen, respectively (Busquet et al., 2005; Cardozo et al., 2004). The eugenol thwarts cessation of large peptides into smaller one (Busquet et al., 2005).

Synergism is noticed when eugenol and cinnamaldehyde were given together in preventing peptidolysis, deamination and refining the basis of amino acids and the peptides of small type to mass (host) and the microbes. Hence, combination of

phytonutrients is expected to produce interaction benefits by interfering in the same metabolic pathway.

Capsicum protected in rumen is observed to change the role of immunity (Liu et al., 2014) and defense mechanism, increase neutrophils, decrease lymphocytes with improved milk production (Oh et al., 2015), respectively.

17 Phytochemicals and the Digestive Microbiota

The mammalian digestive tract serves as a host for multivarious microbial flora and fauna, comprises mainly of bacteria, fungi, archaea, and viruses, and thus forms the intestinal microbiota. These environmentally acquired microorganisms can shape host physiology through their metabolic activities. Many vertebrates feed on nutrients abundant in multifarious enzymes that are not digested by their intestinal enzymes, relying on the various chemical functions of microbiota. The microbiota of intestinal tracts plays a very important part in painful bowel disease in both in human populations and faunae (Oakley et al., 2014). In broiler chickens, feeding oleoresins of Capsicum and *C. longa* reduced the adverse effects of necrotic enteritis (NE), by altering the gut microbiome and thus documenting the part of dietary *Capsicum frutescens* and *Curcuma longa* in combating NE (Kim et al., 2015).

Fifty percent of the energy is obtained from the microbial metabolites in fermenters of foregut, namely, cattle (Callaway et al., 2003), including VFAs. The fermenters of hindgut farm animals, namely pigs, poultry etc., on the other hand obtain only 5–10% of energy requirements from various products of fermentation, since fermentation occurs mostly in the cecum and large intestines. In ruminants or monogastrics, these differences appear to be significant in terms of performance, since the composition of gastrointestinal microbiota is essential for enhanced animal production and the effect of plant-based chemicals on microbiotas used in animal feed are plausible reasons for the affirmative effects observed in the studies. Several favorable plant properties are found in certain bioactive compounds, which are also synthesized as antimicrobial compounds to fight microbial infections. Phytochemicals are known to be important in inducing wide range of health-promoting effects and thus improving animal production. Commercial feeding of tannins and EOs acts as growth promoters in domesticated animal species by altering the gut microbiota in different ways.

Polyphenolic compounds such as tannins have the property of complexing many macromolecules like proteins, carbohydrates such as starch, cellulose, or glycogen and heavy metal ions, are often added in diets of ruminants, namely, fodder, sorghum etc. Both hydrolyzable and condensed tannins are commonly incorporated to increase animal production. Few of the tannins are potent antimicrobials and produce iron deficiency due to interaction with important proteins such as cell wall enzymes of the vulnerable microbe and act as either eliminators or growth restrictors of bacteria (Scalbert et al., 2002; Redondo et al., 2014, 2015), Gram-positive bacteria are extremely vulnerable to tannins (Elizondo et al., 2010). Tannins modify gastrointestinal progressions both by dietary protein binding in ruminants and by changing the rumen microbial composition and improving the growth of few

beneficial bacteria, which vary based on the polyphenols' molecular structures (Min & Rhee, 2015; Carrera-Quintanar et al., 2018). The information on in vivo interactions between plant tannins and rumen bacteria is inadequate.

18 Essentiality of Developing Phytochemicals as Substitutes/Synergists to Antimicrobials and Procedures Therein

Phytochemistry or chemical research of accepted products is the pillar of the commercial herbal productiveness (Carrera-Quintanar et al., 2018). By endorsing the application of herbs in contemporary medication, the creation of bioactivity-directed characterization and isolation of new phytoconstituents should be carried out. New phytoconstituents that have the potential applicants as a "lead" component in the production of imitative referends with modified beneficial action with condensed noxiousness should be identified and evaluated for conversion into medicinal essential drugs (Koparde et al., 2019).

19 Development in Screening Techniques for Phytochemicals

The application of cybernetic analyses employing various cheminformatics, ligand, and structural design has developed into an effective substitute to automated screening tests to detect lead-based structures or biological targets in unearthing of medicines for diseases of inflammation (Katiyar et al., 2012). In discovering the new and synergistic antimicrobial agents, plant-based chemicals show that antimicrobial properties can be tested to inhibit bacterial proteins, namely, peptide deformylase, DNA gyrase B, tyrosine, UDP-galactopyranose mutase, NAD +/− DNA ligase, etc., besides plant-based chemicals that contain efflux pumps or quorum-sensing proteins of microbes, or antibodies that upsurge the immunity (Ramsay et al., 2018). It seems there was exploration for fundamental resemblance along with molecular modelling methods to recognize potential inhibitors of *Shigella flexneri* DNA gyrase (Setzer et al., 2016). Molecular docking has also been helpful in identification of bacterial peptidyl-tRNA hydrolase as a substitute objective of known antibiotics (Rahimi et al., 2016). It was described that antimycotic drug and therapeutic xanthone compounds were recognized with application of amalgamation of chemical mixture and multicomponent scrutiny (Ferguson et al., 2016).

20 Forthcoming Projections and Promises of Flora-Sourced Chemicals

Flora-sourced chemicals function as an imperative birthplace of innovative remedies that now account for 9 out of 10 of the newly discovered drugs. India is one of the top 12 biodiversity hotspots with 45×10^3 different plant species, 15×10^3 to

18×10^3 flowering plants, 23×10^3 fungi, 16×10^3 lichens, 18×10^3 bryophytes, and 13×10^6 marine organisms. From these plants, there are 15×10^3 to 20×10^3 plants with medicinal properties (Bernal & Coy-Barrera, 2015). The global market value of herbal products is 32×10^9 USD with a growth nearly of 9–15%. The commercial herbal products of India generate an average income of INR 2.3×10^9. Herbal exports include medicines from AYUSH merchandises that account for 3% of total Indian herbal drug exports (Ratra & Gupta, 2015a).

21 Conclusion

The use of traditional medicine is widely recognized in our quotidian life, and it is projected that more than 80% of the global populace are still dependent on conventional medicines for basic well-being. The importance of natural products derived from plants and their extraction used by the public was appreciated and documented from ancient times. Researchers and clinicians pay close attention to secondary metabolites found in plants as a result of their antibiotic action sans affecting any antimicrobial obduracy. The antimicrobials of plant origin have been widely used as preventive and therapeutic solutions against many infectious diseases. On the contrary, antimicrobials at times result in serious aftereffects and are very expensive. Hence, interest is in increasing trend in the use of medicinal plants as an alternative medicine. Several species of plants have already been widely reported indicating possible beneficial effects. However, new emerging infections, diseases, and rapid emergence of new bacteria and viruses are urging researchers to continue exploring the environment with natural novel products. Plants certainly play a key role in controlling drug-resistant microorganism. However, these practical principles found in plants should be adopted in order for further research to translate this information into potential therapies.

To conclude the era of antimicrobial-resistant "superbugs," the advancement with novel antibacterial compounds is of paramount importance and herbal plants with active drug ingredients could be an attractive source of future antimicrobials. It is clear that the phytochemicals found in plants epitomize a potential foundation for real, low-cost, and benign agent against harmful microbes.

References

Ahmad, I., & Beg, A. Z. (2001). Antimicrobial and phytochemical studies on 45 Indian medicinal plants against multi-drug resistant human pathogens. *Journal of Ethnopharmacology, 74*, 113–123.

Ahmad, A., Ghosh, A., Schal, C., & Zurek, L. (2011). Insects in confined swine operations carry a large antibiotic resistant and potentially virulent enterococcal community. *BMC Microbiology, 11*(1), 23. Accessed on 5 October 2022.

Allen, H. K., Trachsel, J., Looft, T., & Casey, T. A. (2014). Finding alternatives to antibiotics. *Annals of the New York Academy of Sciences, 1323*(1), 91–100.

Ama'bile-Cuevas, C. F. (2003). Gathering of resistance genes in gram negative bacteria: An overview. In C. F. Ama'bile-Cuevas (Ed.), *Multidrug resistant bacteria* (pp. 9–31). Horizon Scientific Press.

Aminov, R. I. (2010). A brief history of the antibiotic era: Lessons learned and challenges for the future. *Frontiers in Microbiology, 1*, 134.

Arora, R., Chawla, R., Marwah, R., Arora, P., Sharma, R. K., Kaushik, V., Goel, R., Kaur, A., Silambarasan, M., Tripathi, R. P., & Bharadwaj, J. R. (2010). Potential of complementary and alternative medicine in preventive management of novel H1N1 flu (SwineFlu) pandemic: Thwarting potential disasters in the bud. *Evidence-Based Complementary and Alternative Medicine, 2011*, 1–16. https://doi.org/10.1155/2011/586506. Accessed on 5 October 2022.

Atanasov, A. G., Blunder, M., Fakhrudin, N., Liu, X., Noha, S. M., Malainer, C., Kramer, M. P., Cocic, A., Kunert, O., Schinkovitz, A., Heiss, E. H., Schuster, D., Dirsch, V. M., & Bauer, R. (2013). Polyacetylenes from *Notopterygium incisum*–new selective partial agonists of peroxisome proliferator-activated receptor-gamma. *PLoS One, 8*, e61755. Accessed on 5 October 2022.

Bebell, L. M., & Muiru, A. M. (2014). Antibiotic use and emerging resistance: How can resource-limited countries turn the tide? *Global Heart, 9*(3), 347–358.

Beceiro, A., Tomas, M., & Bou, G. (2013). Antimicrobial resistance and virulence: A successful or deleterious association in the bacterial world? *Clinical Microbiology Reviews, 26*, 185–230.

Benchaar, C., Petit, H. V., Berthiaume, R., Ouellet, D. R., Chiquette, J., & Chouinard, P. Y. (2007). Effects of essential oils on digestion, ruminal fermentation, rumen microbial populations, milk production, and milk composition in dairy cows fed alfalfa silage or corn silage. *Journal of Dairy Science, 90*, 886–897.

Bernal, F. A., & Coy-Barrera, E. (2015). Molecular docking and multivariate analysis of xanthones as antimicrobial and antiviral agents. *Molecules, 20*, 13165–13204.

Busquet, M., Calsamiglia, S., Ferret, A., Cardozo, P. W., & Kamel, C. (2005). Effects of cinnamaldehyde and garlic oil on rumen microbial fermentation in a dual flow continuous culture. *Journal of Dairy Science, 88*, 2508–2516.

Busquet, M., Calsamiglia, S., Ferret, A., & Kamel, C. (2006). Plant extracts affect in vitro rumen microbial fermentation. *Journal of Dairy Science, 89*, 761–771.

Byarugaba, D. K. (2004). Antimicrobial resistance in developing countries and responsible risk factors. *International Journal of Antimicrobial Agents, 24*(2), 105–110.

Callaway, T. R., Edrington, T. S., Rychlik, J. L., Genovese, K. J., Poole, T. L., Jung, Y. S., Bischoff, K. M., Anderson, R. C., & Nisbet, D. J. (2003). Ionophores: Their use as ruminant growth promotants and impact on food safety. *Current Issues in Intestinal Microbiology, 4*, 43–51.

Calsamiglia, S., Castillejos, L., & Busquet, M. (2006). Alternatives to antimicrobial growth promoters in cattle. *Recent Advances in Animal Nutrition, 39*, 129.

Cardozo, P. W., Calsamiglia, S., Ferret, A., & Kamel, C. (2004). Effects of natural plant extracts on ruminal protein degradation and fermentation profiles in continuous culture. *Journal of Animal Science, 82*, 3230–3236.

Carrera-Quintanar, L., López Roa, R. I., Quintero-Fabián, S., Sánchez-Sánchez, M. A., Vizmanos, B., & Ortuño-Sahagún, D. (2018). Phytochemicals that influence gut microbiota as prophylactics and for the treatment of obesity and inflammatory diseases. *Mediators of Inflammation, 2018*, 9734845.

Castanon, J. I. R. (2007). History of the use of antibiotic as growth promoters in European poultry feeds. *Poultry Science, 86*, 2466–2471.

Cech, N. B., Junio, H. A., Ackermann, L. W., Kavanaugh, J. S., & Horswill, A. R. (2012). Quorum quenching and antimicrobial activity of goldenseal (*Hydrastis canadensis*) against methicillin-resistant *Staphylococcus aureus* (MRSA). *Planta Medica, 78*(14), 1556–1561.

Chang, H. H., Cohen, T., Grad, Y. H., Hanage, W. P., O'Brien, T. F., & Lipsitch, M. (2015). Origin and proliferation of multiple-drug resistance in bacterial pathogens. *Microbiology and Molecular Biology Reviews, 79*(1), 101–116.

Chen, M. C., Hao, Z., Tian, Y., Zhang, Q. Y., Gao, P. J., & Jin, J. L. (2013). Different effects of six antibiotics and ten traditional Chinese medicines on Shiga toxin expression by *Escherichia coli* O157:H7. *Evidence-Based Complementary and Alternative Medicine, 2013*, 8. Article ID 121407.

Chiva-Blanch, G., & Badimon, L. (2017). Effects of polyphenol intake on metabolic syndrome: Current evidences from human trials. *Oxidative Medicine and Cellular Longevity, 2017*, 5812401.

Cox, S. D., Mann, C. M., & Markham, J. L. (2001). Interactions between components of the essential oil of *Melaleuca alternifolia*. *Journal of Applied Microbiology, 91*, 492–497.

Davies, J., & Davies, D. (2010). Origins and evolution of antibiotic resistance. *Microbiology and Molecular Biology Reviews, 74*, 417–433.

De Filippo, C., Cavalieri, D., Di Paola, M., Ramazzotti, M., Poullet, J. B., Massart, S., Collini, S., Pieraccini, G., & Lionetti, P. (2010). Impact of diet in shaping gut microbiota revealed by a comparative study in children from Europe and rural Africa. *Proceedings of the National Academy of Sciences of the United States of America, 107*(33), 14691–14696. https://doi.org/10.1073/pnas.1005963107. Accessed on 5 October 2022.

Djeridane A, Yousfi M, Nadjemi B, Boutassouna D, Stocker P, Vidal N. (2006). Antioxidant activity of some Algerian medicinal plants extracts containing phenolic compounds. *Food Chemistry, 97*, 654–660.

Dunphy, L. J., Yen, P., & Papin, J. A. (2019). Integrated experimental and computational analyses reveal differential metabolic functionality in antibiotic-resistant *Pseudomonas aeruginosa*. *Cell Systems, 8*, 3–14.

Elizondo, A. M., Mercado, E. C., Rabinovitz, B. C., & Fernandez-Miyakawa, M. E. (2010). Effect of tannins on the in vitro growth of *Clostridium perfringens*. *Veterinary Microbiology, 145*, 308–314.

Fairbrother, J. M., Nadeau, É., & Gyles, C. L. (2005). *Escherichia coli* in postweaning diarrhea in pigs: An update on bacterial types, pathogenesis, and prevention strategies. *Animal Health Research Reviews, 6*, 17–39.

Fakhrudin, N., Waltenberger, B., Cabaravdic, M., Atanasov, A. G., Malainer, C., Schachner, D., Heiss, E. H., Liu, R., Noha, S. M., Grzywacz, A. M., Bison, J. M., Awad, E. M., Schuster, D., Breuss, J. M., Rollinger, J. M., Bochkov, V., Stuppner, H., & Dirsch, V. M. (2014). Identification of plumericin as a potent new inhibitor of the NF-kappaB pathway with anti-inflammatory activity in vitro and in vivo. *British Journal of Pharmacology, 171*, 1676–1686. Accessed on 5 October 2022.

Ferguson, P. P., Holloway, W. B., Setzer, W. N., McFeeters, H., & McFeeters, R. L. (2016). Small molecule docking supports broad and narrow spectrum potential for the inhibition of the novel antibiotic target bacterial Pth1. *Antibiotics (Basel), 5*(2), 16.

Ferme, D., Banjac, M., Calsamiglia, S., Busquet, M., Kamel, C., & Avguštin, G. (2004). The effects of plant extracts on microbial community structure in a rumen-simulating continuous-culture system as revealed by molecular profiling. *Folia Microbiologia (Praha), 49*, 151–155.

Frankič, T., Levart, A., & Salobir, J. (2010). The effect of vitamin E and plant extract mixture composed of carvacrol, cinnamaldehyde and capsaicin on oxidative stress induced by high PUFA load in young pigs. *Animal, 4*, 572–578.

Gadde, U., Kim, W. H., Oh, S. T., & Lillehoj, H. S. (2017). Alternatives to antibiotics for maximizing growth performance and feed efficiency in poultry: A review. *Animal Health Research Reviews, 18*, 26–45.

Gupta, P. D., Daswani, P. G., & Birdi, T. J. (2014). Approaches in fostering quality parameters for medicinal botanicals in the Indian context. *Indian Journal of Pharmacology, 46*, 363–371.

Hoiby, N., Bjarnsholt, T., Givskov, M., Molin, S., & Ciofu, O. (2010). Antibiotic resistance of bacterial biofilms. *International Journal of Antimicrobial Agents, 35*, 322–332.

Hu, X. Q., Sun, Y., Lau, E., Zhao, M., & Su, S. B. (2016). Advances in synergistic combinations of Chinese herbal medicine for the treatment of cancer. *Current Cancer Drug Targets, 16*(4), 346–356.

Ioannidis, A., Magana, M., Bologa, C. G., Oprea, T. I., Paulsen, I. T., & Tegos, G. P. (2015). Defining the microbial effluxome in the content of the host-microbiome interaction. *Frontiers in Pharmacology, 6*, 31.

Kapil, A. (2005). The challenge of antibiotic resistance: Need to contemplate. *The Indian Journal of Medical Research, 121*, 83–89.

Katiyar, C., Gupta, A., Kanjilal, S., & Katiyar, S. (2012). Drug discovery from plant sources: An integrated approach. *Ayu, 33*(1), 10–19.

Kim, J. E., Lillehoj, H. S., Hong, Y. H., Kim, G. B., Lee, S. H., Lillehoj, E. P., & Bravo, D. M. (2015). Dietary *Capsicum* and *Curcuma longa* oleoresins increase intestinal microbiome and necrotic enteritis in three commercial broiler breeds. *Research in Veterinary Science, 102*, 150–158.

Koparde AA., Doijad RC., & Magdum CS (2019). *Natural Products in Drug Discovery* [Online First], IntechOpen.

Kumar, S., & Singh, B. R. (2013). An overview of mechanisms and emergence of antimicrobials drug resistance. *Advances in Animal and Veterinary Sciences, 1*, 7–14.

Lee, S. H., Lillehoj, H. S., Hong, Y. H., Jang, S. I., Lillehoj, E. P., Ionescu, C., Mazuranok, L., & Bravo, D. (2010a). *In vitro* effects of plant and mushroom extracts on immunological function of chicken lymphocytes and macrophages. *British Poultry Science, 51*, 213–221. Accessed on 6 October 2022.

Lee, S. H., Lillehoj, H. S., Jang, S. I., Kim, D. K., Ionescu, C., & Bravo, D. (2010b). Effect of dietary curcuma, capsicum, and lentinus on enhancing local immunity against *Eimeria acervulina* infection. *Journal of Poultry Science, 47*, 89–95. https://doi.org/10.2141/jpsa.009025. Accessed on 6 October 2022.

Li, B., & Webster, T. J. (2017). Bacteria antibiotic resistance: New challenges and opportunities for implant-associated orthopaedic infections. *Journal of Orthopaedic Research, 36*(1), 22–32.

Lillehoj, H. S., & Lee, K. W. (2012). Immune modulation of innate immunity as alternatives-to-antibiotics strategies to mitigate the use of drugs in poultry production. *Poultry Science, 91*, 1286–1291.

Lillehoj, H. S., Kim, D. K., Bravo, D. M., & Lee, S. H. (2011). Effects of dietary plant derived phytonutrients on the genome-wide profiles and coccidiosis resistance in the broiler chickens. *BMC Proceedings, 5*, S34.

Lister, P. D., Wolter, D. J., & Hanson, N. D. (2009). Antibacterial-resistant *Pseudomonas aeruginosa*: Clinical impact and complex regulation of chromosomally encoded resistance mechanisms. *Clinical Microbiology Reviews, 22*, 582–610.

Liu, Y., Song, M., Che, T. M., Almeida, J. A. S., Lee, J. J., Bravo, D., Maddox, C. W., & Pettigrew, J. (2013). Dietary plant extracts alleviate diarrhea and alter immune responses of weaned pigs experimentally infected with a pathogenic. *Journal of Animal Science, 91*, 5294–5306.

Liu, Y., Song, M., Che, T. M., Lee, J. J., Bravo, D., Maddox, C. W., & Pettigrew, J. E. (2014). Dietary plant extracts modulate gene expression profiles in ileal mucosa of weaned pigs after an infection. *Journal of Animal Science, 92*, 2050–2062.

Manzanilla, E. G., Perez, J. F., Martin, M., Kamel, C., Baucells, F., & Gasa, J. (2004). Effect of plant extracts and formic acid on the intestinal equilibrium of early-weaned pigs. *Journal of Animal Science, 82*, 3210–3218.

Memish, Z. A., Venkatesh, S., & Shibi, A. M. (2003). Impact of travel on international spread of antimicrobial resistance. *International Journal of Antimicrobial Agents, 21*(2), 135–142.

Miller, L. H., & Su, X. (2011). Artemisinin: Discovery from the Chinese herbal garden. *Cell, 146*(6), 855–858.

Min, Y. W., & Rhee, P. L. (2015). The role of microbiota on the gut immunology. *Clinical Therapeutics, 37*, 968–975.

Min, B. R., Barry, T. N., Attwood, G. T., & McNabb, W. C. (2003). The effect of condensed tannins on the nutrition and health of ruminants fed fresh temperate forages: A review. *Animal Feed Science and Technology, 106*, 3–19.

Min, B. R., Hart, S. P., Miller, D., Tomita, G. M., Loetz, E., & Sahlu, T. (2005). The effect of grazing forage containing condensed tannins on gastro-intestinal parasite infection and milk composition in Angora does. *Veterinary Parasitology, 130*, 105–113.

Narayanan, S., Raja, S., Ponmurugan, K., Kandekar, S. C., Natarajaseenivasan, K., Maripandi, A., & Mandeel, Q. A. (2011). Antibacterial activity of selected medicinal plants against multiple antibiotic resistant uropathogens: A study from Kolli Hills, Tamil Nadu, India. *Beneficial Microbes, 2*, 235–243. Accessed on 5 October 2022.

Nofrarias, M., Manzanilla, E. G., Pujols, J., Gibert, X., Majo, N., Segalés, J., & Gasa, J. (2006). Effects of spray-dried porcine plasma and plant extracts on intestinal morphology and on leukocyte cell subsets of weaned pigs. *Journal of Animal Science, 84*, 2735–2742.

Oakley, B. B., Lillehoj, H. S., Kogut, M. H., Kim, W. K., Maurer, J. J., Pedroso, A., Lee, M. D., Collett, S. R., Johnson, T. J., & Cox, N. A. (2014). The chicken gastrointestinal microbiome. *FEMS Microbiology Letters, 360*, 100–112.

Oh, J., Giallongo, F., Frederick, T., Pate, J., Walusimbi, S., Elias, R. J., Wall, E. H., Bravo, D., & Hristov, A. N. (2015). Effects of dietary Capsicum oleoresin on productivity and immune responses in lactating dairy cows. *Journal of Dairy Science, 98*, 6327–6339.

Pan, S. Y., Litscher, G., Gao, S. H., Zhou, S. F., Yu, Z. L., Chen, H. Q., Zhang, S. F., Tang, M. K., Sun, J. N., & Ko, K. M. (2014). Historical perspective of traditional indigenous medical practices: The current renaissance and conservation of herbal resources. *Evidence-Based Complementary and Alternative Medicine, 2014*, 525340. https://doi.org/10.1155/2014/525340. Accessed on 5 October 2022.

Panche, A. N., Diwan, A. D., & Chandra, S. R. (2016). Flavonoids: An overview. *Journal of Nutritional Science, 5*, e47.

Pang, Z., Raudonis, R., Glick, B. R., Lin, T. J., & Cheng, Z. (2019). Antibiotic resistance in *Pseudomonas aeruginosa*: Mechanisms and alternative therapeutic strategies. *Biotechnology Advances, 37*, 177–192.

Potroz, M., & Cho, N. (2015). Natural products for the treatment of trachoma and Chlamydia trachomatis. *Molecules, 20*, 4180–4203.

Prance, G. T. (2007). Ethnobotany, the science of survival: A declaration from Kauai. *Economic Botany, 61*, 1.

Quave, C. L., Estévez-Carmona, M., Compadre, C. M., Hobby, G., Hendrickson, H., Beenken, K. E., & Smeltzer, M. S. (2012). Ellagic acid derivatives from *Rubus ulmifolius* inhibit *Staphylococcus aureus* biofilm formation and improve response to antibiotics. *PLoS One, 7*(1), e28737.

Quave, C. L., Lyles, J. T., Kavanaugh, J. S., Nelson, K., Parlet, C. P., Crosby, H. A., Heilmann, K. P., & Horswill, A. R. (2015). Castanea sativa (European Chestnut) leaf extracts rich in ursene and oleanene derivatives block *Staphylococcus aureus* virulence and pathogenesis without detectable resistance. *PLoS One, 10*(8), e0136486.

Rahimi, H., Najafi, A., Eslami, H., Negahdari, B., & Moghaddam, M. M. (2016). Identification of novel bacterial DNA gyrase inhibitors: An *in-silico* study. *Research in Pharmaceutical Sciences, 11*, 250–258.

Ramsay, R. R., Popovic-Nikolic, M. R., Nikolic, K., Uliassi, E., & Bolognesi, M. L. (2018). A perspective on multi-target drug discovery and design for complex diseases. *Clinical and Translational Medicine, 7*(1), 3.

Ratra, M., & Gupta, R. (2015a). Future prospects and aspects of herbal drug discovery in herbal medicines. *Columbia Journal of Pharmaceutical Sciences, 2*(2), 16–21.

Ratra, M., & Gupta, R. (2015b). Comprehensive review on pharmacological profile of *Alangium salvifolium*: A medicinal plant. *UK Journal of Pharmaceutical Biosciences, 3*(3), 22.

Redondo, L. M., Chacana, P. A., Dominguez, J. E., & Miyakawa, M. E. F. (2014). Perspectives in the use of tannins as alternative to antimicrobial growth promoter factors in poultry. *Frontiers in Microbiology, 5*, 118.

Redondo, L. M., Dominguez, J. E., Rabinovitz, B. C., Redondo, E. A., & Miyakawa, M. E. F. (2015). Hydrolyzable and condensed tannins resistance in *Clostridium perfringens*. *Anaerobe, 34*, 139–145.

Reygaert, W. C. (2018). An overview of the antimicrobial resistance mechanisms of bacteria. *AIMS Microbiology, 4*, 482–501.

Richet, H. M., Mohammed, J., McDonald, L. C., & Jarvis, W. R. (2001). Building communication networks: International network for the study and prevention of emerging antimicrobial resistance. *Emerging Infectious Diseases, 7*, 319–322.

Sahoo, N., & Manchikanti, P. (2013). Herbal drug regulation and commercialization: An Indian industry perspective. *Journal of Alternative and Complementary Medicine, 19*(12), 957–963.

Scalbert, A., Morand, C., Manach, C., & Rémésy, C. (2002). Absorption and metabolism of polyphenols in the gut and impact on health. *Biomedicine & Pharmacotherapy, 56*, 276–282.

Settle, T., Leonard, S. S., Falkenstein, E., Fix, N., Van Dyke, K., & Klandorf, H. (2014). Effects of a phytogenic feed additive versus an antibiotic feed additive on oxidative stress in broiler chicks and a possible mechanism determined by electron spin resonance. *International Journal of Poultry Science, 13*, 62.

Setzer, M. S., Sharifi-Rad, J., & Setzer, W. N. (2016). The search for herbal antibiotics: An in-silico investigation of antibacterial phytochemicals. *Antibiotics, 5*(3), 30. https://doi.org/10.3390/antibiotics5030030. Accessed on 5 October 2022.

Shah, A., & Krishnamurthy, R. (2013). Swine flu and its herbal remedies. *International Journal of Engineering Science, 2*, 68–78.

Sharma, C., Rokana, N., Chandra, M., Singh, B. P., Gulhane, R. D., Gill, J., Ray, P., Puniya, A. K., & Panwar, H. (2018). Antimicrobial resistance: Its surveillance, impact, and alternative management strategies in dairy animals. *Frontiers in Veterinary Science, 4*, 237. https://doi.org/10.3389/fvets.2017.00237. Accessed on 5 October 2022.

Silbergeld, E. K., Price, L., & Graham, J. (2008). Industrial farm animal production, antimicrobial resistance and human health. *Annual Review of Public Health, 29*, 151–169.

Spellberg, B., Powers, J. H., Brass, E. P., Miller, L. G., & Edwards, J. E. J. (2004). Trends in antimicrobial drug development: Implications for the future. *Clinical Infectious Diseases, 38*, 1279–1286.

Srivastava, R. K. (2018). Need of nutraceuticals/functional food products for health benefits to world-wide people. *Journal of Biotechnology and Biomedical Science, 1*(4), 1–13.

Stefanovic, O., & Comic, L. (2012). Synergistic antibacterial interaction between *Melissa officinalis* extracts and antibiotics. *Journal of Applied Pharmaceutical Science, 2*, 1–5.

Teissedre, P. L., & Waterhouse, A. L. (2000). Inhibition of oxidation of human low-density lipoproteins by phenolic substances in different essential oils varieties. *Journal of Agricultural and Food Chemistry, 48*, 3801–3805.

Tiwari, R., & Tiwari, G. (2011). Use of antibiotics: From preceding to contemporary. *Scholars' Research Journal, 1*, 59–68.

Tiwari, R., Chakraborty, S., Dhama, K., Rajagunalan, S., & Singh, S. (2013). Antibiotic resistance – An emerging health problem: Causes, worries, challenges and solutions – A review. *International Journal of Current Research, 5*, 1880–1892.

Vila, J., & Pal, T. (2010). Update on antimicrobial resistance in low-income countries: Factors favouring the emergence of resistance. *The Open Infectious Diseases Journal, 4*, 38–54.

Wallace, R. J., Arthaud, L., & Newbold, C. J. (1994). Influence of Yucca shidigera extract on ruminal ammonia concentrations and ruminal microorganisms. *Applied and Environmental Microbiology, 60*, 1762–1767.

WHO. (2002). *WHO traditional medicine strategy 2002–2005*. WHO/EDM/TRM/2002.1.

WHO. (2017). *World Health Organization model list of essential medicines*. WHO. Amended August 2017; 20th List.

Wielinga, P., & Schlundt, J. (2012). *Combating the risk of antimicrobial resistance in animals for the benefit of human health in Denmark*. A case study of emerging risks related to AMR for the International Risk Governance Council (IRGC).

Wink, M. (2008). Evolutionary advantage and molecular modes of action of multicomponent mixtures used in phytomedicine. *Current Drug Metabolism, 9*, 996–1009.

Wink, M., Ashour, M. L., & El-Readi, M. Z. (2012). Secondary metabolites from plants inhibiting ABC transporters and reversing resistance of cancer cells and microbes to cytotoxic and antimicrobial agents. *Frontiers in Microbiology, 3*, 1–15.

Deterring the Transmission of AMR in the Environment: A Chinese Perspective

Ziming Han, Yu Zhang, and Min Yang

Contents

Abstract

Solutions to antimicrobial resistance (AMR) require interdisciplinary cooperation, which needs the joint efforts of researchers of medical, microbiological, and environmental science. China is a developing country with a large population and rapidly growing economy, and has taken efforts to handle AMR challenges. Herein, we summarized the Chinese actions on deterring the trans-

Z. Han · Y. Zhang (✉) · M. Yang (✉)
State Key Laboratory of Environmental Aquatic Chemistry, Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences, Beijing, China

University of Chinese Academy of Sciences, Beijing, China

National Engineering Research Center of Industrial Wastewater Detoxication and Resource Recovery, Beijing, China
e-mail: zmhan_st@rcees.ac.cn; zhangyu@rcees.ac.cn; yangmin@rcees.ac.cn

M. P. Mothadaka et al. (eds.), *Handbook on Antimicrobial Resistance*,
https://doi.org/10.1007/978-981-19-9279-7_52

mission of AMR in the environment. Effluents from the pharmaceutical manufacturing industry, including effluent and fermentation residues from antibiotic production processes, were the hotspot of antibiotic and antibiotic resistance gene (ARG) discharge. Enhanced hydrolysis pretreatment technology was developed for selective removal of antibiotic potency from pharmaceutical effluent and fermentation residues. This technology has been applied in the pharmaceutical industry in China, achieving the simultaneous reduction of chemical oxygen demand (COD) and ARGs. Livestock-related environment was also an important controlling point of AMR dissemination, and the transfer of clinically important ARGs from animal to human has been proved widely. Temperature is a key operational parameter of treatment approaches of anaerobic digestion or composting for livestock waste, and hyperthermophilic pretreatment was recommended for better reduction of fecal bacteria and clinically important ARGs. In addition, application of drugs in livestock farming is prohibited in China, which also significantly contributed to the reduced ARG prevalence in livestock-related environment.

Keywords

Antimicrobial resistance · Control strategy · One Health · Pharmaceutical manufacturing · Livestock waste

1 Introduction

Antimicrobial resistance (AMR) is a global crisis that needs global cooperation and concerted actions among different fields and different countries. As per the "One Health" perspective, AMR disseminates across human, animal, and environment (Walsh, 2018). Reports indicated that the environment could act as emission sources, transfer routes, and even a natural reservoir, which plays a critical role in global AMR crisis (Han et al., 2020; Zhang et al., 2022). For example, antibiotics as well as fecal bacteria harboring antibiotic resistance genes (ARGs) will be introduced to the environment through the effluent and solid waste discharged from antibiotic manufacturing, livestock production, and hospitals. It is difficult and extremely expensive to remove antibiotics and ARGs that are discharged and pollute the environment. Minimizing releases from the main discharge sources and determining antibiotic emanation limits are priorities for preventing environmental AMR development (Zhang et al., 2022).

China has developed its own National Action Plan on AMR (2016–2020 and 2022–2025) with contributions from multisectoral departments. From the views of environmental engineering and management, many important actions to cope with challenges of AMR have been conducted and have received positive impacts in China. Herein, the actions in China, with the aim of decreasing AMR dissemination from the pharmaceutical industry and animal farming to the environment, were

summarized and recommended for better deterring the transmission of AMR in the environment reduction.

2 Deterring the Transmission of AMR During Antibiotic Production Processes

In the pharmaceutical manufacturing industry, microbial fermentation is a common process for producing bulk antibiotics. As the largest producer in the world, China annually produces 1.3 million tons of bulk antibiotics encompassing more than 70 kinds of antibiotics. Effluent and solid waste from antibiotic production contain high-level remaining antibiotic (several ten thousand mg/L or mg/kg level), which are critically higher than the common concentration level of antibiotics in natural environmental media (Larsson & Flach, 2021; Zhang et al., 2022). Although antibiotic pollution from antibiotic production process is less widespread and mainly occurs in developing countries such as China and India, the high-level selective pressure could intensively promote AMR development and course global consequences (Larsson, 2014). Controlling emission of antibiotics and ARGs from antibiotic production processes needs critical engineering approach and environmental management optimization.

2.1 AMR Development During Biological Treatment of Antibiotic Production Effluent

The widely used biological treatment systems, usually an amalgamation of oxygen-independent breakdown and galvanized slurry method, receive pharmaceutical sewer water containing extremely high concentrations of antibiotics. On the one hand, effluent treatment efficacy did not perform well because of the disturbance of functional microorganisms and adverse inhibition of biological activities under high stress of antibiotics (He et al., 2020; Luan et al., 2020). On the other hand, heavy bacterial multidrug resistance would be developed in pharmaceutical effluent biological management arrangements (Liu et al., 2012; Zhang et al., 2013; Zhao et al., 2019). Multidrug-resistant bacteria were dominant in the lower reaches of rivers because of effluent discharge from the oxytetracycline and penicillin production facilities (Larsson et al., 2007; Li et al., 2009; Li et al., 2010; Li et al., 2011). Pharmaceutical manufacturing effluents, together with their receiving waterbodies, have been recognized as the antibiotic resistance hotspots, which was the critical control point of AMR in the environment (Berendonk et al., 2015).

Aerobic biofilm reactors spiked with antibiotics were conducted to explore the antibiotic resistance development mechanism under high antibiotic pressures. One research investigated the integrons in microbial community from two reactors of biofilm handling synthetic pharmaceutical sewer waters spiked with streptomycin and oxytetracycline, respectively (Huyan et al., 2020). The proportion of class 1 integron (*intI1*) that contained antibiotic resistance gene cassettes increased

significantly under antibiotic concentrations over 1 mg/L. Multiple ARGs in gene cassettes mainly included aminoglycosides, macrolides, trimethoprim, and β-lactam. The *intI1* was positively selected and might have actively acquired new gene cassettes in the presence of high concentrations of antibiotics. Another study by Shi et al., 2020 reported that a tetracycline resistance gene – *tet*(E) – is supported by Tn*6433*, a novel transposon with systematic enhancement in oxytetracycline stress of 0–50 mg/L. Tn*6433* was initially detected in the chromosome of *Aeromonas*, but subsequently detected on plasmids, including pAeca1-a variants and pAeca2 with an increase in oxytetracycline concentrations. The core region of the Tn*6433*-*tet*(E) structure consisted of a switch and steadfast component, integron of class I type, principal genes inside, and a Tn*1722*/Tn*501*-type transposon, conserved significantly. Occurrence of such DNA structure on chromosome as well as on plasmids revealed that Tn*6433* mediates the rearrangement of *tet*(E) from chromosome to plasmids at enhanced levels of oxytetracycline pressures. Studies mentioned above showed that rapid ARG horizontal gene transfer among the environmental bacterial community mainly through enriching mobile genetic elements was responsible for AMR dissemination under extreme antibiotic-selective pressure in pharmaceutical effluent. Therefore, the most effective way to minimize the transmission of AMR is to remove the antibiotic antibacterial activity (potency) from effluent before biological treatment.

2.2 Establishment of Enhanced Hydrolysis Pretreatment Technology of Antibiotic Production Effluent

The antibiotic at very high levels in pharmaceutical effluent prevents the functions of natural handling arrangements and results in the spread of drug-unresponsive microbes and ARGs. Therefore, to minimize the release of antibiotics and ARGs from the manufacturing effluents and ensure effluent biological treatment function, it is necessary to develop a novel technology removing the antibiotic potency before the pharmaceutical effluent enters the biological treatment system.

Taking advantage of the fact that the potency of many fermentative antibiotics will lose due to hydrolysis reaction in solution, a pretreatment method before biological treatment for antibiotic production effluent has been established employing an improved method of hydrolysis with an increase in temperature, optimizing the pH or other settings. The enhanced hydrolysis pretreatment has been proven to be able to remove tetracycline, oxytetracycline, and their antibacterial activity from production effluent (Yi et al., 2016; Yi et al., 2017). There are currently dozens of fermentative antibiotics produced in bulk in the pharmaceutical industry, which have different molecular structures and active functional groups. Understanding of the hydrolysis characteristics and mechanisms of different types of fermentative antibiotics is essential to promote the application of this technology in industrial effluent treatment. Thus, a total of 14 different fermentative antibiotics belonging to six classes were studied based on their hydrolysis characteristics and annual outputs (Tang et al., 2020a). It took 1 h with amplified hydrolysis to extricate strength of

eight antimicrobial drugs among the targeted groups. They include bacitracin, chloramphenicol, colistin, erythromycin, penicillin V, spiramycin, streptomycin, and vancomycin. Based on the parameterized model number 3 method, a rapid forecast method was devised by linking the improved efficacy of hydrolysis and the energy gap (ΔE) of the antibiotic structures. A significant correlation ($p < 0.01$) between predicted results and experimental values for the training and test sets indicated its robust nature. The studies revealed that reactivity of antibiotic structures is closely connected with efficacy of the hydrolysis. Accordingly, the fermentative antibiotics could be divided into two types of difficult-to-hydrolyze and easy-to-hydrolyze antibiotics, which provided a valuable method for creating a comprehensive improved hydrolysis pretreatment approach for diverse fermentative effluents of antibiotic production (Tang et al., 2020a). Enhanced hydrolysis by using heterogeneous solid acid/base catalysts under relatively mild reaction conditions (e.g., CaO/MgO solid base catalysts) compared with the enhanced homogeneous reaction was also developed for the pretreatment technology before biological treatment systems (Tang et al., 2019). By using the CaO/MgO solid base, 98% of streptomycin as well as its antibacterial activity from production effluent [streptomycin: 2200 mg/L, chemical oxygen demand (COD_{Cr}): 10,200 mg/L] within 2 h was achieved.

On the other hand, for hard-to-hydrolyze antibiotics with high ΔE, kanamycin was selected as the research target, the removal efficiency and mechanism of residual kanamycin and antibacterial activity by hydrothermal treatment were investigated (Tang et al., 2020b). It was found that hydrolysis temperature was a key parameter impacting kanamycin degradation. The half-life ($t_{1/2}$) of degradation could be condensed to 87-fold with the treatment of hydrothermal temperature raised to 180 °C from 100 °C. Five transformation products were identified by UPLC-QTOF-MS, and their antibacterial activities were lower than kanamycin. Hydrothermal treatment could remove 98% of kanamycin antibacterial activity from the initial COD_{Cr} (~100,000 mg/L) effluents at the time of production. This indicates that despite the presence of higher levels of organic matrices, it is possible for the efficient removal of kanamycin from production effluents. Besides, the application of pretreatment hydrothermal kind resulted in the yield of methane production enhancement up to 2.3 times as evidenced by glycolytic inhibition screening tests. The results showed that hydrothermal reaction was an optional and efficient pretreatment technology for difficult-to-hydrolyze effluents of antibiotic production, especially when organic matrices are at higher levels.

To explore whether enhanced hydrolysis pretreatment could mitigate the AMR development in subsequent biological treatment system, the performance and antibiotic resistance of anaerobic digestion treating synthetic effluent spiked with oxytetracycline were explored by comparing two simulative parallel-operated up-flow anaerobic sludge bed (UASB) reactors with and without enhanced hydrolysis pretreatment (He et al., 2020). The organic loading rate of reactors was kept at 10 g/L/d, and the oxytetracycline concentration increased from 0 to 200 mg/L in 15 months. For reactor-treating effluent that was pretreated by enhanced hydrolysis within 6 h at a temperature of 85 °C, the exclusion frequency of COD and ARG abundance was similar to those of control treatment without oxytetracycline. For the reactor without

the pretreatment, the COD removal was about 90% under oxytetracycline concentrations ranging from 0 to 100 mg/L; however, the reactor collapsed under higher concentrations of oxytetracycline. According to the results of metagenomics sequencing and bioinformatic analysis, ARG abundance enhanced to 2.6×10^{-1} from 1.3×10^{-1} copies/cell with oxytetracycline accumulation from 0 to 5 mg/L, remained unchanged at concentration ranging from 25 to 100 mg/L, and further increased to 4.8×10^{-1} and 1.3 copies/cell at oxytetracycline concentrations of 150 and 200 mg/L, respectively. In a full-scale UASB with influent oxytetracycline levels of near 200 mg/L, poor COD removal and significant ARG enrichment were also found, which further validate the results of synthetic effluent. This study showed that the simultaneous regulation of orthodox impurities such as COD and ARGs could possibly be accomplished in biological treatment by using pretreatment of heightened hydrolysis.

2.3 Application of Enhanced Hydrolysis Pretreatment Technology in the Pharmaceutical Industry

To explore the feasibility of enhanced hydrolysis pretreatment for actual pharmaceutical effluent, a pilot system was established in a pharmacological industrial unit for treating production discharge of oxytetracycline, which consisted of enhanced hydrolysis pretreatment and a UASB reactor in succession. The performance of this pilot system was equated with an unhampered oxygen-independent system functioned analogous at the same industrial unit in Hebei Province (Yi et al., 2017). The operational condition of enhanced hydrolysis was under 85 °C and 6 h without adjusting the pH of effluent. The influent COD was approximately 11,000 mg/L. The COD removal rate of this pilot system could be 83%, 79%, and 69% under organic loading rates of 3, 5, and 6 kg COD/m^3/d, respectively. At the same time, it was observed that by adopting enhanced hydrolysis pretreatment and UASB reactor, the oxytetracycline concentration was reduced to a mere 0.6 mg/L from 900 mg/L, and the potency was also less than 0.8 mg/L after the treatment. In addition, such pretreatment action could also mitigate the antibiotic resistance development. The relative abundance of the total tetracycline (*tet*) genes and *intI1* gene in glycolytic slurry on day 96 decreased significantly compared with the UASB system without the pretreatment ($P < 0.01$). Different from the pilot system, the maximal COD removal and organic loading rate could be around 50% and 1 kg/m^3/d, respectively, with the dilution of actual oxytetracycline production effluent with COD of 3720 mg/L as the influent in the full-scale anaerobic system. The relative abundance of total tetracycline genes in full-scale anaerobic sludge was five times higher than that of the pilot-scale system.

Enhanced hydrolysis pretreatment strategy has been successfully used in actual full-scale antibiotic production effluent in production sites of pharmaceuticals. It was applied in the update of oxytetracycline manufacturing effluent treatment in two sites (800 m^3/d and 1000 m^3/d, respectively) in Hebei Province, China. The ARG relative abundance in the effluent biological treatment systems could be reduced by more

than 83%, oxytetracycline removal reached more than 99%, and the challenge of biological inhibition was also solved. Pretreatment of antibiotic production effluent before biological treatment based on enhanced hydrolysis has contributed to the "*Technical Brief on Water, Sanitation, Hygiene and Effluent Management to Avert Infections and Diminish the Blowout of AMR*" issued by the WHO Blue Book and Environment-Health-Safety (EHS) Guideline for Pharmaceutical Industry in China, which will also provide scientific guidance for AMR management in the global pharmaceutical industry.

2.4 Decreasing Environmental AMR Impact of Antibiotic Fermentation Residues

Beside production effluent, antibiotic fermentation residues (i.e., fermentation mycelia residues) will also produce and discharge during the fermentative production of bulk antibiotics. China generates nearly ten million tons of wet antibiotic fermentation residues containing antibiotics or related metabolites. In the early stages of antibiotic production, a huge amount of residues was directly processed into animal feed or feed additives. On February 9, 2002, the Ministry of Agriculture and the Ministry of Health jointly issued the "*Catalogue of Drug Varieties Prohibited from Use in Feed and Animal Drinking Water*" (No. 176, Announcement of the Ministry of Agriculture, China). Antibiotics were included in the catalog. At the same time, it was forbidden to make feed or feed additives directly from untreated antibiotic residues because of the lack of accurate and precise safety tests and potential hazards. Since August 23, 2002, it has been illegal to use residues as feed or feed additive directly. In 2008, "*mother liquor and reaction or medium wastes in the production of chemical raw materials*" were included in the revised National Hazardous Waste List in China, further restricting the use of antibiotic residues as feed additives and organic fertilizers. Resource recycling and harmless treatment of antibiotic residues are urgently needed by the pharmaceutical industry (Han et al., 2024).

Research, development, and effective implementation of safe and efficient comprehensive utilization technology of antibiotic residues has become a big challenge facing the current biopharmaceutical industry. The huge environmental pressure has prompted many large pharmaceutical companies across the country to carry out active and effective exploratory work in the field of harmless disposal of antibiotic residues. In January 2018, approved by the Ministry of Ecology and Environment, a "*State Environmental Protection Engineering Center,*" named as safe handling and source consumption of antimicrobial drug excesses (hereinafter referred to as the "*Residues Treatment and Disposal Center*"), was formally established by Chuanning Biotechnology Co., Ltd., Yili City, Xinjiang, China. In view of the current situation of the large production of antibiotic residues with high environmental risk but lack of management measures, the center will carry out technical research and industrial promotion of harmless treatment and resource utilization of antibiotic residues. It is committed to the evaluation of biosafety and environmental ecological benefits of

Table 1 Utilization and treatment technology of antibiotic fermentation residues in domestic pharmaceutical factory

Category	Factory	Process	Destination
Penicillin	**Pharmaceutical Group**Co., Ltd.	Drying – temporary storage	Inactivate and composting
	**Pharmaceutical group Corporation	Drying – dry hyphae temporary storage	The mycelial part is used as protein powder for production and the other part as organic fertilizer
	** Biotechnology Co., Ltd.	In-plant high-temperature hydrolysis/drying treatment	Organic fertilizer resource for planting/recycling
Streptomycin	**Pharmaceutical ** Co., Ltd.	Drying – temporary storage	Inactivate and composting
Tetracycline hydrochloride	** Pharmaceutical Co., Ltd.	Dehydration	Mixed and incineration
Mycophenolic acid	** Pharmaceutical Co., Ltd.	Transport	All landfilled by local sanitation
Erythromycin thiocyanate	**Pharmaceutical Co., Ltd.	Dehydration	Mixed and incineration
	**Biotechnology Co., Ltd.	In-plant high-temperature hydrolysis/drying treatment	Organic fertilizer resource for planting/recycling
Spectinomycin	**Pharmaceutical Co., Ltd.	Dehydration	Organic fertilizer
Streptomycin sulfate	**Pharmaceutical Co. Ltd	Dehydration treatment/partial drying	Drying part sealed and the rest incinerated.
Avermectin	**Pharmaceutical Group Co., Ltd.	Drying	Organic fertilizer
Cephalosporins	**Pharmaceutical Co., Ltd.	Drying	Organic fertilizer
	**Biological Products Co., Ltd.	Drying	Organic fertilizer for sale
	**Biotechnology Co., Ltd.	In-plant high-temperature hydrolysis/drying treatment	Organic fertilizer resource for planting/recycling

harmless (mainly for AMR) and resource of antibiotic residues and provide suggestions for the treatment and management of antibiotic residues in the whole industry.

The utilization and treatment of antibiotic residues from 10 pharmaceutical companies in China are listed in Table 1. It can be concluded that the current domestic utilization and treatment technologies of residues mainly focus on incineration, landfill, and fertilizer. Enhanced hydrolysis-based hydrothermal technology has been also adopted in three full-scale facilities in China for recycling waste

cephalosporin, penicillin A, and erythromycin fermentation residues (Han et al., 2022). The antibiotic content in fermentation residues could be efficiently removed by diverse thermal treatment methods, and this further mitigates the environmental AMR development consequences during the disposal or utilization of antibiotic fermentation residues (Luan et al., 2021).

China's revised "National Hazardous Waste List" and other policies and regulations clearly stipulate that antibiotic residues need final disposal according to hazardous waste. Its treatment and disposal as organic fertilizer, feed, or feed additive are strictly prohibited. At present, incineration is the most common method of hazardous waste disposal, which needs very expensive equipment, high operating cost, as well as the secondary pollution. It has been a concern and consideration of the government, enterprises, and researchers to eliminate the harm of residues from pollution (antibiotics) from the technical level, realize the harmless treatment of residues to prevent the AMR development, and turn residues into valuable resources.

3 Deterring the Transmission of AMR in the Livestock-Related Environment

Livestock and poultry breeding is another key emission source of AMR in the environment. High abundance and rapid transfer of ARGs in animal manure have been widely reported (Udikovic-Kolic et al., 2014). High doses of veterinary antibiotics and metal particularly copper and zinc used in animal farming to promote animal growth and control diseases have promoted the AMR development (Zhao et al., 2020). Thus, livestock-related environment is the hotspot and critical control point of AMR.

3.1 Dissemination of Clinically Important ARGs from Animal to Environment

Animal farming-related environment is one of the most important reservoirs of clinically important ARGs. The most attractive research targets included New Delhi metallo-β-lactamase gene ($bla_{\text{NDM-1}}$) reported firstly by Yong et al., 2009, *Klebsiella pneumoniae* carbapenemase gene (bla_{KPC}), CTX-M-type ESBL genes ($bla_{\text{CTX-M}}$) (D'Andrea et al., 2013), an ABC transporter gene for linezolid and florfenicol cross-resistance (*optrA*) (Wang et al., 2015), gene associated with *plasmid-mediated colistin resistance* gene (*mcr*-1) (Lei et al., 2017; Liu et al., 2016; Wang et al., 2017a; Wang et al., 2017b), and mobile tigecycline resistance genes (*tet*(X) variants, especially *tet*(X3/4)) (He et al., 2019; Sun et al., 2019). Before utilization, anaerobic digestion and composting are usually applied to treat animal manure waste. Thus, environmental research on AMR from livestock farming will require more information on the occurrence and fate of the above clinically important ARGs.

Escherichia coli, a representative *Enterobacteriaceae* bacterium, and related ESBL genes, such as clinically important $bla_{\text{CTX-M}}$ are detected in both animals and humans worldwide. The occurrence and transfer characteristics of $bla_{\text{CTX-M}}$ in several out and out and laboratory-based anaerobic digesters handling swine wastes in ambient and mesophilic settings were studied by both molecular and culture-based approaches (Tian et al., 2022). Real-time TaqMan polymerase chain reaction revealed ubiquitous CTX-M-1 and CTX-M-9 group genes in four geographically different full-scale anaerobic systems in China. Although anaerobic digestion could reduce the complete copiousness of $bla_{\text{CTX-M}}$ groups 1 and 9 by 0.63–2.24 and 0.08–1.30 log, 10^2–10^3 and 10^4 level copies/mL of $bla_{\text{CTX-M}}$ groups 1 and 9 could still be detected in the effluent, respectively. By nonselective culture, a total of 223 *E. coli* strains were isolated from raw swine waste, lab, and full-scale anaerobic effluents, with 79 isolates resistant to the third-generation cephalosporin cefotaxime indicating the high detection of $bla_{\text{CTX-M}}$-carrying *E. coli* in the anaerobic systems. Then, by selective agar plates supplemented with cefotaxime, a total of 141 $bla_{\text{CTX-M}}$-carrying *E. coli* were isolated from the anaerobic digesters, which all conferred resistance to cefotaxime. The $bla_{\text{CTX-M-14}}$ carried by prevailing replicas of *E. coli* ST6802 and ST155 were the most important subtype in the sewage. In addition, the $bla_{\text{CTX-M-14}}$ was located on ΔIS*26*-$bla_{\text{CTX-M-14}}$-ΔIS*903B*-fosA3 genetic element on the conjugative IncHI2- and IncFIB-type plasmids. By conjugation assays using filter mating method, plasmids containing $bla_{\text{CTX-M-14}}$ transmitted at the rate of 10^{-3}–10^{-2} cells per recipient cell and the $bla_{\text{CTX-M-14}}$-harboring plasmids were genetically stable. This study revealed the prevalence of $bla_{\text{CTX-M}}$ genes in both large- and small-scale oxygen-independent runoffs of piggery exudes in China and called on additional efforts for deterring the transmission of ARGs from livestock to the environment.

One of the last-resort drugs for treating extremely drug-unresponsive microbial infections is tigecycline, and *tet*(X3) -*tet*(X6) are the newly emerging plasmid-mediated tigecycline-obdurate genes. To understand the emergence and behavior of mobile *tet*(X)-variant genes in animal manure-based organic fertilizers, a large-scale investigation was conducted across eight provinces in China (Dai et al., 2022). It was found that *tet*(X4) was the principal mobile *tet*(X)-variant gene in new compost, natural, and thermophilic composting products with both utmost occurrence and abundance. Mobile *tet*(X)-variant genes, particularly *tet*(X4), could also be detected in the unloading soil subsequent composting fertilizer application, which revealed the diffusion from compost to soil. Harmonized samples of fresh manures and products treated by natural or thermophilic composting were also collected to explore the variation of *tet*(X)-variant gene abundance before and after the treatments. No noteworthy decrease in mobile *tet*(X)-variant genes by natural composting was found; however, thermophilic composting exhibited strong effectiveness in eradicating *tet*(X)-variant genes. Notably, compared with other mobile *tet*(X)-variant genes, *tet*(X4) demonstrated the decline to a lowermost level after thermophilic composting. Thus, it is required to consider how to enhance the removal of *tet*(X4) that is persisting in animal farming environments.

The *optr*A gene, a florfenicol resistance gene mainly carried by Gram-positive *Enterococci*, was selected in livestock-related environment because of the use of phenicol antibiotic florfenicol in animal farming. The *optrA* gene also confers resistance to linezolid, which is a kind of critically important antibiotic in the clinic. The occurrence and dissemination of *optrA* gene in lab- and full-scale mesophilic glycolytic digesters treating swine wastes in Beijing, China, were investigated, and a total of 339 enterococci strains were isolated (Yang et al., 2020). By PCR screening, *optr*A was detected in *enterococci* isolates in the influent and effluent for 74% and 39%, respectively. Based on the minimum inhibitory concentrations (MICs) of the enterococci strain, the influent and effluent-originated strains were unresponsive at 86%, 78%, and 78% and at 56%, 65%, and 13% to chloramphenicol, florfenicol, and linezolid, respectively. The phenotype study of strains was consistent with the genotype results. This study observed that the *optr*A gene harbored by *enterococci* persisted in oxygen-independent systems of swine exudes of both laboratory and full-sale effluent structures. In sum, a sound process and operational controlling strategy are urgently needed to prevent the spread of unresponsiveness to antibiotics, especially the clinically important ARGs such as $bla_{\text{CTX-M}}$, *tet*(X)-variant genes, and *optr*A from the livestock waste treatment systems.

3.2 Environmental Engineering Attempts in Preventing AMR Dissemination in Livestock-Related Environment in China

Anaerobic treatment temperature is the key parameter of anaerobic digestion, which might be associated with the reduction in fecal bacteria and ARGs. Multiple tools, including the high-throughput sequencing, bioinformatic analysis, community-wide Bayesian Source Tracker method, and culture-based methods, revealed that temperature is the key factor affecting the persistence of fecal bacteria in lab-scale anaerobic effluent under different temperatures (Tian et al., 2021). To analyze the different proportion of fecal bacteria in anaerobic digestion effluents at different temperatures, based on the LEfSe method, swine fecal bacterial taxa could be divided into "lost" and "survivor" in unwanted dung. The "survivor" profusion was negatively connected with anaerobic digestion temperature ($P < 0.006$). Moreover, "*variation partition analysis (VPA)*" revealed that temperature will elucidate nearly 30% of variations in effluent bacterial community.

To further explore the impact of anaerobic digestion temperature on the reduction of clinically important ARGs, the antibiotic-resistant *E. coli*, CTX-M-type ESBL genes and other important ARGs of mesophilic, thermophilic, and hyperthermophilic–mesophilic two-stage anaerobic digestion system treating chicken waste and swine waste were investigated, respectively, by isolation, antimicrobial susceptibility test, and whole-genome sequencing. It was found that anaerobic digestion operated at 50 °C (the thermophilic) could effectively remove culturable *E. coli* and related $bla_{\text{CTX-M}}$ genes compared with 37 °C (the mesophilic), but linezolid-resistant *Enterococci* remained in anaerobic effluent. Pretreatment at 70 °C (the hyperthermophilic pretreatment) could remove not only the culturable *E. coli* and

related $bla_{\text{CTX-M}}$ genes, but also the linezolid-resistant Enterococci and associated ARGs. By metagenomic sequencing, CTX-M-type ESBL genes were undetected after the hyper-thermophilic–mesophilic anaerobic digestion. The hyper-thermophilic–mesophilic anaerobic digestion reduced 99% of fecal bacteria and 94% of potential bacterial hosts of ESBL genes. Herein, the hyper-thermophilic–mesophilic two-stage anaerobic digestion system could block both vertical and horizontal transfers of $bla_{\text{CTX-M}}$ genes and other clinically important ARGs, indicating the management of operating temperature in anaerobic digestion or composting will be one of the potential engineering means to minimize the transmission of the clinically important ARGs from the animal waste treatment systems.

In fact, tackling the global challenge of antimicrobial resistance demanded a "One Health" perspective, which emphasized the close connections among human, animal, and environment. One of the most exciting stories is the proscription on the application of antibiotics as growth promoters in animal feed in China. Following the discovery of *mcr-1* in sectors of animals and humans (Wang et al., 2017a), colistin as an animal growth promoter was prohibited by the "*Ministry of Agriculture and Rural Affairs,*" China. It has been reported that the taking out strategy and the lessened application of colistin in livestock farming resulted in a substantial impact on plummeting bacterial colistin obduracy in microbes of clinical and animal sectors in China (Wang et al., 2020). The government of China took a decision to prohibit application of antimicrobial drugs as promoters of growth in feeds of animals recently. In a responsible and prudent manner, it is widely recommended that employment of antimicrobial drugs in all sectors including animal husbandry, fisheries, and agriculture is only need-based, namely, infectious disease management (treatment and control) and not for the prevention or promotion of growth.

4 Conclusion

The environment is playing a growing role, which is a hotspot of intensive investigation. Pharmaceutical industry, livestock farming, and hospitals are the key emission sources of high concentrations of antibiotics, antibiotic-resistant bacteria, and ARGs in the environment. Pharmaceutical effluent contains very high concentrations of antibiotics, while livestock farming and hospital effluent not only contains high levels of antibiotics but also contains important antibiotic resistance pathogens and genes. Therefore, it is urgently needed to strengthen the control strategy of these key emission sources and establish relevant emission standards and technical guidelines. Since AMR development in the environment is extremely complex, a holistic framework and corresponding coordinated action plan should be established to deter environmental AMR development. Coordinated monitoring, research, and actions are also required. Contamination of antibiotics and development of AMR during the treatment of antibiotic production effluents and residues has been uncovered, and a novel technology to minimize the release of these pollutants from the pharmaceutical manufacturing sites was developed in China, which will provide scientific guidance for AMR management in the global pharmaceutical industry. Another success story

is that the colistin has been banned as an animal growth promoter in China. Through a large-scale comparative study, a substantial impact on diminishing colistin unresponsiveness in clinical settings of humans and in animals has been observed in China with the implementation of withdrawal policy and the reduced levels of application of colistins. It is expected that more countries would accelerate their national action about antibiotics usage as growth promoters. The National Action Plans on AMR in China have been issued with contributions from more than ten departments in 2006 and 2022, respectively. China is now advocating creating a civic society with a common future for humanity; thus, it will play a more important role in coping with AMR challenges in international cooperation.

Acknowledgments The authors thank the National Nature Science Foundation of China (NSFC) project (grant number 32141002 and 22076203).

References

Berendonk, T. U., Manaia, C. M., Merlin, C., Fatta-Kassinos, D., Cytryn, E., Walsh, F., Bürgmann, H., Sørum, H., Norström, M., Pons, M.-N., Kreuzinger, N., Huovinen, P., Stefani, S., Schwartz, T., Kisand, V., Baquero, F., & Martinez, J. L. (2015). Tackling antibiotic resistance: The environmental framework. *Nature Reviews Microbiology, 13*(5), 310–317.

D'Andrea, M. M., Arena, F., Pallecchi, L., & Rossolini, G. M. (2013). CTX-M-type β-lactamases: A successful story of antibiotic resistance. *International Journal of Medical Microbiology, 303*(6), 305–317.

Dai, S., Liu, D., Han, Z., Wang, Y., Lu, X., Yang, M., & Zhang, Y. (2022). Mobile tigecycline resistance gene *tet*(X4) persists with different animal manure composting treatments and fertilizer receiving soils. *Chemosphere, 307*, 135866.

Han, Z., Zhang, Y., An, W., Lu, J., Hu, J., & Yang, M. (2020). Antibiotic resistomes in drinking water sources across a large geographical scale: Multiple drivers and co-occurrence with opportunistic bacterial pathogens. *Water Research, 183*, 116088.

Han, Z., Feng, H., Luan, X., Shen, Y., Ren, L., Deng, L., Joakim Larsson, D. G., Gillings, M., Zhang, Y., & Yang, M. (2022). Three-year consecutive field application of erythromycin fermentation residue following hydrothermal treatment: Cumulative effect on soil antibiotic resistance genes. *Engineering, 15*(8), 78–88.

Han, Z., Luan, X., Feng, H., Deng, Y., Yang, M., & Zhang, Y. (2024). Metagenomic insights into microorganisms and antibiotic resistance genes of waste antibiotic fermentation residues along production, storage and treatment processes. *Journal of Environmental Sciences, 136*, 45–55.

He, T., Wang, R., Liu, D., Walsh, T. R., Zhang, R., Lv, Y., Ke, Y., Ji, Q., Wei, R., Liu, Z., Shen, Y., Wang, G., Sun, L., Lei, L., Lv, Z., Li, Y., Pang, M., Wang, L., Sun, Q., Fu, Y., Song, H., Hao, Y., Shen, Z., Wang, S., Chen, G., Wu, C., Shen, J., & Wang, Y. (2019). Emergence of plasmid-mediated high-level tigecycline resistance genes in animals and humans. *Nature Microbiology, 4*(9), 1450–1456.

He, Y., Tian, Z., Yi, Q., Zhang, Y., & Yang, M. (2020). Impact of oxytetracycline on anaerobic effluent treatment and mitigation using enhanced hydrolysis pretreatment. *Water Research, 187*, 116408.

Huyan, J., Tian, Z., Zhang, Y., Zhang, H., Shi, Y., Gillings, M. R., & Yang, M. (2020). Dynamics of class 1 integrons in aerobic biofilm reactors spiked with antibiotics. *Environment International, 140*, 105816.

Larsson, D. G. J. (2014). Pollution from drug manufacturing: Review and perspectives. *Philosophical Transactions of the Royal Society, B: Biological Sciences, 369*(1656), 20130571.

Larsson, D. G. J., & Flach, C.-F. (2021). Antibiotic resistance in the environment. *Nature Reviews Microbiology, 20*(5), 257–269.

Larsson, D. G. J., de Pedro, C., & Paxeus, N. (2007). Effluent from drug manufactures contains extremely high levels of pharmaceuticals. *Journal of Hazardous Materials, 148*(3), 751–755.

Lei, L., Wang, Y., Schwarz, S., Walsh, T. R., Ou, Y., Wu, Y., Li, M., & Shen, Z. (2017). *mcr-1* in Enterobacteriaceae from companion animals, Beijing, China, 2012-2016. *Emerging Infectious Diseases, 23*(4), 710–711.

Li, D., Yang, M., Hu, J., Zhang, J., Liu, R., Gu, X., Zhang, Y., & Wang, Z. (2009). Antibiotic-resistance profile in environmental bacteria isolated from penicillin production effluent treatment plant and the receiving river. *Environmental Microbiology, 11*(6), 1506–1517.

Li, D., Li, Z., Yu, J., Cao, N., Liu, R., & Yang, M. (2010). Characterization of bacterial community structure in a drinking water distribution system during an occurrence of red water. *Applied and Environmental Microbiology, 76*(21), 7171–7180.

Li, D., Qi, R., Yang, M., Zhang, Y., & Yu, T. (2011). Bacterial community characteristics under long-term antibiotic selection pressures. *Water Research, 45*(18), 6063–6073.

Liu, M., Zhang, Y., Yang, M., Tian, Z., Ren, L., & Zhang, S. (2012). Abundance and distribution of tetracycline resistance genes and Mobile elements in an oxytetracycline production effluent treatment system. *Environmental Science & Technology, 46*(14), 7551–7557.

Liu, Y. Y., Wang, Y., Walsh, T. R., Yi, L. X., Zhang, R., Spencer, J., Doi, Y., Tian, G., Dong, B., Huang, X., Yu, L. F., Gu, D., Ren, H., Chen, X., Lv, L., He, D., Zhou, H., Liang, Z., Liu, J. H., & Shen, J. (2016). Emergence of plasmid-mediated colistin resistance mechanism MCR-1 in animals and human beings in China: A microbiological and molecular biological study. *The Lancet Infectious Diseases, 16*(2), 161–168.

Luan, X., Zhang, H., Tian, Z., Yang, M., Wen, X., & Zhang, Y. (2020). Microbial community functional structure in an aerobic biofilm reactor: Impact of streptomycin and recovery. *Chemosphere, 255*, 127032–127032.

Luan, X., Han, Z., Shen, Y., Yang, M., & Zhang, Y. (2021). Assessing the effect of treated erythromycin fermentation residue on antibiotic resistome in soybean planting soil: In situ field study. *Science of the Total Environment, 779*, 146329.

Shi, Y., Tian, Z., Gillings, M. R., Zhang, Y., Zhang, H., Huyan, J., & Yang, M. (2020). Novel transposon Tn*6433* variants accelerate the dissemination of *tet*(E) in Aeromonas in an aerobic biofilm reactor under oxytetracycline stresses. *Environmental Science & Technology, 54*(11), 6781–6791.

Sun, J., Chen, C., Cui, C. Y., Zhang, Y., Liu, X., Cui, Z. H., Ma, X. Y., Feng, Y., Fang, L. X., Lian, X. L., Zhang, R. M., Tang, Y. Z., Zhang, K. X., Liu, H. M., Zhuang, Z. H., Zhou, S. D., Lv, J. N., Du, H., Huang, B., Yu, F. Y., Mathema, B., Kreiswirth, B. N., Liao, X. P., Chen, L., & Liu, Y. H. (2019). Plasmid-encoded *tet*(X) genes that confer high-level tigecycline resistance in *Escherichia coli*. *Nature Microbiology, 4*(9), 1457–1464.

Tang, M., Dou, X., Tian, Z., Yang, M., & Zhang, Y. (2019). Enhanced hydrolysis of streptomycin from production effluent using CaO/MgO solid base catalysts. *Chemical Engineering Journal, 355*, 586–593.

Tang, M., Gu, Y., Wei, D., Tian, Z., Tian, Y., Yang, M., & Zhang, Y. (2020a). Enhanced hydrolysis of fermentative antibiotics in production effluent: Hydrolysis potential prediction and engineering application. *Chemical Engineering Journal, 391*, 123626.

Tang, M., Li, F., Yang, M., & Zhang, Y. (2020b). Degradation of kanamycin from production effluent with high-concentration organic matrices by hydrothermal treatment. *Journal of Environmental Sciences, 97*, 11–18.

Tian, T., Qiao, W., Han, Z., Wen, X., Yang, M., & Zhang, Y. (2021). Effect of temperature on the persistence of fecal bacteria in ambient anaerobic digestion systems treating swine manure. *Science of the Total Environment, 791*, 148302.

Tian, T., Dai, S., Liu, D., Wang, Y., Qiao, W., Yang, M., & Zhang, Y. (2022). Occurrence and transfer characteristics of $bla_{\text{CTX-M}}$ genes among *Escherichia coli* in anaerobic digestion systems treating swine waste. *Science of the Total Environment, 834*, 155321.

Udikovic-Kolic, N., Wichmann, F., Broderick, N. A., & Handelsman, J. (2014). Bloom of resident antibiotic-resistant bacteria in soil following manure fertilization. *Proceedings of the National Academy of Sciences of the United States of America, 111*(42), 15202–15207.

Walsh, T. R. (2018). A one-health approach to antimicrobial resistance. *Nature Microbiology, 3*(8), 854–855.

Wang, Y., Lv, Y., Cai, J., Schwarz, S., Cui, L., Hu, Z., Zhang, R., Li, J., Zhao, Q., He, T., Wang, D., Wang, Z., Shen, Y., Li, Y., Feßler, A. T., Wu, C., Yu, H., Deng, X., Xia, X., & Shen, J. (2015). A novel gene, *optrA*, that confers transferable resistance to oxazolidinones and phenicols and its presence in enterococcus faecalis and enterococcus faecium of human and animal origin. *Journal of Antimicrobial Chemotherapy, 70*(8), 2182–2190.

Wang, Y., Tian, G.-B., Zhang, R., Shen, Y., Tyrrell, J. M., Huang, X., Zhou, H., Lei, L., Li, H.-Y., Doi, Y., Fang, Y., Ren, H., Zhong, L.-L., Shen, Z., Zeng, K.-J., Wang, S., Liu, J.-H., Wu, C., Walsh, T. R., & Shen, J. (2017a). Prevalence, risk factors, outcomes, and molecular epidemiology of *mcr-1*-positive Enterobacteriaceae in patients and healthy adults from China: An epidemiological and clinical study. *The Lancet Infectious Diseases, 17*(4), 390–399.

Wang, Y., Zhang, R., Li, J., Wu, Z., Yin, W., Schwarz, S., Tyrrell, J. M., Zheng, Y., Wang, S., Shen, Z., Liu, Z., Liu, J., Lei, L., Li, M., Zhang, Q., Wu, C., Zhang, Q., Wu, Y., Walsh, T. R., & Shen, J. (2017b). Comprehensive resistome analysis reveals the prevalence of NDM and MCR-1 in Chinese poultry production. *Nature Microbiology, 2*(4), 16260.

Wang, Y., Xu, C., Zhang, R., Chen, Y., Shen, Y., Hu, F., Liu, D., Lu, J., Guo, Y., Xia, X., Jiang, J., Wang, X., Fu, Y., Yang, L., Wang, J., Li, J., Cai, C., Yin, D., Che, J., Fan, R., Wang, Y., Qing, Y., Li, Y., Liao, K., Chen, H., Zou, M., Liang, L., Tang, J., Shen, Z., Wang, S., Yang, X., Wu, C., Xu, S., Walsh, T. R., & Shen, J. (2020). Changes in colistin resistance and *mcr-1* abundance in *Escherichia coli* of animal and human origins following the ban of colistin-positive additives in China: An epidemiological comparative study. *Lancet Infectious Diseases, 20*(10), 1161–1171.

Yang, X.-X., Tian, T.-T., Qiao, W., Tian, Z., Yang, M., Zhang, Y., & Li, J.-Y. (2020). Prevalence and characterization of oxazolidinone and phenicol cross-resistance gene *optrA* in enterococci obtained from anaerobic digestion systems treating swine manure. *Environmental Pollution, 267*, 115540.

Yi, Q., Gao, Y., Zhang, H., Zhang, H., Zhang, Y., & Yang, M. (2016). Establishment of a pretreatment method for tetracycline production effluent using enhanced hydrolysis. *Chemical Engineering Journal, 300*, 139–145.

Yi, Q., Zhang, Y., Gao, Y., Tian, Z., & Yang, M. (2017). Anaerobic treatment of antibiotic production effluent pretreated with enhanced hydrolysis: Simultaneous reduction of COD and ARGs. *Water Research, 110*, 211–217.

Yong, D., Toleman, M. A., Giske, C. G., Cho, H. S., Sundman, K., Lee, K., & Walsh, T. R. (2009). Characterization of a new metallo-β-lactamase gene, $bla_{\text{NDM-1}}$, and a novel erythromycin esterase gene carried on a unique genetic structure in Klebsiella pneumoniae sequence type 14 from India. *Antimicrobial Agents and Chemotherapy, 53*(12), 5046–5054.

Zhang, Y., Xie, J., Liu, M., Tian, Z., He, Z., van Nostrand, J. D., Ren, L., Zhou, J., & Yang, M. (2013). Microbial community functional structure in response to antibiotics in pharmaceutical effluent treatment systems. *Water Research, 47*(16), 6298–6308.

Zhang, Y., Walsh, T. R., Wang, Y., Shen, J., & Yang, M. (2022). Minimizing risks of antimicrobial resistance development in the environment from a public one health perspective. *China CDC Weekly, 4*(49), 1105–1109.

Zhao, R., Feng, J., Liu, J., Fu, W., Li, X., & Li, B. (2019). Deciphering of microbial community and antibiotic resistance genes in activated sludge reactors under high selective pressure of different antibiotics. *Water Research, 151*, 388–402.

Zhao, Y., Yang, Q., Zhou, X., Wang, F., Muurinen, J., Virta, M., & Brandt, K. (2020). Antibiotic resistome in the livestock and aquaculture industries: Status and solutions. *Critical Reviews in Environmental Science and Technology, 51*, 1–38.

Anti-virulence to Counter the AMR Conundrum: Principles and Strategies

Shaheer Peeralil, Sreejith Viyyokkaran Narayanan, Toms C. Joseph, and Kuttanappilly Velayudham Lalitha

Contents

Abstract

Antibiotic resistance for a large diversity of clinically significant pathogens has been observed with increasing frequency over the past several decades. This pervasive issue is one of the most serious public health concerns of this century. Clinicians and researchers have been persuaded to develop innovative techniques to manage harmful microorganisms to address this problem. The bacterial virulence genes are mostly in the monitoring purview of quorum sensing signal molecules, called autoinducers, and are expressed only when the signal is beyond

S. Peeralil (✉) · S. V. Narayanan · K. V. Lalitha
Microbiology Fermentation and Biotechnology Division, Central Institute of Fisheries Technology, Cochin, Kerala, India

T. C. Joseph
Microbiology Fermentation and Biotechnology Division, ICAR-Central Institute of Fisheries Technology (ICAR-CIFT), Kochi, Kerala, India

M. P. Mothadaka et al. (eds.), *Handbook on Antimicrobial Resistance*,
https://doi.org/10.1007/978-981-19-9279-7_44

a threshold which is determined by the bacterial cell density. Hence, interfering with these signals will effectively silence the operon that harbors virulence genes, without being detrimental to bacteria so that there is no selective pressure. This interpolation with quorum sensing paths of microbes, known as antipathogenic and/or anti-virulence strategies, is one of several such promising approaches which mainly focus on disruption of bacterial pathogenicity and does not involve bacterial killing. Inhibition of indicator molecules is accomplished by signal dilapidation, use of natural or synthetic analogues, and various other strategies which are described in this chapter. Also discussed are the modes of action of bacterial quorum sensing and an overview of potential sources of bioactive components as QS inhibitors.

Keywords

AHL · AIP · Antibiotics · Anti-virulence · Quorum sensing

1 Introduction

Antimicrobial resistance (AMR) is the establishment of antibiotic unresponsiveness of microbes to antimicrobial agents such as antibiotics and antivirals mainly by its misuse and overuse. The extensive haphazard application of antimicrobial drugs not only resulted in emergence of drug resistance but also has led to growth of superbugs (Hinchliffe et al., 2018). AMR is increasingly becoming the most serious global threats in this century, both economically as well as in terms of health. Reports indicate that microbial infections associated with drug unresponsiveness results in minimum of 10 million mortalities per year with an economic cost of more than $100 trillion by 2050 (although these predictions are contested) (de Kraker et al., 2016). About 90% of deaths due to AMR is occurring in low-income countries and middle-income countries (LIC & MIC) in Africa and Asia. This warrants for an immediate multisectoral action so that accomplishing the Sustainable Development Goals (SDGs) does not remain in the bay. The WHO in 2017 suggested a global framework for fighting AMR in three major areas such as R&D, access, and stewardship ("World Health Organization and Food and Agriculture Organization of the United Nations," 2017). A paradigm shifts in management of infectious diseases and alternative approaches that are free from antibiotic drugs are major lasting solutions to fight AMR. Studies on how intercellular bacterial signaling controls the virulence features has revealed a promising approach to bacterial-mediated diseases (LaSarre & Federle, 2013). Among this, one promising example is the therapeutic method that targets bacterial "quorum sensing (QS)," a process that facilitates bacterial communication which helps in the synchronizing virulence factors expression. The establishment of a host infection by bacteria is the result of synchronized communication signaled by quorum sensing that brings together the whole population and make changes in gene expression pattern. This chapter builds on QS, one of the most widely investigated anti-virulence therapy targets as an extremely promising method to combat the problem of drug resistance. Moreover,

most antibiotics are bacteriostatic or bactericidal, which leads to an acute adaptive pressure responsible for bacterial antibiotic resistance by rapid genotypic and phenotypic responses. The QS inhibitors are neither bacteriostatic nor bactericidal and can inhibit bacterial virulence without imposing any selective pressure.

Control of virulence gene expression by QS has a significant impact, and that led scientists to study the mode of action of QS in microbes at length, especially at the molecular level. Studies in the last four decades have revealed that the virulence factors formed by various botanical, faunal, and human pathogens are organized by QS system (Defoirdt, 2018). These virulence factors can alter the balance of host defense mechanism. With the help of various adhesion and invasion factors, bacterial pathogens colonize on the host surface and secrete various enzymes and toxins that cause tissue damage and inflammatory responses and when left uncontrolled leads to death. These virulence factors can be divided into a number of functional types: adherence and colonization factors, invasion factors that help bacterium invade host cells, capsules and other surface components that protect them from opsonization and phagocytosis, endotoxins that cause lethal effect on host system, exotoxins (e.g., neurotoxins, cytotoxins, and enterotoxins) that form a class of poisons that is among the most potent of all toxic substances, and siderophores that facilitates intake of metal ions.

Often, the synthesis of virulence features is orchestrated by extracellular signaling molecules that mediate communication between cells in a population and are called autoinducers (AIs). *Vibrio fischeri* is a marine bacterium that uses AIs to control bioluminescence (Eberhard et al., 1981). Likewise, quorum sensing systems centered on the generation and identification of "acylated homoserine lactones (AHLs)" were later discovered in various Gram-negative bacteria. Different chemical and structural variations are observed in AIs produced by different bacterial communities (Ng & Bassler, 2009). These AIs may either be encased into vesicles for trafficking between cells or in most cases secreted by the cells. These signaling molecules that facilitate communication between the bacterium can be hacked so that virulence factors are not produced. This forms the core of this chapter, and the principles, strategies, and available bioactive compounds are discussed herein.

2 Influence of QS Systems on Bacterial Pathogenicity

The QS system depends on three important principles; firstly, the production of AIs by the bacterial population which is determined by the cell density. Secondly, AIs are detected by receptors which are cytoplasmic or in most cases attached to membrane kind, and lastly, recognition of autoinducers is required to bring about QS controlled gene expression or repression. Mostly the genes for the receptors, enzymes for the synthesis of autoinducers, and transcription factors are encrypted in the identical operon which is below the regulatory control of QS system and thus forming a feed-forward loop (Rutherford & Bassler, 2012). One important aspect of quorum sensing is the levels of AIs that is directly proportional to the cell density. The change in the level of AIs in the environment helps the bacterium track the cell density, and the coordinated virulence gene expression occurs only when the autoinducer concentration is beyond the threshold.

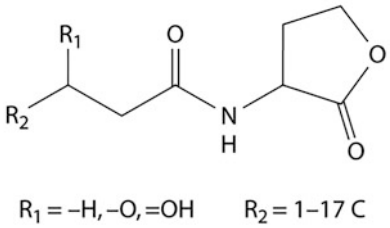


Fig. 1 Structures of acyl homoserine lactones (AHLs)

QS autoinducers include "N-acylhomoserine lactone (AHL), furanosyl borate diester (AI-2), 4,5-dihydroxy-2,3-pentanedione (DPD), cis-11-methyl-2 dodecenoic acid (diffusible signal factor, DSF), 3-hydroxypalmitic acid methyl ester (3OH-PAME), diketopiperazines (DKP), 2- heptyl 3-hydroxy-4-quinolone (PQS) and 4-hydroxy 2-heptylquinoline (HHQ) to molecules of high molecular weight such as oligopeptides (AIPs-Auto inducer peptides)" (Haque et al., 2019). The substrate for making "acyl homoserine lactones (AHLs)" are mainly "S-adenosyl methionine (SAM)" (Wei et al., 2011). The chemical arrangement of these AHL molecules consist of a homoserine lactone ring with saturated or unsaturated acyl side chain (size ranging from 1 to 17 carbons) which is linked covalently through an amide bond (Fig. 1) (Atkinson et al., 2006). In Gram-negative bacteria, AHL synthesis is catalyzed by the clan of "LuxI homologue proteins" which uses the relevant "acyl carrier protein (ACP)" that provides the acyl side chain and "S-adenosyl methionine (SAM)," that delivers "homoserine lactone moiety" (Eberl, 1999). When the concentration of AHL molecules touches the edge, it binds to the receptor molecules which initiates a signaling torrent that results in activation of a transcriptional regulator that mediates the target gene expression (Fuqua et al., 1994).

2.1 Gram-Positive Bacteria

Gram-positive and Gram-negative bacteria possess varied types of QS mechanisms. In Gram-positive bacteria, the QS signals are "autoinducing peptides (AIPs)" ranging the size of 5–17 amino acids that may be linear or cyclized. After the production of AIPs inside the cells, these are posttranslationally modified and secreted. In Gram-positive microbes, these indications are detected by an arrangement of binary scheme constituting a "feeler kinase and rejoinder regulator." On binding of the AIPs, the sensor kinase autophosphorylates a key deposit of histidine. The phosphoryl cluster from the histidine deposit is further transferred on to an aspartate deposit on the response regulator which then activates the QS response genes. The two-component system proteins, the AIP precursors (pro-AIPs), and transporters are encoded in an operon which is activated by the phosphorylated response regulator thereby causing a positive feedback loop of QS response (Rutherford & Bassler, 2012). AIPs have been located in several Gram-positive bacteria. In *Staphylococcus*

aureus, one of the widely studied Gram-positive QS systems, gene expression is controlled through "Agr (Accessory gene regulator)" system. The Agr system possesses the regulatory control over two promoters in "Agr locus, P2 and P3" that yields "RNAII and RNAIII" transcripts. The RNAII encrypts AgrA, the response regulator and "AgrC, the transmembrane histidine kinase" along with AgrD and AgrB that codes for proAIP and proAIP processing machinery (De Kievit & Iglewski, 2000). The RNAIII transcript is 514 bp long and codes for many regulatory functions including upregulation of virulence genes like α-hemolysin and downregulation of protein A (*spa*) and repressor of toxins (*rot*) (George & Muir, 2007). It is logical to think that proteins associated in connection and defense (collagen, fibronectin binding protein, protein A) are particularly required during primary stages of infection and at enhanced levels of cell density the Agr QS system downregulates these proteins and upregulates the secretory proteins associated with virulence.

2.2 Gram-Negative Bacteria

In Gram-negative *V. fischeri*, the marine bacterium known for its symbiotic association with squids, the proteins associated with bioluminescence are encrypted in the "*luxICDABEG*" operon which is under the direct control of "LuxI-LuxR" QS system. The *LuxI* is an AI synthase that codes for "N-3-oxohexanoyl-homoserine lactone (3-oxo-C6 HSL)," the autoinducer and LuxR controller of transcription that dimerizes with the autoinducer which then attaches to the location of promoter of "*luxICDABEG"* operon activating the transcriptional machinery thereby causing the production of proteins like luciferase that are significant in the biochemical pathway of bioluminescence (Boylan et al., 1989; Schaefer et al., 1996). QS in Gram-negative bacteria depends mainly on N-acyl homoserine lactone (AHLs) as signaling molecules (Galloway et al., 2011). The study of bacterial genomes showed that homologs of LuxI and LuxR are frequently encountered on proteobacterial genome (Case et al., 2008). One of the extensively studied systems among the Gram-negative bacteria is the QseC/QseB system, the QseC being the membrane-bound sensor histidine kinase and QseB the transcription factor. Apart from being a receptor of AHL, the QseC also detects the host adrenergic molecules enabling the expression of virulence system after ensuring a conducive host ecosystem (Clarke et al., 2006). The QseC/QseB system or its homologs are widely present in human pathogens including *Shigella flexneri, Citrobacter koseri, Enterobacter, Salmonella typhimurium, Salmonella enterica sub*sp. *Enterica, Haemophilus influenzae, Chromobacterium violaceum, Yersinia mollaretti, Klebsiella pneumoniae, Pasteurella multocida, Coxiella burnetii, Burkhholderia phymatum, Legionella pneumophila, Bordetella pertussis, Pseudomonas aeruginosa, Yersinia pestis, Vibrio sp.,* and more (Rasko et al., 2008).

2.3 Multichannel QS Systems

The members of the genus *Vibrio* are known for their multichannel QS system which recognizes different autoinducer signals and produce a graded response in the gene expression pattern with respect to the autoinducers detected. The peculiar feature of the system is that all signal cascades finally affect the expression of same transcriptional regulator protein, although at different levels. One of the best studied multichannel systems belongs to that of *V. harveyi.* An understanding of such multichannel systems that respond to similar signals from different species helps formulating intervention strategies that may target different pathogens in the environment.

The QS in *V. harveyi* is a three-channel system controlled by three molecules, the "HAI-1 (Harveyi autoinducer-1) an acyl HSL synthesized by LuxM," "AI-2 (a furanosyl borate diester) formed by LuxS" and "CAI-1 (cholera autoinducer) synthesized by CqsA." These signals are detected by LuxN, LuxQ, and cqsS receptor proteins at the cell surface. These receptors have both kinase and phosphatase activity. On binding with autoinducers, the receptor proteins act as phosphatases and dephosphorylates LuxO thereby inactivating it. The LuxO protein is involved in the creation of small regulatory RNAs (called qrr-quorum regulatory RNAs) which destabilizes the mRNA involved in the synthesis of transcriptional regulator LuxR (Tu & Bassler, 2007). Hence, when the autoinducers are present, the LuxO is inactive and qrrs are not produced, and therefore, LuxR is synthesized. Free from autoinducers, the activity of kinase of receptors phosphorylates the LuxO, allowing the synthesis of qrr which inhibits the production of LuxR. All most all genes of *V. harveyi* QS regulon is controlled by LuxR (Waters & Bassler, 2006; Defoirdt et al., 2007).

One of the well-known features controlled by *V. harveyi* QS system is bioluminescence. Many other virulence aspects of the bacterium such as metalloproteases, siderophores, phospholipase, chitinase, extracellular toxins, and components of type III secretory system are also under the control of the QS system (Defoirdt et al., 2007). The QS system also helps the bacterium to induce pathogenicity as many of its virulence factors are under its direct control. Studies indicate pathogenicity of *V. harveyi* toward the brine shrimp *Artemia franciscana* is mediated through AI-2. In the absence of luxS, AI-2 synthase gene, or luxP the AI-2 receptor, the bacterium was unable to induce pathogenicity (Defoirdt & Sorgeloos, 2012). Luminescent vibriosis is one of the common bacterial infections caused by the organism, and studies have confirmed the pathogenic effects of *V. harveyi* in penaeid shrimp (Peeralil et al., 2020).

An interesting question would be, why the bacterium needs three different signals if all signals regulate the same transcriptional factor? One hypothesis is that three AIs would prevent noise screening from other similar molecules in a multispecies populated environment. It is also possible that different combinations of AIs induce the expression of different set of genes. Apparently, variable LuxR synthesis occurs for different combination of AIs. This is explained by the promoter affinity model. In fact, the promoter affinity toward the transcription factor LuxR varies considerably

among the promoters and accordingly the genes under these promoters could be divided into three classes. Class I gene promoters have very low affinity to LuxR and get activated only at highest concentration of LuxR, which happens only during the corresponding binding of HAI-1 and AI-2. Whereas, the class II gene promoters have an intermediate affinity to LuxR and show additive response to autoinducers. The class III gene promoters have high affinity toward LuxR that respond fully in the occurrence of HAI-1 or AI-2. Thus, with each AI input, the qrr expression varies at different levels of LuxR and downstream gene expression. The CAI-1 signal is understood to be the weakest of the signals that it activates the gene promoters with the highest affinity (Waters & Bassler, 2006).

This variability in the gene expression pattern has been demonstrated in a beautiful experiment where the light produced by luminescence in response to various combinations of AIs was measured in terms of relative light units. In response to the AI inputs "(No AI, AI-2, HAI-1, AI-2+HAI-1)," four levels of lights based on the intensity were measured. Each AI input contributed an incremental change in the light intensity and the highest was observed in the concurrent occurrence of all AIs (Mok et al., 2003; Waters & Bassler, 2006).

The concentrations of AIs also provide the bacterium discrete information about the environment as the combination of available autoinducers in various environments would be different. In a mixed species environment, the levels of AI-2 and CAI-1 will be higher than HAI-1. In a gastrointestinal environment, the levels of AI-2 will be less due to the fact that bacteria like *E. coli* have AI-2 import and degradation mechanisms (Xavier & Bassler, 2005). Hence, a low level of AI-2 and significant levels of HAI-1 and CAI-1 would act as an indicator of gastrointestinal environment (Waters & Bassler, 2006).

3 Exploiting Quorum Sensing Interference Strategies as an Anti-Virulence Therapy

One of the major advantages of interfering with the QS system is that it allows the control of pathogenicity while not being bactericidal and reduces the selective pressure which often results in the emergence of resistance. Interference with the QS system can be achieved by any of the following strategies: (i) interfering with the biosynthesis of Ais, i.e., targeting the AI producing enzymes; (ii) inactivation of secreted signal biomolecules, i.e., degradation of AIs using quorum quenching enzymes; and (iii) interfering with the signal detection, i.e., using structural analogs which compete with the signal molecule.

3.1 Interfering with the Biosynthesis of Signal Compounds

As mentioned earlier, the acyl-HSL, the major autoinducer in Gram-negative bacteria is produced from the substrate "S-adenosyl methionine (SAM)" and "acyl carrier proteins (ACP)" that provide the acyl group of the acyl-HSL, and the process is

catalyzed by LuxI homologue of proteins. Interfering with the synthesis of "S-adenosyl methionine" or acyl carrier proteins can effectively hinder the synthesis of acyl-HSL. FabI is an enzyme related to alcohol dehydrogenases family and is involved in the synthesis of acyl-ACP. These short-chain alcohol dehydrogenases can be inhibited by antibacterial like triclosan and diazirine's (LaSarre & Federle, 2013). TofI a homolog of LuxI in *Burkholderia glumae* is shown to be inhibited by suppresser "J8-C8," a fundamental equivalent of "C8-HSL" which acts as an aggressive suppressor of "octanoyl-ACP" substrate (Chung et al., 2011). The making of QS signal molecules "PQS (3,4 dihydroxy-2-heptylquinoline) and HHQ (2-heptyl-4-hydroxyquinoline)" of the Pseudomonas quinolone system starts with the enzyme anthranilyl-CoA ligase (PsqA) which can be inhibited by sulfonyladenosine compounds and halogenated anthranilate analogs (LaSarre & Federle, 2013). Similarly, ambuic acid is shown to interfere with the biosynthesis of autoinducer peptides (AIPs) in *S. aureus* (Todd et al., 2017). Even though these strategies appear to be promising, there are some limitations while targeting the signal biosynthesis. These target enzymes are always present inside the cell, and for the inhibitory substances to access these enzymes, they must cross the cell wall barrier. Even then, many bacteria have mechanisms that remove these "toxic" substances from the cytoplasm. For, e.g., *P. aeruginosa* removes triclosan through a multidrug efflux pump (LaSarre & Federle, 2013).

3.2 Inactivation of Secreted Signal Using AHL-Modifying/Degrading Enzymes

Alteration or dilapidation of QS indicator molecules is top among QS interference mechanism assessments. These modifications or degradations are often achieved through specific enzymes which are often produced by bacteria to compete with other species in a multi-populated ecosystem. The three classes of quorum quenching enzymes have been extensively studied are lactonases, acylases, and oxidoreductases that target acyl-HSL (Fetzner, 2015).

3.2.1 Lactonases

The hydrolysis of ester bond of homoserine lactone rings can be achieved through enzymes called lactonases. As the target- the lactone rings, are unique to all AHL's the lactonases can be used against a large number of AHL's. The lactonases were first identified in Bacillus, and the gene found to be responsible for the inactivation of AHL was designated as autoinducer inactivation gene (aiiA). This group of lactonases have their place in the superfamily of Metallo-β-lactamases. Similar lactonases were also identified in *Agrobacterium tumefaciens, Klebsiella pneumoniae, Rhizobium* sp., and *Arthrobacter* sp. There are other lactonases belonging to other protein families including α/β hydrolases, GDSL hydrolases, glycosyl hydrolases, dienelactone hydrolases, and phosphotriesterases that has been identified from different bacterial genera. The presence of lactonases has been reported from eukaryotes as well. Of particular importance is the PON family of lactonases

reported in mammals (humans). The mammalian enzyme paraoxonases (PON 1–3) were identified to be primarily lactonases with several distinct physiological functions including prevention against lipid peroxidation and oxidative stress, bioactivation of drugs, detoxification of reactive molecules, and hydrolyzing toxic oxidative metabolites of organophosphorus insecticides (Martinelli et al., 2013; Levy et al., 2019). PON-1 is known for its link to cardiovascular diseases due to its role in diminishing lipid peroxide buildup on low-density lipoproteins (Shunmoogam et al., 2018). Apart from these physiological functions, different observations have demonstrated the part of PON-1 in AHL degradation in epithelial cells (Stoltz et al., 2007; LaSarre & Federle, 2013).

3.2.2 Acylases

The acylases (amidohydrolase) act by amide bond hydrolysis which separates the acyl chain (fatty acid) and the lactone ring. The diversity of AHLs is a function of acyl groups which may vary in length and structure. Hence, the acylases that target the acyl group are more specific toward each class of AHLs. AHL acylases related to N-terminal nucleophile (Ntn) hydrolases protein superfamily known for their property of amide bond hydrolysis. Acylases was first reported in *Variovorax paradoxus* where it was shown to cleave C4-HSL and release fatty acid from HSL (Leadbetter & Greenberg, 2000). A well-studied member of the family of proteins is penicillin acylase which catalyzes the diacylation of β-lactam antibiotics. Penicillin acylase and AHL acylase have similarities in their mode of action, and cross-reactivity between PGA (penicillin G acylase) and AHL acylase has also been reported. AHL acylases have been characterized from various bacterial species viz. *Acinetobacter, A. tumefaciens, Brucella melitensis, Deinococcus radiodurans, Kluyvera citrophila, P. aeruginosa, Pectobacterium atrospeticum, Ralstonia* sp., *Shewanella,* and *Streptomyces*. The phylogenetic analysis of these AHL acylases shows that most of them belong to either of the four groups viz., PGA (penicillin G acylase), AAC (aculeacin A acylase), PVA (penicillin V acylase), and AmiE (amidase family)" (LaSarre & Federle, 2013; Utari et al., 2017).

3.2.3 Oxidoreductases

The oxidoreductases render a signal molecule inactive by modifying the acyl side chain through oxidation or reduction reactions. The modification will reduce the distinction in identification of the indicator molecules consequently blocking the activation of QS genes. Cytochrome P450 monooxygenase of *Bacillus megatarium* was demonstrated to be efficient in the oxidation acyl-HSL. Specifically the enzyme hydroxylates the "ω-1, ω-2, and ω-3 carbon atoms" of the acyl chain which reduces the quorum sensing activity by 18-fold as compared to the parental signal compound (Chowdhary et al., 2007). Another oxidoreductase enzyme, "NADP-dependent short chain dehydrogenase/reductase" was shown to reduce the pyocyanin production, biofilm formation, motility, and pathogenicity of *P. aeruginosa*. The enzyme is involved in the amelioration of "N-(3-oxo-dodecanoyl)-2-homoserine lactone (3-oxo-C12-HSL)" (Bijtenhoorn et al., 2011).

3.3 Interfering with the Signal Detection

This strategy makes use of AHL antagonists that bind to the AHL receptor resulting in an inefficient signal-receptor complex that hinder the expression of QS genes. Understanding the crystal structure of the receptor and its interaction with signal compound is important in designing novel ligands that act as agonist or antagonist. Computational screening of wide number of natural and synthetic ligands with the help of molecular docking programs also aid in the discovery of new ligands. Structural analogs of AHLs could be synthesized, and modifications can be made in the acyl chain, the lactone moiety, or the central amide connective function. It is proved that the size of the acyl chain is crucial in binding to the receptor. The correspondents of AHL with acyl chain that is elongated in nature have the more inhibitory effect, and they are often antagonistic to AHLs with shorter acyl chains. Luminescence of *V. fischeri* could be inhibited using synthetic analogs of 3-oxo-C6-HSL with the C3 to C2 oxo group removed, whereas when the chain length is increased by two carbon atoms (3-oxo-C8-HSL), the analog turned out to be agonistic. The study of AHL analogs in the plant pathogen *Erwinia carotovora* demonstrated that increasing the length by one carbon atom reduced the QS activity by 50%. Addition of a phenyl group on the acyl chain of 3-oxo-C4-HSL gives the new ligand a competitive advantage over the native 3-oxo-C6-HSL in binding to LuxR (Hentzer & Givskov, 2003; Stevens et al., 2011). Various synthetic AHL analogues, namely, "Furanyl hydrazide, macrolides, cyclohexanone, N-(indole-3-butanoyl)-L-HSL, and lactam analogs, viz., 3OC12HSL, N-(heptyl-sulfanyl-acetyl)-L-HSL, 3-oxo-C12-(2aminophenol), 3-nitro phenylacetonyl HL (C14), and diastereomeric 2-methoxycyclopentyl" have been effective against *P. aeruginosa* QS systems (Kalia, 2013).

4 Quorum Sensing Inhibitors

4.1 Screening the Quorum Sensing Inhibitor Compounds

A quorum sensing inhibitor shall ideally be a small molecule with the ability to reduce QS-dependent gene expression and should be specific toward the targeted QS system and chemically stable, not affect the growth of target bacterium so as to induce selective pressure and not have any adverse effect on the host. The bioactive compounds of the nature are the ever sought for remedies, and researches keep exploring the natural compounds – from biotic and abiotic sources – for the presence of quorum sensing inhibitors. A very efficient way to test these compounds is by using reporter strains that exhibit quorum sensing responses like pigment production or luminescence. *C. violacium* is such a reported strain with the ability to produce violacein pigment in response to AHLs. A mutant strain of this bacterium *C. violacium* CV026 have a mutated *cviI* gene (homolog of *luxI*) and is unable to produce the native AHL. When the bacterium is supplemented with AHL of acyl chain length eight carbon atom or lesser, it produces the pigment. To test a

compound for its quorum sensor inhibitory activity, the compound is plated along with *C. violacium* induced by AHLs. If the test compound has an inhibitory effect on AHL, the pigmented plate will have a clear zone of nonpigmentation around the test compound (McClean et al., 1997). In Staphylococcal AIP reporters, β-galactosidase gene is inserted into the *agr* operon which harbors the virulence genes. If the test compound has AIPs, the QS system is activated, and the virulence genes are expressed. The β-galactosidase gene inserted among the virulence gene also gets expressed, and this facilitates the identification by observing the color (Nielsen et al., 2010).

4.2 Quorum Sensing Inhibitors from Nature

Several quorum sensing inhibitors (QSI) have been extensively studied, which are isolated from natural products and would be promising resources in drug development. Quorum sensing inhibitors from the microbes include enzymes that degrade/modify the QS signal molecules, the major categories of which are already discussed in the previous sections. AHL-acylases have been recorded to occur in *Ralstonia* sp., *P. aeruginosa, P. syringae, Streptomyces* sp., and *Tenacibaculum maritimum* (Kalia, 2013). AHL-lactonases were documented from various *Bacillus* sp. such as *B. subtilis, B. cereus*, and *B. thuringiensis* (Noor et al., 2022). Other rich sources of QSI are phytochemicals and compounds from aquatic and marine sources.

4.2.1 QSI from Plants

Parts of plants or crude plant extracts have been used in traditional medical practice to fight diseases, although their mechanism of action was quite obscure. It is widely accepted that phytochemicals are rich source of antibacterials and antiviral compounds. Corroborating this notion is the result from several studies that report antibacterial activity on agar diffusion or microbroth diffusion assays. This direct screening is often the initial step in the identification of phytochemical compounds from which the promising ones are further subjected to QS inhibitory tests on plates with reporter bacteria or by microdilution where the pigment production is measured based on optical density. The plant extracts with inhibitory effects can be subjected to fractionation using HPLC followed by structural analysis using mass spectrometry and nuclear magnetic resonance. This is followed by in silico analysis and molecular docking studies (Deryabin et al., 2019).

Plants are considered as a rich natural resource of quorum quenching agents. Plant metabolites are shown to inhibit pathogenic bacteria. Essential oils from plants, namely, *Eucalyptus radiate, Citrus reticulate*, *Eucalyptus globulus,* and *Thymus vulgar*, have inhibitory effect of quorum sensing (Luís et al., 2017). Food extracts from different plants such as fruits, vegetables, and fresh herbs and spices also have been confirmed as strong QSI (Deryabin et al., 2019).

In plants, the sulfur-containing compounds include organosulfur compounds akin to allicin and ajoene seen in garlic, onion, and leeks and isothiocyanates from vegetables like cabbages, kales, and broccoli. Allicin and ajoene were shown to

have a suppressive effect on biofilm of *P. aeruginosa* and QS-dependent virulence factors (Fong et al., 2017). Allyl isothiocyanate reduces the biofilm formation and exhibit QS inhibition in the reported organism *C. violacium.* Diallyl sulfide present in garlic has suppressive effect on *P. aeruginosa* QS system lasR resulting in the reduced production of pyocyanin, biofilm matrix, and extracellular polysaccharide (Li et al., 2018). Erucin, natural isothiocyanate from broccoli, is also antagonist to *P. aeruginosa* lasR (Ganin et al., 2012). Isothiocyanate Iberin from horseradish extract inhibits biofilm formation and rhamnolipid production by *P. aeruginosa* (Tan et al., 2014). Some plants have terpene synthase gene that helps in the synthesis of hydrocarbon organic compounds. Monoterpenes found in many plant-derived essential oils have the ability to inhibit *C. violacium* QS system. Monoterpenes like limonene, myrcene, γ-terpene, and α-pinene have suppressive effect on *P. aeruginosa* QS system (Deryabin et al., 2019). Cinnamon essential oil has cinnamaldehyde which belongs to the family of phenylpropanols and significantly repress las and rhl QS of *P. aeruginosa* that produce protease, elastase, and pyocyanin. In *P. fluorescens,* the inhibition of QS is brought about by the binding of cinnamaldehyde to LuxR-type transcriptional regulators. Another phenylpropene compound, eugenol, harbored in extracts herbs and cloves can suppress QS-mediated gene expression and suppress the biosynthesis of elastase, pyocyanin, pyoverdine, protease, rhamnolipids, and extracellular polysaccharides in *P. aeruginosa* and MDR clinical isolates (Deryabin et al., 2019). The functional activity of eugenol comes from its binding to LasR-type receptors. Benzoic acid derivative vanillin and vanillic acid inhibits biofilm formation in *A. hydrophila* and controls making of short and long sequence autoinducer. Biofilm and QS-regulated virulence of *S. marcescens* is significantly affected by Kiwi pulp extract-based vanillic acid. Curcumin belonging to the family of diarylheptanoids has high affinity toward LuxI-type proteins. It reduces the QS-mediated production of pyocyanin, activity of elastase and protease, biofilm formation, and AHL creation in *P. aeruginosa* (Rudrappa & Bais, 2008). Flavonoids are compounds with variable phenolic structures and are ubiquitous in plants. Citrus extract flavonoids have been shown to inhibit QS synchronized *V. harveyi* bioluminescence (Deryabin et al., 2019).

4.2.2 QSI from Fungi

In the environment, fungi are known to release secondary metabolites that inhibit the development of bacteria. These fungal secondary metabolites are antibiotics, and some antibiotics are naturally quorum sensing inhibitor, and to search for QSI in fungi would seem to be ironic. However, there are secondary metabolites, besides antibiotics produced that have inhibitory effects of QS System. Secondary metabolites from *Penicillium* spp. like patulin and penicillic acid have been found to produce (Rasmussen et al., 2005). Fungi of the family Ascomycota and Basidiomycota are shown to have lactonase activity that degrades 3OC6HSL and C6HSL (Uroz & Heinonsalo, 2008). Several extracellular enzymes produced by fungi such as cellulases, amylases, and proteases have the ability to degrade bacterial

biofilms. Lactonase enzyme activity has been reported in Basidiomycete yeast *Trichosporon loubieri. Tremella fuciformis* (Basidiomycota) is known to contain heteroglucan and triterpenes. The crude extract from this fungus is shown to inhibit AHL activity of *C. violaceum.* Other fungal species demonstrated to have QSI activity include *Auricularia auricula, Phellinus igniarius, Ganoderma lucidum, Fusarium graminearum, and Lasiodiplodia* sp. (Sharma & Jangid, 2015).

4.2.3 Marine Organism-Based QSIs

Marine ecosystems are reservoirs of myriad array of biomolecules produced by algae, cyanobacteria, sponges, cnidarians, and bryozoans, and wide range of QSI compounds were identified in extracts of cyanobacteria, marine algae, sponges, and invertebrates. Hanaucins produced by marine cyanobacterium Leptolyngbya showed inhibitory activity on *V. harveyi* bioluminescence (LaSarre & Federle, 2013). The Great Barrier Reef of Australia is one of the complex ecosystems in the world and has vast species diversity. Study of extract of marine organisms for the Great Barrier Reef revealed that 23% of the 284 extracts exhibited quorum sensing inhibitory activity. The secondary metabolites of sponges like "manoalide monoacetate, moanoalide, and secomanoalide" had strong QS inhibitory activity (Skindersoe et al., 2008). QSI compounds were reported from Cnidaria (including jellyfish, sea anemones, and corals), bryozoans (sea mats or sea mosses) as well.

4.2.4 Antibody-Based QSI

The mammalian immune system produces antibody in response to allergens. Quorum sensing signal molecules, especially AHLs, are non-proteinaceous, low-molecular-weight compounds and hardly elicit immune response and produce antibodies against them. Lactam-containing haptens whose structure mimics AHL-side chains were used for the development of monoclonal antibodies, and it was shown to suppress the in vitro synthesis of *P. aeruginosa* QS-controlled pyocyanin (Kaufmann et al., 2006). In another study, AHL (3OC12HSL) conjugated with bovine serum albumin was used for immunizing mice. The level of free 3OC12HSL in serum and lung homogenate were lower than that of nonimmunized mice, and the immunization greatly increased the survival rate. Also the cytotoxic effects of 3OC12HSL over macrophages were reduced in immunized mice (Miyairi et al., 2006). Significant number of studies of both in vitro and in vivo types documented the synthesis and evaluation of quorum quenching antibodies. Monoclonal antibodies produced against AIPs, signal molecules in Gram-positive microbes suppressed the *S. aureus*-based QS system. A hapten (AP4) resembling the AIP-4 signal molecule was used to create monoclonal antibodies, and one among the 20 prepared monoclonal antibodies had high binding affinity toward AIP-4. This monoclonal antibody (AP4-24H11) was able to suppress the expression of agr QS system of *S. aureus*-associated exoprotein syntheses including virulence factors. Further, it was shown that AP4-24H11 increased the biofilm formation, a feature which is known to be adversely controlled by QS in *S. aureus* (Park et al., 2007).

5 Challenges in Anti-Virulence Therapies

Even though the QS signals are mainly involved in the virulence of many pathogens, their roles in the physiological functions are rarely considered. According to some recent studies, AI-2 has an important part in regulating expression of genes associated with metabolism, cell division, morphogenesis and DNA repair, and biofilm formation which is a mode of adaptation to environmental stress. Correspondingly, the production of these signaling molecules was demonstrated to be significant for human gut microbiome in their potential to stick to, form biofilms, and produce metabolites that helps reduce the intestinal colonization of pathogens such as *Vibrio cholerae,* Salmonella, etc. Therefore, QSIs used ambitiously to control pathogens may directly or indirectly affect the gut microbiome and might cause disturbance of gut microbiome homeostasis. In addition, some studies have shown the involvement of QS signals in inducing pro-inflammatory and pro-apoptotic responses in eukaryotic cells. In some pathogens, not all virulence features are controlled by QS system, and targeting the QS activity may lead to increase in the pathogenicity features. For instance, biofilm formation is negatively regulated by the QS system in many pathogens, namely, *Staphylococcus epidermidis, Staphylococcus aureus, Streptococcus mutans Helicobacter pylori, Vibrio cholera,* and *Bacillus cereus*, and hence, targeting the QS activity increases the aggregation of biofilms.

6 Conclusion

Nearly a century ago, the discovery of antibiotics altered the course of human history, and there are no such wonder molecules that could replace the antibiotics as of now. However, growing concern over the ever-increasing antibiotic resistance among the pathogens necessitates research in new dimensions and exploring the unexplored. Studies in basic sciences help in understanding the molecular mechanisms of pathogenesis which provides space for intervention. Research over the recent decades showed how QS controls the virulence in animal, plant, and human pathogens and opened new vistas for possible therapeutic interventions. The basic principles of how quorum sensing inhibition is made possible by various organism's show the way for the extensive studies for new biomolecules. These biomolecules may in the near future save us from MDR pathogens or at least could be used in the MDR developing zones in the environments like the aquaculture farms. As this strategy do not cause any bactericidal activity, it would not create a selective pressure resulting in resistance development, and these agents could be best utilized in the environment. Since many crucial virulence-associated processes in pathogenic bacteria still remain unclear, research on basic science of the molecular mechanisms underlying quorum sensing system, interaction of inhibitory molecules, host-pathogen interactions, effect on gut microbiome, harmful effects if any on the indigenous flora when taken to the environment, and other related aspects toward dealing with challenges shall be subjected to research.

References

Atkinson, S., Chang, C.-Y., Sockett, R. E., Cámara, M., & Williams, P. (2006). Quorum sensing in yersinia enterocolitica controls swimming and swarming motility. *Journal of Bacteriology, 188*, 1451–1461. https://doi.org/10.1128/JB.188.4.1451-1461.2006

Bijtenhoorn, P., Mayerhofer, H., Müller-Dieckmann, J., Utpatel, C., Schipper, C., Hornung, C., et al. (2011). A novel metagenomic short-chain dehydrogenase/reductase attenuates pseudomonas aeruginosa biofilm formation and virulence on caenorhabditis elegans. *PLoS One, 6*, e26278. https://doi.org/10.1371/Journal.Pone.0026278

Boylan, M., Miyamoto, C., Wall, L., Graham, A., & And Meighen, E. (1989). Lux C, D and E genes of the *Vibrio fischeri* luminescence operon code for the reductase, transferase, and synthetase enzymes involved in aldehyde biosynthesis. *Photochemistry and Photobiology, 49*, 681–688. https://doi.org/10.1111/J.1751-1097.1989.TB08441.X

Case, R. J., Labbate, M., & Kjelleberg, S. (2008). AHL-driven quorum-sensing circuits: Their frequency and function among the Proteobacteria. *The ISME Journal, 24*(2), 345–349. https://doi.org/10.1038/ismej.2008.13

Chowdhary, P. K., Keshavan, N., Nguyen, H. Q., Peterson, J. A., González, J. E., & Haines, D. C. (2007). Bacillus megaterium CYP102A1 oxidation of acyl homoserine lactones and acyl homoserines. *Biochemistry, 46*, 14429–14437. https://doi.org/10.1021/BI701945J

Chung, J., Goo, E., Yu, S., Choi, O., Lee, J., Kim, J., et al. (2011). Small-molecule inhibitor binding to an N-acyl-homoserine lactone synthase. *Proceedings of the National Academy of Sciences of the United States of America, 108*, 12089–12094. https://doi.org/10.1073/PNAS.1103165108/-/DCSUPPLEMENTAL

Clarke, M. B., Hughes, D. T., Zhu, C., Boedeker, E. C., & Sperandio, V. (2006). The QseC sensor kinase: A bacterial adrenergic receptor. *Proceedings of the National Academy of Sciences of the United States of America, 103*, 10420–10425. https://doi.org/10.1073/PNAS.0604343103

De Kievit, T. R., & Iglewski, B. H. (2000). Bacterial quorum sensing in pathogenic relationships. *Infection and Immunity, 68*, 4839–4849. https://doi.org/10.1128/IAI.68.9.4839-4849.2000/ASSET/0908D8CE-0CBE-47E2-8B90-12F53FBE8167/ASSETS/GRAPHIC/II0900701003.JPEG

de Kraker, M. E. A., Stewardson, A. J., & Harbarth, S. (2016). Will 10 million people die a year due to antimicrobial resistance by 2050? *PLoS Medicine, 13*, 1002184. https://doi.org/10.1371/JOURNAL.PMED.1002184

Defoirdt, T. (2018). Quorum-sensing systems as targets for antivirulence therapy. *Trends in Microbiology, 26*, 313–328. https://doi.org/10.1016/J.TIM.2017.10.005

Defoirdt, T., & Sorgeloos, P. (2012). Monitoring of Vibrio harveyi quorum sensing activity in real time during infection of brine shrimp larvae. *The ISME Journal, 6*, 2314. https://doi.org/10.1038/ISMEJ.2012.58

Defoirdt, T., Boon, N., Sorgeloos, P., Verstraete, W., & Bossier, P. (2007). Quorum sensing and quorum quenching in Vibrio harveyi: Lessons learned from in vivo work. *The ISME Journal, 21*(2), 19–26. https://doi.org/10.1038/ismej.2007.92

Deryabin, D., Galadzhieva, A., Kosyan, D., & Duskaev, G. (2019). Plant-derived inhibitors of AHL-mediated quorum sensing in bacteria: Modes of action. *International Journal of Molecular Sciences, 20*. https://doi.org/10.3390/IJMS20225588

Eberhard, A., Burlingame, A. L., Eberhard, C., Kenyon, G. L., Nealson, K. H., & Oppenheimer, N. J. (1981). Structural identification of autoinducer of Photobacterium fischeri luciferase. *Biochemistry, 20*, 2444–2449. https://doi.org/10.1021/BI00512A013

Eberl, L. (1999). N-acyl homoserinelactone-mediated gene regulation in gram-negative bacteria. *Systematic and Applied Microbiology, 22*, 493–506. https://doi.org/10.1016/S0723-2020(99)80001-0

Fetzner, S. (2015). Quorum quenching enzymes. *Journal of Biotechnology, 201*, 2–14. https://doi.org/10.1016/J.JBIOTEC.2014.09.001

Fong, J., Yuan, M., Jakobsen, T. H., Mortensen, K. T., Delos Santos, M. M. S., Chua, S. L., et al. (2017). Disulfide bond-containing Ajoene analogues as novel quorum sensing inhibitors of Pseudomonas aeruginosa. *Journal of Medicinal Chemistry, 60*, 215–227. https://doi.org/10.1021/ACS.JMEDCHEM.6B01025/SUPPL_FILE/JM6B01025-SI002.CSV

Fuqua, W. C., Winans, S. C., & Greenberg, E. P. (1994). Quorum sensing in bacteria: The LuxR-LuxI family of cell density- responsive transcriptional regulators. *Journal of Bacteriology, 176*, 269–275. https://doi.org/10.1128/jb.176.2.269-275.1994

Galloway, W. R. J. D., Hodgkinson, J. T., Bowden, S. D., Welch, M., & Spring, D. R. (2011). Quorum sensing in Gram-negative bacteria: Small-molecule modulation of AHL and AI-2 quorum sensing pathways. *Chem. Rev. 111*, 28–67. https://doi.org/10.1021/CR100109T/ASSET/CR100109T.FP.PNG_V03.

Ganin, H., Rayo, J., Amara, N., Levy, N., Krief, P., & Meijler, M. M. (2012). Sulforaphane and erucin, natural isothiocyanates from broccoli, inhibit bacterial quorum sensing. *MedChemComm, 4*, 175–179. https://doi.org/10.1039/C2MD20196H

George, E. A., & Muir, T. W. (2007). Molecular mechanisms of agr quorum sensing in virulent staphylococci. *Chembiochem, 8*, 847–855. https://doi.org/10.1002/CBIC.200700023

Haque, S., Yadav, D. K., Bisht, S. C., Yadav, N., Singh, V., Dubey, K. K., et al. (2019). Quorum sensing pathways in gram-positive and -negative bacteria: Potential of their interruption in abating drug resistance. *Journal of Chemotherapy, 31*, 161–187. https://doi.org/10.1080/1120009X.2019.1599175

Hentzer, M., & Givskov, M. (2003). Pharmacological inhibition of quorum sensing for the treatment of chronic bacterial infections. *The Journal of Clinical Investigation, 112*, 1300. https://doi.org/10.1172/JCI20074

Hinchliffe, S., Butcher, A., & Rahman, M. M. (2018). The AMR problem: Demanding economies, biological margins, and co-producing alternative strategies. *Palgrave Communications, 41*(4), 1–12. https://doi.org/10.1057/s41599-018-0195-4

Kalia, V. C. (2013). Quorum sensing inhibitors: An overview. *Biotechnology Advances, 31*, 224–245. https://doi.org/10.1016/j.biotechadv.2012.10.004

Kaufmann, G. F., Sartorio, R., Lee, S. H., Mee, J. M., Altobell, L. J., Kujawa, D. P., et al. (2006). Antibody interference with N-acyl Homoserine lactone-mediated bacterial quorum sensing. *Journal of the American Chemical Society, 128*, 2802. https://doi.org/10.1021/JA0578698

LaSarre, B., & Federle, M. J. (2013). Exploiting quorum sensing to confuse bacterial pathogens. *Microbiology and Molecular Biology Reviews, 77*, 73–111. https://doi.org/10.1128/MMBR.00046-12

Leadbetter, J. R., & Greenberg, E. P. (2000). Metabolism of acyl-homoserine lactone quorum-sensing signals by Variovorax paradoxus. *Journal of Bacteriology, 182*, 6921–6926. https://doi.org/10.1128/JB.182.24.6921-6926.2000/ASSET/660BF097-B3D9-4186-901F-B2F2FAF1BD90/ASSETS/GRAPHIC/JB2400663007.JPEG

Levy, D., Reichert, C. O., & Bydlowski, S. P. (2019). Paraoxonases activities and polymorphisms in elderly and old-age diseases: An overview. *Antioxidants, 8*, 118. https://doi.org/10.3390/ANTIOX8050118

Li, W. R., Ma, Y. K., Shi, Q. S., Xie, X. B., Sun, T. L., Peng, H., et al. (2018). Diallyl disulfide from garlic oil inhibits Pseudomonas aeruginosa virulence factors by inactivating key quorum sensing genes. *Applied Microbiology and Biotechnology, 102*, 7555–7564. https://doi.org/10.1007/S00253-018-9175-2

Luís, Â., Duarte, A., Pereira, L., & Domingues, F. (2017). Chemical profiling and evaluation of antioxidant and anti-microbial properties of selected commercial essential oils: A comparative study. *Medicines (Basel, Switzerland), 4*, 36. https://doi.org/10.3390/MEDICINES4020036

Martinelli, N., Consoli, L., Girelli, D., Grison, E., Corrocher, R., & Olivieri, O. (2013). Paraoxonases: Ancient substrate hunters and their evolving role in ischemic heart disease. *Advances in Clinical Chemistry, 59*, 65–100. https://doi.org/10.1016/B978-0-12-405211-6.00003-6

McClean, K. H., Winson, M. K., Fish, L., Taylor, A., Chhabra, S. R., Camara, M., et al. (1997). Quorum sensing and *Chromobacterium violaceum*: Exploitation of violacein production and

inhibition for the detection of N-acylhomoserine lactones. *Microbiology, 143*(Pt 12), 3703–3711. https://doi.org/10.1099/00221287-143-12-3703

Miyairi, S., Tateda, K., Fuse, E. T., Ueda, C., Saito, H., Takabatake, T., et al. (2006). Immunization with 3-oxododecanoyl-L-homoserine lactone-protein conjugate protects mice from lethal Pseudomonas aeruginosa lung infection. *Journal of Medical Microbiology, 55*, 1381–1387. https://doi.org/10.1099/JMM.0.46658-0

Mok, K. C., Wingreen, N. S., & Bassler, B. L. (2003). Vibrio harveyi quorum sensing: A coincidence detector for two autoinducers controls gene expression. *The EMBO Journal, 22*, 870–881. https://doi.org/10.1093/EMBOJ/CDG085

Ng, W. L., & Bassler, B. L. (2009). Bacterial quorum-sensing network architectures. *Annual Review of Genetics, 43*, 197–222. https://doi.org/10.1146/ANNUREV-GENET-102108-134304

Nielsen, A., Nielsen, K. F., Frees, D., Larsen, T. O., & Ingmer, H. (2010). Method for screening compounds that influence virulence gene expression in Staphylococcus aureus. *Antimicrobial Agents and Chemotherapy, 54*, 509–512. https://doi.org/10.1128/AAC.00940-09

Noor, A. O., Almasri, D. M., Basyony, A. F., Albohy, A., Almutairi, L. S., Alhammadi, S. S., et al. (2022). Biodiversity of N-acyl homoserine lactonase (aiiA) gene from Bacillus subtilis. *Microbial Pathogenesis, 166*, 105543. https://doi.org/10.1016/J.MICPATH.2022.105543

Park, J., Jagasia, R., Kaufmann, G. F., Mathison, J. C., Ruiz, D. I., Moss, J. A., et al. (2007). Infection control by antibody disruption of bacterial quorum sensing signaling. *Chemistry & Biology, 14*, 1119. https://doi.org/10.1016/J.CHEMBIOL.2007.08.013

Peeralil, S., Joseph, T. C., Murugadas, V., Akhilnath, P. G., Sreejith, V. N., & Lalitha, K. V. (2020). Vibrio harveyi virulence gene expression in vitro and in vivo during infection in black tiger shrimp Penaeus monodon. *Diseases of Aquatic Organisms, 139*, 153–160. https://doi.org/10.3354/dao03475

Rasko, D. A., Moreira, C. G., De, R. L., Reading, N. C., Ritchie, J. M., Waldor, M. K., et al. (2008). Targeting QseC signaling and virulence for antibiotic development. *Science, 321*, 1078. https://doi.org/10.1126/SCIENCE.1160354

Rasmussen, T. B., Skindersoe, M. E., Bjarnsholt, T., Phipps, R. K., Christensen, K. B., Jensen, P. O., et al. (2005). Identity and effects of quorum-sensing inhibitors produced by Penicillium species. *Microbiology, 151*, 1325–1340. https://doi.org/10.1099/mic.0.27715-0

Rudrappa, T., & Bais, H. P. (2008). Curcumin, a known phenolic from Curcuma longa, attenuates the virulence of Pseudomonas aeruginosa PAO1 in whole plant and animal pathogenicity models. *Journal of Agricultural and Food Chemistry, 56*, 1955–1962. https://doi.org/10.1021/JF072591J

Rutherford, S. T., & Bassler, B. L. (2012). Bacterial quorum sensing: Its role in virulence and possibilities for its control. *Cold Spring Harbor Perspectives in Medicine, 2*. https://doi.org/10.1101/cshperspect.a012427

Schaefer, A. L., Val, D. L., Hanzelka, B. L., Cronan, J. E., & Greenberg, E. P. (1996). Generation of cell-to-cell signals in quorum sensing: Acyl homoserine lactone synthase activity of a purified Vibrio fischeri LuxI protein. *Proceedings of the National Academy of Sciences of the United States of America, 93*, 9505. https://doi.org/10.1073/PNAS.93.18.9505

Sharma, R., & Jangid, K. (2015). Fungal quorum sensing inhibitors. In V. C. Kalia (Ed.), *Quorum sensing vs quorum quenching: A battle with no end in sight* (pp. 237–257). Springer. https://doi.org/10.1007/978-81-322-1982-8_20

Shunmoogam, N., Naidoo, P., & Chilton, R. (2018). Paraoxonase (PON)-1: A brief overview on genetics, structure, polymorphisms and clinical relevance. *Vascular Health and Risk Management, 14*, 137–143. https://doi.org/10.2147/VHRM.S165173

Skindersoe, M. E., Ettinger-Epstein, P., Rasmussen, T. B., Bjarnsholt, T., De Nys, R., & Givskov, M. (2008). Quorum sensing antagonism from marine organisms. *Marine Biotechnology, 10*, 56–63. https://doi.org/10.1007/s10126-007-9036-y

Stevens, A. M., Queneau, Y., Soulère, L., Von Bodman, S., & Doutheau, A. (2011). Mechanisms and synthetic modulators of AHL-dependent gene regulation. *Chemical Reviews, 111*, 4–27. https://doi.org/10.1021/cr100064s

Stoltz, D. A., Ozer, E. A., Ng, C. J., Yu, J. M., Reddy, S. T., Lusis, A. J., et al. (2007). Paraoxonase-2 deficiency enhances Pseudomonas aeruginosa quorum sensing in murine tracheal epithelia. *American Journal of Physiology. Lung Cellular and Molecular Physiology, 292*. https://doi.org/10.1152/AJPLUNG.00370.2006

Tan, S. Y. Y., Liu, Y., Chua, S. L., Vejborg, R. M., Jakobsen, T. H., Chew, S. C., et al. (2014). Comparative systems biology analysis to study the mode of action of the isothiocyanate compound iberin on Pseudomonas aeruginosa. *Antimicrobial Agents and Chemotherapy, 58*, 6648–6659. https://doi.org/10.1128/AAC.02620-13/SUPPL_FILE/ZAC011143400SO1.PDF

Todd, D. A., Parlet, C. P., Crosby, H. A., Malone, C. L., Heilmann, K. P., Horswill, A. R., et al. (2017). Signal biosynthesis inhibition with ambuic acid as a strategy to target antibiotic-resistant infections. *Antimicrobial Agents and Chemotherapy, 61*. https://doi.org/10.1128/AAC.00263-17/SUPPL_FILE/ZAC008176432S1.PDF

Tu, K. C., & Bassler, B. L. (2007). Multiple small RNAs act additively to integrate sensory information and control quorum sensing in Vibrio harveyi. *Genes & Development, 21*, 221–233. https://doi.org/10.1101/GAD.1502407

Uroz, S., & Heinonsalo, J. (2008). Degradation of N-acyl homoserine lactone quorum sensing signal molecules by forest root-associated fungi. *FEMS Microbiology Ecology, 65*, 271–278. https://doi.org/10.1111/J.1574-6941.2008.00477.X

Utari, P. D., Vogel, J., & Quax, W. J. (2017). Deciphering physiological functions of AHL quorum quenching acylases. *Frontiers in Microbiology, 8*, 1123. https://doi.org/10.3389/FMICB.2017.01123/BIBTEX

Waters, C. M., & Bassler, B. L. (2006). The Vibrio harveyi quorum-sensing system uses shared regulatory components to discriminate between multiple autoinducers. *Genes & Development, 20*, 2754–2767. https://doi.org/10.1101/GAD.1466506

Wei, Y., Perez, L. J., Ng, W. L., Semmelhack, M. F., & Bassler, B. L. (2011). Mechanism of vibrio cholerae autoinducer-1 biosynthesis. *ACS Chemical Biology, 6*, 356–365. https://doi.org/10.1021/cb1003652

World Health Organization, and Food and Agriculture Organization of the United Nations. (2017). *Global framework for development & stewardship to combat antimicrobial resistance*. Available at: https://www.who.int/publications/m/item/global-framework-for-development-stewardship-to-combat-antimicrobial-resistance-draft-roadmap Accessed 23 Mar 2023.

Xavier, K. B., & Bassler, B. L. (2005). Regulation of uptake and processing of the quorum-sensing autoinducer AI-2 in *Escherichia coli*. *Journal of Bacteriology, 187*, 238. https://doi.org/10.1128/JB.187.1.238-248.2005

CRISPR and CAS Editing Tools Employment in the Control of AMR Pathogens

Shivasharanappa Nayakvadi, Shivaramu Keelara, Paula J. Fedorka-Cray, and B. R. Shome

Contents

Abstract

Antibiotic-resistant bacteria have significantly emerged as a result of the widespread use of antibiotics in human and veterinary treatment. Antimicrobial resistance is the biggest problem in the coming decades, and developing alternative antibiotics is the only solution for its mitigation. CRISPR Cas9 (clustered regularly interspaced short palindromic repeats)-Cas (CRISPR-associated) is a powerful, precise gene-editing technique employed in medicine, agriculture, and many other fields for editing specific sequences of genes. Since antibiotic resistance is primarily spread by mobile genetic elements/plasmids, which carry hundreds of genes conferring resistance to even lost resort antibiotics, eliminating plasmids in drug-resistant bacteria is challenging. CRISPR Cas9 has recently

S. Nayakvadi (✉) · B. R. Shome
ICAR-National Institute of Veterinary Epidemiology and Disease Informatics (NIVEDI), Bengaluru, India
e-mail: shivasharanappa.n@icar.gov.in; Bibek.Shome@icar.gov.in

S. Keelara · P. J. Fedorka-Cray
Population Health and Pathobiology Department at the College of Veterinary Medicine, North Carolina State University, Raleigh, NC, USA
e-mail: skeelar@ncsu.edu; pjcray@ncsu.edu

M. P. Mothadaka et al. (eds.), *Handbook on Antimicrobial Resistance*,
https://doi.org/10.1007/978-981-19-9279-7_50

been employed to edit different multidrug-resistant pathogens in human and veterinary medicine. This method allows for the selective removal of superbugs from complicated microbial communities without causing harm to the surrounding microflora. CRISPR and CRISPR-associated (Cas) proteins constitute a strong adaptive immune system against foreign nucleic acids such as bacteriophages and plasmids in bacteria and archaea. CRISPR Cas9 essentially consists of short-guide RNA and the endonuclease Cas9 that together form a complex. The Cas9 protein is directed to the target site that complements the sgRNA by the sgRNA-Cas9 complex. Through either homologous recombination (HR) or non-homologous end joining (NHEJ), the cell will then repair the double-strand break (DSB) that is generated in the target sequence. This mechanism is being explored to eliminate AMR pathogens selectively. CRISPR Cas9 can be programmed to incorporate into disinfectants topical or oral formulations with suitable delivery mechanisms to cure bacterial infections.

Keywords

CRISPR · Cas9 · Antimicrobial resistance · Antibiotics · sgRNA · ESBLs

Abbreviations

ACRs	Anti-CRISPR (Acr) genes
AFM	Atomic force microscopy
AMR	Antimicrobial resistance
ARC	Antibiotic resistance cassette
Cas	CRISPR-associated endonuclease enzyme
CGE	Centre of Genomic Epidemiology
Cpf1	CRISPR from *Prevotella* and *Francisella*, and now called as Cas12a
CRISPR	Clustered regularly interspaced short palindromic repeats
CRISPRa	CRISPR-mediated gene activation
CRISPRi	CRISPR interference
crRNA	CRISPR RNA
dCas9	Dead Cas9 (catalytic dead Cas9)
DSB	Double-strand break (double-strand DNA break)
dsDNA	Double-stranded DNA
gRNA	Guide RNA
HDR	Homology-directed repair
HR	Homologous recombination
INDELS	Insertions and deletions
MDR	Multidrug resistance
NCBI	National Center for Biotechnology Information
NGS	Next-generation sequencing
NHEJ	Non-homologous end joining
PAM	Protospacer-adjacent motif

RNAi	RNA interference
RNP	Ribonucleoprotein
S.p.	*Streptococcus pyogenes*
sgRNA	Single-guide RNA
ssDNA	Single-stranded DNA
tracrRNA	Transactivating CRISPR RNA
ZFN	Zinc finger nuclease

1 Introduction

For developing the groundbreaking gene-editing method known as CRISPR-Cas, co-discoverers Emmanuelle Charpentier and Jennifer Doudna received the 2020 Nobel Prize in Chemistry. Since its discovery in 1987, CRISPR has evolved from "curious sequences of unknown biological purpose" into a potential tool for genome editing. In 2015, *Science Magazine* termed CRISPR Cas9 as "breakthrough of the year." In 2017, CRISPR Cas9 editing was captured live by Shibata et al. (2017) by atomic force microscopy (AFM).

Genome engineering started initially with zinc finger nucleases (ZFN) and TALENS. Later, CRISPR evolved as a powerful tool with several applications in medicine, agriculture, and other synthetic biology fields. Clustered regularly interspaced short palindromic repeats (CRISPR) and CRISPR-associated (Cas) proteins constitute a strong adaptive immune system against foreign nucleic acids such as bacteriophages and plasmids. CRISPR in bacteria and archaea specifically recognizes the complementary DNA sequences on target DNA and degrades/cleaves the gene. A double-stranded DNA (dsDNA) nuclease called Cas9 was discovered in *Streptococcus pyogenes*. Cas9 can be programmed to cleave practically any desired DNA sequence (van der Oost et al., 2014; Jinek et al., 2012). The CRISPR-Cas systems, which were initially found in the *E. coli* genome in 1987, are present in roughly 46% of bacteria and numerous archaea (Ishino et al., 1987), but their function in the elimination of resistant bacteria was elucidated in 2007 (Barangou et al., 2007; Barangou & Ousterout, 2017; Sorek et al., 2013). CRISPR arrays are defined as alternating stretches of short, noncontiguous DNA repeats separated by variable spacer sequences that form peculiar loci in the bacterial and archaeal genomes (Horvath & Barrangou, 2010). The CRISPR-associated (Cas) genes surround these repeat sequences, which are generally 20–38 nucleotides long (Makarova et al., 2011) that encode a diverse family of proteins such as nucleases involved in interaction with nucleic acids (Makarova et al., 2015). In particular, the DNA nuclease Cas9 may be programmed to modify and cleave any desired DNA sequence in bacteria (Jinek et al., 2012). *Staphylococcus aureus* and *Escherichia coli* were modified by Bikard et al. (2014) and Citorik et al. (2014) to cleave the resistance genes using plasmids that coded for Cas9 and sgRNAs. The findings prove that CRISPR Cas9 promotes the cytotoxicity of resistant bacteria in a sequence-specific way.

2 Principle of CRISPR Cas Antimicrobials

Antibiotic resistance is mainly mediated by mobile genetic elements (MGE) or plasmids, which are the principal means of spreading and accumulating resistance genes (also called resistant determinants) in bacteria and the environment. For example, antibiotic resistance is almost always through plasmid-mediated in emerging pathogens like ESBL *E. coli* and carbapenem-resistant *Klebsiella* spp. The other unique feature of plasmids is that they can co-transfer two different resistant genes among bacterial pathogens. Targeting these resistant genes in plasmids to induce mutations or sequence alterations may lead to disruption of their function. Through CRISPR Cas9, antibiotic resistance determinants can be targeted based on sequence-specific editing, which induces alternations or mutations, leading to cell death or plasmid removal. The mechanism is called plasmid curing. In case of bacteriophage-mediated CRISPR-Cas9 editing, it renders the resistant bacteria sensitive to antibiotics or CRISPR can selectively cause cell death of AMR pathogens.

3 Components of CRISPR-CAS

Short-Guide RNA The "single-guide RNA additionally" is referred to as sgRNA. It is also known as guide RNA or gRNA. The gRNA is a particular RNA sequence that identifies the target DNA region in the bacterial genome and drives Cas9 to that place editing. CRISPR RNA (crRNA), which is an 18–20 nucleotide sequence complementary to the target DNA, and a scaffold of tracr RNA, which acts as a binding site for the Cas9 nuclease, are the two key portions of the gRNA (Fig. 1). The efficiency of gRNA depends on the GC content of the guide sequence and should be 50–80%. High GC content will cause stabilization of the CRISPR-Cas9 complex. Ideally, the length of sgRNA should be 18–20 nucleotides that can be synthetically generated or made in vitro or in vivo from a DNA template. For the purpose of gRNA design optimization, there are additional core guidelines. Avoiding poly-T sequences, retaining a low amount of GC content, and adding a G immediately upstream of the PAM (i.e., a GNGG motif) are some examples in this context. The nucleotide region (10–12 bp) adjacent to the PAM, called the seed region, is crucial for CRISPR Cas9 activity as this region binds the DNA first following recognition of the PAM (Liu et al., 2016). Off-target effects of sgRNAs

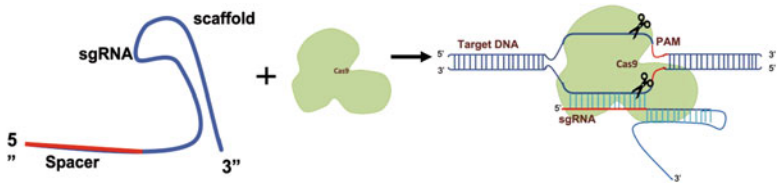


Fig. 1 Components of CRISPR Cas9 system mainly consist of sgRNA, scaffold, spacer, and Cas9, which forms complex at binds at PAM region on target DNA

are the most undesirable features in CRISPR Cas9 experiments and several sgRNA design tools have been designed to address this issue (Hiranniramol et al., 2020)

tracrRNA Trans-activating CRISPR RNA (tracrRNA) is a small trans-encoded RNA that helps with CRISPR RNA (crRNA) to form a complex and Emmanuelle Charpentier first isolated it from *Streptococcus pyogenes.*

crRNA Short-guide RNA is made up of two main components: CRISPR RNA (crRNA), which has a 17–20 nucleotide sequence complementary to the target DNA, and tracrRNA, which acts as a scaffold for Cas nuclease binding.

PAM PAM, which stands for protospacer adjacent motifs, is a brief (2–6 base pair) DNA region that comes after the target DNA sequence and is crucial for Cas nuclease cleavage. PAM has the form of the nucleotide sequence 5′-NGG-3′, where "N" can be any basic nucleotide and is followed by two guanine ("G") nucleobases.

Protospacer and Spacer Protospacer is DNA on the target DNA sequence and the spacer is RNA, which is a part of guide RNA base pairs with the target sequence.

Cas9 Cas9 is a 160 kD protein whose primary job is to cut DNA, which changes the biological genome. Cas9 is a dual RNA-guided DNA endonuclease enzyme connected to the CRISPR that was identified from *S. pyogenes* (Jinek et al., 2012). *S. pyogenes* uses Cas9 to cleave the foreign DNA, such as bacteriophage or plasmid DNA, and CRISPR to memorize foreign DNA. Cas9 conducts this inquiry by unwinding foreign DNA and searching for locations complementary to the short-guide RNA's 18–20 nucleotide spacer region (sgRNA).

Cas12a In 2015, another type of Cas protein called Cas12a was characterized by the bacterium *Francisella novicida* (Zetsche et al., 2015). It differs from Cas9 in a number of ways, such as the way that it cuts dsDNA; Cas9 produces "blunt" cuts in dsDNA. Additionally, efficient targeting of Cas12a requires only a crRNA, whereas Cas9 requires both a crRNA and a transactivating crRNA (tracrRNA). Cas12a is used in multiplexed genome editing in a much more target-specific manner as a result of these benefits. Compared with Cas9, which only cuts three base pairs upstream of the PAM site, Cas12a cleaves DNA 18–23 base pairs downstream from the PAM site, allowing for several rounds of DNA cleavage and indels that are repaired by the NHEJ pathway.

Cas13 The RNA-guided RNA endonuclease Cas13a from the bacteria *Leptotrichia shahii* was first identified in 2016. It only breaks single-stranded RNA and is directed by crRNA. Similar to other ssRNA molecules, Cas13 binds to the target and cleaves it (Abudayyeh et al., 2016)

dCas9 It is a mutant form of Cas9 that is deficient in endonuclease domains, leading to decreased activity. However, it can still bind to guide RNA and can be used in CRISPR interference (CRISPRi) and CRISPR activation (CRISPRa). In CRISPRi, dCas9 binds to its target DNA but does not cleave it. This mere binding will prevent the cell's transcription by inhibiting/activating the gene expression.

Off-Target Activity Because the gRNA cannot bind Cas9 at the intended genomic regions, Cas9 edits the target gene at undesirable locations.

On-Target Activity Due to gRNA selectivity, Cas9 cleaves at a desired place on the target genome.

Phagemids Plasmids that contain a phage origin of replication and can be packaged into replication-ineffective phage particles are known as phagemids.

3.1 How Does CRISPR Cas9 Editing Work?

CRISPR-based genome editing essentially required two basic components: a guide RNA and Cas9 endonuclease both forms as ribonucleoprotein complex (RNP). This complex can be delivered into the cells by means of different delivery vectors such as plasmids, phages, and nanoparticles.

The CRISPR-Cas9 system, which combines a sgRNA with the endonuclease Cas9, directs the enzyme to a target site that is complementary to the sgRNA, facilitating site-specific cleavage (Sander & Joung, 2014). The cell then uses either HR or NHEJ to repair the DSB that was produced by an enzyme (Figs. 2 and 3) (Iyama & Wilson III, 2013).

CRISPR-Cas9 is a powerful tool for editing genes in bacteria. It was explored to edit virulence genes and antibiotic-resistant genes to eliminate ESBL-producing *Escherichia coli* (Kim et al., 2016) depicted in Fig. 4. Numerous investigations revealed that the CRISPR-Cas system is cytotoxic, which can cause cell death due to the insertion of irreversible chromosomal lesions or INDELS, which result in NHEJ repair (Citorik et al., 2014; Gholizadeh et al., 2020).

Since 2013, other researchers have separately shown how CRISPR may be used to successfully modify genes that cause antibiotic resistance. Multiple drug-resistant microorganisms were altered utilizing the CRISPR/Cas9 technique. Some examples are; *Escherichia coli* (Jiang et al., 2013; Gomaa et al., 2014; Zerbini et al., 2017), *Bacillus subtilis* (Westbrook et al., 2016), kanamycin-resistant *S. aureus* in a mouse skin colonization model and MRSA *S. aureus* (Bikard et al., 2014), *Klebsiella pneumoniae* (Citorik et al., 2014), *Streptococcus pneumoniae* (Jiang et al., 2013), and *Salmonella enterica* (Gomaa et al., 2014). Zhang et al. (2017) developed a novel technique to edit bacterial genomes by employing CRISPR/Cas9 and an antibiotic resistance cassette (ARC). The transformants are chosen on plates containing antibiotics. Here, ARC is first injected close to the target locations. The Cas9 enzyme then proceeded to break the ARC sequence, and homology-directed repair was used

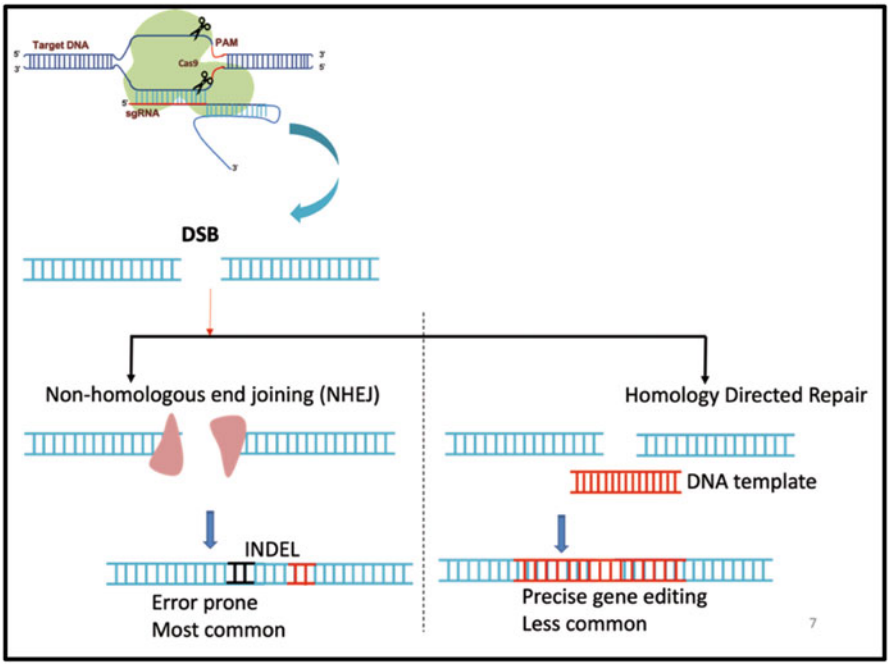


Fig. 2 Mechanism of CRISPR editing in bacteria and DNA repair

to replace it with any modified DNA sequences. This method did not depend on PAM sequences within the genome.

4 Experimental Steps Involved in CRISPR-Cas9-Mediated Editing of ESBL *E. coli*

The steps for plasmid-mediated CRISPR editing in *E. coli* are as follows.

Designing short-guide RNAs against the target genes that will be edited, cloning them into the CRISPR vector, delivering them by transformation into test and control isolates, and then validating the CRISPR edit by PCR and sequencing are the fundamental processes. When CRISPR is delivered using a plasmid, these steps are necessary. When sgRNA-Cas9 or ribonucleoprotein complexes are packed inside phages for phage-mediated delivery, the complexes disrupt the target gene upon entry. Depending on the method of gene editing, RNP complexes might develop inside or outside the target cells. It is also possible to encapsulate these RNP complexes in nanoparticles and deliver them to the cells.

The first and foremost requirement for CRISPR experiments is to identify and locate the target antibiotic-resistant genes on either bacterial plasmid or chromosome by using whole-genome sequence (WGS) data or partial gene sequence data. For detecting AMR genes in bacteria, at least 50 freely available bioinformatics tools are

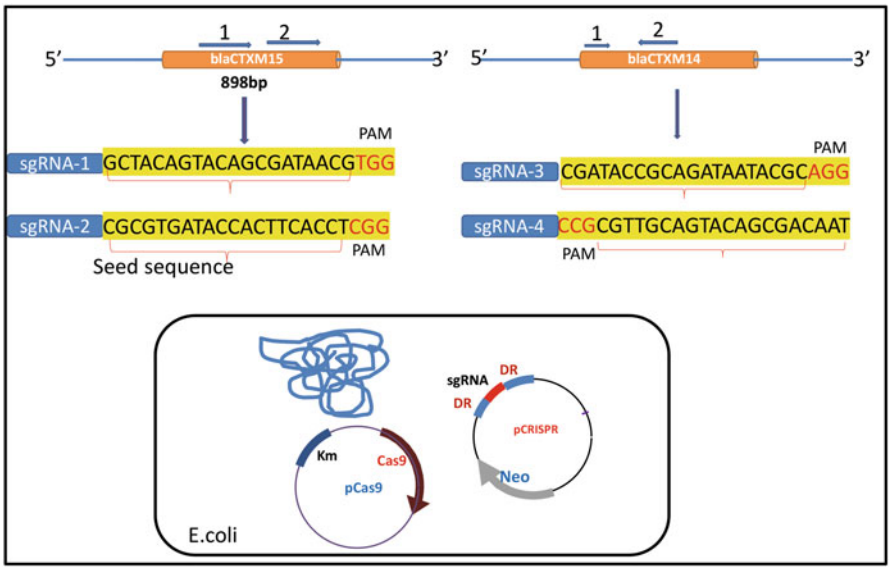


Fig. 3 Typically short-guide RNAs consist of 18–20 nucleotides adjacent to PAM regions. Multiple-guide RNAs can be designed and cloned into CRISPR plasmids and delivered into target cells

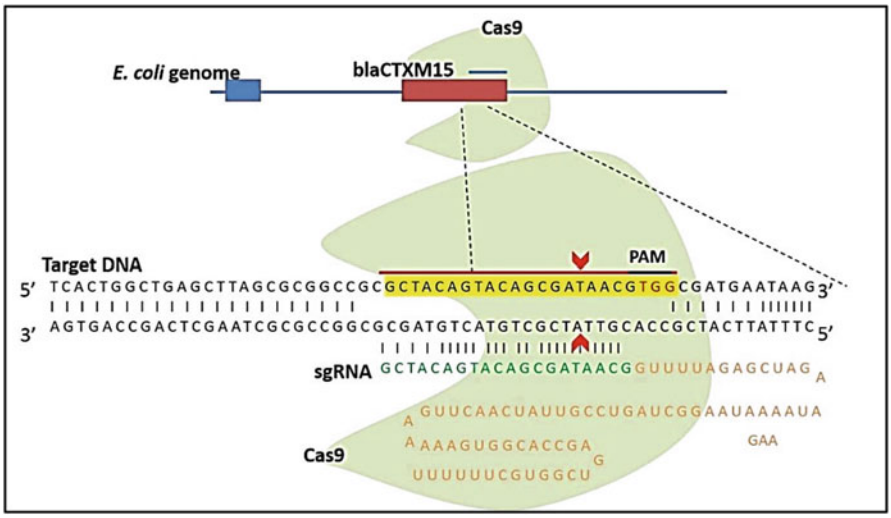


Fig. 4 CRISPR-Cas9 targeting *blaCTXM-15* in ESBL *E. coli*

available. ARG-ANNOT, CARD, MEGARes, KmerResistance, AMRFinder, SRST2, Genefinder, ARIBA, and ResFinder are just a few examples. A few of them are briefly explained here:

- CLC Workbench (https://www.qiagenbioinformatics.com/products/clc-main-workbench/) (paid): CLC Microbial Genomics Module 4.0 (or later) can be used to identify AMR markers on the genomes of pathogenic bacteria as well as point mutations in genome.
- CGE – Centre of Genomic Epidemiology (https://cge.cbs.dtu.dk/services/) (free). The plasmid finder and Res finder tools can be used to identify antibiotic resistance gene sequences from whole-genome sequence data of drug-resistant bacteria.
- CARD (Comprehensive Antibiotic Resistance Database) (https://card.mcmaster.ca/analyze/rgi) (free). Resistance Gene Identifier (RGI) can be used to predict resistomes from protein or nucleotide sequence data of bacteria
- VR profile (http://bioinfo-mml.sjtu.edu.cn/VRprofile/index.php) (free): The VRprofile website was designed to find extended mobilome-related gene clusters in newly sequenced bacterial genomes as well as virulence and/or antibiotic resistance genes in bacteria.
- ICE finder or ICEberg (http://202.120.12.136:7913/ICEberg2/index.php) (free): This is particularly helpful in identifying acquired resistance (AR) (acquired antibiotic resistance determinants) in bacteria.

After selecting desired antibiotic-resistant genes to be edited, the following essential steps are involved in CRISPR Cas9-mediated editing of target genes:

(a) *sgRNA Design*

Numerous bioinformatic techniques and a thorough evaluation are available for the construction of short-guide RNA (Cui et al., 2018; Wilson et al., 2018). To construct effective sgRNA, it is crucial to choose the right tool. Below are a few instances:

- CRISPOR
- CHOPCHOP
- Cas-OFFinder
- E-CRISP
- Benchling
- Synthego

For instance, the Harvard University-developed CHOPCHOP program (http://chopchop.cbu.uib.no/) offers more than 200 whole-genome sequence datasets of prokaryotes and eukaryotes with properties like PAM sites, efficiency score, off targets, and estimated number of mismatches. Designing an appropriate gRNA should be based on these traits.

(b) *Cloning of sgRNA into pCRISPR Vector with pCAS9*

It is possible to clone sgRNAs in CRISPR plasmids using a variety of procedures that have been improved by numerous investigations (Jiang et al., 2013). There are several processes involved, including the isolation of genomic DNA, restriction digestion of CRISPR DNA with BsaI, separation, and elution. Prior to transformation into DH5 cells and test isolates, primers for cloning and

ligation of short-guide RNAs should be developed to be complementary to one another.

(c) *Delivery*: Several studies utilized the following four main delivery vehicles/strategies to transport CRISPR Cas components into the target cells to get desired effects. These are mainly
- Plasmid-mediated
- Bacteriophage-mediated
- sgRNA-Cas9 protein complex delivery
- Nanoparticles

Bacteriophage-Mediated Delivery of CRISPR The most successful means of introducing CRISPR Cas into bacterial cells are phages. Here, bacteriophage particles are used to transport the CRISPR-sgRNA construct along with Cas9, producing targeted and effective antimicrobial effects against bacteria that contain plasmid or chromosomal target sequences (Fig. 5). CRISPR constructs delivered by bacteriophages affect the target cell in a sequence-dependent way. Target sequences that are on chromosomes have a cytotoxic effect; those that are episomal or in the cytoplasm result in cell death or plasmid loss. Phage administration of CRISPR Cas

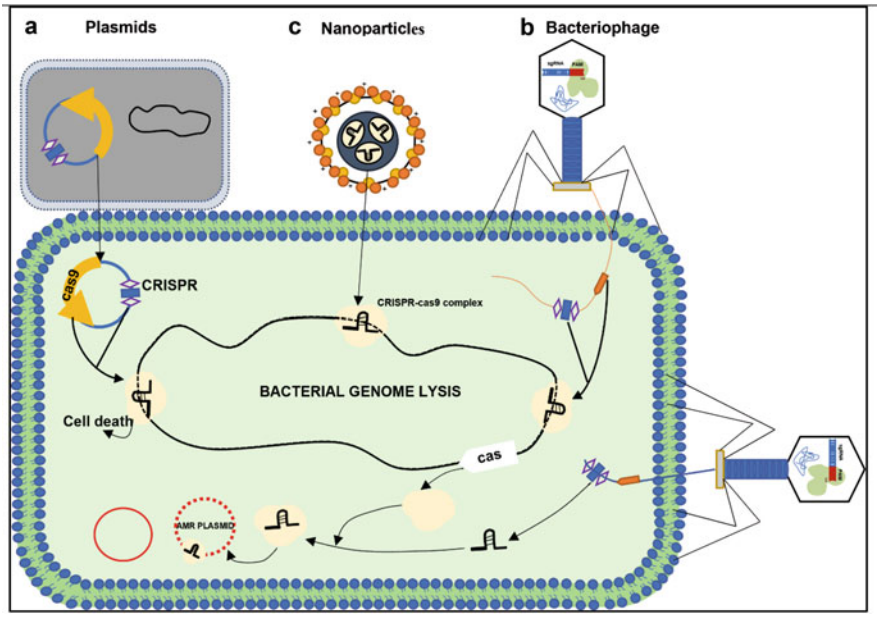


Fig. 5 Delivery vehicles of CRISPR-Cas antimicrobials. (**a**) To selectively damage the bacterial chromosomal genome and destroy or cure the AMR plasmid, bacteriophages use expressed CRISPR Cas. This kills or resensitizes the AMR pathogens. (**b**) Bacterial genomes are destroyed or plasmids are cured when CRISPR Cas9 are injected into the target bacterial cells via a plasmid. (**c**) Nanoparticle-based delivery of CRISPR Cas9 complexes directly into the bacterial cells is also an effective method to destroy the AMR pathogens

antimicrobials to eradicate AMR bacteria or delete drug-resistant plasmids has been proven effective in a number of investigations. In order to target the methicillin-resistance gene mecA in virulent strains of *S. aureus*, Bikard et al. (2014) constructed ΦNM1 CRISPR Cas9 in NM1 phage. This resulted in a significant decrease in mecA-carrying *S. aureus* in mixed cultures. A temperate and lytic phage designed by Yosef et al. (2015) was used to resensitize bacteria to β-lactam drugs.

Bacteriophages have huge genome sizes, thus adding CRISPR-Cas components to a phage genome could make it more difficult to replicate and assemble the phage (Hua et al., 2017). A unique phage is needed to treat a specific infection because of its limited host range, which is another limiting aspect of phage-mediated delivery. Phage delivery raises safety concerns for the transmission of virulence factor genes since it may transmit both the CRISPR-Cas machinery and chromosomal segments from the host (Penadés et al., 2015).

Plasmid-Mediated Delivery The delivery of CRISPR Cas9 into the target cells for editing desired sequences in drug-resistant bacteria can be accomplished via conjugative plasmids, which can transmit genetic material between bacteria cells (Fig. 5). Here, receptor-mediated attachment is absent unlike in phage-mediated delivery. The advantage here is that resistance due to mutations in the receptors in phage–host uptake will not occur. However, narrow host range and low-delivery efficiency are a few challenges encountered in plasmid-mediated delivery (Pursey et al., 2018). The dissemination of resistance genes is facilitated by conjugation, a crucial method of bacterial gene transfer (Harriso & Brockhurst, 2012; Köstlbacher et al., 2021). Removing carbapenemase resistance genes like blaNDM and blaKPC, resensitizing the resistant bacteria to carbapenems, and having positive therapeutic effects on clinical isolation of carbapenem-resistant Enterobacteriaceae are all possible with the introduction of a plasmid vector containing the pCasCure system into carbapenem-resistant Enterobacteriaceae (Kang et al., 2017b). The CRISPR-Cas system can be efficiently transferred to *E. coli* using the targeted antimicrobial plasmids (TAPs), which can also be used to resensitize recipient cells containing the pOXA48 gene and prevent the spread of drug resistance (Reuter et al., 2021).

Nanoparticles or Nanocomplexes With the development of nanotechnology, it is now possible to deliver Cas proteins and CRISPR directly into target cells using a range of nanoparticle types, such as cationic polymer-based, inorganic, and gold nanoparticles (Rahimi et al., 2020). It was shown that the methicillin-resistant gene may be successfully disrupted in MRSA in vitro by adding a cationic polymer-based nanosized CRISPR complex (Kang et al., 2017a, b). However, nanoparticles-based CRISPR-Cas delivery is still in its infancy stage because to difficulties with increasing encapsulation rate and efficient delivery into complex pathogens like *Mycobacterium tuberculosis*, which has exceptionally thick and highly impenetrable cell walls (Chiaradia et al., 2017).

Validation of CRISPR-Edited Cells This is a crucial step to relook at the precise genome efficiency of gRNA constructs, Cas9 delivery, transformation, etc. CRISPR

genome editing results in mixed cell populations, with only a small subset have desired gene edits. To determine which cells have the desired CRISPR knockout or targeted mutation, number of assays available such as PCR, cleavage assays, Sanger's sequencing, and, most importantly, NGS. Here, quick assessment is needed whether CRISPR has edited a significant number of the cells or not. A mismatch cleavage experiment is used to evaluate this for indels. This is typically looked into for HDR as a shift in the restriction pattern at the point of interest. A smaller PCR result can be seen when deletions are present. We can verify intended genome modifications, including insertion, deletion, and mutation events, after producing the desired gRNA construct and delivering Cas9 into target cells.

Cells that have both a gRNA and Cas9 introduced become mixed cell types. Depending on the kind of cell, these cells experience double-strand breaks (DSBs), which are then repaired by NHEJ or homology-directed repair (HDR). This can be detected by using PCR with primers flanking at the insertion/deletion site. In case of plasmid transformation, colonies can be picked from selective antibiotic agar plates and colony PCR can be performed to confirm sgRNA insertion.

PCR, coupled with restriction digestion, is the best method to confirm positive clones in mixed cell populations. Using CRISPR internal primers, LB agar plates with positive colonies can be further sequenced by Sanger's sequencing. The sgRNA sequence in CRISPR can be confirmed using online web tools (e.g., Synthego) specifically developed to validate the cloning of sgRNAs into CRISPR plasmid. If fluorescent protein markers are present on plasmids, FACS can be employed to enrich the cells that received Cas9 and sgRNA. Protein expression via Western blot can also be used as a further form of validation.Next-generation sequencing (NGS) is a powerful tool to validate edits (quantitatively) and to simultaneously detect off-targets in edited cells. NGS is the best option if many samples are to be validated and simultaneously look at off-target changes. Amplicon sequencing by NGS can identify and quantify the insertions and deletions that result from NHEJ in bacteria following CRISPR-induced DSB and is considered superior to all other methods for CRISPR validation. Table 1 describes the different methods of CRISPR validation.

Table 1 Various validation methods of CRISPR-mediated gene editing

Type of change on target DNA	Validation assay	Qualitative/ quantitative	Scale
Deletions	PCR	Quantitative	Low to high throughput
Insertions	RFLP-PCR	Quantitative	Low to high throughput
Insertions and deletions (INDELS) and single mismatches	Mismatch cleavage assay	Semi-quantitative	Low to high throughput
INDELS, mismatches	Sanger's sequencing	Qualitative and quantitative	Low
INDELS, all edits, off targets	Next-generation sequencing	Qualitative and quantitative	Low to high throughput

5 Approaches of CRISPR Cas-Mediated Editing of AMR Pathogens

CRISPR can be used as an antimicrobial with two approaches: pathogen-focused and gene-focused, depending on where the target gene is located. A pathogen-focused method focuses on particular sequence sections of the bacterial chromosome. Due to its cytotoxic effect on the entire cell, this strategy causes bacterial cell death. A plasmid carrying one or more drug resistance genes is targeted in the gene-based strategy, which results in the elimination of the plasmid and resensitization of the bacterial population to antibiotics. Since antibiotic resistance genes are frequently migratory and can spread between different bacterial species, eliminating AMR genes from any host should be the primary goal of CRISPR-based editing. Here, the efficiency of gene editing is limited due to the number of off-targets on target DNA sequence and poor DNA repair mechanism in bacteria (NHEJ).

To overcome these setbacks, another efficient method of CRISPR Cas9 coupled with recombineering has been considered one of the best approaches for bacterial genome editing tested successfully in *E. coli* (Ronda et al., 2016). Briefly stated, the strain that needs to be modified is first genetically modified to express the Cas9 nuclease and the λ Red machinery, and then it is co-transformed with (i) a CRISPR plasmid encoding the guide RNA, which anneals with the chromosomal region that needs to be modified and promotes a site-specific DNA cleavage by the Cas9, and (ii) a donor DNA, which promotes the DSB repair λ Red-mediated homologous recombination (HR), thereby introducing the desired and efficient mutation. The presence of the λ Red machinery plays an important role and increases the efficiency of editing.

Recent studies have shown that CRISPR Cas13a-based antibiotics exceed CRISPR Cas9-based antibiotics at killing carbapenem-resistant *E. coli* and methicillin-resistant *S. aureus* (Kiga et al., 2020). Irrespective of the location of their target genes, they exhibit robust antibacterial activity. When the Cas13a protein and a crRNA targeting the carbapenem-resistant gene blaIMP-1 were introduced in vitro, the quantity of recovered bacterial cells carrying the resistance gene on the chromosome or plasmid was reduced by 2–3 logs. When the blaIMP-1 gene is only found on the chromosome, the introduction of Cas9 and a crRNA result in a 3-log reduction in the number of bacterial cells (Kiga et al., 2020).

6 Challenges and Limitations of the CRISPR Cas Antimicrobials

Key questions raised about the CRISPR-based antimicrobials are many. For example, how long is CRISPR Cas going to edit inside the cells? Can it can be reprogrammed? Stopped? Or removed from bacterial cells? if editing is taking place, is it causing cell death of cells with desired genes or randomly killing all the microbial community? What are the translational methods to develop new-generation CRISPR antimicrobials and what are the different delivery strategies

one can think of for effective delivery of CRISPR systems into bacteria? This robust technology provides new opportunities to eradicate drug-resistant superbugs; however, there are hundreds of mutations in the sequences of AMR pathogens, and a single or few CRISPR sequences may not be sufficient to target all the mutations. There are a few limitations and challenges discussed here.

The Delivery Vectors Although CRISPR can be delivered by plasmids, phages, phagemids, or nanocomplexes in a variety of optimized methods, however, these vehicles have their own limitations. Most phage species have a narrow host range, which limits them from targeting multiple species of bacteria. Phage-mediated delivery of CRISPR Cas9 approach is also of limited effect in spatially complex bacterial communities, including pathogenic and commensals. To overcome these challenges, few studies proved using engineered phages in terms of expanding their host range, etc. (Citorik et al., 2014; Pursey et al., 2018). In case of plasmid-mediated delivery also, similar restrictions like narrow host range, uptake, and their establishment into the bacterial cells are major drawbacks.

Resistance Against CRISPR-Cas: In recent studies, few colonies were able to survive by avoiding genome targeting the studies carried out by Citorik et al. (2014) and Gomaa et al. (2014). It was suggested that this was caused by the formation of resistance against CRISPR Cas antimicrobials in the escaping colonies, which may have resulted from spontaneous mutations in the Cas genes or the target sequences. Other researchers also found that the target host genomes had anti-CRISPR (Acr) genes and that the expression and activity of the Cas protein were suppressed (Yan et al., 2021).

Small proteins called Acrs can disable CRISPR Cas by binding with its essential parts, such as sgRNA, Cas, or crRNA. Prokaryotes include a wide variety of proteins that can render nearly all CRISPR Cas system types inactive (Marino et al., 2020; Davidson et al., 2020). According to an analysis of 600 drug-resistant *P. aeruginosa* genomes, more than 30% of them possessed at least one acr gene, which could restrict the antibacterial effect of CRISPR-Cas systems (van Belkum et al., 2015).

Mutations in the Cas genes were discovered by sequencing escaped/unedited colonies from CRISPR Cas-mediated genome targeting (Xu et al., 2021). Resistance to CRISPR may also be brought on by mutations in the bacterial chromosomal sequence or widely distributed variants of AMR genes (for instance, $>$150 variants of ESBLs). While developing CRISPR Cas antimicrobials, quick and robust detection of specific AMR genes is necessary to ensure the exact targeting and effective destruction of AMR genes (Cui et al., 2020).

Intracellular Pathogens Intracellular pathogens, such as *Mycobacterium tuberculosis, Salmonella enterica*, and *Burkholderia* spp., replicate within the cells and are capable of escaping from CRISPR Cas-mediated action due to their cell membrane thickness and permeability of host cells. Selecting suitable delivery vehicles such as liposomes containing phages and avirulent bacterial strains that can transport

CRISPR into intracellular pathogens through eukaryotic plasma membranes may yield successful results (Duan et al., 2021; Yan et al., 2021).

7 Applications of CRISPR Cas Antimicrobials

The synthesis of cell walls, DNA replication, or protein synthesis is among the key processes in bacteria that are the targets of numerous antibiotics currently in use. Therefore, their mechanism of killing is generic in nature, leading to the removal of both pathogenic and commensal bacteria. The overuse or underuse of antibiotics in the medical and veterinary fields has contributed to the creation of multidrug-resistant (MDR) bacteria. Currently, there are no medications that can target virulent or antibiotic-resistant bacteria. Additionally, the current antibiotics lack specificity and contribute to infections linked to antibiotic use. To avoid these issues and eliminate the superbugs in a sequence-specific way, innovative therapies like CRISPR antimicrobials are urgently and critically needed (Beisel et al., 2014; Palacios Araya et al., 2021).

The potential advantages of CRISPR antimicrobials over traditional ones are many and are evident from more than 2000 research publications that proved their efficacy over the last decade. Since most bacteria lack a functional non-homologous end-joining system, as was mentioned above, a double-strand break in the genome caused by CRISPR-Cas9 is lethal in nature. A plasmid is lost when this DSB takes place, which may potentially result in cell death. CRISPR also enables CRISPR antimicrobials to target specific sequences in a single virulent or drug-resistant bacterial species, or even an antibiotic resistance gene, with a higher degree of precision. Importantly, the presence of normal microflora helps the niche recolonize, minimizing the likelihood of opportunistic infection with organisms like *Clostridium difficile*.

Recently, Citorik et al. (2014) and Bikard et al. (2014) created plasmid-based CRISPR antibiotics. Citorik et al. (2014) used plasmid and phagemid delivery systems to specifically target beta-lactam and quinolone resistance genes in *E. coli*. Even though a high-copy plasmid had this resistance gene encoded, these CRISPR antimicrobials were able to successfully resensitize a population of β-lactam-resistant bacteria to the antibiotic. CRISPR phagemids were discovered to be deadly exclusively for resistant bacteria in the case of quinolone resistance, which is mediated by DNA gyrase. In a mouse skin colonization model, Bikard et al. (2014) used the phagemid system to specifically target virulent *S. aureus*. They discovered that this reduced the proportion of virulent *S. aureus* from 50% to 11% in under 24 hours. A relatively large number of phagemids would be required for an in vivo therapy to effectively remove resistant bacteria because they do not have a high rate of replication. Otherwise, a tiny pool of these bacteria will reappear and spread the infection.

Kim et al. (2016) used the CRISPR Cas9 approach to specifically kill *E. coli* that produces the extended-spectrum beta-lactamase (ESBL) by regaining its antibiotic sensitivity. In this study, over 1000 SHV and TEM-type ESBL mutants were examined for conserved target sequences. These target sequences were then used

to resensitize ESBL cells to antibiotics. There are several ESBL gene variants; therefore, focusing on a particular mutant will have limited clinical benefit. These promising precision antibiotics have already been created by a number of synthetic biology businesses around the world, including Locus Biosciences, Intellia Therapeutics, and Eligo Bioscience. These, though, are still in the preclinical stages. To treat bacterial infections, Locus Biosciences has created phage-mediated CRISPR therapies (crPhage™).

More research is needed to evaluate the therapeutic efficacy of CRISPR antimicrobials and put them into clinical practice in both human and veterinary medicine. Few in vivo infection investigations have been carried out to evaluate the effectiveness of CRISPR-based antibiotics. For instance, a study utilizing a mouse skin colonization model revealed that using CRISPR/Cas9 to target *S. aureus* significantly reduced *S. aureus* skin colonization when compared to alternative treatment methods (Citorik et al., 2014). In another study, treating *Galleria mellonella* infected with enterohemorrhagic *E. coli* with carbenicillin is superior to treating it with a CRISPR antibacterial (Bikard et al., 2014). The development of a delivery vector with a wide host range and the deployment of a multiplex strategy employing CRISPR-Cas to target several sequences to lessen the possibility of resistance are problems that should be the focus of future research (Pursey et al., 2018)

8 Conclusions and Prospects

In terms of delivery methods and editing effectiveness, CRISPR Cas antimicrobials confront enormous difficulties. They may be superior to traditional antimicrobials once these difficulties are overcome. They may turn out to be cutting-edge antibiotics for the management of intricate microbial ecosystems in the future. In order to stop the spread of AMR diseases, they can also be effective at differentiating between harmful and helpful bacteria. Only a few in vivo studies of this kind have been done, and more need to be done, in order to demonstrate this. When combined with an effective delivery method, CRISPR Cas9 antimicrobials allow selective elimination of MDR microorganisms.

Additionally, the multiplex functionality of CRISPR-Cas systems could be used to simultaneously target several species while achieving multiple sequences of the same organism to stop the emergence of resistant strains. The CRISPR Cas9 technology has the potential to significantly alter the microbiome in humans, animals, and the environment. It can be programmed to be included in disinfectants, topical ointments, and oral formulations with appropriate delivery mechanisms. Topical formulations containing CRISPR molecules can be developed in veterinary medicine to treat skin infections or mastitis in dairy cattle caused by MDR bacteria. CRISPR-edited mutants can be incorporated into probiotics to selectively target drug-resistant bacteria in the gut while leaving normal microflora alone.

Legislative and social challenges associated with CRISPR should be considered when implementing this technology as gene editing methods should be strictly regulated with clear guidelines for safe use. CRISPR sequences are also found naturally in plasmids/bacterial chromosomes and should be used with caution in

the environment because they can cause significant environmental and public health problems. Nonetheless, reaping the benefits of CRISPR antimicrobials should be the long-term goal in addressing the emerging problem of AMR in both human and veterinary medicine.

References

Abudayyeh, O. O., Gootenberg, J. S., Konermann, S., Joung, J., Slaymaker, I. M., & Cox, D. B. (2016). C2c2 is a single-component programmable RNA-guided RNA-targeting CRISPR effector. *Science, 353*(6299), aaf5573.

Barangou, R., & Ousterout, D. G. (2017). Repurposing CRISPR-Cas systems as DNA-based smart antimicrobials. *Cell & Gene Therapy Insights, 3*, 63–72.

Barangou, R., Fremaux, C., Deveau, H., Richards, M., Boyaval, P., Moineau, S., et al. (2007). CRISPR provides acquired resistance against viruses in prokaryotes. *Science, 315*(5819), 1709–1712.

Beisel, C. L., Gomaa, A. A., & Barrangou, R. (2014). A CRISPR design for next-generation antimicrobials. *Genome Biology, 15*(11), 516.

Bikard, D., Euler, C. W., Jiang, W., Nussenzweig, P. M., Goldberg, G. W., Duportet, X., et al. (2014). Exploiting CRISPR-Cas nucleases to produce sequence-specific antimicrobials. *Nature Biotechnology, 32*(11), 1146–1150.

Chiaradia, L., Lefebvre, C., Parra, J., Marcoux, J., Burlet-Schiltz, O., Etienne, G., et al. (2017). Dissecting the mycobacterial cell envelope and defining the composition of the native mycomembrane. *Scientific Reports, 7*(1), 1–12.

Citorik, R. J., Mimee, M., & Lu, T. K. (2014). Sequence-specific antimicrobials using efficiently delivered RNA-guided nucleases. *Nature Biotechnology, 32*(11), 1141–1145.

Cui, Y., Xu, J., Cheng, M., Liao, X., & Peng, S. (2018). Review of CRISPR/Cas9 sgRNA design tools. *Interdisciplinary Sciences, Computational Life Sciences, 10*(2), 455–465.

Cui, Z. H., Zheng, Z. J., Tang, T., Zhong, Z. X., Cui, C. Y., Lian, X. L., et al. (2020). Rapid detection of high-level Tigecycline resistance in Tet(X)-producing *Escherichia coli* and Acinetobacter spp. based on MALDI-TOF MS. *Frontiers in Cellular and Infection Microbiology, 10*, 583341.

Duan, C., Cao, H., Zhang, L. H., & Xu, Z. (2021). Harnessing the CRISPR-Cas systems to combat antimicrobial resistance. *Frontiers in Microbiology, 12*, 716064.

Gholizadeh, P., Köse, Ş., Dao, S., Ganbarov, K., Tanomand, A., Dal, T., et al. (2020). How CRISPR-Cas system could be used to combat antimicrobial resistance. *Infection and Drug Resistance, 13*, 1111.

Gomaa, A. A., Klumpe, H. E., Luo, M. L., Selle, K., Barrangou, R., & Beisel, C. L. (2014). Programmable removal of bacterial strains by use of genome-targeting CRISPR-Cas systems. *MBio, 5*(1), e00928.

Harriso, E., & Brockhurst, M. A. (2012). Plasmid-mediated horizontal gene transfer is a coevolutionary process. *Trends Microbiology, 20*(6), 262–267.

Hiranniramol, K., Chen, Y., Liu, W., & Wang, X. (2020). Generalizable sgRNA design for improved CRISPR/Cas9 editing efficiency. *Bioinformatics, 36*(9), 2684–2689.

Horvath, P., & Barrangou, R. (2010). CRISPR/Cas, the immune system of bacteria and archaea. *Science, 327*, 167–170.

Hua, Y., Wang, C., Huang, J., & Wang, K. (2017). A simple and efficient method for CRISPR/Cas9-induced mutant screening. *Journal of Genetics and Genomics, 44*(4), 207–213.

Ishino, Y., Shinagawa, H., Makino, K., Amemura, M., & Nakata, A. (1987). Nucleotide sequence of the iap gene, responsible for alkaline phosphatase isozyme conversion in Escherichia coli, and identification of the gene product. *Journal of Bacteriology, 169*, 5429–5433.

Iyama, T., & Wilson, D. M., III. (2013). DNA repair mechanisms in dividing and non-dividing cells. *DNA Repair, 12*(8), 620–636.

Jiang, W., Bikard, D., Cox, D., Zhang, F., & Marraffini, L. A. (2013). RNA-guided editing of bacterial genomes using CRISPR-Cas systems. *Nature Biotechnology, 31*(3), 233–239.

Jinek, M., Chylinski, K., Fonfara, I., Hauer, M., Doudna, J. A., & Charpentier, E. (2012). A programmable dual-RNA-guided DNA endonuclease in adaptive bacterial immunity. *Science, 337*, 816–821.

Kang, T., Li, F., Baik, S., Shao, W., Ling, D., & Hyeon, T. (2017a). Surface design of magnetic nanoparticles for stimuli-responsive cancer imaging and therapy. *Biomaterials, 136*, 98–114.

Kang, Y. K., Kwon, K., Ryu, J. S., et al. (2017b). Nonviral genome editing based on a polymer-derivatized CRISPR nanocomplex for targeting bacterial pathogens and antibiotic resistance. *Bioconjugate Chemistry, 28*(4), 957–967.

Kiga, K., Tan, X. E., Ibarra-Chávez, R., Watanabe, S., Aiba, Y., Sato'o, Y., et al. (2020). Development of CRISPR-Cas13a-based antimicrobials capable of sequence-specific killing of target bacteria. *Nature Communications, 11*(1), 1–11.

Kim, J. S., Cho, D. H., Park, M., Chung, W. J., Shin, D., Ko, K. S., & Kweon, D. H. (2016). CRISPR/Cas9-mediated re-sensitization of antibiotic-resistant Escherichia coli harboring extended-spectrum beta-lactamases. *Journal of Microbiololgy and Biotechnology, 26*(2), 394–401.

Köstlbacher, S., Collingro, A., Halter, T., Domman, D., & Horn, M. (2021). Coevolving plasmids drive gene flow and genome plasticity in host-associated intracellular bacteria. *Current Biology, 31*(2), 346–357.

Liu, X., Homma, A., Sayadi, J., Yang, S., Ohashi, J., & Takumi, T. (2016). Sequence features associated with the cleavage efficiency of CRISPR/Cas9 system. *Scientific Reports, 6*, 19675.

Makarova, K. S., Haft, D. H., Barrangou, R., Brouns, S. J., Charpentier, E., et al. (2011). Evolution and classification of the CRISPR/Cas systems. *Nature Reviews Microbiology, 9*, 467–477.

Makarova, K. S., Wolf, Y. I., Alkhnbashi, O., Costa, F., Shah, S., et al. (2015). An updated evolutionary classification scheme for CRISPR-Cas systems. *Nature Reviews Microbiology, 13*, 722–736.

Palacios Araya, D., Palmer, K. L., & Duerkop, B. A. (2021). CRISPR-based antimicrobials to obstruct antibiotic-resistant and pathogenic bacteria. *PLoS Pathogens, 17*(7), e1009672.

Penadés, J. R., Chen, J., Quiles-Puchalt, N., Carpena, N., & Novick, R. P. (2015). Bacteriophage-mediated spread of bacterial virulence genes. *Current Opinion in Microbiology, 23*, 171–178.

Pursey, E., Sünderhauf, D., Gaze, W. H., Westra, E. R., & van Houte, S. (2018). CRISPR-Cas antimicrobials: Challenges and future prospects. *PLoS Pathogens, 14*(6), e1006990.

Rahimi, H., Salehiabar, M., Charmi, J., Barsbay, M., Ghaffarlou, M., Razlighi, M. R., & Webster, T. J. (2020). Harnessing nanoparticles for the efficient delivery of the CRISPR/Cas9 system. *Nano Today, 34*, 100895.

Reuter, A., Hilpert, C., Dedieu-Berne, A., et al. (2021). Targeted-antibacterial-plasmids (TAPs) combining conjugation and CRISPR/Cas systems achieve strain-specific antibacterial activity. *Nucleic Acids Research, 49*(6), 3584–3598.

Rodrigues, M., McBride, S., Hullahalli, K., Palmer, K., & Duerkop, B. (2019). Conjugative delivery of CRISPR-Cas9 for the selective depletion of antibiotic-resistant enterococci. *Antimicrobial Agents and Chemotherapy, 63*(11), e01454–e01419.

Ronda, C., Pedersen, L. E., Sommer, M. O., & Nielsen, A. T. (2016). CRMAGE: CRISPR optimized mage recombineering. *Scientific Reports, 6*(1), 1–11.

Sander, J. D., & Joung, J. K. (2014). CRISPR-Cas systems for editing, regulating and targeting genomes. *Nature Biotechnology, 32*(4), 347–355.

Shibata, M., Nishimasu, H., Kodera, N., Hirano, S., Ando, T., Uchihashi, T., & Nureki, O. (2017). Real-space and real-time dynamics of CRISPR-Cas9 visualized by high-speed atomic force microscopy. *Nature Communications, 8*(1), 1–9.

Sorek, R., Lawrence, C. M., & Wiedenheft, B. (2013). CRISPR-mediated adaptive immune systems in bacteria and archaea. *Annual Review of Biochemistry, 82*(1), 237–266.

Van Belkum, A., Soriaga, L. B., LaFave, M. C., Akella, S., Veyrieras, J. B., Barbu, E. M., et al. (2015). Phylogenetic distribution of CRISPR-Cas systems in antibiotic-resistant *Pseudomonas aeruginosa*. *MBio, 6*(6), e01796–e01715.

Van der Oost, J., Westra, E. R., Jackson, R. N., & Wiedenheft, B. (2014). Unraveling the structural and mechanistic basis of CRISPR-Cas systems. *Nature Reviews Microbiology, 12*, 479–492.

Westbrook, A. W., Moo-Young, M., & Chou, C. P. (2016). Development of a CRISPR-Cas9 tool kit for comprehensive engineering of *Bacillus subtilis*. *Applied and Environmental Microbiology, 82*(16), 4876–4895.

Wilson, L. O., O'Brien, A. R., & Bauer, D. C. (2018). The current state and future of CRISPR-Cas9 gRNA design tools. *Frontiers in Pharmacology, 9*, 749.

Xu, Z., Li, Y., Cao, H., Si, M., Zhang, G., Woo, P. C., & Yan, A. (2021). A transferrable and integrative type IF Cascade for heterologous genome editing and transcription modulation. *Nucleic Acids Research, 49*(16), e94–e94.

Yan, J., Kang, D. D., & Dong, Y. (2021). Harnessing lipid nanoparticles for efficient CRISPR delivery. *Biomaterials Science, 9*(18), 6001–6011.

Yosef, I., Manor, M., Kiro, R., & Qimron, U. (2015). Temperate and lytic bacteriophages programmed to sensitize and kill antibiotic-resistant bacteria. *Proceedings of the National Academy of Sciences, 112*(23), 7267–7272.

Zerbini, F., Zanella, I., Fraccascia, D., König, E., Irene, C., Frattini, L. F., et al. (2017). Large scale validation of an efficient CRISPR/Cas-based multi gene editing protocol in *Escherichia coli*. *Microbial Cell Factories, 16*(1), 68.

Zetsche, B., Gootenberg, J. S., Abudayyeh, O. O., Slaymaker, I. M., Makarova, K. S., Essletzbichler, P., Volz, S. E., Joung, J., van der Oost, J., Regev, A., Koonin, E. V., & Zhang, F. (2015). Cpf1 is a single RNA-guided endonuclease of a class 2 CRISPR-Cas system. *Cell, 163*(3), 759–771.

Zhang, H., Cheng, Q. X., Liu, A. M., Zhao, G. P., & Wang, J. (2017). A novel and efficient method for bacteria genome editing employing both CRISPR/Cas9 and an antibiotic resistance cassette. *Frontiers in Microbiology, 8*, 812.

Economic Impact of Antimicrobial Resistance and Projected Future Trends

Ranjit Kumar Nadella, Ezhil S. Nilavan, and Mukteswar Prasad Mothadaka

Contents

Abstract

The application of antimicrobials for treating infectious diseases in humans has been carried out since the twentieth century. However, recently there has been a huge surge in the development of resistance by the pathogenic microorganisms toward these antibiotics. This has adversely affected the treatment process and the cost of treatment has also gone up significantly. The impact of resistance on economics can be viewed from different viewpoints as described in this chapter. Multiple factors drive the antibiotic resistance dissemination in the microorganism

R. K. Nadella (✉)
MFB Division, ICAR-Central Institute of Fisheries Technology, Willingdon Island, Cochin, Kerala, India
e-mail: nranjeetkumar@gmail.com; Ranjit.Nadella@icar.gov.in

E. S. Nilavan
Microbiology, Fermentation and Biotechnology, ICAR-Central Institute of Fisheries Technology (ICAR-CIFT), Cochin, Kerala, India
e-mail: theezhilnilavan@gmail.com; Ezhil.Nilavan@icar.gov.in

M. P. Mothadaka
Microbiology, Fermentation and Biotechnology Division, ICAR-Central Institute of Fisheries Technology (ICAR-CIFT), Cochin, Kerala, India
e-mail: prasadmm@hotmail.com; Mm.Prasad@icar.gov.in

M. P. Mothadaka et al. (eds.), *Handbook on Antimicrobial Resistance*,
https://doi.org/10.1007/978-981-19-9279-7_45

of different ecosystems. Several strategies have been devised and used effectively to mitigate the burden of antibiotic resistance from economic perspective. This chapter briefly deals with the economic impact of antibiotic resistance, its assessment, and different measures to be taken to minimize the effect of antibiotic resistance from economic point of view. In addition, it also emphasizes the future trends of antibiotic resistance and the possible mitigation measures.

Keywords

Antibiotic resistance · Economic impact · Impact Assessment · Future trends · Control measures

1 Introduction

Antimicrobials are the drugs used to treat infectious diseases caused by organisms, that is, bacteria, viruses, and fungi. Inappropriate use of these chemical agents may result in the development of resistance, called antimicrobial resistance (AMR), and the organisms are called AMR organisms (Founou et al., 2017). In this situation, AMR has become one of the major threats to the community health systems and shows a detrimental impact on the economy of developed and developing countries (Prestinaci et al., 2015; Founou et al., 2017). In the future, infectious diseases will be difficult to treat with antibiotics, thereby presenting a huge challenge to the healthcare systems (Chokshi et al., 2019). Infections from antimicrobial resistance lead to prolonged illnesses as well as an increase in hospital treatment costs and higher cost of alternate medicines for better treatment, finally resulting in mortality because of treatment failure (Shrestha et al., 2018). European countries spend 9 billion euros each year for activities related to AMR (Llor & Bjerrum, 2014; ECDC, 2017). Also, the Center for Disease Control and Prevention, USA, (CDC) has estimated that the United States spends about 20 billion dollars annually in the healthcare sector toward major funds for the treatment of antimicrobial resistance (CDC, 2013). In the United States, approximately 23,000 mortalities occur annually due to diseases caused by antibiotic-resistant bacteria. It is projected that worldwide by 2050 annually 50,000 deaths may be caused due to antibiotic resistance (CDC, 2019).

India is considered one of the largest reservoirs of drug-resistant bacteria, especially in the pathogenic populations belonging to both Gram-positive bacteria and Gram-negative bacteria (Dixit et al., 2019). The available data on clinical pathogens indicate the rising rates of antibiotic resistance, especially resistance to *Staphylococcus aureus* isolates for methicillin, which increased from 29% in 2008 to 47% by 2014 (Walia et al., 2015). India spends almost 5% of its gross GDP on hospitals and public healthcare facilities, of which the government spends only one-fourth of the total spending (MHFW, 2017). Among the different microorganisms exhibiting antimicrobial resistance, there is a significant threat from the resistance shown by bacteria, particularly in infectious bacteria (Prestinaci et al., 2015). The effects of antibiotic resistance in humans include compromised immunity and delayed responses in the body to act against the infectious organisms and the drastic effects

on the susceptible population undergoing major surgery, dialysis, or under continuous chemotherapy (CDC, 2013). In addition, the antibiotic resistance affects patients with chronic conditions like rheumatoid arthritis, asthma, and diabetes (CDC, 2019). Finally, the antibiotic resistance decreases the effectiveness of administered antibiotics in the body, thereby forcing the medical specialists to prescribe the last resort class of antibiotics, viz., carbapenem. Using carbapenem can result in major side effects and is also a hugely expensive drug to administer (Eurosurveillance Editorial Team, 2015).

2 Factors Accelerating the Rate of Antimicrobial Resistance

The factors that play a main role in accelerating the antibiotic resistance rate in microorganisms are (1) improper and excessive use of antibiotics, (2) application of antimicrobial agents in agriculture and allied fields, (3) rise in income earning levels, (4) increase in the frequency of international travel, (5) biological factors, and (6) gaps in knowledge in the treatment of pathogenic microorganisms.

a) **Improper and Excessive Use of Antibiotics**

Antibiotic resistance is a worldwide human health problem that has been augmented by the improper and excessive of antibiotics (Llor and Bjerrum, 2014). Antibiotics are generally used by people even in treating minor health issues at the recommendation of a doctor. The dose and frequency differ from what is normally required. Due to the people's unawareness, they use antibiotics improperly or sometimes excessively, which results in the development and dissemination of antibiotic resistance (Dadgostar, 2019).

b) **Application of Antimicrobial Agents in Agriculture and Allied Fields**

Application of antibiotics can be seen in different food production systems such as agriculture and allied sectors such as veterinary, aquaculture, and horticulture (Hollis and Ahmed, 2013; Chantziaras et al., 2014; Nadella et al., 2021a; Falkiner, 1998). Uncontrolled use of these antibiotics is the main reason for the development of resistance in microorganisms. There is a huge transfer of resistance determinants from microorganisms of aquatic origin to terrestrial-origin bacteria and, finally, to human commensal bacteria through genetic determinants (Cabello et al., 2013).

c) **Rise in Income Earning Levels**

In the past two decades, global usage of antibiotics has increased by 60–65% mainly because of overconsumption in the developed and developing countries due to an increase in income (Klein et al., 2018; Chaw et al., 2018). In addition, the income earning levels of middle- and low-income countries have also increased significantly, resulting in an increase in the usage of antibiotics (Dall, 2019).

d) **Increase in the Frequency of International Travel**

At present, there is an increase in the frequency of international travel for various reasons such as trade, tourism, sports, and media (CDC, 2019). This increase in travel is associated with the exchange of food and drinking habits of

people from different communities that gets mixed. Thus, in addition to this, the transmission of disease-causing bacterial pathogens has also been noted that directly correlates with the use of antibiotics (Frost et al., 2019).

e) **Biological Factors**

Antimicrobial resistance develops in bacteria through two different ways, viz., through mutations in chromosomal material or through the transfer of resistance determinants through extrachromosomal materials such as plasmids, integrons, and transposons (Read and Woods, 2014). The plasmids are small fragments of DNA that are circular in shape and responsible for the transfer of resistance with the help of other mobile genetic elements (Li et al., 2019). In aquatic environs, the rate of transfer of resistance genes mediated through integrons and transposons was found to be higher (Nadella et al., 2021b).

f) **Gaps in Knowledge in the Treatment of Pathogenic Microorganisms**

Knowledge of the prevalence of the disease-causing pathogens, treatment methods, drugs used for treatment, and the levels of antibiotic resistance is up to the level. The gaps in information on these factors should be addressed thoroughly before suggesting mitigation measures for the impact of antibiotic resistance in both healthcare scenarios and animal-rearing systems worldwide (CDC, 2019).

3 Different Viewpoints on the Impact of AMR on Economics

The impact of AMR on the economic situation of an individual or society depends on various factors, and it is important to consider the different viewpoints from physicians' perspective, patients' requirements, healthcare and hospital businesses, drug industry, and, finally, the public toward antimicrobial resistance to assess the economic impact (McGowan, 2001).

A) **Physicians' Perspective**

At present, from a medical, healthcare, and economic point of view, the medical physician (doctor) is considered the most important for treating patients suffering from minor and/or major health problems. The main problem faced by doctors is that the drugs prescribed by them are very effective early in treatment, but as antibiotic resistance develops, the treatment becomes ineffective in treating the same disease. Hence, doctors should prescribe correct medicine to control the infections, which will effectively reduce the economic burden on patients.

B) **Patients' Requirements**

The economic impact of AMR is also measured in terms of patients' requirements since in situations where they suffer from long-lasting illnesses, extra cost need to be paid for the treatment of an antibiotic-resistant microorganism because the patients are required to pay extra money to avail better healthcare services and medicines.

C) **Healthcare and Hospital Businesses**

The economic expenditure for healthcare businesses toward antimicrobial resistance is involved in the procedures that preserve the effectiveness of antibiotics and other antimicrobial agents, including the costs for diverse drugs and

healthcare facilities, availability of healthcare specialists, medical goods, equipment, and institutions to devise and execute the plans to handle AMR.

D) **Pharmaceutical or Drug Manufacturing Companies**

Currently, pharmaceutical industries and other drug manufacturing companies manufacturing antimicrobial agents, vaccines, etc., for the treatment, management, and prevention of contagious diseases are mainly focused on targeted outcomes laid on product sales, which are short term and profitable in nature. But the industry should also focus on the long-term effects of antimicrobial resistance by introducing the sale of new products that will decrease the impact of antibiotic resistance on the public and adopt a two-sided approach, that is, to maintain the life of the present antimicrobial products in addition to the newly introduced products of AMR as well as set up the industry for specific drugs that are more effective and easily marketable.

E) **Public (Society) View**

Finally, for a better understanding of the economic impact of AMR, public (society's) view on healthcare facilities and goods should be considered important. The goal is to provide good health and adequate healthcare facilities to the entire population, which requires a longer time frame to achieve. Antibiotics and other antimicrobial drugs will be considered valuable resources by the public since they are used to both prevent and treat pathogenic infections, thereby providing economic benefits to the society. From the public viewpoint, proper use of antimicrobial agents for treating an infection would lead to a satisfactory decrease in the cost incurred on AMR. In contrast, overuse or inappropriate use of antibiotic drugs may lead to a significant increase in burden from AMR, which ultimately has a detrimental effect on the resources of the society.

4 Economic Impact of AMR and Its Assessment

The overall economic impact of AMR is calculated from the cost involved in treating an infection or a disease caused by drug-resistant microorganisms, termed "treatment cost," minus the cost incurred toward the prevention of such infections, termed "prevention cost" (Chrischilles & Scholz, 1999). The analysis of cost incurred toward the prevention or treatment of AMR should count on all the resources that had suffered drastically due to the infection. The income spent on lab tests, radiological studies, bronchoscopic, and any other diagnostics should be included as treatment cost, which the healthcare organizations (hospitals) lay on the patients for the treatment of diseases acquired through AMR. Similarly, apart from diagnostics procedures, the patients need to spend more money on purchasing antibiotics, therapeutic agents, and antibacterial drugs. This affects the patients severely, resulting from both direct cost (hospital cost, medicines cost) involved in availing healthcare services and indirect cost (money spent on other services for maintaining post-treatment). AMR can also affect not only the patients but also has severe effects on income because the pharmaceutical or drug manufacturing companies sometimes reduce the availability of the antimicrobial drugs and the salaries paid to the workers will also be added to the final cost of the medicine. The economic impact of AMR

depends on all these factors and always shows an increasing trend. Most of the studies carried out to assess the economic impact of AMR did not include these factors. The studies considered hospital charges, costs for specific treatments, additional diagnostic tests, and time of stay in hospital, which are easy to collect to assess the economic impact of patients infected with drug-resistant organisms compared with susceptible organism. Some studies also considered the cost incurred for prolonged illness and mortality resulting from drug-resistant infections. In addition, a few studies assessed the economic impact of AMR outside the healthcare facilities. A few studies are also needed to assess the impact of AMR on the whole community setting receiving antimicrobial treatment (Eandi & Zara, 1998).

The economic impact of AMR on a given drug has different aspects (Liss & Batchelor, 1987). The benefits of using an antimicrobial drug administered should be compared with the benefits of using an alternative drug when the main drug is not available. This helps in deciding the value of an antimicrobial drug and also increases the cost incurred for treating an infection. For example, the expenditure incurred by a patient for treating an infection with drug (X)-resistant bacteria is compared with the money spent by a patient for treating an infection with the same drug (X)-susceptible bacteria. The main problem arising from this kind of comparison is that there is non-availability of the same homogenous group that can be used as a reference (Harris et al., 2000; Rennie & Luft, 2000).

5 Measures To Be Taken to Minimize AMR and Its Economic Impact

To date, several strategies have been developed and numerous approaches have tried to deal with AMR and its control. Professional societies, governmental agencies, and independent review groups suggest various measures to minimize the harmful effects of resistance, including academic awareness plans, increased surveillance for AMR microorganisms, improved disease surveillance programs for the patients affected by AMR, execution of control measures for AMR, development of vaccine for AMR bacteria, and appropriate use of AMR drugs in prophylaxis and treatment. These strategies can be assessed for reducing AMR and the cost involved in its treatment. For the assessment of the overall impact of AMR on the economics of any country in terms of AMR control, the costs involved in each one of these strategies should be included.

6 Future Trends of AMR and Possible Solutions for AMR Control

Antibiotics and antimicrobial drugs have been commonly used in several areas of medicine since their discovery in 1929 for treating diseases, preventing infections, or improving growth and metabolism. Inappropriate use of antibiotics for the prevention or treatment of AMR has significantly increased the bacterial resistance, especially in the pathogenic microbial populations worldwide (Ali et al., 2018). This has

led to the worst situation where the human mortalities have reached about 50,000 annually in the United States and Europe (Simlai et al., 2016). Jansen et al., 2018 have reported that every year about ten million deaths occur because of bacterial resistance surpassing the deaths caused by cancer, which is at present 8.2 million deaths. The list consisting of AMR bacterial pathogens for humans published by the WHO in 2017 includes 12 pathogenic bacteria that are resistant to the beta-lactam group of antibiotics (WHO, 2017). The focus is now on discovering novel drugs or antibiotic substances that sets up a new target for upcoming research activities.

In clinical settings while assessing AMR, understanding the impact of the environment will surely lead to better clues as to what is equally important for AMR assessment of natural microbial populations that are relentlessly wide open to antibiotic excesses through polluted manure, sewage, and wastewater. Additionally, the future trends in research on AMR need to go beyond communications between bacterial communities to understand the parts of different kingdom interactions in AMR. Scholastic considerations will tilt toward quantifying the intra-community interactions between bacteria, which is highly significant (Bottery et al., 2021).

6.1 Aquatic Resources

Healthcare-associated infections (HAIs) are one of the most important problems faced by the patients, affecting 7% of patients in developed countries annually. The increase in AMR bacteria has been recognized as one of the major worldwide health trials, resulting in nearly 23,000 annual mortalities in the United States alone. Household items such as hand railings, bed posts, and door knobs have been identified as the sources of AMR. On the contrary, aquatic resources and related items were never taken seriously as the probable causes of HAIs. Taking this cue methodical assessment made by Hayward et al. (2022), we should investigate the part of aquatic and water-related devices in the spread of AMR bacteria responsible for HAIs, deliberating common aquatic devices, pathogens, and observation stratagems. The review indicated that AMR strains of previously described waterborne pathogens, including *Pseudomonas aeruginosa, Mycobacterium* spp., and *Legionella* spp., were commonly isolated. At the same time, MRSA and Enterobacteriaceae of carbapenem resistance that were not frequently occurring were also seen to occur in aquatic sources. Biofilms were recognized as a potential source for the distribution of genes accountable for existing functions. The major constraints recognized are the absence of uniformity among environmental surveillance possibility, isolation procedure, and description of AMR. There is a need to develop wide-ranging worldwide environmental surveillance strategies and implement them to monitor AMR pathogens, to identify the impending threats before waterborne infection outbreaks occur. The methodical inspection and guideline of concentrated focuses in water bodies are deficient worldwide, and it will be tough to accomplish and quantify advancement if the importance of aquatic sources in the fight against AMR is not fully recognized. The global potable water deficiency scenario is shown in Fig. 1.

The data indicate that 785,341,734 of 7,920,408,313 people or 9.915% of the world's population do not have access to potable water. The lack of potable water

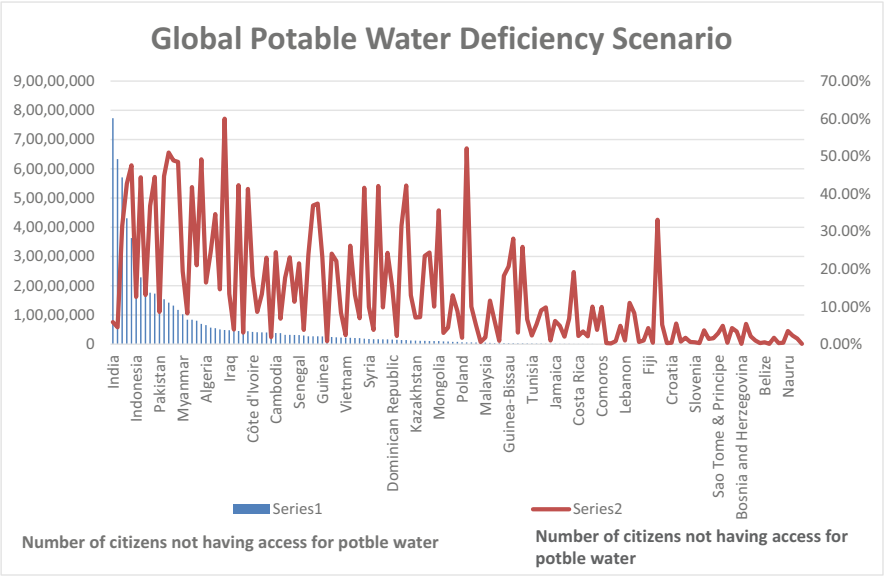


Fig. 1 Global scenario of deficiency of potable water. (Source; AQUASTAT - FAO's Global Information System on Water and Agriculture World Population Prospects: The 2019 Revision - United Nations Population Division, United Nations Statistics Division & World Bank. Accessed on 19.01.2023)

facility for the nearly 785 million people in the world is resulting in HAIs. These populations constitute low-income groups and do not have access to proper treatment for HAIs. This, in turn, will have a serious impact on the quality-adjusted life-years (QALYs), disability-adjusted life-years (DALY), and ill life-years (ILY) with infections of super bugs.

Currently, there is an excess use of available antimicrobial agents in humans, agriculture, and allied activities, resulting in the development and emergence of AMR bacteria and also resistant commensal bacteria, which ultimately lead to the spread of AMR genes into the aquatic environment (Nitsch Osuch et al., 2016). In case of ESKAPE pathogens, this phenomenon is regularly noticed, leading to an alarming situation where these bacterial pathogens have the ability to tolerate and show improved growth in the presence of these antibiotic substances (Boucher et al., 2009). There is an urgent requirement for the discovery of novel antibiotics and antimicrobial substances that can restrict the spread of AMR and safeguard the beneficial microorganisms.

The general strategies for mitigation of antibiotic resistance are shown in Fig. 2. The bacterial pathogens have developed in most of the drugs used in the treatment of diseases. There is an urgent need for novel drugs to which the bacterial pathogens are less resistant. Different sources or unexplored places may be searched for the availability of novel drugs. Another strategy to mitigate antibiotic resistance is to limit the usage of chemical drugs to limit the exposure of pathogenic

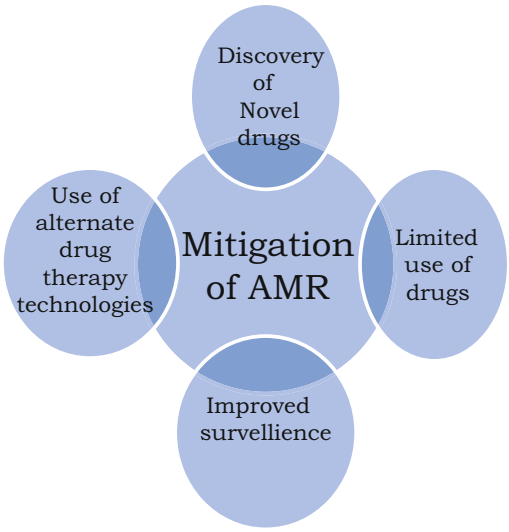


Fig. 2 Strategies to be followed for mitigation of antimicrobial resistance

microorganisms to the antibiotics. The general tendency of the people is to use antibiotics for mild treatment, which is also responsible for the development of resistance. Hence, the drugs should be used as per the medical practitioner's recommendations Proper disease surveillance strategies need to be devised and implemented at field level so that disease outbreaks can be easily identified. Proper identification of disease-causing agents is of utmost importance in recommending the drugs required for the treatment of diseases. Lastly, adopting ecofriendly alternative drug therapy technologies that can limit the use of antibiotics should be highly encouraged by the federal authorities so that the antimicrobial resistance can be addressed properly. Application of CRISPR–Cas9, nanotechnology, and bacteriophage therapy may be seen as potential techniques for mitigating bacterial resistance, which can be applied individually or in combination with others (Lima et al., 2019). The application of novel tools to treat AMR bacteria needs to be fastened as there is already a massive development of resistance to the currently effective antibiotics. This could include combinations between different techniques or in relation to available combination antibiotic substances to fight the emerging problem of AMR.

A) **CRISPRs**

Application of short repeats of palindromic interspaced regularly clustered sequences (CRISPRs), which is an adaptive mechanism of immune system, was first reported in microorganisms such as primitive archaea and prokaryotic bacteria. The mechanism involved in this technology is that CRISPR-Cas systems use small sequences of RNA to identify target (template) DNA and in combination with the enzyme (Cas) can denature the nucleic acids that require the same protein for binding and cleavage. Researchers have effectively used this

simple technique and developed a new molecular technique based on the available CRISPRs in natural organisms. CRISPRs can have various applications: they can act as cytotoxic structures that can kill AMR bacteria since they are antimicrobial in nature and can also provide immunity against AMR bacterial plasmids (Hsu et al., 2014; Sorek et al., 2013). In medical applications, CRISPR can be used to target and kill specific selective bacterial strains based on sequence information, thus creating new opportunities to fight against the drug-resistant pathogenic bacterial infections, which is advantageous than the other antimicrobial approaches that provide only limited solutions (Gomaa et al., 2014). In combination with nanotechnology, CRISPR–Cas can be effectively delivered at the site of action against target-specific AMR bacteria.

B) **Nanotechnology Application Against AMR Bacteria**

Nanotechnology is an emerging technique that can be applied to the synthesis of novel antibiotics in smaller sizes, which can result in increased contact surface area with the bacterial pathogens that result in improved absorption, increased bioavailability, faster delivery at the site of action, faster entry into the cell, and enhanced adhesion to the mucosal surface (Zaidi et al., 2017). In addition, nanoparticles can be coupled with antibiotic particles to synthesize newly controlled drug delivery systems that can be used to target encapsulated drugs (Jamil and Imran, 2018). Nanoparticles' (especially silver) mode of action is to affect the respiration in the bacteria by generating reactive oxygen species, ultimately resulting in killing bacteria (Shahverdi et al., 2007). This approach can be used in combination with antibiotic drugs that affect the inhibition of protein synthesis, alteration in the cell wall of bacteria, and finally rupture the cell wall (Kumar et al., 2018). The main concern with the application of nanoparticles coupled with antimicrobial drugs is that there is the possibility of stimulation of the resistant gene transfer to the susceptible bacteria, making them drug-resistant. Nanoparticles can act on the bacterial cell in two ways, that is, microbicidal (puncturing the cell wall) or microbiostatic (arresting the growth of bacterial cell). In addition, nanotechnology can also be used to resolve the problems associated with the solubility of antimicrobial drugs by means of encapsulation that improves membrane permeability, increased blood circulation time, and increased efficacy at the site of action (Rodzinski et al., 2016). Nanoparticles can be effectively used to treat infectious diseases, especially in locations where the pathogens evade the host immune responses by hiding or mimicking as host surface. But application of nanotechnology to mitigate antimicrobial resistance of bacterial pathogens requires extensive data on toxicity and preclinical and clinical studies, and also there is a need for proper guidelines and regulations (Zaidi et al., 2017).

C) **Use of Bacteriophages for AMR Control**

Bacteriophages (small viruses) are the most abundant (>1000 types) biological entities that infect bacterial cells (Chibani-Chennoufi et al., 2004). Bacteriophages are omnipresent in the environment and show high specificity toward an individual bacterial species and can be considered natural predator organisms to

bacteria. Even though the bacteriophages were discovered more than 100 years ago, now only the attention is diverted toward using the phages as an alternative to antibiotics as they are capable of killing drug-resistant bacterial cells (Summers, 2012).

The mechanism of action of phages is to attach to the outer cell wall membrane of the bacteria by using specific receptors. They usually show greater tissue permeability and do not show any harmful effects on the growth of the beneficial intestinal microbes and also do not stimulate secondary microbial infections. Generally, the phage particles can accumulate in high concentrations and grow exponentially where there is a need only if the bacterial host exists (Harada et al., 2018). The primary requirement for phage therapy is that the bacterium responsible for infection needs to be isolated for which specific lytic or lysogenic phage can be isolated and identified. As the phages are protein particles, they can be easily recognized by the cells of immune system that can result in decreased therapeutic efficacy (Chan and Abedon, 2012) and are prone to destruction or denaturation. Phage particles can develop bacterial resistance due to nonadsorption of particles, damage of viral genes by bacterial restriction endonucleases, and coating of phage membrane with the mucilage produced by bacterial cell (Wittebole et al., 2013). The phage particles can be protected by encapsulating with nanoparticles and make them invisible to the immune and digestive systems or binding with material support and making them (Balcão et al., 2013, 2014; Rios et al., 2018). There are some mechanisms through which the bacteria can resist the action of phages. Primarily the bacterial cells resist phages through the modification occurring in the cell surface such as concealment, conformational change of cell membrane, and downregulation of receptor cells on cell membrane (Azam & Tanji, 2019). Bacteria sometimes exhibit sensory mechanisms that detect binding of phages through molecular interactions to get entry into the host cells before binding (Debarbieux, 2014). In some cases, mutations or structural modifications of the receptors to which phages bind also responsible for the resistance.

Although there is a huge potential for the application of bacteriophages for the treatment and prevention of infections caused by AMR bacteria, very limited data are available on the clinical trials carried out in humans and are regulated by authorities such as the FDA and EMA (Rios et al., 2016). It is well known that phages were mainly used as a novel therapy agent in treating bacterial infections in humans. After its discovery in 1915 by F. Twort, the phages were first used in clinical studies in 1917, which yielded very good results in controlling bacterial infections. Again, research on the utility of phages was expanded from 1980 onward due to the emergence of multidrug resistance in bacteria. Since then, there have been several reports available on the utility of phages in different fields such as agriculture, veterinary, food safety, and industrial applications. In agriculture, the use of phages for controlling infections is in an early stage as most of the research conducted concentrated on the discovery of novel phages and their production in huge number by using cost-effective technologies.

Table 1 Utility of phages in different fields to control bacterial pathogens

S. no.	Field of application	Pathogen	Phage studied	Reference
1	Agriculture	*Ralstonia solanacearum*	φRSSKD1; φRSSKD2	Addy et al., 2016
2	Agriculture	*Pseudomonas syringae*	Three-phage cocktail	Susianto et al., 2014
3	Agriculture	*Pseudomonas syringae*	Five-phage cocktail	Rombouts et al., 2016
4	Agriculture	*Xanthomonas oryzae*	φXo411	Lee et al., 2006
5	Terrestrial animal	*E. coli*	TPR7	Rahmani et al., 2015
6	Terrestrial animal	Methicillin-resistant *S. aureus*	MR-10	Chhibber et al., 2013
7	Terrestrial animal	*Salmonella enterica*	Phage cocktail	Wall et al., 2010
8	Aquaculture	*V. harveyi*	*Siphoviridae* phage	Wang et al., 2017
9	Aquaculture	*Flavobacterium columnare*	Nine phages	Prasad et al., 2011
10	Marine corals	*Thalassomonas loyana*	Phage BA3	Atad et al., 2012

Similarly, the pages were used to study the control of infection in in vivo studies conducted in mouse models. In case of food industry, the meat gets cross-contaminated by the bacterial pathogens, and phages can be used singly, cocktail, or combined with other beneficial organisms to reduce food-related illness. In aquaculture, several reports are also available on the use of phage therapy for controlling the disease-causing pathogenic bacteria in both fishes and shellfishes. A few reports on the utility of phages in different fields to control pathogenic bacterial infections are given in Table 1.

In the future, the phages can be successfully applied under One Health approach to mitigate the problem of antibiotic resistance in bacteria in different food production sectors, and environment and human health sectors. In combination, therapies involving phages with other beneficial agents can be successfully used especially to control infections with bacterial biofilms. The combination therapies are always beneficial in nature as the fitness costs related to mitigating antibiotic resistance depend on multiple factors. In some cases, phages can be administered along with enzymes to improve the activity against pathogenic bacteria. Modifications can also be done in phage genome to improve their efficacy in the field of synthetic biology. The phages are host-specific in their activity since they cannot exhibit their activity on multiple pathogens. However, this can be overcome by tailoring the phage genome against multiple hosts by swapping receptor-binding proteins using different engineering approaches.

7 Conclusion

Currently, antibiotic resistance has been regarded as one of the key risks to public health systems and can exhibit unfavorable impact on the economics of several countries. This results in increase in hospital treatment cost as well as medicines. Several factors are responsible for the negative impact of AMR on the economic situation of an individual or society. Several strategies should be adopted to minimize the impact of AMR on economics. The most promising technologies are CRISPR–Cas9, nanotechnology, and bacteriophage therapy, which can be brought to use at the field level for better results.

References

Addy, H. S., Azizi, N. F., & Mihardjo, P. A. (2016). Detection of bacterial wilt pathogen and isolation of its bacteriophage from banana in Lumajang area, Indonesia. *International Journal of Agronomy, 2016*, 5164846.

Ali, J., Rafiq, Q. A., & Ratcliffe, E. (2018). Antimicrobial resistance mechanisms and potential synthetic treatments. *Future Science OA, 4*(4), Fso290. https://doi.org/10.4155/fsoa-2017-0109

Atad, I., Zvuloni, A., Loya, Y., & Rosenberg, E. (2012). Phage therapy of the white plague-like disease of *Favia favus* in the red sea. *Coral Reefs, 31*, 665–670.

Azam, A. H., & Tanji, Y. (2019). Bacteriophage-host arm race: An update on the mechanism of phage resistance in bacteria and revenge of the phage with the perspective for phage therapy. *Applied Microbiology and Biotechnology, 103*(5), 2121–2131.

Balcão, V. M., Barreira, S. V. P., Nunes, T. M., Chaud, M. V., Tubino, M., & Vila, M. M. D. C. (2014). Carbohydrate hydrogels with stabilized phage particles for bacterial biosensing: Bacterium diffusion studies. *Applied Biochemistry and Biotechnology, 172*, 1194–1214. https://doi.org/10.1007/s12010-013-0579-2

Balcão, V. M., Moreira, A. R., Moutinho, C. G., Chaud, M. V., Tubino, M., & Vila, M. M. (2013). Structural and functional stabilization of phage particles in carbohydrate matrices for bacterial biosensing. *Enzyme and Microbial Technology, 53*, 55–69. https://doi.org/10.1016/j.enzmictec.2013.03.001

Bottery, M. J., Matthews, J. L., Wood, A. J., Johansen, H. K., Pitchford, J. W., & Friman, V. P. (2021). Inter-species interactions alter antibiotic efficacy in bacterial communities. *The ISME Journal*. https://doi.org/10.1038/s41396-021-01130-6

Boucher, H. W., Talbot, G. H., Bradley, J. S., Edwards, J. E., Gilbert, D., Rice, L. B., et al. (2009). Bad bugs, no drugs: No ESKAPE! An update from the Infectious Diseases Society of America. *Clinical Infectious Diseases, 48*(1), 1–12. https://doi.org/10.1086/595011

Cabello, F. C., Godfrey, H. P., Tomova, A., Ivanova, L., Dölz, H., Millanao, A., & Buschmann, A. H. (2013). Antimicrobial use in aquaculture re-examined: Its relevance to antimicrobial resistance and to animal and human health. *Environmental Microbiology, 15*(7), 1917–1942.

CDC. (2013). Antibiotic resistance threats in the United States. https://www.cdc.gov/drugresistance/pdf/ar-threats-2013-508.pdf

CDC. (2019). Antibiotic resistance: A global threat features CDC. https://www.cdc.gov/features/antibiotic-resistance-global/index.html

Chan, B. K., & Abedon, S. T. (2012). Phage therapy pharmacology phage cocktails. In A. I. Laskin, S. Sariaslani, & G. M. Gadd (Eds.), *Advances in applied microbiology* (Vol. 78, pp. 1–23). Elsevier Academic Press. https://doi.org/10.1016/B978-0-12-394805-2.00001-4

Chantziaras, I., Boyen, F., Callens, B., & Dewulf, J. (2014). Correlation between veterinary antimicrobial use and antimicrobial resistance in food-producing animals: A report on seven countries. *Journal of Antimicrobial Chemotherapy, 69*(3), 827–834.

Chaw, P. S., Höpner, J., & Mikolajczyk, R. (2018). The knowledge, attitude and practice of health practitioners towards antibiotic prescribing and resistance in developing countries – A systematic review. *Journal of Clinical Pharmacy and Therapeutics, 43*(5), 606–613.

Chhibber, S., Kaur, T., & Sandeep, K. (2013). Co-therapy using lytic bacteriophage and linezolid: Effective treatment in eliminating methicillin resistant *Staphylococcus aureus* (MRSA) from diabetic foot infections. *PLoS One, 8*, e56022.

Chibani-Chennoufi, S., Bruttin, A., Dillmann, M.-L., & Brüssow, H. (2004). Phage–host interaction: An ecological perspective. *Journal of Bacteriology, 186*(12), 3677–3686. https://doi.org/10.1128/JB.186.12.3677-3686.2004

Chokshi, A., Sifri, Z., Cennimo, D., & Horng, H. (2019). Global contributors to antibiotic resistance. *Journal of Global Infectious Diseases, 11*(1), 36–42. https://doi.org/10.4103/jgid.jgid_110_18

Chrischilles, E. A., & Scholz, D. A. (1999). Dollars and sense: A practical guide to cost analysis for hospital epidemiology and infection control. *Clinical Performance and Quality Health Care., 7*, 107–111.

Dadgostar, P. (2019). Antimicrobial resistance: Implications and costs. *Infection and Drug Resistance, 12*, 3903–3910.

Dall, C. (2019). Global antibiotic use rises, fueled by economic growth CIDRAP. Available from: http://www.cidrap.umn.edu/news-perspective/2018/03/global-antibiotic-use-rises-fueled-economic-growth. Accessed September 16, 2020.

Debarbieux, L. (2014). Bacterial sensing of bacteriophages in communities: The search for the Rosetta stone. *Current Opinion in Microbiology, 20*, 125–130.

Dixit, A., Kumar, N., Kumar, S., & Trigun, V. (2019). Antimicrobial resistance: Progress in the decade since emergence of New Delhi metallo-β-lactamase in India. *Indian Journal of Community Medicine, 44*, 4–8.

Eandi, M., & Zara, G. P. (1998). Economic impact of resistance in the community. *International Journal of Clinical Practice, 95*(Suppl), 27–38.

ECDC. (2017). Surveillance of Antimicrobial Resistance in Europe. https://www.ecdc.europa.eu/sites/default/files/documents/EARS-Net-report-2017-update-jan-2019.pdf

Eurosurveillance Editorial Team (2015). WHO member states adopt global action plan on antimicrobial resistance. *Eurosurveillance 20*: 21137

Falkiner, F. R. (1998). The consequences of antibiotic use in horticulture. *The Journal of Antimicrobial Chemotherapy, 41*(4), 429–431.

Founou, R. C., Founou, L. L., & Essack, S. Y. (2017). Clinical and economic impact of antibiotic resistance in developing countries: A systematic review and meta-analysis. *PLoS One, 12*, e0189621. https://doi.org/10.1371/journal.pone.0189621

Frost, I., Van Boeckel, T. P., Pires, J., Craig, J., & Laxminarayan, R. (2019). Global geographic trends in antimicrobial resistance: The role of international travel. *Journal of Travel Medicine, 26*(8), taz036.

Gomaa, A. A., Klumpe, H. E., Luo, M. L., Selle, K., Barrangou, R., & Beisel, C. L. (2014). Programmable removal of bacterial strains by use of genome-targeting CRISPR–Cas systems. *MBio, 5*(1), e00928–e00913. https://doi.org/10.1128/mBio.00928-13

Harada, L. K., Silva, E. C., Campos, W. F., Del Fiol, F. S., Vila, M., Dąbrowska, K., et al. (2018). Biotechnological applications of bacteriophages: State of the art. *Microbiological Research, 212–213*, 38–58. https://doi.org/10.1016/j.micres.2018.04.007

Harris, J. D., Samore, M., & Carmeli, Y. (2000). Control group selection is an important but neglected issue in studies of antibiotic resistance. *Annals of Internal Medicine, 133*, 159.

Hayward, C., Brown, M.H. and Whiley, H., 2022. Hospital water as the source of healthcare-associated infection and antimicrobial-resistant organisms. *Current Opinion in Infectious Diseases, 35*(4), 339–345.

Hollis, A., & Ahmed, Z. (2013). Preserving antibiotics, rationally. *New England Journal of Medicine, 369*(26), 2474–2476.

Hsu, P. D., Lander, E. S., & Zhang, F. (2014). Development and applications of CRISPR–Cas9 for genome engineering. *Cell, 157*(6), 1262–1278. https://doi.org/10.1016/j.cell.2014.05.010

Jamil, B., & Imran, M. (2018). Factors pivotal for designing of nanoantimicrobials: An exposition. *Critical Reviews in Microbiology, 44*(1), 79–94. https://doi.org/10.1080/1040841X.2017.1313813

Jansen, K. U., Knirsch, C., & Anderson, A. S. (2018). The role of vaccines in preventing bacterial antimicrobial resistance. *Nature Medicine, 24*(1), 10–19. https://doi.org/10.1038/nm.4465

Klein, E. Y., Van Boeckel, T. P., Martinez, E. M., Pant, S., Gandra, S., Levin, S. A., & Laxminarayan, R. (2018). Global increase and geographic convergence in antibiotic consumption between 2000 and 2015. *Proceedings of the National Academy of Sciences, 115*(15), E3463–E3470.

Kumar, M., Curtis, A., & Hoskins, C. (2018). Application of nanoparticle technologies in the combat against anti-microbial resistance. *Pharmaceutics, 10*(1), 11. https://doi.org/10.3390/pharmaceutics10010011

Lee, C. N., Lin, J. W., Chow, T. Y., Tseng, Y. H., & Weng, S. F. (2006). A novel lysozyme from *xanthomonas oryzae* phage varphixo411 active against *xanthomonas* and *stenotrophomonas*. *Protein Expression and Purification, 50*, 229–237.

Li, Q., Chang, W., Zhang, H., Hu, D., & Wang, X. (2019). The role of plasmids in the multiple antibiotic resistance transfer in ESBLs-producing Escherichia coli isolated from wastewater treatment plants. *Frontiers in Microbiology, 10*, 633.

Lima, R., Del Fiol, F. S., & Balcão, V. M. (2019). Prospects for the use of new technologies to combat multidrug-resistant bacteria. *Frontiers in Pharmacology, 10*, 692. https://doi.org/10.3389/fphar.2019.00692

Liss, R. H., & Batchelor, F. R. (1987). Economic evaluations of antibiotic use and resistance-a perspective: Report of task force 6. *Reviews of Infectious Diseases, 9*(Suppl 3), S297–S312.

Llor, C., & Bjerrum, L. (2014). Antimicrobial resistance: Risk associated with antibiotic overuse and initiatives to reduce the problem. *Therapeutic Advances in Drug Safety, 5*(6), 229–241. https://doi.org/10.1177/2042098614554919

McGowan, J., Jr. (2001). Economic impact of antimicrobial resistance. *Emerging Infectious Diseases, 7*(2), 286–292.

Ministry of Health and Family Welfare. National Health Policy, 2017. Available from: http://164.100.158.44/showfile.php?lid=4275

Nadella, R. K., Panda, S. K., Rao, B. M., Prasad, K. P., Raman, R. P., & Mothadaka, M. P. (2021a). Antibiotic resistance of culturable heterotrophic bacteria isolated from shrimp (*Penaeus vannamei*) aquaculture ponds. *Marine Pollution Bulletin, 172*, 112887.

Nadella, R. K., Panda, S. K., Rao, B. M., Prasad, K. P., Raman, R. P., & Mothadaka, M. P. (2021b). Multi-drug resistance, integron and transposon-mediated gene transfer in heterotrophic bacteria from *Penaeus vannamei* and its culture environment. *Environmental Science and Pollution Research*. https://doi.org/10.1007/s11356-021-18163-1

Nitsch-Osuch, A., Gyrczuk, E., Wardyn, A., Życinska, K., & Brydak, L. (2016). Antibiotic prescription practices among children with influenza. *Advances in Experimental Medicine and Biology, 905*, 25–31. https://doi.org/10.1007/5584_2015_198

Prasad, Y., Kumar, A. D., & Sharma, A. K. (2011). Lytic bacteriophages specific to *Flavobacterium columnare* rescue catfish, *Clarias batrachus* (linn.) from columnaris disease. *Journal of Environmental Biology, 32*, 161–168.

Prestinaci, F., Pezzotti, P., & Pantosti, A. (2015). Antimicrobial resistance: A global multifaceted phenomenon. *Pathog Glob Health, 109*(7), 309. https://doi.org/10.1179/2047773215Y.0000000030

Rahmani, R., Zarrini, G., Sheikhzadeh, F., & Aghamohammadzadeh, N. (2015). Effective phages as green antimicrobial agents against antibiotic-resistant hospital *Escherichia coli*. *Jundishapur Journal of Microbiology, 8*, e17744.

Read, A. F., & Woods, R. J. (2014). Antibiotic resistance management. *Evolution, Medicine, and Public Health, 2014*(1), 147.

Rennie, D., & Luft, H. S. (2000). Pharmacoeconomic analyses – Making them transparent, making them credible. *JAMA, 283*, 2158–2160.

Rios, A. C., Moutinho, C. G., Pinto, F. C., Del Fiol, F. S., Jozala, A., Chaud, M. V., et al. (2016). Alternatives to overcoming bacterial resistances: State-of-the-art. *Microbiological Research, 191*, 51–80. https://doi.org/10.1016/j.micres.2016.04.008

Rios, A. C., Vila, M. M. D. C., Lima, R., Del Fiol, F. S., Tubino, M., Teixeira, J. A., et al. (2018). Structural and functional stabilization of bacteriophage particles within the aqueous core of a W/O/W multiple emulsion: A potential biotherapeutic system for the inhalational treatment of bacterial pneumonia. *Process Biochemistry, 64*, 177–192. https://doi.org/10.1016/j.procbio.2017.09.022

Rodzinski, A., Guduru, R., Liang, P., Hadjikhani, A., Stewart, T., Stimphil, E., et al. (2016). Targeted and controlled anticancer drug delivery and release with magnetoelectric nanoparticles. *Scientific Reports, 6*, 20867. https://doi.org/10.1038/srep20867

Rombouts, S., Volckaert, A., Venneman, S., Declercq, B., Vandenheuvel, D., Allonsius, C. N., Van Malderghem, C., Jang, H. B., Briers, Y., & Noben, J. P. (2016). Characterization of novel bacteriophages for biocontrol of bacterial blight in leek caused by *Pseudomonas syringae* pv. *Porri*. *Frontiers in Microbiology, 7*, 279.

Shahverdi, A., Fakhimi, A., Shahverdi, H., & Minaian, S. (2007). Synthesis and effect of silver nanoparticles on the anti-bacterial activity of different antibiotics against S*taphylococcus aureus* and *Escherichia coli*. *Nanomedicine: Nanotechnology, Biology and Medicine, 3*, 168–171. https://doi.org/10.1016/j.nano.2007.02.001

Shrestha, P., Cooper, B. S., Coast, J., et al. (2018). Enumerating the economic cost of antimicrobial resistance per antibiotic consumed to inform the evaluation of interventions affecting their use. *Antimicrobial Resistance and Infection Control, 7*(1), 98. https://doi.org/10.1186/s13756-018-0384-3

Simlai, A., Mukherjee, K., Mandal, A., Bhattacharya, K., Samanta, A., & Roy, A. (2016). Partial purification and characterization of an antimicrobial activity from the wood extract of mangrove plant *Ceriops decandra*. *EXCLI Journal, 15*, 103–112.

Sorek, R., Lawrence, C. M., & Wiedenheft, B. (2013). CRISPR-mediated adaptive immune systems in bacteria and archaea. *Annual Review of Biochemistry, 82*, 237–266. https://doi.org/10.1146/annurev-biochem-072911-172315

Summers, W. C. (2012). The strange history of phage therapy. *Bacteriophage, 2*(2), 130–133. https://doi.org/10.4161/bact.20757

Susianto, G., Farid, M. M., Dhany, N. R., & Addy, H. S. (2014). Host range for bacteriophages that infect bacterial blight pathogen on soybean. *Procedia Environmental Sciences, 20*, 760–766.

Walia, K., Ohri, V. C., & Mathai, D. (2015). Antimicrobial Stewardship Programme of ICMR. Antimicrobial stewardship programme (AMSP) practices in India. *The Indian Journal of Medical Research, 142*, 130–138.

Wall, S. K., Zhang, J. Y., Rostagno, M. H., & Ebner, P. D. (2010). Phage therapy to reduce preprocessing *Salmonella* infections in market-weight swine. *Applied and Environmental Microbiology, 76*, 48–53.

Wang, Y., Barton, M., Elliott, L., Li, X., Abraham, S., O'Dea, M., & Munro, J. (2017). Bacteriophage therapy for the control of *Vibrio harveyi* in greenlip abalone (*Haliotis laevigata*). *Aquaculture, 473*, 251–258.

Wittebole, X., de Roock, S., & Opal, S. M. (2013). A historical overview of bacteriophage therapy as an alternative to antibiotics for the treatment of bacterial pathogens. *Virulence, 4*(8), 1–10. https://doi.org/10.4161/viru.25991

World Health Organization. (2017). Global priority list of antibiotic-resistant bacteria to guide research, discovery, and development of new antibiotics.

Zaidi, S., Misba, L., & Khan, A. U. (2017). Nano-therapeutics: A revolution in infection control in post antibiotic era. *Nanomedicine, 13*(7), 2281–2301. https://doi.org/10.1016/j.nano.2017.06.015

Stakeholders' Role for Addressing Global Threat of Antimicrobial Resistance: A Multisectoral One Health Approach

Prejit Nambiar and K. Asha

Contents

Abstract

The role of antibiotics in human and animal medicine has been an important pillar of modern medicine. Microbes developing resistance to antibiotics are currently a catastrophic global public health crisis that needs immediate attention. The

P. Nambiar (✉)
Centre for One Health Education, Advocacy, Research and Training (COHEART), KVASU, Pookode, Wayanad, Kerala, India
e-mail: prejit@kvasu.ac.in

K. Asha
Department of Veterinary Public Health, KVASU, Pookode, Wayanad, Kerala, India

M. P. Mothadaka et al. (eds.), *Handbook on Antimicrobial Resistance*,
https://doi.org/10.1007/978-981-19-9279-7_47

response to reduce such resistance involves regular surveillance, prudent drug usage, infection control measures, and identifying new drug molecules or alternative agents. There is an urgent need to enhance the effectiveness of these interventions as the drug-resistant pathogens circulate through various sources like food animals and the environment. This calls for an urgent need to promote antimicrobial stewardship to counter antimicrobial resistance (AMR). By combating AMR, countries can prevent unwarranted deaths, alleviate economic losses, and contribute to societal and economic development. A collaborative action of diverse stakeholders involved in the control of AMR is the best approach which is gaining more and more attention. The main objective of the chapter is to identify the stakeholders' involved in addressing threats due to AMR and to describe their roles and contributions. All stakeholders need to work collaboratively under One Health umbrella in order to save all species in the planet from the catastrophic public health crisis of AMR.

Keywords

AMR · Infection · One Health · Stakeholders' collaboration

1 Antimicrobial Resistance (AMR): A Multifaceted Complexity

Antimicrobial resistance (AMR) is a potential catastrophic public health and animal health crisis severely affecting the economies world over and is rated as one among the top ten threats to health (WHO, 2019). They account for nearly 0.7 million deaths per year leading to a loss of US$ 3.4 trillion in GDP by the end of 2030 (WHO, 2018). In United States alone, more than 2.8 million individuals are infected each year with AMR organisms and 35,900 persons succumb to death due to these infections (CDC, 2019). AMR arises in bacteria through a wide variety of mutations and gene transfer actions, which are ingrained with the global environment (Graham et al., 2019). The magnitude of impact of AMR is critical, and if it continues over a few more years, the general infections and injuries that were easily treatable may once again kill millions. Resistant bacteria and associated genes can transfer between man, animals, and environment, and transmission route is difficult to decipher. AMR is thus a very complex, serious, and contentious issue especially affecting the community health, environment, food security, and gross economy of the country. If no effective action is quickly put into practice, the global antibiotic use in the year 2030 shall considerably increase by 200 per cent compared to that in the year 2015 (Klein et al., 2018).

In livestock sector, the estimates of global consumption of antimicrobials are also projected to increase to 200k tonnes by 2030 (Van Boeckel et al., 2017). The increased occurrence of AMR bacteria is attributed to the overuse or misuse of antimicrobial agents, lack of infection and disease prevention measures, and the inadequate access to quality, affordable medicines or diagnostics. This problem is

aggravated by certain providers of questionable competence in the healthcare sector whose irrational usage worsens the situation (Ahmed et al., 2009; Hoque et al., 2020). Apart from this, several factors, viz, self-medication, access to antibiotics without prescription, and lack of knowledge about how and when to use antibiotics, shall lead to more microbes developing resistance (Barker et al., 2017; Chandy et al., 2013). Indiscriminate drug use in animal husbandry and aquaculture especially for growth promotion and disease control can lead to the spread of AMR pathogens. Antibiotics that are crucial to human health are commonly used for growth promotion in poultry (Brower et al., 2017) which if left unchecked may lead to post-antibiotic era of medicine where treatments from minor surgery to major transplants could become impossible (Shallcross et al., 2015). This chapter presents a "snap shot" of how stakeholders' collaborative approach can effectively tackle AMR issues and is intended to emphasize One Health approach to reduce the imminent danger of AMR calamity in the planet for all the species.

2 The Interconnectedness

AMR organisms are ubiquitous in natural ecosystems and do not distinguish geographic or species borders. The increased usage of antimicrobials in animals is practiced to meet the growing requirement for animal protein among the human populations (Van Boeckel et al., 2019). Such shift to high-protein diets has been facilitated by industrial livestock production systems, in which antimicrobials are used regularly to uphold health and productivity (Silbergeld et al., 2008). The human problems of AMR cannot be comprehended precisely without taking into account nonhuman factors, and hence there is a need to divert attention to the inevitable multispecies entanglement in the understanding of health (Rock, 2017). There is growing evidence that AMR genes, once evolved in bacteria of any kind anywhere, can spread indirectly through a myriad of pathways to other kinds of bacteria anywhere else (Thomas, 2002). It cannot be neglected that environment serves as an important reservoir for many AMR pathogens (Pornsukarom & Thakur, 2017) and are also the predominant contributor to the spread in humans and animals, in particular in high-risk areas where there are several waste waters streams (European Commission, 2017). While considering the concept of interconnectedness, the role of the environment, predominantly sewage water, in the spread of resistant bacteria is increasingly gaining attention. Liquid waste represents a potential route for dispersing such organisms from animal agriculture to human habitation and the risks to the environment depend heavily on the type and level of wastewater treatment (Graham et al., 2019). Apart from above sources, literature reveals that there are several wildlife species that carry resistant organisms in a wide range of habitats, which further raises the question of their role in AMR dynamics at the interface between human, domestic animals, and natural ecosystems (Marion et al., 2016). The authors also revealed that carnivorous and omnivorous species are commonly under the risk of carrying such resistant organism. Among avian species, the raptors and gulls present high colonization rates (Marion et al., 2016). The speed

at which resistance emerges and spreads from livestock, and the persistence of the resistance in the environment, depends on many factors including the type of pathogen, the class of antibiotic used, the treatment approach, and the general environmental conditions for livestock production (Hoelzer et al., 2017). Soil contamination can also lead to evolution of multidrug-resistant organisms through metabolic processes (Grenni et al., 2018). Further, in aquatic systems there are evidences of the resistance gene exchanges that occur between environmental bacteria and human pathogens (Wellington et al., 2013), and the same is evident from sources of food, animals, and by direct contact (Laxminarayan et al., 2014). Lazarus et al. (2015) could demonstrate significant share of extended spectrum cephalosporin-resistant *E. coli* from human extraintestinal infections that originate from food animals. Similar research on animals carrying and transmitting of AMR genes are reported from several regions (Hamna et al., 2019; Akarsh et al., 2019; Suma et al., 2019).

3 Multisectoral One Health Intervention

As AMR is considered to be a multifaceted problem, there is a need in a multifaceted solution (White & Hughes, 2019; Prejit, 2020). The usage of antibiotics in animal and human systems cannot be neglected, but at the same time resistance to antimicrobial agents poses severe threat not only to the health of human and animal but also to the global ecosystem (OIE, 2016). Since AMR is due to the naturally occurring survival instinct of the bacteria, total elimination might be difficult; however, there are possibilities to decrease it to a level that no longer causes a threat to humanity. Apart from humans, the other sectors that are usually affected by AMR include animal health and welfare, agriculture, animal husbandry, fisheries, food supply and production, environmental health, water and sanitation, trade and tourism, etc. Tackling such hazards should go beyond the conventional sensitization of members of the public to include a One Health intervention involving collaboration and the development of various alliances and partnerships (Nweneka et al., 2009). Such transdisciplinary interactions are often needed for effective early warning, surveillance, and response for AMR pathogens (Prejit, 2017). Immediate action is required across industries and sectors to mitigate, prevent, and control AMR. Poor coordination of efforts can hamper the effectiveness of the response for AMR control. There is a need to construe that the issue of AMR respects no species barriers, and a coordinated One Health response will only help to achieve sustainability of the control efforts (Hernando et al., 2019; Hoque et al., 2020, Prejit, 2020). The regulations and policies that support rational use of medicines are essential for effective interventions to uphold the progress and spread of AMR (Hoque et al., 2020). Such a cross-sectoral collaboration of stakeholders at the regional, national, and global level targets stronger political commitments to building sustainable resilience against public health threats (Kieny et al., 2014; Mohan et al., 2018). The poor response to control AMR pathogen has served as an important catalyst for

increased efforts to comply with WHO's International Health Regulations (IHR), OIE's Performance of Veterinary Services (PVS), and Global Health Security Agenda (GHSA) goals. The World Health Assembly urged the member countries of the WHO to develop national action plans as a result of which India's Ministry of Health and Family Welfare implemented the National Action Plan (NAP) on AMR established on the principle of One Health approach and released in April 2017 (Gandra et al., 2017). Formulating such action plans for the region or state can be the preliminary step for implementation of control strategies. They help to recognize relevant stake holders and also help in the effective utilization of infrastructure and other resources as well as networking of laboratories. Since the implementation of NAP, India has made significant progress in health security interventions and the southern state of Kerala was first to implement state-level action plan to contain AMR threat (Prejit, 2020). The guidelines on antibiotic use in food animals and humans, as well as its use in aquaculture, fisheries, wildlife, and environment, are desired when implementing One Health approach to AMR (Thakur & Gray, 2019).

The prerequisite for multisectoral approach is a strong political commitment among members, knowledge and leadership among sectors, firm support from FAO/WHO and WOAH at all stages of development, and team work of all the stakeholders. A multidisciplinary group for AMR containment can accommodate members from all the key stakeholders, including health service department, physicians, veterinary clinicians, fisheries professionals, agriculture and public health scientists, official of drug regulator authority and food safety sector, wildlife specialists, environmentalists, epidemiologists, and microbiologists.

4 Stakeholders' Role

The National Action Plan has defined the role of various stakeholders to act against antimicrobial resistance and is based on a collaborative approach between various sectors and professionals to effectively prevent AMR. This includes defined stakeholders from the provider and the consumer sides. From the provider side, the important stakeholders are policy makers, researchers, health practitioners, pharmacists and pharmaceutical industries, laboratory workers and diagnostic industries, physicians, and veterinarians. From the consumer side, the stakeholders are patients, farmers, and the society. The lists of various stakeholders and their roles are depicted in Table 1.

The availability of data to assess the burden of AMR needs to be based on monitoring information, for which availability differs greatly between various states or regions. All the sectors need to collectively act to undertake AMR data collection, both actively and passively. Passive activities involve voluntary or mandatory reporting of cases, either animal or humans, that warrants a consultation with a specialist. Active systems involve "searching" for evidence of disease through routine or periodic collection of data (Kalluri et al., 2007). The NAP implementation to prevent and contain AMR shall be based on an expert interdisciplinary national

Table 1 Stakeholders role to combat AMR

Sl No.	Stakeholder	Role to combat AMR
1.	International Organizations (WHO, OIE, FAO, etc.)	Providing top-level leadership on Global Health matters including AMR (e.g., tripartite agreement)
2.	Relevant ministries, government, and policy makers	Providing best amenities and policies needed for the country to address AMR challenges. Involves leadership at central, state, and district levels to meet the requirements for AMR control
3.	Public health stakeholders	To encourage rational use of antibiotics in general population and to ascertain control measures
4.	Veterinary and fisheries stakeholders	To provide quality veterinary and aquaculture services by abiding on legislation on the use of drugs to prevent antimicrobial resistance in animal/fisheries production
5.	Environment stakeholders	To minimize the environment role in transferring multidrug-resistant pathogens and antimicrobial residues and the impact so created
6.	Research and academic institutes	To prioritize research plans in identifying resistance to antimicrobials and design strategies for infection control
7.	Research funding agencies	To provide resources to generate new knowledge, solutions, and implementation strategies on AMR
8.	Food safety stakeholders	To regulate the unwarranted usage of antibiotics in foods and enforce regulation for residue limits
9.	Society, farmers, and food producers	To follow good farm practices and biosecurity to limit the use of antimicrobials
10.	Laboratories	For routine surveillance of AMR pathogens and generating resistant organism or resistant gene data base
11.	Tertiary hospitals	To care for the patients to prevent them from harboring drug-resistant pathogens and implementing infection prevention and control strategies
12.	Pharmaceutical industries and drug controllers	For the quality assurance of antibiotics and also for developing new drugs as well as policy to check that industry waste does not contaminate environment
13.	Media	To act as a reliable source of information to generate awareness as well as clarifying myths that are spread in other media
14.	Consumers	To demand for responsible use of antibiotics in various food production systems

steering committee and facilitated through advisory or expert groups (Kumar et al., 2013). Best approach to control AMR is stakeholders' collaborative action coupled with Antimicrobial Stewardship which is "a coherent set of actions which promote using antimicrobials responsibly" (Dyar et al., 2017). Apart from this, focus shall be on behavioral approaches that involve strategies to control infection prevention to those focusing on responsible use of antimicrobials (Dar et al., 2016). The role of

various stakeholders is given below, and further details can be retrieved from Global Action Plan detailed by WHO.

4.1 International Organizations

The international organizations such as FAO, WOAH, and WHO provide technical support to implement national action plan on AMR, support and review countries' existing policy, networks, and collaborations on AMR, and recognize priority areas as well as provide technical guidance in coordination mechanisms. They also guide in harmonized surveillance of AMR, worldwide monitoring of usage of antimicrobials along with the support to fight against the circulation of poor quality or counterfeit products. On similar lines, the Codex Alimentarius contains certain guiding principles on minimization of drug-resistant organisms in animal husbandry. These organizations encourage the discovery of new drugs with unique antimicrobial properties, inventions of rapid diagnostic tests, and improve perception of ways to influence antibiotic usages in the medical and veterinary sectors including new vaccine development to prevent infection. WHO has established an Advisory Group on integrated surveillance of AMR to upkeep global efforts to curtail the burden of AMR associated in all food-producing animals (Fletcher, 2015). WHO has also established a free database software called WHONET for the data management and analysis of antimicrobial susceptibility test results.

4.2 Relevant Ministries, Government, and Policy Makers

Combating AMR necessitates strong political will and leadership quality. The federal government must take the lead in tackling the problem of antimicrobial resistance and assign responsibility to agencies to develop relevant policies for enforcement. The concerned agencies need to design an operational plan to implement and monitor National Action Plan on AMR and provide budgetary allocation for its operationalization, further conduct impact and cost-benefit analysis in order to measure the success factor of the new policy implementation measures. Government needs to promote integrated policies on the responsible prescription of medicines in animal and human patients, and recommend new guidelines for antimicrobial use in crops and food production. To make this possible, the government should implement NAP on AMR designed on One Health approach and sensitize all stakeholders to adopt the practice. Such practices can be coordinated locally by district administrators who will serve as front-runner to facilitate activities of a particular district in the state with the involvement of relevant health service and animal husbandry departments. This is especially suitable for countries where health is considered as a state subject.

4.3 Public Health Stakeholders

The public health stakeholders are people or organizations who invest their time and energy in public health-related activities and promptly act on the results and recommendations. They include physicians, health providers, health-related managers, administrative staff, health-based research workers, field health workers, health advocacy groups, and coalition members. They encourage rational use of antibiotics and ascertain appropriate patient care. They have to take necessary steps to stop over the counter sale of antibiotics and guarantee continuous access to essential drugs of guaranteed quality at hospitals (Kumar et al., 2013). They play a vital role in inspecting inappropriate prescription and dispensing of medicines. They identify the type of infection, design vaccination strategies, and suggest infection prevention and control (IPC) practices in hospital settings that can limit the spread of drug-resistant disease. They formulate offline and online training programs for doctors at all levels. To cite an example, in India the Indian Council of Medical Research invited a qualified group of prescribers in a workshop to augment their knowledge on AMR and engaged them in preparing guidelines for rational prescription of antimicrobials (Chandy et al., 2014). They can train all nurses, health workers, and pharmacists in antibiotic protocols, antibiotic use or abuse, and its resistance. The public health stakeholders by virtue of their experience should convince policy makers to establish microbiology laboratories in all major government hospitals and undertake routine AMR surveillance.

5 Veterinary and Fisheries Stakeholders

Veterinary stakeholders working under government sector require to focus on quality veterinary services by abiding on legislation on the use of drugs especially to prevent the development of resistant pathogens. They should implement region-specific guidelines on proper management of waste in farms, food processing units, feed manufacturers, slaughter houses, and veterinary hospitals. They should help farmers to enforce farm biosecurity guidelines and good manufacturing practices for processing units that involve meat or dairy. Similarly, fisheries professionals need to advocate prudent usages of antibiotics in fish and other aquaculture. These stakeholders shall discourage the use of growth promoters in animal/fish production, especially antibiotics which are important to human health, as well as slowly phase out its use. They should also devise strategies to optimize the practice of using antimicrobials in food animals and develop policy to register or license the farms, slaughter houses, factories, fish-processing units, meat or dairy animal processing units, feed-manufacturing units, healthcare facilities, and veterinary care units. They are the stakeholders who can ensure proper disposal measures of antibiotics, monitor antibiotic residues in animal-based foods or seafood, and also create awareness programs for farmers.

6 Environment Stakeholders

The environment stakeholders include government representatives from forest and other environment-associated sectors, institutes working on environmental health and safety, NGOs, environmental scientists, landowners (of farm producers), and the users of natural resources. Antibiotics in the environment could be considered as a hazardous chemical, and it calls for environmental stakeholders to adopt an eco-centric waste management approach. Stakeholders associated with environmental health need to develop strategies to minimize the role of environment in transferring multidrug-resistant pathogens and antimicrobial residues. They need to build capacity for surveillance of antimicrobial resistance in the environment, quantify antibiotic residues, identify environmental sources of infection, and improve housekeeping and environment management (including sewage treatment facilities). They involve in spearheading surveillance of AMR bacteria in waste/effluents, litter, manure, and soil, particularly from hot spots. These stakeholders are responsible for development of standards for residual antibiotics in the environment and issue waste management guidelines including biomedical waste management.

7 Research and Academic Institutes

The research institutes and universities have a key role to prioritize research plans in identifying resistance to antimicrobials and design strategies for infection control. They can improve awareness among school children on use and abuse of antibiotics and importance of resistance. Institutes in countries like Indonesia and Malaysia have hosted dedicated webpages for consumer education on appropriate use of antimicrobials and launched separate campaigns in schools (Hoque et al., 2020). In order to create mass sensitization programs in schools and colleges, the stakeholders required to organize events such as the commemoration of world antimicrobial awareness week (WAAW). In research areas, they can define research priorities for AMR innovations, develop new drugs, innovate diagnostics, and validate vaccines. These stakeholders can also plan capacity-building activities for students, house-surgeons, and doctors at all levels of the healthcare system. Academic institutes can provide expert advice on farm biosecurity measures and undertake research to develop an understanding of the dynamics of AMR transmission at human-animal-environment interface. They can generate data to assess the burden of AMR that includes mortality, morbidity, and economic analysis of impact of AMR in their country. They can design research interventions in respect of developing newer drugs or develop alternatives to antimicrobials and adjunct remedies for infectious diseases. The research centers and university that work under the mandate of improving animal health have the responsibility of scaling up the diagnostic tests in animal diseases, provide resources to field veterinarians on the antimicrobial classes best suited to treat and control the disease, and utilize research findings on policy interventions at national context. They also implement appropriate surveillance strategies in the animal health sectors to generate up-to-date epidemiological

information, baseline data, and record trends on antimicrobial resistance. Such stakeholders should discourage subtherapeutic use of antimicrobial agents in poultry, piggery, agriculture, and aquaculture practices as growth-promoters (Kumar et al., 2013). They need to update food animal farmers and practitioners on prevalent AMR issues.

8 Research-Funding Agencies

Public funding agencies for research on AMR are another key stakeholder who will provide resources to generate new knowledge, solutions, and implementation strategies on AMR. It is observed recently there are limited or no new antibiotic classes entering the market which threatens the society to enter into a preantibiotic era. To address this issue, certain funding agencies, such as the Joint Programming Initiative on Antimicrobial Resistance (JPIAMR), were established (Kelly et al., 2016). Research on AMR warrants new investment from a range of sources, and more informed resource allocation is needed to make a true impact.

9 Food Safety Stakeholders

The food safety stakeholders of the country are responsible to regulate the usage of antibiotics in crops, strengthen the detection of residue of antibiotics in food, enforce regulation of maximum residual limits, monitor the levels of antibiotics used in foods, and check plant-derived food produced for residual antibiotics. They take steps to prevent use of antibiotics for human use as well as antibiotics for growth promotion.

10 Society, Farmers, and Food Producers

Society, farmers, and food producers need to bear in mind that the AMR is a grave threat to human health in the near future. Farmers be aware that AMR microbes can pose risk of mortality and reduce animal performance, thereby affecting the economic returns from livestock production and generate possibly higher food prices for consumers in the long run. To prevent infections on farms, farmers and food producers all follow good practices (that include good husbandry, biosecurity, high-quality feed, proper hygiene, vaccination, appropriate waste, or manure management). The usage of antimicrobials should only be done after the advice of a veterinarian or crop specialist and be administered responsibly to minimize such threats. Alternatives to antimicrobials, like vaccines, should be considered.

11 Laboratories

Laboratories are the stakeholders who conduct routine surveillance for pathogens and their resistance or susceptibility testing, and develop guidelines for collection of samples, its transportation, processing, and quality assurance for AMR testing. For example, some of the labs in India undertake routine AMR surveillance for pathogens like *E. coli*, *Klebsiella* spp., *Salmonella*, *Staphylococcus aureus,* and *Enterococcus* spp. from food animals and their product which help to establish AMR database for human, animal, food, and environment.

12 Tertiary Hospitals

Tertiary hospitals are the stakeholders who should care for their patients to prevent them from harboring drug-resistant or nosocomial pathogens and also care for their staffs who are at risk to harbor AMR pathogens by implementing IPC program and establishing policy to reduce the transfer of AMR gene occurring through occupational exposures to hospital personnel. They focus on improved hand hygiene in hospitals and ensure administrative oversight of IPC activities. These hospitals act as an important stakeholder when they adhere to antibiotic usage guidelines and antimicrobial stewardship program to warrant suitable prophylaxis and use of antibiotics.

13 Pharmaceutical Industries and Drug Controllers

Pharmaceutical industry must have a strong research and development wing to check for the quality assurance of manufactured and/or imported pharmaceuticals and also for developing new drugs. They should ensure that the waste generated is disposed of safely without contaminating the soil and the environment. Drug controllers play a vital role in implementing and monitoring the sale of antibiotics adhering to state and national guidelines, conducting centralized prescription audits, monitoring the quality of antimicrobials, and screening for the occurrence of antibiotics in feeds used in veterinary sector and aquaculture. They also conduct constructive interactions with the pharmaceutical industry for encouraging the development of new drugs and vaccines (Kumar et al., 2013).

14 Media

Reliable media (Print/electronic/social media) sources act as a potential stakeholder for creation of awareness and play a critical role in restricting the panic of AMR among public and advocating its correct use. The trained media professionals can help to adequately convey information not only about the harmful effects of antibiotic misuse to the individual, but also about the way in which it impinges on the

wider society (Nweneka et al., 2009). For example, the television provides a dual route (spoken word and creative images) for conveying AMR message to farmers, food producers, and the general public. Equally important is the need to ensure that those in the front line of communication – namely, health journalists – have sufficient tools and skills to perform their task (Nweneka et al., 2009).

15 Consumers

Educated and well-informed consumers are the key stakeholders to demand the health, industry, and food-producing units for responsible use of antibiotics. When it comes to labeling a food as "antibiotic free," all consumers gain confidence that they are consuming a food which is free from hazardous chemicals. That itself is a motivation for the food industry to deliver products as per consumer requirements. In certain scenario when antibiotic-free labeled food is difficult to obtain, such consumer will be glad to note if they observe a label indicating "no routine antibiotic used" which denotes that the animal has been raised without the use of non-therapeutic antibiotics. Present-day consumers seek more knowledge in the antibiotics use and thus avoid self-medication as well as buying drugs over the counter. This role is what is generally anticipated from a consumer to be a part of the AMR control campaign.

16 Conclusion

To control AMR, One Health approach has been widely advocated and at many a global scenarios (White & huges, 2019); it was also implemented. Such a cross-sectoral transdisciplinary collaboration at the animal–human–ecosystems interface involving the different stakeholders shall be an ideal approach to address AMR. The rationale behind this chapter was to recognize such stakeholders involved in the prevention and control of AMR, discuss their roles, and elaborate on their possible contributions to control AMR. The literature synthesis revealed that different stakeholders have very important roles in their respective domains to tackle with AMR issues. International organizations (such as FAO, OIE, and WHO) provide top-level leadership for AMR control through measures such as political commitment, intersectoral responsibility, adoption of national action plans, and intersectoral collaboration and research for development of new drugs, diagnostic tools, and vaccines. From the research till date, it is clear that a sustainable solution to mitigate globally increasing AMR is fueled by stakeholder's collaboration coupled with responsible antimicrobial stewardship. Such coordinated One Health action shall control not only AMR threats but also other public health crisis including the current pandemic of COVID-19.

References

Ahmed, S. M., Hossain, M. A., & Chowdhury, M. R. (2009). Informal sector providers in Bangladesh: How equipped are they to provide rational healthcare? *Health Policy and Planning, 24*(6), 467–478.

Akarsh, K. L., Prejit, N., Asha, K., Vergis, J., Vinod, V. K., & Hema, P. A. (2019). Occurrence and characterization of extended spectrum β-lactamase producing *E. coli* and *Salmonella* spp. from raw milk samples in Wayanad district, Kerala, India. *International Journal of Current Microbiology and Applied Sciences, 8*(12), 2089–2098.

Barker, A. K., Brown, K., Ahsan, M., Sengupta, S., & Safdar, N. (2017). Social determinants of antibiotic misuse: A qualitative study of community members in Haryana, India. *BMC Public Health, 17*(1), 333 [Online]. https://doi.org/10.1186/s12889-017-4261-4. Accessed 18 Dec 2020.

Brower, C. H., Mandal, S., Hayer, S., Sran, M., Zehra, A., Patel, S. J., Kaur, R., Chatterjee, L., Mishra, S., Das, B. R., Singh, P., Singh, R., Gill, J. P. S., & Laxminarayan, R. (2017). The prevalence of extended-spectrum β-lactamase producing multidrug-resistant *Escherichia coli* in poultry chickens and variation according to farming practices in Punjab, India. *Environmental Health Perspectives*, *125*(7), 077015 [Online]. https://doi.org/10.1289/EHP292. Accessed 18 Dec 2020.

CDC. (2019). *Biggest threats and data, 2019* [Online]. Available at: https://www.cdc.gov/drugresistance/biggest-threats.html. Accessed 22 Dec 2020.

Chandy, S. J., Mathai, E., Thomas, K., Faruqui, A. R., Holloway, K., & Lundborg, C. S. (2013). Antibiotic use and resistance: Perceptions and ethical challenges among doctors, pharmacists and the public in Vellore, South India. *Indian Journal of Medical Ethics, 10*(1), 20–27 [Online] https://doi.org/10.20529/IJME.2013.005. Accessed 22 Dec 2020.

Chandy, S. J., Michael, J. S., Veeraraghavan, B., Abraham, O. C., Bachhav, S. S., & Kshirsagar N. A. (2014). ICMR programme on antibiotic stewardship, prevention of infection and control (ASPIC). *The Indian Journal of Medical Research, 139*(2), 226 [Online]. PMID: 24718396. Accessed 22 Dec 2020. PMCID: PMC4001333.

Dar, O. A., Hasan, R., Schlundt, J., Harbarth, S., Caleo, G., Dar, F. K., Littmann, J., Rweyemamu, M., Buckley, E. J., Shahid, M., Kock, R., Li, H. L., Giha, H., Khan, M., So, A. D., Bindayna, K. M., Kessel, A., Pedersen, H. B., Permanand, G., Zumla, A., & Heymann, D. L. (2016). Exploring the evidence base for national and regional policy interventions to combat resistance. *Lancet (London, England), 387*(10015), 285–295 [Online]. https://doi.org/10.1016/S0140-6736(15)00520-6. Accessed 22 Dec 2020.

Dyar, O. J, Huttner, B., Schouten, J., & Pulcini C. (2017). ESGAP (ESCMID Study Group for Antimicrobial Stewardship). What is antimicrobial stewardship? *Clinical Microbiology and Infection, 23*(11), 793–798 [Online]. https://doi.org/10.1016/j.cmi.2017.08.026. Accessed 24 Dec 2020.

European Commission. (2017). *A European One Health action plan against antimicrobial resistance (AMR)* [Online]. Available at: https://ec.europa.eu/health/amr/sites/amr/files/amr_action_plan_2017_en.pdf. Accessed 24 Dec 2020.

Fletcher, S. (2015). Understanding the contribution of environmental factors in the spread of antimicrobial resistance. *Environmental Health and Preventive Medicine, 20*, 243–252.

Gandra, S., Jyoti, J., Anna, T., Anjana, S. L., & Ramanan, L. (2017). *Scoping report on antimicrobial resistance in India*. Center for Disease Dynamics, Economics and Policy [Online]. Available at: https://cddep.org/wp-content/uploads/2017/11/scoping-report-on-antimicrobial-resistance-in-india.pdf. Accessed 24 Dec 2020.

Graham, D. W., Gilles, B., Megan, W. B., James, D., Filomena, G., Adina, H., Laura, H. K., Paul, S. M., Morgan, S., Shabbir, S., Randall, S. S., Tara, C. S., Carina, S., & Thomas, E. W. (2019). Complexities in understanding antimicrobial resistance across domesticated animal, human, and environmental systems. *Annals of the New York Academy of Sciences, 1441*, 17–30.

Grenni, P., Ancona, V., & Barra, C. A. (2018). Ecological effects of antibiotics on natural ecosystems: A review. *Microchemical Journal, 136*, 25–39 [Online]. https://doi.org/10.1016/j.microc.2017.02.006. Accessed 26 Dec 2020.

Hamna, H., Prejit, Asha, K., Vergis, J., Deepa, P. M., Suma, N., & Hema, P. A. (2019). Nasal carriage of *Staphylococcus aureus* and MRSA among companion animals and livestock in Wayanad district, India. *International Journal of Current Microbiology and Applied Sciences, 8*(12), 1254–1260.

Hernando, A. S., Coque, T. M., Baquero, F., & Martínez, J. L. (2019). Defining and combating antibiotic resistance from One Health and global health perspectives. *Nature Microbiology, 4*, 1432–1442.

Hoelzer, K., Wong, N., Thomas, J., Talkington, K., Jungman, E., & Coukell, A. (2017). Antimicrobial drug use in food- producing animals and associated human health risks: What, and how strong, is the evidence? *BMC Veterinary Research, 13*, 211.

Hoque, R., Ahmed, S. M., Naher, N., Islam, M. A., Rousham, E. K., Islam, B. Z, & Hassan, S. (2020). Tackling antimicrobial resistance in Bangladesh: A scoping review of policy and practice in human, animal and environment sectors. *PLoS ONE, 15*(1), e0227947 [Online]. https://doi.org/10.1371/journal.pone.0227947. Accessed 26 Dec 2020.

Kalluri, S., Gilruth, P., Rogers, D., & Szczur, M. (2007). Surveillance of arthropod vector-borne infectious diseases using remote sensing techniques: A review. *PLoS Pathogens, 3*, 1361–1371.

Kelly, R., Zoubiane, G., Walsh, D., Ward, R., & Goossens, H. (2016). Public funding for research on antibacterial resistance in the JPIAMR countries, the European Commission, and related European Union agencies: A systematic observational analysis. *Lancet Infectious Diseases, 16*(4), 431–440 [Online]. https://doi.org/10.1016/S1473-3099(15)00350-3. Accessed 28 Dec 2020.

Kieny, M. P., Evans, D. B., Schmets, G., & Kadandale, S. (2014). Health-system resilience: Reflections on the Ebola crisis in western Africa. *The Bulletin of the* World Health Organization*, 92*(12), 850 [Online]. https://doi.org/10.2471/BLT.14.149278. Accessed 28 Dec 2020.

Klein, E. Y., Van Boeckel, T. P., Martinez, E. M., Pant, S., Gandra, S., Levin, S.A., Goossens, H., & Laxminarayan, R. (2018). Global increase and geographic convergence in antibiotic consumption between 2000 and 2015. P*roceedings of the National Academy of Sciences of the United States of America, 115*, E3463–E3470.

Kumar, S. G., Adithan, C., Harish, B. N., Sujatha, S., Roy, G., & Malini, A. (2013). Antimicrobial resistance in India: A review. *Journal of Natural Science, Biology, and Medicine, 4*(2), 286–291 [Online]. https://doi.org/10.4103/0976-9668.116970. PMID: 24082718; PMCID: PMC3783766. Accessed 16 Dec 2020.

Laxminarayan, R., Duse, A., Wattal, C., Zaidi, A. K. M., Wertheim, H. F. L., Sumpradit, N., Vlieghe, E., Hara, G. L., Durand, C. G., & Aires, B. (2014). Antibiotic resistance—The need for global solutions. *Lancet Infectious Diseases, 13*, 1057–1098 [Online]. https://doi.org/10.1016/S1473-3099(13)70318-9. Accessed 22 Dec 2020.

Lazarus, B., Paterson, D. L., Mollinger, J. L., & Rogers, B. A. (2015). Do human extraintestinal *Escherichia coli* infections resistant to expanded-spectrum cephalosporins originate from food-producing animals? A systematic review. *Clinical Infectious Diseases, 60*(3), 439–452. [Online]. https://doi.org/10.1093/cid/ciu785. Accessed 12 Dec 2020.

Marion, V., Sylvain, G., Franck, P., Patrick, D., Lionel, B., Nicolas, R., Audrey, A., Salim, A., Helene, J., Michel, G. C., Frederic, T., & Francois, R. (2016). Antimicrobial resistance in wildlife. *Journal of Applied Ecology, 53*, 519–529.

Mohan, P. J., Chifumbe, C., Mirfin, M., Dan, K., Oliver, H., Tenaw, A., Martha, E., Bayobuya P., & Heran, G. (2018). Multidisciplinary and multisectoral coalitions as catalysts for action against antimicrobial resistance: Implementation experiences at national and regional levels. *Global Public Health, 13*(12), 1781–1795 [Online]. https://doi.org/10.1080/17441692.2018.1449230. Accessed 17 Dec 2020.

Nweneka, C. V., Ndey, T., & Anibal, S. (2009). Curbing the menace of antimicrobial resistance in developing countries. *Harm Reduction Journal, 6*, 31 [Online]. https://doi.org/10.1186/1477-7517-6-31. Accessed 27 Dec 2020.

OIE, World Organization for Animal Health. (2016). *The OIE strategy on antimicrobial resistance and the prudent use of antimicrobials*. [Online]. Available at: https://www.oie.int/en/oie-strategy-tackles-the-threat-of-antimicrobial-resistance-amr-in-animals/. Accessed 17 Dec 2020.

Pornsukarom, S., & Thakur, S. (2017). Horizontal dissemination of antimicrobial resistance determinants in multiple Salmonella serotypes following isolation from the commercial swine operation environment after manure application. *Applied Environmental Microbiology, 83*(20), e01503–e01517 [Online]. https://doi.org/10.1128/AEM.01503-17. Accessed 19 Dec 2020.

Prejit. (Ed.). (2017). *Veterinary public health: One health* (1st ed.). New Delhi Publishers.

Prejit. (2020). India to envision one health movement for confronting emerging health threats: From concept to approach toward institutionalization. *International Journal of One Health, 6*(2), 165–176.

Rock, M. J. (2017). Who or what is 'the public' in critical public health? Reflections on post-humanism and anthropological engagements with One Health. *Critical Public Health, 27*(3), 314–324.

Shallcross, L. J., Howard, S. J., Fowler, T., & Davies, S. C. (2015). Tackling the threat of antimicrobial resistance: From policy to sustainable action. *Philosophical Transactions of the Royal Society, B370*, 20140082 [Online]. https://doi.org/10.1098/rstb.2014.0082. Accessed 22 Dec 2020.

Silbergeld, E. K., Graham, J., & Price, L. B. (2008). Industrial food animal production, antimicrobial resistance, and human health. *Annual Review of Public Health, 29*, 151–169.

Suma, N., Prejit, Asha, K., Jess, V., Hamna, H., & Hema, P. A. (2019). Occurrence of ESBL producing *Yersinia enterocolitica* among companion animals in Wayanad district. *The Pharma Innovation Journal, 8*(9), 182–187.

Thakur, S., & Gray, G. C. (2019). The mandate for a global "one health" approach to antimicrobial resistance surveillance. *American Journal of Tropical Medicine and Hygiene, 100*, 227 [Online]. https://doi.org/10.4269/ajtmh.18-0973. Accessed 10 Dec 2020.

Thomas, F. O. (2002). Emergence, spread, and environmental effect of antimicrobial resistance: How use of an antimicrobial anywhere can increase resistance to any antimicrobial anywhere else. *Clinical Infectious Diseases, 34*, S78–S84.

Van Boeckel, T. P., Glennon, E. E., Chen, D., Gilbert, M., Robinson, T. P., Grenfell, B. T., Levin, S. A., Bonhoeffer, S., & Ramanan, L. (2017). Reducing antimicrobial use in food animals. *Science Insights, 357*, 6358.

Van Boeckel, T. P., João, P., Reshma, S., Cheng, Z., Julia, S., Nicola, G. C., Marius, G., Sebastian, B., & Ramanan, L. (2019). Global trends in antimicrobial resistance in animals in low- and middle-income countries. *Science, 365*, eaaw1944.

Wellington, E. M., Boxall, A. B., Cross, P., Feil, E. J., Gaze, W. H., Hawkey, P. M., Johnson-Rollings, A. S., Jones, D. L., Lee, N. M., Otten, W., Thomas, C. M., & Williams, A. P. (2013). The role of the natural environment in the emergence of antibiotic resistance in gram-negative bacteria. *Lancet Infectious Diseases, 13*(2), 155–165.

White, A., & Hughes, J. M. (2019). Critical importance of a one health approach to antimicrobial resistance. *EcoHealth, 16*(3), 404–409.

WHO (World Health Organization). (2018). *The Thirteenth General programme of Work, 2019–2023, promote health, keep the world safe, serve the vulnerable* [Online]. Available at: https://apps.who.int/iris/bitstream/handle/10665/324775/WHO-PRP-18.1-eng.pdf. Accessed 27 Dec 2020.

WHO (World Health Organization). (2019). *Ten threats to global health in 2019* [Online]. Available at: https://www.who.int/news-room/spotlight/ten-threats-to-global-health-in-2019. Accessed 29 Dec 2020.

AMR Global Scourge: Literacy as an End-to-End Containment Measure

Mukteswar Prasad Mothadaka,
Chandragiri Nagarajrao Ravishankar, and Joykrushna Jena

Contents

M. P. Mothadaka (✉)
Visakhapatnam Research Centre of ICAR-CIFT, ICAR-Central Institute of Fisheries Technology (ICAR-CIFT), Visakhapatnam, Andhra Pradesh, India

Microbiology, Fermentation and Biotechnology Division, ICAR-Central Institute of Fisheries Technology (ICAR-CIFT), Cochin, Kerala, India
e-mail: mm.prasad@icar.gov.in; prasadmm@hotmail.com

C. N. Ravishankar
ICAR-Central Institute of Fisheries Education (ICAR-CIFE), Maharashtra, Mumbai, India
e-mail: ravishankar.cn@icar.gov.in

J. Jena
Division of Fisheries Science, Indian Council of Agricultural Research (ICAR), New Delhi, India
e-mail: ddgfs.icar@gov.in

M. P. Mothadaka et al. (eds.), *Handbook on Antimicrobial Resistance*,
https://doi.org/10.1007/978-981-19-9279-7_46

Abstract

Currently, the AMR problems and solutions therein are widely discussed and containment measures are pragmatized in different ways and means with the sole aim of solving this universal scourge. From the Nobel lecture of Alexander Fleming in 1945 to the Rustav Aminov Report in 2010, to date AMR problems continue to attract attention. From the COVID-19 pandemic treatment to meeting important targets of SDG, the AMR is dominating academics, research, socio-cultural economic, healthcare of both humans and animals, and the environment, leading us to adopt the "One Health" approach. Attempts were made to understand the cognizable levels of AMR knowledge among medical and nonmedical students, types of research being carried out, and deficiencies therein, while addressing the health literacy levels of patients and assessing survey models have a long way to go. Healthcare, pharmacy, agriculture, animal husbandry, fisheries and aquaculture, and the environment are the various areas identified to improve antibiotic literacy and to initiate, develop, and spread literacy in relation to antibiotics. The ways and means to achieve antibiotic literacy were also provided. Antibiotic literacy is the best example for investing in humans than in finances that deliver the quickest possible results against antimicrobial resistance in the shortest period of time.

Keywords

Antimicrobial resistance · Antibiotics · Literacy

1 Introduction

The present world population is 7,919,643,644,213 (as of 12 January, 2022) and is likely to reach 9,735,033,990 by 2050, with a density of 65 per square kilometer, median age of 36 years, and 6,679,756,162 (68.6%) people in urban areas. This will put a huge pressure on resources while meeting the demographic demands of health, cleaner environments, and aquatic requirements in terms of potable water for agriculture purposes, food, and nutritional security. This, in turn, can put stress on agriculture and animal husbandry to enhance production and productivity in unit time and space. One of the unseen and unfelt major hindrances in meeting these demands is the complex problem of antimicrobial resistance.

In recent decades, there has been a remarkable increase in the use of antibiotics in both the human and animal sectors. This has resulted in antimicrobial resistance in

bacteria and the concomitant increase in drug-related infections (DRIs). It is estimated that these infections will cause 0.01 billion mortalities annually worldwide if they are not controlled by the middle of this century, with a potential economic cost of US$ 20 trillion. The surreptitious development of AMR worldwide is rallying its stride. The economically underdeveloped and developing countries are more vulnerable to this scourge. This is also a significant hazard to biosecurity. The AMR microbes are ubiquitous in nature and spread from pond to plate, healthcare facilities, work places, animal husbandry, agriculture, humans, soil, and seas. In technologically and economically advanced countries like the United States, nearly 2 million people suffer from infections related to AMR, resulting in 0.23 million mortalities annually. The recent studies indicate that there are more chances of further deterioration of the DRI in the future. Alexander Fleming predicted these issues in his Nobel lecture delivered on December, 11, 1945:

> The time may come when penicillin can be bought by anyone in the shops. Then there is the danger that the ignorant man may easily under dose himself and by exposing his microbes to non-lethal quantities of the drug make them resistant. Here is a hypothetical illustration. Mr. X. has a sore throat. He buys some penicillin and gives himself, not enough to kill the streptococci but enough to educate them to resist penicillin. He then infects his wife. Mrs. X gets pneumonia and is treated with penicillin. As the streptococci are now resistant to penicillin the treatment fails. Mrs. X dies. Who is primarily responsible for Mrs. X's death? Why Mr. X whose negligent use of penicillin changed the nature of the microbe. Moral: If you use penicillin, use enough.

The testimony on antibiotic resistance by Rustav Aminov in 2010 shows the intricacy of the impending peril: "It is not a single grand challenge; it is rather a complex problem requiring concerted efforts of microbiologists, ecologists, health care specialists, educationalists, policy makers, legislative bodies, agricultural and pharmaceutical industry workers, and the public to deal with. In fact, this should be of everyone's concern, because, in the end, there is always a probability for any of us at some stage to get infected with a pathogen that is resistant to antibiotic treatment."

Antibiotics, the most important and dominant component of global well-being, are predicted to lose their shine by 2050, resulting in 0.35 billion moralities from superbug infections. In most of the developed countries, the absence of motivating strategic devices to assimilate the growth of inexpensive and good antimicrobials is an undeniable fact. In the WAAW observed during November 18–19, 2020, the inconsistencies in treatment related to AMR were discussed as a part of European Patients' Rights Day, where it was noted that due to AMR the healthcare development of the century will be totally retrogressive in handling communicable diseases.

The development of antimicrobial resistance is not a new phenomenon regarding bacteria, but the recent acceleration is posing intimidating conditions. The hazard discernment of the US CDC on AMR specifies that "*employ* antibiotics *expect resistance*" (CDC, 2013, p. 14). The AMR sources are encompassed inside and outside surroundings, pets, domestic animals, and hospitals (Fortun, 2012; Orzech & Nichter, 2008). The employment of antibiotics as beneficial controller didn't serve the purpose, instead the AMR spread everywhere due to antibiotics (Oravcova et al., 2013; Rose et al., 2013).

The World Bank statement "Drug Resistant Infections: A Threat to Our Economic Future" states that, enhanced AMR will result in drug-related infections that cannot be treated, leading to prolonged hospitalization and seriously impacting LIG countries to a tune of 5% GDP losses by 2050. These conditions also increase the poverty levels by 3 million. Unlike the financial crises that occur at the global level wherein the chances of recovery are more, AMR-related infections leave no chance of recovery. The impact of AMR persists without projections for a cyclical recovery (World Bank, 2016; Adeyi et al., 2017).

1.1 COVID-19 Adding Fuel to the Fire of AMR

The worsening trends in SARS-CoV-2/COVID-19 treatment were due to the excessive use of antibiotics as shown by the treatment of 70–80% of hospitalized COVID-19 patients in the United States who were prescribed antibiotics despite only $<10\%$ patients having secondary bacterial infections. Similarly, in Italy, the Italian National Institute of Health states that out of 3335 mortalities due to COVID-19, nearly 86% were treated with antibiotics when only 12% required treatment for secondary infections (Svet et al., 2020).

Butler et al. (2021) indicate that using azithromycin to treat patients with unconfirmed COVID-19 is not a valid reason to decrease recovery time or avoid hospitalization. They also reported enhanced levels of azithromycin usage during the COVID-19 period.

There is a significant time lapse in taking into consideration the seriousness of the AMR problem, and the reasons include (Landecker, 2016)

1. Changing the prevailing drug.
2. Accessibility of alternative drugs.
3. It was believed that AMR is limited to defiant patients.
4. The mutants of drug resistance are sporadic and restricted to succeeding generations.
5. The nature of AMR genetic inheritance is vertical.
6. Instead of analyzing its complexity, it was treated as a reaction to the new drugs.
7. Not giving significant importance as a major test to the model.
8. The nature of resistant genes was not known.
9. Lack of molecular understanding of the AMR.
10. Bioinformatics and its applications are completely unknown.

Antibiotics are called "societal drugs" because when an individual starts using a particular antibiotic for specific ailment, for instance, throat infection, the samples taken from the household showed resistance to the antibiotic in all family members (Levy, 1998). Similarly, the persistent and wide usage of oxytetracycline for

prophylactic purposes in aquaculture has been proposed to be treated as an "aquaculture drug" (Prasad & Ravishankar, 2018).

More often than not what is forsaken are the ways and means to decrease the requirements of antibiotics. The occurrence of infections is attributed to inadequacy of water, improper sanitation, and unhygienic conditions (WASH). The reports from the World Bank and the Chatham House confirmed that the root cause of antibiotic usage in underdeveloped and developing nations is improper hygiene. Pragmatic care in WASH in under-resourced areas can reduce antibiotic use and the resultant reduction in AMR due to reduction in antibiotics use.

Self-medication is becoming a progressively significant part of healthcare. It takes patients to a better unconventional state in decision-making in managing inconsequential ailments, thereby encouraging enablement (Hughes et al., 2001). The advantages of self-treatment for healthcare systems are that it simplifies improved practice of medical assistances, escalates admittance to prescription, and possibly underwrites decreasing recommended medication expenditures connected in public subsidized well-being programs. However, it self-significantly undermines accompanying hazards, viz., misdiagnosis, over- and improper use of drug dosage, protracted period of use, drug interfaces, and polypharmacy. Senior citizens and immunocompromised cases with existing comorbidities, especially aged population, are more prone to these hazards. Observing structures, trust among patients, doctors, and posologists, and the setting up of edification and evidence to all related to safe self-treatment are recommended to maximize advantage and minimize risk. This is one school of thought at the advent of the twenty-first century (Hughes et al., 2001). The self-treatment assessment broadened encompassing different groups and countries. It became more relevant in the recent pandemic, wherein the use of antibiotics indicates more of a hazardous medication in the absence of any secondary infections (Table 1).

1.2 Concerns on the Impact of AMR on SDG

The direct and major impacts of AMR include (1) reduced effectiveness of the accessible drugs, (2) beginning of controlling, (3) decline in quality-adjusted life-years (QALY) and rate of recovery of the patients affected, (4) enhanced healthcare procedures and concomitant stay in hospitals, (5) amplification in expenditure for the healthcare organizations, and (6) reduced confidence in drugs and medication (Gajdács et al., 2021).

The indirect impacts of AMR are (1) multifaceted invasive interferences, (2) transplantation of organs, (3) chemotherapy of cancer, (4) intensive care, and (5) neonatology (Gajdács et al., 2021).

One of the fundamental needs for successful implementation of antibiotic literacy is to make sure it reaches everyone worldwide in colloquial terms that are easy to use and available in all languages and dialects.

Table 1 Country-wise case studies on self-treatment/medication with antibiotics

S. no.	Type of study	Country of study	Reference
1	Habit and deviations of antibiotics in Latin America.	Latin America	Wolff (1993)
2	Self-treatment practices in pastoral Maharashtra.	India	Phalke et al. (2006)
3	Self-treatment with antimicrobial drugs in Europe.	Europe	Grigoryan et al. (2006)
4	Self-treatment among residents of urban resettlement colony, New Delhi.	India	Lal et al. (2007)
5	Self-treatment with antibiotics in Jordanian population	Jordan	Al-Azzam et al. (2007)
6	Evaluation of self-treatment of antibiotics in a Jordanian population.	Jordan	Sawair et al. (2008)
7	Occurrence and outline of self-treatment use in coastal regions of South India.	India	Balamurugan and Ganesh (2011)
8	Study of self-treatment among patients go to public pharmacies in Erode, India	India	Samuel et al. (2011)
9	Self-treatment practice among undergraduate medical students in a tertiary care medical college, West Bengal.	India	Banerjee and Bhadur (2012)
10	Assessment of self-treatment antibiotics use pattern among patients attending public pharmacies in rural India, Uttar Pradesh.	India	Ahmad et al. (2012)
11	Health Literacy; WHO Regional Office for Europe: Copenhagen, Denmark	WHO, Denmark	Kickbusch et al. (2013)
12	Social determining factors of health facts pursuing among Chinese adults in Hong Kong	Hong Kong, China	Wang et al. (2013)
13	Assessment of self-treatment practices in rural area of town Sahaswan at Northern Northern India.	India	Ahmad et al. (2014)
14	Public knowledge, beliefs and behavior on antibiotic use and self-treatment in Lithuania.	Lithuania	Pavyde et al. (2015)
15	Awareness and outlooks towards antibiotic use and resistance – a latent class analysis of a Swedish population-based sample.	Sweden	Vallin et al. (2016)

(continued)

Table 1 (continued)

S. no.	Type of study	Country of study	Reference
16	Self-treatment and self-prescription with antibiotics in the Middle East –do they really happen? A systematic review of the prevalence, possible reasons, and outcomes.	Middle East	Alhomoud et al. (2017)
17	Practice of self-treatment with antibiotics in the Colombo district, Sri Lanka.	Sri Lanka	Senadheera et al. (2017)
18	Public knowledge and perception about antimicrobials and antimicrobial resistance in Japan: a national questionnaire survey in 2017.	Japan	Kamata et al. (2017)
19	Forms of self-treatment amongst medical and nonmedical university students in Jordan.	Jordan	Alshogran et al. (2018)
20	Valuation of information, approach and behavior towards antibiotic use in primary health care patients in Fayoum Governorate, Egypt.	Egypt	El Sherbiny et al. (2018)
21	Online health facts and public familiarity, approaches, and behaviours concerning antibiotics in the UK: Multiple regression analysis of Wellcome Monitor and Eurobarometer Data.	The United Kingdom	Anderson (2018)
22	Antibiotic use, understanding and health literacy among the general population in Berlin, Germany and its surrounding rural areas.	Germany	Salm et al. (2018)
23	Inappropriately prescribed and over-the-counter antimicrobials in the Brazilian Amazon Basin: We need to promote more rational use even in remote places.	Brazil	Muri-Gama et al. (2018)
24	Self-treatment of antibiotics: Examining practice among university students at the Malaysian National Defence University.	Malaysia	Haque et al. (2019)

(continued)

Table 1 (continued)

S. no.	Type of study	Country of study	Reference
25	Incidence and outline of antibiotic self-treatment practice in an urban population of Kerala, India: a cross-sectional study.	India	Rajendran et al. (2019)
26	Use of antibiotics without a prescription in the U.S. population: a scoping review.	The United States	Grigoryan et al. (2019)
27	Antibiotic use and resistance in hospitals: time-series analysis strategy for determining and prioritising interventions.	Providing hospitals in use of nonlinear time-series analysis for antibiotic use and controlling resistance	Jirjees et al. (2020)
28	Identification of thresholds in relationships between specific antibiotic use and carbapenem-resistant *Acinetobacter baumannii* (CRAb) incidence rates in hospitalized patients in Jordan.	Jordan	Hayajneh et al. (2021)
29	The prevalence and patterns of self-treatment with antibiotics in Jordan: a community-based study.	Jordan	Nusair et al. (2021)
30	Is health literacy associated with antibiotic use, knowledge and awareness of antimicrobial resistance among non-medical university students in Egypt? A cross-sectional study.	Egypt	Mostafa et al. (2021)
31	Self-treatment and antimicrobial resistance: a survey of students studying healthcare programmes at a tertiary institution in Ghana.	Ghana	Ofori et al. (2021)
32	A cross-sectional survey of the knowledge, attitudes, and practices of antimicrobial users and providers in an area of high-density livestock-human population in Western Kenya.	Kenya	Kemp et al. (2021)

(continued)

Table 1 (continued)

S. no.	Type of study	Country of study	Reference
33	Perception and practice of self-treatment with antibiotics among medical students in Sudanese universities: a cross-sectional study.	Sudan	Elmahi et al. (2022)
34	The impact of health literacy on self-treatment: a cross-sectional outpatient study.	Jordan	Muflih et al. (2022)
35	Self-medication practices and its determinants in healthcare professionals during the Coronavirus Disease-2019 pandemic: cross-sectional study.	Nigeria	Okoye et al. (2022)
COVID-related studies			
36	Antibiotic use in patients with COVID-19: A 'snapshot' Infectious Diseases International Research Initiative (ID-IRI) survey.	Survey in 23 countries from 82 different hospitals	Beovi'c et al. (2020)
37	Bacterial and fungal coinfection in individuals with coronavirus: a rapid review to support COVID-19 antimicrobial prescribing.	In this study, 1007 abstracts and 18 full-length papers were screened	Rawson et al. (2020)
38	Clinical course and outcomes of critically ill patients with SARS-CoV-2 pneumonia in Wuhan, China: a single-centered, retrospective, observational study.	China	Yang et al. (2020)
39	Rapid spread and control of multidrug-resistant Gram-negative bacteria in COVID-19 patient care units.	The United States	Patel et al. (2021)
40	Public health literacy, knowledge, and awareness regarding antibiotic use and antimicrobial resistance during the COVID-19 pandemic: a cross-sectional study.	Jordan	Muflih et al. (2021)

2 Assessing AMR Understanding Among Medical Students

2.1 European Outlook

The major concern is, are the medical students geared up to prescribe antibiotics reasonably and responsibly? An assessment was made on the self-preparedness of final-year medical students from European universities using a comprehensive set of topics on the responsible use of antibiotics. A cross-sectional, multicenter, web-based survey was conducted. Final-year medical students from European universities were eligible to take part in this survey. The readiness marks were computed for individual students, and the average marks were computed to the ranks of medical colleges and countries. Evaluations were also made with country-level data on antimicrobial resistance of common (4) microbial pathogens.

The survey revealed that out of a total of 7328 responses that included 60.47% of qualified medical colleges from all the countries (100%), the undergraduates were adequately equipped on an average of more than 70% of 71.2% of subjects evaluated, with 54.8% to 84.8% in Portugal and Latvia, respectively (Dyar et al., 2018).

The percentage needed for more education on judicious antibiotics use or common antibiotic use was the lowest in Sweden (20.3%) the highest in Slovakia (94.3%), with a mean of 66.1%. A strong and inverse correlation was observed in preparedness scores, that is, Spearman's rank correlation coefficient of 0.72 out of 29, where $P < 0.001$. The incidence of antibiotic nonsusceptible bacteria at higher degrees was associated with lesser responsiveness marks and superior reports of subjects (self) under study who needed further instructions (P, 0.01) (Dyar et al., 2018).

The survey revealed that the majority of European final-year medical students felt the prerequisite for additional instructions on antibiotics use to facilitate their forthcoming preparation as junior doctors. The survey also identified the forms of readiness on precise topics that were significantly consistent across countries and correlated with both perceived requirements for further education and levels of antibiotic resistance among common bacteria (Dyar et al., 2018).

2.2 Japanese Scenario

In Japan, the AMR NAP was introduced in 2016, and a study on antibiotic literacy, namely, responsiveness, information, and assertiveness in relation to the antibiotics use, was conducted with professionals (practicing doctors) and nonprofessionals (common people) as the target group from September 2019 to February 2020. The Okayama University Medical School students enrolled for this purpose. The data were collected using a paper-based questionnaire of 11 questions on antibiotic literacy with a response rate of 93.8%, that is, 661 of 705 students. The study indicated that 92.6% of the students were cognizant of antibiotics inhibiting growth of bacteria and 6.5% (43) were aware of the AMR action plan in Japan. The study suggested the need for enhanced level of literacy on antimicrobial use in addressing AMR and endorsing antimicrobial stewardship (Hagiya et al., 2020).

3 Egyptian Case Study with University Students sans Medical Background

A one-of-its-kind study in low-income countries (LICs) and low-middle-income countries (LMICs) was conducted in Egypt to evaluate the relationship between different stages of health literacy and antibiotic employment, information on antibiotics, and cognizance of antimicrobial resistance among the university students without medical background (Mostafa et al., 2021). The survey was based on the established questionnaires that were used to investigate diverse populations and the Egyptian Health Literacy Survey (HLS-EU-Q16). The Multi-Country Public Awareness Survey of World Health Organization-Antimicrobial Resistance was also used. The use of self-administered investigation could help circumvent generally anticipated responses and permitted examination of a number of characteristics relevant to students' acquaintance with antibiotic use that earlier lessons endorsed addressing, but a detailed enquiry did not appear practicable by means of the existing study proposal (Mostafa et al., 2021). The authors felt that the cross-sectional-type investigation and the suitable sample size of 508 nonmedical university students are not a total reflection of the wider student communities without medical background in Egypt; however, this fact-finding study laid the foundation for shielding the gap in familiarity about the link between university students' health literacy, antibiotic use, familiarity with antibiotics, and responsiveness of antibiotic resistance. The conceivable hazard aspects found in the study could direct forthcoming surveys and support legislators in planning interventions for AMR control, which can attend to the precise requirements of academia scholars in LICs and LMICs in which over-the-counter sale and use of antibiotics without prescription is a common feature.

4 Identification of Research Types and Lacunae in Information

One of the top 10 global security threats is antimicrobial resistance, that can have profound effects on food security, healthcare, and development. The AMR hazard is on par with threats of extremism and climate change (Stig Wall, 2019).

As a part of assessing the knowledge gaps and the strategies to prevent ABR, the study explored screening the published data of last two decades, the extent of peer-reviewed and unconventional works that referred to the AMR problem and with "prevention" at its core (Wall, 2019).

Wall's (2019) review encompassed important information sources, namely, EBSCO, MEDLINE, SCOPUS, and Clarivate Web of Science, searched from 2000 to 2017. The search of the wider investigation ground "antibiotic or antimicrobial resistance" resulted in 431,355 hits. Reduction in the pursuit on "prevention of antibiotic or antimicrobial resistance" resulted in 1062 of the remaining titles of which 622 were unique titles. Further search of the 622 titles revealed that 420 abstracts were read, of which 282 papers were read completely. An extra 53 mentions were recognized from these documents, and 64 printed included the

years 2018 and 2019 with a concluding scoping review database of 399 documents (Wall, 2019). While categorizing, the published papers on various subject areas led to the emergence of domain structure that could serve as proxy for the research community involved in AMR research. More than half of the 399 research articles published in the last four years showed that the AMR research area is evolving.

There is a need to strengthen epidemiological modelling and also there is a dire requirement for additional and enhanced investigation arrangements, particularly in LICs and MICs. (Wall, 2019). The availability of voluminous data is observed in use and misuse of antibiotics at the regional and country levels. Lack of evidence was noted in didactic and supervisory programs. Numerous evaluations addressed the information of the common man and prescribers. Strategic instructions are communicated in many disturbing reports from domestic and global establishments.

The literature survey showed that it is more descriptive in nature than theoretical determinations. The dire requirement for essential methods was observed when it came to better comprehension and elucidation of AMR scenario from a behavioral viewpoint. A plan for an epidemiological underlying network behind ABR is proposed that may help recognize access topics for possible interventions.

5 Addressing the Health Literacy Levels of AMR

A study carried out by Philip Howard and Gillian Hawksworth in 2019 (presented at the Pharmacy Together 2019 conference on November 29, 2019). observed that in the United Kingdom more than 40% in the 16–65-year age group do not have the skills to read and discern healthcare information. The authors also felt that one way to overcome this impediment is to have enhanced levels of health literacy wherein they defined it as "the ability of individuals to gain access to, understand and use information in ways which promote good health."

5.1 Patient Information Leaflet (PIL)

The objectives of the study were

1. Development of patient information leaflet (PIL) on antibiotic courses and AMR with health literacy techniques.
2. Screening the patients with PIL.
3. To find out the use of PIL in enhancing patient information on antibiotics.
4. To assess ease in comprehension of the fact and figures supplied to patients on health literacy procedures.
5. To examine the efficacy of PIL as a means of organized treatment by working public pharmacologists by recognizing whether they thought it was a beneficial treatment tool that reinforced their preparation.
6. Is it in the connivance of druggists that patients aided from the written counseling given to them *in lieu* of the usual unwritten (vocal) practice?

The authors obtained necessary approval of ethics in this study. Using the updated Royal Pharmaceutical Society Specification for Community Pharmacy and health literacy techniques that included Patients Schooling Resources Valuation Method, a PIL and patient survey was established (Howard & Hawksworth, 2019; Beck et al., 2020).

They pooled information from eight community pharmacies compliant to the study for a period of 35 days. The PIL used as a part of treatment method by druggists was given to the patients at the time of providing short-course antibiotics. Suitable sampling was carried out with personal interaction surveys using PIL forms. Patients too completed the survey at the completion of the study period.

Response from Patients: A total of 106 fully completed patient surveys were received in the study that confirmed that all patients had experience of exposure to antibiotics on previous occasions. The study showed that before exposure to PIL, 13% had significant knowledge of AMR, >50% had some knowledge, and 20% had heard about AMR but not completely aware, and 9% had no knowledge of AMR. The study also observed correlation between education level and knowledge of AMR, wherein 90% university graduates were in the category of "some/a lot knowledge" about AMR compared with ~66% college and 6th Form and more than 50% school dropouts (Howard & Hawksworth, 2019; Beck et al., 2020). Regarding the proper use of antibiotics, all the respondents felt that PIL is really useful in enhancing their knowledge. Also, 81% felt that PIL enriched their understanding of antibiotics showed inclination to change their attitude, while more than 50% said that they completed the required dosage of antibiotics at all times. All the respondents responded that PIL is easy to follow, and 90% who used antibiotics felt that treatment with PIL was easier to comprehend than other treatments (Howard & Hawksworth, 2019; Beck et al., 2020). The response from pharmacists indicates all (8) completed the surveys, of which 6 continued the survey even in the absence of the examiners. On a hedonic scale of 5, 5 indicates improved significantly and 1 indicates no significant impact at all in assessing the use of PIL. While upgrading the patient's information on the suitable use of antibiotics, 62.5% of the druggists scored 80–100%, demonstrating that in their opinion PIL can enhance the knowledge of patients. Also, 25% of the druggists scored 60% and 12.5% reported a score of 2. Regarding the time taken to comprehend PIL, 62.5% of the pharmacists felt that it is similar to that of standard counseling, 37.5% felt that it takes more time to comprehend, and 25% felt that it was useful (Howard & Hawksworth, 2019; Beck et al., 2020).

Some of the important findings of the study of Howard and Hawksworth (2019) and Beck et al. (2020) are provided next.

Despite the sample size of the study being small and the patients were not taking the antibiotics for the first time, the authors deduced that

1. There is an urgent requirement for providing information on antibiotics at the supply point.
2. The information on PIL needs to be very simple for communication employing health literary techniques in enhancing the knowledge of AMR.

3. Use of right antibiotics taking into consideration the practice of druggists as an organized treatment method.
4. Written PIL has more impact than oral counseling that resulted in acquiring information on AMR when the information is provided at the time of supply of the antibiotic.
5. The preliminary study indicated that patients' behavior toward responsible use of antibiotic can be altered when the organized method is based on health knowledge procedures.
6. It is suggested to use different layouts of PIL for various associates (Howard & Hawksworth, 2019; Beck et al., 2020).

6 Assessing the Evolution of Survey Methods in AMR

In the assessment of knowledge and awareness of antibiotic use and AMR, different methods are being used by various organizations. Many reviews aimed on the research outcomes on the levels of knowledge and AMR awareness. But evaluation of survey methods and data tools were not taken care of as they contribute significantly to logical conclusions. However, Kosiyaporn et al.'s review (2020) assessed the methods of the study and procedures of prevailing population-based surveys and explored the important components that define the levels of knowledge and awareness of antibiotic use and AMR in the general population. For this purpose, they explored the available literature on population-based surveys that pursued knowledge and awareness of antibiotic use and AMR in the general population employing the databases, viz., Ovid, MEDLINE, EMBASE, PsycINFO, and Scopus, domestic journals, and grey literature sources. The review period was from January 2000 to December 2018 on population-based cross-sectional studies that were published in English or Thai. The quality assessment was carried out using the "Appraisal Tool for Cross-Sectional Studies" (AXIS).

A total of 22 studies were analyzed that had strongly focused on the objective of screening people's levels of knowledge, awareness, attitudes, and behavior relating to antibiotic use and awareness of AMR. Regarding population-based cross-sectional surveys, the (22) studies have followed suitable procedures pertaining to research queries. The review indicated that more than 50% appropriately designed the methodologies that met laid-down standards. However, the remaining did not follow proper sample size, viz., estimations, sample frames, and selection biases. The review identified four themes for the common queries in the surveys: knowledge and awareness in antibiotics use, knowledge and awareness of AMR, behavior related to antibiotic use, and cross-cutting topics such as self-medication.

In the study, four important features in good practices of antibiotic use and awareness surveys were (1) objective of survey shall be clear; (2) sampling techniques are scientific based on ensuring the representativeness; (3) appropriate approaches for enrolment of samples and survey administration methods; and (4) trustworthy sampling sizes to avoid biases. The review emphasized the necessity

to design questionnaire on the healthcare systems that have access to health services and antibiotics.

A better use and implementation of the survey findings in antibiotics use and the AMR is possible only when they principally produce public health interventions and target specific groups to mitigate the AMR problems.

The central emphasis of the 2021 G7 summit was to address the silent role of the AMR pandemic reflected in another study. Employing antimicrobials below the required levels is not only of pitiable clinical consequences but also a major driver of AMR. To achieve antimicrobial security, a balancing act on research efforts on AMR between new drug development and the plans to safeguard usefulness and enhance efficiency of available antimicrobial agents is necessary. With this premise, Charani et al. (2021) reviewed existing proof and multistage involvement with varied global stakeholders that include those associated with community health, healthcare, research and development, policy, and patient advocacy, and the authors recognized urgencies in research for appropriate use of antibiotics for human use through four comprehensive subjects, viz., (1) program and tactical planning; (2) medicine administration and recommending arrangements; (3) expertise in optimizing and recommending; and (4) framework, beliefs, and behaviors. It is stated that the viability of the progress depends on the development of suitable interventions that are affordable and suitable to the context, ease in data usage, and counseling systems throughout the healthcare situations; assisting proper supporting suitable and accessible scientific inventions. Fulfilling this approach for AMR investigations on the appropriate use of antimicrobial in people could add to justifiable worldwide well-being (Charani et al., 2021).

7 CARB of US NAP: 2020–2025

The US Government NAP for Combating Antimicrobial Resistant Bacteria (CARB) for the period 2020–2025 presented coordinated and strategic action plans for the betterment of all its citizens by altering the progression of antibiotic resistance. The plan of action is based on US CARB of 2014, and the first NAP was released in 2015 by intensifying proof-centered actions that have revealed reduction in AMR, namely, augmenting the use of antibiotics in human and animal health settings. The strategy is to carry forward the infection prevention and control programs on a priority basis to reduce the spread of infections as well the use of antibiotics. The strategy supports state-of-the-art methods to develop and install diagnostic tests and treatment plans to ensure appropriate care of antibiotics to the patients. One of the important and major aspects of this plan is to implement One Health approach with an extended effort of understanding AMR in the environment Furthermore, the plan emphasizes collecting and using data to assess the source of resistance, fund development of new diagnostics and treatment opportunities, and promote international coordination.

The purpose of AR threat report 2019 was to

1. Assist as a data source on AMR
2. Deliver an up-to-date US A.R affliction assessments for human well-being
3. Highpoint evolving zones of apprehension and further accomplishment required

Persistent hazards (Ex; *Clostridioides difficile,* Acinetobacter & Enterobacterales that are resistant to carbapenem)

Severe hazards (Ex: Campylobacter, Candida, nontyphoidal Salmonella, Salmonella serotype Typhi, Shigella, *Streptococcus pneumoniae,* and tuberculosis of drug-resistant type; ESBL Enterobacterales, VRE).

In relation to dangerous (Ex: Group A Streptococcus, Group B Streptococcus of erythromycin and clindamycin, resistant type, respectively).

Lookout category: The drug-resistant type *Bordetella pertussis* and *Mycoplasma genitalium.*

Some of the vilification exercises well observed by the CDC Director included keeping an end to (CDC, 2019):

No more mention of post-antibiotic era for the reason the so-called miracle drugs are sympatric and most of the patients are torn apart by the invisible enemy. The need of the hour is to be a part of the resolution.

An end to the blame game as each individual, industry, and country is playing an important role in the development of AMR. Hence, each has a role and responsibility in combating this scourge to a logical conclusion.

No more dependency on new antibiotics as they are not being developed for so many reasons such as time taken, cost-effectiveness, and slow pace in their advent to market. The simplest belligerent tactic to implement is to keep these germs at arm's length of prevention than controlling.

The AMR is in the "backyard" of each community, country, hospital, food, and environment. It is worldwide without any place of escape. At the same time, everyone can avoid it washing hands to prevent diseases and also using antibiotics.

8 Swedish Resourcefulness

Sweden is the first country to appoint an AMR ambassador. According to the Minister for Health and Social Affairs Lena Hallengren: "Antimicrobial resistance has long been an important issue for the Government. It is a serious and growing public health problem in Sweden and around the world. Sweden has a great deal of knowledge and experience in this area, and we are now raising the bar even higher by appointing an ambassador to strengthen Sweden's work internationally" (Swedish Press Release, 2022).

The world's first AMR ambassador is Dr. Malin Grape, currently heading Public Health Agency, Unit for Antibiotics and Infection Control, who has huge experience both at the national and international levels on matters related to antimicrobial resistance. For instance, Dr. Grape was in charge of the Public Health Agency to shape and advance GLASS (Global Antimicrobial Resistance and Use Surveillance System) of the WHO and has considerable experience of working on the projects of the European Joint Action on Antimicrobial Resistance and Healthcare-Associated

Infections (JAMRAI). Upon her appointment as AMR ambassador, Dr. Grape said: "I am tremendously honoured by this appointment. It feels very important to work on what the WHO considers to be one of the greatest global threats to human health. It's an issue that concerns us all and that we must work with at international level to have an impact" (Swedish Press Release, 2022).

A perusal on these Swedish AMR-related contributions indicates that the Swedish government has been working on AMR both with the EU and at the international level. In WHO, Sweden and the United Kingdom co-drafted a resolution 8 years earlier (2014). Also, both Sweden and the United Kingdom co-founded the Ministerial Alliance of Champions against Antimicrobial Resistance, with Australia, Brazil, Canada, China, France, South Africa, and United States as members. The Swedish Minister for Health and Social Affairs Lena Hallengren is responsible internationally for endorsing AMR-containing efforts as a co-chair of the UN Global Leaders Group on AMR. Dr. Malin Grape holds a master's degree in pharmacy and a doctorate in AMR research with wide-ranging experience both at the national and international levels (Swedish Press Release, 2022).

9 India's Initiatives

9.1 Matter of Contemplation

India's Prime Minister Narendra Modi initiated Mann Ki Baat (Inner Thoughts or Matter of Contemplation) on October 3, 2014, a program in which he addressed the people through AIR (All India Radio), Doordarshan (DD) National, and DD News. In the 22nd episode of Mann Ki Baat on July 31, 2016, the Prime Minister addressed the nation regarding the scourge of antimicrobial resistance. He reminded the people that in the struts and frets of life quotidian they have forsaken their own selves. He advised the people not to take an antibiotic for a quick relief from any illness. He added: "It sure may provide prompt relief but then this kind of. accidental antibiotic intake results in serious health hazard." He also advised the people to not take antibiotics for temporary relief but *to take only with the prescription of a physician*. The antibiotics should only be taken when prescribed by the doctors. Antibiotics intake without prescription can lead to severe problems. He added: "Despite the fact that the relief is quick upon intake of antibiotics at random, the same leads to development of resistance to those antibiotics by microorganisms." He also said that "due to the development of resistance the antibiotic loses its effect and to combat to the particular disease efforts needs to start afresh that means new drugs needs to be developed for which years of research is required. In the process of taking longer periods of time for the development of new drugs, the disease causes more health problems and more expenses. For the same reason, we need to be conscious on this." Then he added: "One other manifestation of the antibiotic consumption is that whenever doctor prescribes a specific dose, more often than not the patients tend to stop half way of the dosage because there is relief from the symptoms of the disease. What they do not realise is that when the dosage is incomplete the particular

disease causing microbes are not completely eliminated. And they develop resistance to the antibiotics under use." For instance, if the doctor prescribes 15 tablets for 5 days, then the patients need to follow as prescribed. He requested everyone to take medicines as prescribed by the doctor because an incomplete treatment is more harmful as it will help microbes survive and cause more problems in the future. He also stressed on the ill-effects of overconsumption of antibiotics as it helps the bacteria to develop resistance. Hence, it is important to consume the right amount of antibiotics and at the right time. Abandoning treatment midway makes the bacteria more resistant and the disease difficult to treat in the future. The microorganisms causing tuberculosis and malaria mutate and make most of the antibiotics in treatment ineffective, is what is called "resistance." He reminded the citizens to look for the red-colored part of an antibiotic strip and be conscious of the same.

9.2 Swachh Bharat Mission (SBM)

The Government of India started the Clean India Mission Swachh Bharat Mission (SBM), Swachh Bharat Abhiyan, throughout the country in 2014 to eradicate open excretion and develop the process of controlling the solid discards. One of the important objectives of Phase I of the mission included making conscious effort and bring in a behavioral transformation vis-à-vis cleanliness practices and capacity building at the grass root level. Phase I of SBM ended on October 2, 2019, with significant changes across the country.

9.3 Swachh Bharat Mission U-II

One of the major problems in countries like India, in which every sixth person walking on the Earth is Indian, is the handling of solid wastes that includes human exudates, especially of open-air excretion, which is a serious environmental issue in terms of hygiene and is also a source of transmission of diseases. To manage this, the Government of India started the Swachh Bharat Mission II (SBM-U 2.0).

The seriousness of the problem was evident by the monetary expenditure approved for SBM-Urban 2.0. In Phase I off SBM, the fiscal approval was INR0.62009 trillion, and Phase II approval is INR 1.41600 trillion, which is 2.28 times the importance given to tackle the problem.

9.4 The Aims of Clean India Mission II

1. Complete eradication of excreting in open areas, together with excretal slush controlling in all towns with a population of 100,000 or below.
2. Abolition of dangerous material entry into drains and to putrefying tanks.

3. To ensure wastewater is treated properly to prevent pollution of waterbodies.
4. It is envisaged that in the end all the intended municipalities will get the Garbage Free Certification of three-star category.

Deliverables under SBM-U-2.0 include

1. All the constitutional cities to attain first level of Open Excretion Free Areas (OEFA).
2. All the towns with a population 100,000 and below to reach level II of Open Excretion Free Areas (OEFA).
3. Establishment of schemes and practices to facilitate safe treatment of wastewater with best recycling ways so that no polluted water is left to further contaminate other water sources.

The root cause of the occurrence and transmission of all communicable diseases is the absence of managing properly solid wastes and unclean environs, resulting in increased expenses in treating diseases, and medications without prescription of mainly antibiotics of irregular dosage, resulting in the development of antimicrobial resistance. All these problems can be avoided if hygiene prevails in all the environments, and Clean India Mission is the world's largest mission catering to the needs of continuously growing population.

9.5 INFAAR of India

The antibiotic consumption pattern in humans revealed that in 2014 India was ranked at the top, followed by China and the United States, despite the per capita consumption of antibiotics in India being less than that of economically developed countries (Laxminarayan et al., 2016). India was ranked fifth in the world with regard to antibiotics usage in the animal sector in 2010, and it is predicted to enhance by 312% by 2030, thus achieving fourth rank in the world (Van Boeckel et al., 2015).

The FAO and Indian Council of Agriculture Research (ICAR) together organized a meeting at Kolkata, India, on March 7–8, 2017, to enable the formation of a national network of veterinary laboratories for antimicrobial resistance (AMR) in India. The network was named the Indian Network for Fishery and Animals Antimicrobial Resistance (INFAAR). The INFAAR network aims is to document AMR in diverse production organizations, define the transmission of resistant bacterial strains and resistance genes, recognize developments in resistance, and make propositions on bases and sources of resistant bacteria by means of systematic national reconnaissance programs. The vital data generated at the spatial level planned to contribute to frame policies and procedures to prevent and decrease the transmission of AMR in farmed animals and fish, and successively to the humans. INFAAR is essentially a methodological program of the Indian Council of Agricultural Research

(ICAR), being implemented with the cooperation of the Food and Agricultural Organization (FAO) and USAID since August 2018, mainly functional currently with intra-financial resources of ICAR institutions. In this network, the lead institutions are ICAR-National Bureau of Fish Genetic Resources (NBFGR), Lucknow, for fisheries and ICAR Indian Veterinary Research Institute (IVRI), Bareilly, for animal science. INFAAR is presently working with 18 organizations (15 ICAR institutions and 3 State Agriculture Universities) in 20 centers (9 fisheries and 11 livestock center) pan India (Rathore et al., 2020). Currently, INFAAR (1) carries out surveillance of AMR in objective microorganisms isolated from healthy farmed animals and fish/shellfish to compute its affliction and monitor the spatial and sequential developments of AMR in India, and (2) *the second important aspect is to develop attentiveness and accepting the hazards of AMR among the aquaculturists, fishers, veterinary and fish health professionals, and policymakers through effective communication, education, and preparation to encourage the prudent application of antimicrobials in farmed food animals and fish* (Rathore et al., 2020).

Observing the World Antibiotic Awareness Week (WAAW) religiously by all the institutes of ICAR and other INFAAR groups every year has become a common practice. Based on the theme of the year, lectures by experts to scientists, technicians, administrative personnel, scientists of INFAAR groups visiting, schools, colleges, and universities to spread awareness of AMR, organizing quiz programs, essay and elocutions competitions, among students, conducting field level and in hinterland areas have become regular affairs.

9.6 AMR NAP II on Animal Health of India

In 2015, the Global action Plan on AMR was established by the FAO, WOAH, and WHO (WHO, 2015). Based on the aforementioned procedural strategies of global agencies, India promulgated its National Action Plan on AMR of animal health sector with a time period of 2017–2021. In continuation, India plans to establish animal sector NAP II on AMR w.e.f. 2022; however, it was halted as review and revision of the existing NAP-AMR I were not possible due to the COVID-19 pandemic. Hence, a meeting was held on March 23 and 24, 2022, with a group of experts (1) to evaluate the animal health aspect of NAP of India; (2) assess the advancements made in pragmatization of AMR actions and assessment of antimicrobials use in the animal sector as envisaged; (3) recommend furtherance and/or modify activities wherever needed with reference to restricting the AMR in the animal health sector of the forthcoming 2022–2025 NAP II; (4) recognize the connections with human health and environment sectors to blend for superior deliverables; (5) pinpoint new actions and probable executing organizations for animal health part of NAP II of AMR of India; and finally (6) the combined draft of planned activities is planned to be submitted to the national authorities. One more meeting was held in Delhi with a small group of experts on March 25, 2022, to finalize the document submitting to the Government of India.

10 Kerala, Southern State of India, as an Example

The Government of Kerala has promulgated a distinct operation to mark the State "Antibiotic Literate" by 2023 as a measure of reinforcing its Kerala Antimicrobial Resistance State Action Plan (KARSAP). This scheme is prepared as an extent of action strategy of II Navakeralam. In a recent meeting, it was proposed to fortify accomplishment methods through short- and long-term objectives to facilitate successful completion of comprehensive aims of KARSAP within half a decade. It was also decided to form district-level committees of antimicrobial resistance based on the fruitful transformation of "hub-and-spoke" of Ernakulum District and implement the same to other districts of Kerala. The plan is to hold regular trimester meetings with extravagant drives to generate responsiveness at the community level on AMR with a distinct attentive type for the benefits of all school students. All the line departments included health.

The wide-ranging aims envisioning the antibiotic literacy of Kerala intraregion level included

1. Widespread attentiveness of the prominence of right to access food and water free from antibiotic residues.
2. General consciousness of the significance of antibiotics intake only upon prescription by clinicians.
3. Common cognizance of the position of securely discarding the antibiotics that are of no use and with expired dates.
4. Attentiveness in school-going students on the hazards of antimicrobial resistance.
5. Bringing to the knowledge of the classification of antibiotics under WHO-AWaRe to all federal officials.
6. Organizing campaigns of community wakefulness activities vis-à-vis antimicrobial resistance. Infection avoidance and controlling at outpatient and at ground level with the help of Kudumbasree* (Prosperity of the Family) and ASHA**, possibly employing the AMR posters of WHO.

   (*The "Prosperity of the Family," known as Kudumbashree in Malayalam, is a program that intends to alleviate poverty and enablement of women executed by the State Poverty Eradication Mission (SPEM) of Kerala government.)

   (**Accredited Social Health Activists. The ASHAs are volunteers who are compensated with performance-centered inducements positioned for every thousand persons in Kerala.)

11 Cost-Effective Ways in Containing AMR

The AMR development in bacteria is a continuous process. It is not possible to stop the process. On the contrary, the process can be hindered by simple and cost-effective methods. These methods are a part of antibiotic literacy. The faster the

rate and the widespread the antibiotic literacy across the globe, the easier it is to contain the AMR. The growth and expansion of AMR to antibiotics are unavoidable, but can be significantly reduced through comparatively simple measures:

1. WASH in all walks of life. When WASH becomes way of life, the spread of infections will reach bare minimum levels, and thus, it is not necessary to use antibiotics. This, in turn, also leads to a drastic reduction in healthcare expenditures. There will be a significant increase in the quality-adjusted life-years (QALY). There will be a decrease in nonfatal burden of years lost in disability (YLD) and also years of life lost (YLL) due to premature fatalities with overall reduction in disability-adjusted life-years (DALY).
2. Improve the hygiene in hinterlands and rural areas, especially in LMICs and LICs.
3. Supply potable water. One-tenth of the world's population (784 million) have no access to potable water. In the least developed countries, 22% of the healthcare facilities have no potable water. The unsafe water and absence of good sanitation lead to fatalities in children of not living beyond 5 years, resulting in the deaths of one child every 60 s. What is required primarily here is the allotment of federal funds for supply of potable water as every single dollar (US$) devoted to potable water and sanitation results in US$ 5–28 return, which, in turn, can be used for other economic development activities. The aim of the UN's Sustainable Development Goal 6 is to make sure that potable water is available to all people for sustainable management of water and sanitation by 2030.
4. Good Manufacturing Practices (GMP) in the food sector mainly in hygienic handling of food throughout the supply chain.
5. Hygienic food preparation at the domestic and community levels.
6. Improved/altered mode of action of antimicrobials to deter resistance.
7. Development of new antibiotics.
8. Infection control by preventive measures.
9. Updating and proper maintenance of sanitation system.
10. Introduction of economically viable and effective effluent treatment systems.
11. Reduction in employing antibiotics in humans, agriculture, animal husbandry, and aquaculture.
12. Development of rapid detection methods for emerging, re-emerging, and re-re-emerging infections.
13. Better preparation for handling pandemics to avoid use, excess use, and misuse of antibiotics in the prevention and handling of secondary infections.
14. Updating information to clinicians for appropriate prescriptions and suitable prescription of broad-spectrum antibiotics.
15. Patient education in clinical setting.
16. Pharmacists' education in delivering antibiotics.
17. Educating the clinicians, agriculturists, veterinarians, aquaculturists, and environmentalists and policymakers (Figs. 1, 2, and 3).
18. Educating consumers.

These are all a part of antibiotic literacy.

12 Basic Requirements for the Implementation of Antibiotic Literacy

1. Labeling with ISO certification that all edible products are free of antibiotic residues.
2. Preparation of AMR literacy pamphlets in all languages.
3. Talks: radio, TV, panel discussions, lectures by experts, holding seminars and symposia at the regional, national, and international levels to exchange latest developments and encompass all stakeholders.
4. Promulgation of laws
   (I) Prohibiting over-the-counter sale of antibiotics
   (II) Treating antibiotics on par with psychedelic drugs, bringing them under the preview of DEA of different countries
5. Sale of antimicrobials/antibiotics only upon prescription from qualified doctors.
6. Regular inspection of the quality of the antimicrobials and antibiotics sold.

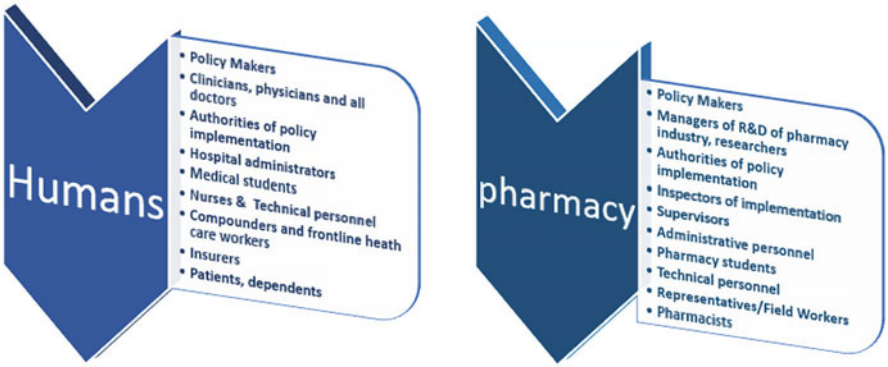


Fig. 1 Avenues for antibiotic literacy initiation, development, and spread: humans and pharmacy

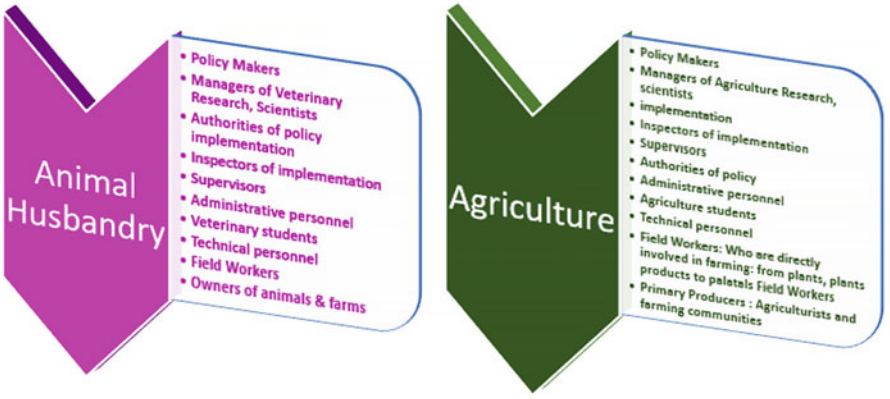


Fig. 2 Avenues for antibiotic literacy initiation, development, and spread: agriculture and animal husbandry

Fig. 3 Avenues for antibiotic literacy initiation, development, and spread: fisheries and aquaculture and environment

7. Clear-cut definitions for all aspects of AMR literacy.
8. All communication in colloquies and easy to follow by all people.
9. Conducting survey across the globe to gain on-ground reality in the stipulated, shortest span of time to avoid wide variations in time period lapse within sampling region and across the regions.
10. There is an urgent need for finalization of framework for antibiotic literacy.
11. Once framework is finalized, the immediate next step is setting up of review time schedules. It can be 3 years intervals for review and modifications on an as-needed basis.
12. Immediate, mid-term, and long-term plans can be made to reach the target of Global Antibiotic Literacy by 2030. This is the target year for SDG too. Antibiotic literacy can be a part of SDG.

13 Conclusion

The successful fight with AMR in a stipulated time is possible only through antibiotic literacy. The top-down or bottom-up approach and the immediate prerequisite are to define the terms clearly, develop framework, and time period for achieving the goal of global antibiotic literacy. The Swedish type of initiative is the need of the hour in other countries, indicating that AMR is a serious global threat to humans, agriculture, animal, and environmental sectors that requires a collective realistic effort from all the countries to contain the scourge. Among the predicted 10 million mortalities by 2050, the economically underprivileged countries are predicted to bear the brunt to the tune of 90% of the mortalities (O'Neill, 2016). To avoid this unseen, unfelt, and slow spread of antimicrobial resistance, a multi-pronged blitzkrieg attempt can only yield results if easy-to-follow and straightforward requirements for the pragmatization of antibiotic literacy are available. Literacy

on antibiotics is one aspect that makes tackling antimicrobial resistance by multi-thronging and multi-pronging at one time.

References

Adeyi, O. O., Baris, E. J., Olga, B., Irwin, A., Berthe, F. C. J., Le Gall, F. G., Marquez, P. V., Nikolic, I. A., Plante, C. A., Schneidman, M., Shriber, D. E., & Thiebaud, A. (2017). *Final report*. World Bank Group. http://documents.worldbank.org/curated/en/323311493396993758/final-report

Ahmad, A., Parimalakrishnan, S., Patel, I., Kumar, N. V., Balkrishnan, T. R., & Mohanta, G. P. (2012). Evaluation of self-medication antibiotics use pattern among patients attending community pharmacies in rural India, Uttar Pradesh. *Journal of Pharmacy Research, 5*, 7658. Accessed 4 Apr 2022.

Ahmad, A., Patel, I., Mohanta, G., & Balkrishnan, R. (2014). Evaluation of self-medication practices in rural area of town Sahaswan at Northern India. *Annals of Medical and Health Sciences Research, 4*, S73–S78.

Al-Azzam, S. I., Al-Husein, B. A., Alzoubi, F., Masadeh, M. M., & Al-Horani, M. A. S. (2007). Self-medication with antibiotics in Jordanian population. *International Journal of Occupational Medicine and Environmental Health, 20*, 373–380.

Alhomoud, F., Aljamea, Z., Almahasnah, R., Alkhalifah, K., Basalelah, L., & Alhomoud, F. K. (2017). Self-medication and self-prescription with antibiotics in the Middle East-do they really happen? A systematic review of the prevalence, possible reasons, and outcomes. *International Journal of Infectious Diseases, 57*, 3. https://doi.org/10.1016/j.ijid.2017.01.014

Alshogran, O. Y., Alzoubi, K. H., Khabour, O. F., & Farah, S. (2018). Patterns of self-medication among medical and nonmedical university students in Jordan. *Risk Management and Healthcare Policy, 11*, 169–176. https://doi.org/10.2147/RMHP.S170181. Accessed 4 Apr 2022.

Anderson, A. (2018). Online health information and public knowledge, attitudes, and behaviours regarding antibiotics in the UK: Multiple regression analysis of Wellcome Monitor and Eurobarometer Data. *PLoS One, 13*, e0204878.

Balamurugan, E., & Ganesh, K. (2011). Prevalence and pattern of self-medication use in coastal regions of South India. *BJMP, 4*(3), a428. Accessed 4 Apr 2022.

Banerjee, I., & Bhadur, T. (2012). Self-medication practice among undergraduate medical students in a tertiary care medical college, West Bengal. *Journal of Postgraduate Medicine, 58*, 127–131. Accessed 4 Apr 2022.

Beck, K. I., Sidrah Ahmed, S., Zaman, S. N., Hawksworth, G., Afzal, F. S., & Howard, P. (2020). Using health literacy techniques to support pharmacist practice when counselling on antibiotics courses. *Access Microbiology, 2*(2). https://doi.org/10.1099/acmi.fis2019.po0058

Beovi'c, B., Doušak, M., Ferreira-Coimbra, J., Nadrah, K., Rubulotta, F., Belliato, M., Berger-Estilita, J., Ayoade, F., Rello, J., & Erdem, H. (2020). Antibiotic use in patients with COVID-19: A 'snapshot'Infectious Diseases International Research Initiative (ID-IRI) survey. *The Journal of Antimicrobial Chemotherapy, 75*, 3386–3390.

Boeckel, V., Brower, C., Gilbert, M., Grenfell, B. T., Simon, A., Robinson, T. P., Teillant, A., & Ramanan Laxminarayan, R. (2015). Global trends in antimicrobial use in food animals. *Proceedings of the National Academy of Sciences of the United States of America, 112*, 5649–5654. Accessed 6 Apr 2022.

Butler, C. C., Dorward, J., Yu, L., Gbinigie, O., Hayward, G., Saville, B. R., Hecke, O. V., Berry, N., Detry, M., Saunders, C., Fitzgerald, M., Harris, V., Patel, M. G., Lusignan, S., Ogburn, E., Evans, P. H., Nicholas, P. B., Thomas, N. P. B., & Hobbs, F. D. R. (2021). Azithromycin for community treatment of suspected COVID-19 in people at increased risk of an adverse clinical

course in the UK (PRINCIPLE): A randomised, controlled, open-label, adaptive platform trial. *The Lancet, 397*(10279), 1063–1074.

CDC. (2019). *Antibiotic resistance threats in the United States*. U.S. Department of Health and Human Services. https://doi.org/10.15620/cdc:82532

Charani, E., McKee, M., Ahmad, R., Balasegaram, M., Bonaconsa, C., Merrett, G. B., Busse, R., Carter, V., Castro-Sanchez, E., Franklin, B. D., Georgiou, P., Hill-Cawthorne, K., Hope, W., Imanaka, Y., Kambugu, A., Leather, A. J. M., Mbamalu, O., McLeod, M., Mendelson, M., Mpundu, M., Timothy, M., Rawson, T. M., Ricciardi, W., Rodriguez-Manzano, J., Singh, S., Tsioutis, C., Uchea, C., Zhu, N., & Holmes, A. H. (2021). Optimising antimicrobial use in humans-review of current evidence and an interdisciplinary consensus on key priorities for research. *The Lancet Regional Health, 7*, 100161. https://doi.org/10.1016/j.lanepe.2021.100161

Dyar, O.J, Nathwani D., , Monne, D.L, Gyssens, I.C., Lundborg, C.S and Pulcini, C (2018). Do medical students feel prepared to prescribe antibiotics responsibly? Results from a cross-sectional survey in 29 European countries. The Journal of Antimicrobial Chemotherapy 73; 2236–2242.

El Sherbiny, N. A., Ibrahim, E. H., & Masoud, M. (2018). Assessment of knowledge, attitude and behavior towards antibiotic use in primary health care patients in Fayoum Governorate, Egypt. *Alexandria Journal of Medicine, 54*, 535–540.

Elmahi, O. K. O., Musa, R. A. E., Shareef, A. A. H., Omer, M. E. A., Elmahi, M. A. M., Altamih, R. A. A., Mohamed, R. I. H., & Alsadig, T. F. M. (2022). Perception and practice of self-medication with antibiotics among medical students in Sudanese universities: A cross-sectional study. *PLoS One, 17*(1), e0263067. https://doi.org/10.1371/journal.pone.0263067. Accessed 4 Apr 2022.

Fortun, K. (2012). Ethnography in late industrialism. *Cultural Anthropology, 27*(3), 446–464.

Gajdács, M., Urbán, E., Stájer, A., & Baráth, Z. (2021). Antimicrobial resistance in the context of the sustainable development goals: A brief review. *European Journal of Investigation in Health, Psychology and Education, 11*, 71–82. https://doi.org/10.3390/ejihpe1101000

Grigoryan, L., Haaijer-Ruskamp, F. M., Burgerhof, J. G., Mechtler, R., Deschepper, R., & Tambic-Andrasevic, A. (2006). Self-medication with antimicrobial drugs in Europe. *Emerging Infectious Diseases*. https://doi.org/10.3201/eid1203.050992. Accessed 4 Apr 2022.

Grigoryan, L., Germanos, G., Zoorob, R., Juneja, S., Raphael, J. L., Paasche-Orlow, M. K., & Traunter, B. W. (2019). Use of antibiotics without a prescription in the U.S. population: a scoping review. *Annals of Internal Medicine*. https://doi.org/10.7326/M19-0505. Accessed 4 Apr 2022.

Hagiya, H., Ino, H., Kazuki, T., Miyoshi, T., Ochi, K., & Otsuka, F. (2020). Antibiotic literacy among Japanese medical students. *The Journal of Infection and Chemotherapy, 26*(10), 1107–1109. https://doi.org/10.1016/j.jiac.2020.06.021

Haque, M., Rahman, N. A. A., McKimm, J., Kibria, G. M., Majumder, M. A. A., Haque, S. Z., Islam, M. Z., Abdullah, S. L. B., Daher, A. M., & Zulkifli, Z. (2019). Self-medication of antibiotics: Investigating practice among university students at the Malaysian National Defence University. *Infection and Drug Resistance, 12*, 1333.

Hayajneh, W. A., Al-Azzam, S., Yusef, D., Lattyak, W. J., Lattyak, E. A., Gould, I., López-Lozano, J. M., Conway, B. R., Conlon-Bingham, G., & Aldeyab, M. A. (2021). Identification of thresholds in relationships between specific antibiotic use and carbapenem resistant *Acinetobacter baumannii* (CRAb) incidence rates in hospitalized patients in Jordan. *The Journal of Antimicrobial Chemotherapy, 76*, 524–530.

Howard, P., & Hawksworth, G. (2019). Paper presented at the Pharmacy Together 2019 conference on 29 November, 2019.

Hughes, C. M., McElnay, J. C., & Fleming, G. F. (2001). Benefits and risks of self-medication. *Drug Safety, 24*(14), 1027–37. https://doi.org/10.2165/00002018-200124140-00002. PMID: 11735659.

Jirjees, F. J., Al-Obaidi, H. J., Sartaj, M., Conlon-Bingham, G., Farren, D., Scott, M. G., Gould, I. M., López-Lozano, J. M., Aldeyab, M. A. (2020). Antibiotic use and resistance in hospitals:

Time-series analysis strategy for determining and prioritising interventions. *Hospital Pharmacy Europe*. https://hospitalpharmacyeurope.com/news/reviews-research/antibiotic-use-and-resistance-in-hospitals-time-series-analysis-strategy-for-determining-and-prioritisinginterventions/. Accessed 4 Apr 2022.

Kamata, K., Tokuda, Y., Gu, Y., Ohmagari, N., & Yanagihara, K. (2017). Public knowledge and perception about antimicrobials and antimicrobial resistance in Japan: A national questionnaire survey in 2017. *PLoS One, 2018*, e0207017. https://doi.org/10.1371/journal.pone.0207017

Kemp, S. A., Pinchbeck, G. L., Fèvre, E. M., & Williams, N. J. (2021). A cross-sectional survey of the knowledge, attitudes, and practices of antimicrobial users and providers in an area of high-density livestock-human population in Western Kenya. *Frontiers in Veterinary Science*. https://doi.org/10.3389/fvets.2021.727365

Kickbusch, I., Pelikan, J. M., Apfel, F., & Tsouros, A. (2013). *Health literacy*. WHO Regional Office for Europe. ISBN 9289000155.

Kosiyaporn, H., Chanvatik, S., Issaramalai, T., Kaewkhankhaeng, W., Kulthanmanusorn, A., Saengruang, N., Witthayapipopsakul, W., Viriyathorn, S., Kirivan, S., Kunpeuk, W., Suphanchaimat, R., Lekagul, A., & Tangcharoensathien, V. (2020). Surveys of knowledge and awareness of antibiotic use and antimicrobial resistance in general population: A systematic review. *PLoS One, 15*(1), e0227973. https://doi.org/10.1371/journal.pone.0227973

Lal, V., Goswami, A., & Anand, K. (2007). Self-medication among residents of urban resettlement colony, New Delhi. *Indian Journal of Public Health, 51*, 249–251. Accessed 4 Apr 2022.

Landecker, H. (2016). Antibiotic resistance and the biology of history. The new biologies. *Body and Society, 22*(4), 19–52.

Laxminarayan, R., Sridhar, D., Blaser, M., Wang, M., & Woolhouse, M. (2016). Achieving global targets for antimicrobial resistance: The un should promote targets, funding, and governance. *Science, 353*(6302), 874–875. https://doi.org/10.1126/science.aaf9286. Accessed 6 Apr 2022.

Levy, S. (1998). The challenge of antibiotic resistance. *Scientific American, 278*(3), 32–39.

Mostafa, A., Abdelzaher, A., Rashed, S., AlKhawaga, S. I., Afifi, S. K., Abdel Alim, S., Mostafa, S. A., & Zidan, T. A. (2021). Is health literacy associated with antibiotic use, knowledge and awareness of antimicrobial resistance among non-medical university students in Egypt? A cross-sectional study. *BMJ Open, 11*, e046453.

Muflih, S. M., Al-Azzam, S., Karasneh, R. A., Conway, B. R., & Aldeyab, M. A. (2021). Public health literacy, knowledge, and awareness regarding antibiotic use and antimicrobial resistance during the COVID-19 pandemic: A cross sectional study. *Antibiotics, 10*, 1107. https://doi.org/10.3390/antibiotics10091107

Muflih, S. M., Bashir, H. N., Khader, Y. S., & Karasneh, R. A. (2022). The impact of health literacy on self-medication: A cross-sectional outpatient study. *Journal of Public Health (Oxford, England), 44*(1), 84–91. https://doi.org/10.1093/pubmed/fdaa188. PMID: 33164078.

Muri-Gama, A. S., Figueras, A., & Secoli, S. R. (2018). Inappropriately prescribed and over-the-counter antimicrobials in the Brazilian Amazon Basin: We need to promote more rational use even in remote places. *PLoS One, 13*(8), e0201579. https://doi.org/10.1371/journal.pone.0201579. PMID: 30075021; PMCID: PMC6075761.

Nusair, M. B., Al-azzam, S. H., & Momani, M. Y. (2021). The prevalence and patterns of self-medication with antibiotics in Jordan: A community-based study. *International Journal of Clinical Practice, 75*, e13665.

O'Neill, J. (2016). Tackling drug-resistant infections globally: Final report and recommendations. Review on Antimicrobial Resistance.

Ofori, A. K., Darko, E., Danquah, C. A., Poku, T. A., & Buabeng, K. O. (2021). Self-medication and antimicrobial resistance: A survey of students studying healthcare programmes at a Tertiary Institution in Ghana. *Frontiers in Public Health*. https://doi.org/10.3389/fpubh.2021.706290

Okoye, O. C., Adejumo, O. A., Opadeyi, A. O., Madubuko, C. R., Ntaji, M., Okonkwo, K. C., Edeki, I. R., Agboje, U. O., Alli, O. E., & Obodo, J. O. O. (2022). Self-medication practices and its determinants in health care professionals during the coronavirus disease-2019 pandemic:

Cross-sectional study. *International Journal of Clinical Pharmacy, 44*, 507. https://doi.org/10.1007/s11096-021-01374-4

Oravcova, V., Zurek, L., Townsend, A., Clark, A., Ellis, J., & Cizek, A. (2013). American crows as carriers of vancomycin-resistant enterococci with vanA gene. *Environmental Microbiology, 16*, 939. https://doi.org/10.1111/1462-2920.12213

Orzech, K., & Nichter, M. (2008). From resilience to resistance: Political ecological lessons from antibiotic and pesticide resistance. *Annual Review of Anthropology, 37*, 267–282.

Patel, A., Emerick, M., Cabunoc, M. K., Williams, M. H., Preas, M. A., Schrank, G., Rabinowitz, R., Luethy, P., Johnson, J. K., & Leekha, S. (2021). Rapid spread and control of multidrug-resistant gram-negative bacteria in COVID-19 patient care units. *Emerging Infectious Diseases, 27*, 1234.

Pavyde, E., Veikutis, V., Maˇciulien, A., Maˇciulis, V., Petrikonis, K., & Stankeviˇcius, E. (2015). Public knowledge, beliefs and behavior on antibiotic use and self-medication in Lithuania. *International Journal of Environmental Research and Public Health, 12*, 7002–7016.

Phalke, V. D., Phalke, D. B., & Durgawale, P. M. (2006). Self-medication practices in rural Maharashtra. *Indian Journal of Community Medicine, 31*, 34–35. Accessed 3 Apr 2022.

Prasad, M. M., & Ravishankar, C. N. (2018). The Antimicrobial Resistance (AMR): The ICAR-CIFT contributions in addressing the problem. In A. Jayathilak, V. Padmanabham, & A. J. Tharakan (Eds.), *21st India International Seafood Show* (pp. 66–70). Souvenir.

Rajendran, A., Kulirankal, K. G., Rakesh, P. S., & George, S. (2019). Prevalence and pattern of antibiotic self-medication practice in an urban population of Kerala, India: A cross-sectional study. *Indian Journal of Community Medicine, 44*(Suppl1), S42–S45. https://doi.org/10.4103/ijcm.IJCM_33_19

Rathore, G., Lal, K. K., Rajesh Bhatia, R., & Jena, J. K. (2020). INFAAR– A research platform for accelerating laboratory-based surveillance of antimicrobial resistance in fisheries and aquaculture in India. *Current Science, 119*(12), 1884–1885. https://doi.org/10.18520/cs/v119/i12/1884-1885

Rawson, T. M., Moore, L. S. P., Zhu, N., Ranganathan, N., Skolimowska, K., Gilchrist, M., Satta, G., Cooke, G., & Holmes, A. (2020). Bacterial and fungal coinfection in individuals with coronavirus: A rapid review to support COVID-19 antimicrobial prescribing. *Clinical Infectious Diseases, 71*, 2459–2468.

Rose, S., Hill, R., Bermudez, L., & Miller-Morgan, T. (2013). Imported ornamental fish are colonized with antibiotic-resistant bacteria. *Journal of Fish Diseases, 36*(6), 533–542.

Salm, F., Ernsting, C., Kuhlmey, A., Kanzler, M., Gastmeier, P., & Gellert, P. (2018). Antibiotic use, knowledge and health literacy among the general population in Berlin, Germany and its surrounding rural areas. *PLoS One, 13*, e0193336.

Samuel, S. S., Prakasam, K. C., & Nandhakumar, N. (2011). Assessment of self-medication among patients attending community pharmacies in Erode, India. *International Journal of Pharmacy & Pharmaceutical Sciences, 3*, 258–262. Accessed 4 Apr 2022.

Sawair, F. A., Baqain, Z. H., Abu Karaky, A., & Abu Eid, R. (2008). Assessment of self-medication of antibiotics in a Jordanian population. *Medical Principles and Practice, 18*, 21–25.

Senadheera, G. P., Sri Ranganathan, S., Gunawardane, N. S., Fernando, G. H., & Fernandopulle, B. M. (2017). Practice of self-medication with antibiotics in the Colombo district, Sri Lanka. *Ceylon Medical Journal*. https://doi.org/10.4038/cmj.v62i1.8439

Svet, Lustig, & Vijay. (2020). COVID-19 is exacerbating antibiotic use – and antimicrobial resistance is rising fast, WHO warns. Health Policy Watch. Posted on 02.06.2020. Accessed 13 Jan 2022.

Swedish Press Release. (2022). Press release from Ministry of Health and Social Affairs "Sweden appoints an ambassador to work against antimicrobial resistance". Published 18 February 2022.

Vallin, M., Polyzoi, M., Marrone, G., Rosales-Klintz, S., Wisell, T. K., & Lundborg, S. C. (2016). Knowledge and attitudes towards antibiotic use and resistance-a latent class analysis of a Swedish population-based sample. *PLoS One, 11*, e0152160. https://doi.org/10.1371/journal.pone.0152160. Accessed 4 Apr 2022.

Wall, S. (2019). Prevention of antibiotic resistance-an epidemiological scoping review to identify research categories and knowledge gaps. *Global Health Action, 12*, 1756191. https://doi.org/10.1080/16549716.2020.1756191

Wang, M. P., Viswanath, K., Lam, T. H., Wang, X., & Chan, S. S. (2013). Social determinants of health information seeking among Chinese adults in Hong Kong. *PLoS One, 8*, e73049.

WHO. (2015). *Global action plan of antimicrobial resistance*. WHO. ISBN 978 92 4 150976 3.

Wolff, M. J. (1993). Use and misuse of antibiotics in Latin America. *Clinical Infectious Diseases, 17*(Suppl 2), S346–S351. https://doi.org/10.1093/clinids/17.supplement_2.s346. PMID: 8274599. Accessed 4 Apr 2022.

World Bank. (2016). By 2050, drug-resistant infections could cause global economic damage on par with 2008 financial crisis. The World Bank Press Release September 20, 2016.

Yang, et al. (2020). Preliminary estimation of the basic reproduction number of novel coronavirus (2019-nCoV) in China, from 2019 to 2020: A data-driven analysis in the early phase of the outbreak. *International Journal of Infectious Diseases, 92*, 214–217.

Index

M. P. Mothadaka et al. (eds.), *Handbook on Antimicrobial Resistance*,
https://doi.org/10.1007/978-981-19-9279-7