Sabu Thomas *Editor*

# Antimicrobial Resistance

## Global Challenges and Future Interventions

Springer

# Antimicrobial Resistance

Sabu Thomas
Editor

# Antimicrobial Resistance

## Global Challenges and Future Interventions

Springer

*Editor*
Sabu Thomas
Cholera and Biofilm Research Laboratory
Rajiv Gandhi Centre for Biotechnology
Thiruvananthapuram, Kerala, India

ISBN 978-981-15-3657-1 ISBN 978-981-15-3658-8 (eBook)
https://doi.org/10.1007/978-981-15-3658-8



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

When Dr. Sabu Thomas, the Editor-in-Chief approached me to write a Foreword for his new book entitled *Antimicrobial Resistance: Global Challenges and Future Interventions* I readily agreed. But with the humid weather, the assignment got procrastinated and other matters took priority. I was jolted back to reality when today's newspaper (The Times of India, January 21, 2020) carried on its front page in huge bold fonts about the drying supply of antibiotics as a cause for concern leading the WHO Director General Tedros Adhanom Ghebreyesus to announce that "Never has the threat of antimicrobial resistance been more immediate and the need for solutions more urgent."

Antimicrobial resistance or AMR as it is more commonly known has today evolved into a major problem, the world over. The major periodicals, journals, books, and magazines carry the bizarre truth of AMR. The most frightening aspect is that AMR could make easily treatable diseases life-threatening and also has the potential of converting every surgery a problem with resistance looming ahead making antibiotics a potential death threat.

This book on AMR, therefore, comes at a very appropriate time and covers topics ranging from origin, evolution, and adaptation of AMR in pathogens to planetary health perspectives to tackle the AMR challenge. Fungal infections have recently emerged as increasingly intractable predicament, and the chapter on antifungal resistance, especially of the intractable and recalcitrant fungal infections, is of particular interest. The topics dealt with recent advances on the influence of AMR on gut microbiota and use of bacterial cell wall recycle inhibitors to combat AMR in bacteria would yield new approaches. All in all, the book covers a wide range of topics on AMR that will attract academicians, clinicians, researchers, and students. In today's complex world of varied health issues, this book serves as a beacon to health care systems all over the world.

Rajiv Gandhi Centre for Biotechnology
Thiruvananthapuram, Kerala, India

G. Balakrish Nair

# Preface

Antimicrobial resistance is a global public health problem. Sir Alexander Fleming had rightly predicted resistance to penicillin, as early as 1945, soon after its wide-scale introduction. Indeed, the discovery of antibiotics has been a milestone and has truly revolutionized medicine, saving countless lives. Unfortunately, the indiscriminate and inappropriate use of antimicrobials in healthcare and livestock has put selection pressure on microbial population leading to the emergence of resistant bugs by way of evolution and genetic jugglery. The WHO released a catalogue of antibiotic-resistant "priority pathogens" to spur incentives and investments for novel antibiotic discovery and research and fears the world may enter "a post-antibiotic era" in which even common infections and minor injuries can lead to mortality. Factors related to human, animal husbandry, agriculture, environment, and fishery sectors drive AMR, which, therefore needs to be tackled in collaboration with all stakeholders under the umbrella of a comprehensive One Health approach. It includes strict infection control and legislation to check antibiotic use, increased global surveillance, and facilitating data sharing, intensification of public funding for R&D, as well as efforts to raise social awareness. We also need to equip ourselves by understanding the resistant pathogens and the complexity of the environment in their emergence and transmission. In accordance with the WHO guidelines, action plans have been implemented by the governments at the Central and State levels in a focused manner to keep a vigil on AMR at the grass root level.

The aim of this book was to assemble an up-to-date progress from the eminent experts in the field. It unfolds by drawing comparisons from the human immune system to understand how microbes defend themselves and how these brilliant strategies have eventually led to resistance. The next chapter discusses in detail the burden and mechanisms of resistance in Gram-negative bacteria towards carbapenems—the last resort antibiotic available. The topics deal with recent advances on the influence of AMR on gut microbiota and the alteration of gut microbial composition due to antibiotics. Subsequent chapters give a comprehensive overview of the occurrence and promulgation of antibiotic resistance genes in the environment, the intricate interdependence of humans, animals, and the environment requiring the concerted action of diverse partners to tackle AMR and introduce alternative remedies in the form of traditional medicine and cell wall recycling inhibitors to reduce antimicrobial use. The chapter on antifungal resistance is of particular interest. The book ends by expounding the harmful effects of sub-lethal

concentration of antibiotics and how microbes evolve resistance to it, and also highlights the application of next-generation sequencing technologies to unravel resistance determinants in microorganisms.

It is hoped that this book will be of extensive use for readers from a variety of backgrounds in the medical field. I extend my sincere gratitude to all who have been part of this endeavor. Foremost, I would like to pen my sincere gratitude to Prof. M. Radhakrishna Pillai, Director, RGCB, and Dr. G. Balakrish Nair, Honorary Distinguished Professor, for their consistent support and encouragement. I would like to extend my sincere gratitude to the Department of Health and Family Welfare, Government of Kerala, for their constant support and providing me a platform to utilize my skill set to control the increasing AMR in the State. As an editor, I am deeply indebted to all the contributors who pursue the AMR research with passion and dedication. I am grateful to the editorial team and all my lab members for their support and valuable suggestions. I sincerely thank Springer Nature Publishers for the excellent assistance in producing this book. I also acknowledge the Department of Biotechnology of the Govt. of India. Indeed, I hope this volume provides valuable information on antimicrobial resistance and boost scientific interest to contribute and remain committed to the global efforts to tackle this challenge in the context of global/national antimicrobial resistance strategic action plan.

Thiruvananthapuram, India Sabu Thomas

# Acknowledgments

I would like to thank Karthika Suryaletha, Lekshmi Narendrakumar, Aparna Shankar, Divya M Prabhakaran, and Devika J Das who have generously given up valuable time to assist in reviewing and editing the book chapters. Their conscientiousness is much appreciated.

# Contents

# About the Editor

**Sabu Thomas, MSc, PhD,** is a senior faculty scientist heading the Cholera and Biofilm Research Group at Rajiv Gandhi Centre for Biotechnology (National Institute under the Department of Biotechnology, Govt. of India). Dr. Thomas has been working for more than two decades in environmental and clinical pathogenic bacteria with special focus on gut and chronic wound-infected pathogens, antimicrobial resistance (AMR), and alternative strategies to curb AMR. Dr. Thomas's team has published more than 75 research articles in prestigious journals at National and International level, 12 book chapters, and edited one book. He has been awarded with various honors such as member in the Global Task Force on Cholera Control of World Health Organization and Fellow Award in Medical Biotechnology of Society for Applied Biotechnology. Currently, he is a member of State Working Committee on Antimicrobial Resistance, and research co-ordinator on AMR activities in Kerala. He was part of the Second Indian Arctic Scientific Expedition team organized by Govt. of India to study the bioprospecting potential of psychrophilic bacteria in the polar region. He is also involved in various reputed organizations across the globe such as Global Foodborne Infections Network, CHOLDInet—Global Laboratory Network for Cholera and other Diarrhoeal Infections, International Society for Infectious Diseases and Freshwater Action Network South Asia.

# The Evolution of Microbial Defence Systems Against Antimicrobial Agents

Archana Madhav, Robert C. Will, and Ankur Mutreja

## 1 Introduction

Antimicrobial resistance (AMR) has repeatedly been discussed in the context of the growing failure of last-line therapeutics. The rapid progression of AMR is also a testament to the success of microbial defence. In this chapter, we invite the reader to step into the shoes of a microbe and visualise a unique approach to understanding AMR as a microbial 'immune response'. We also explore the evolutionary trade-offs in developing antibiotic resistance (ABR) and how it has affected infectivity (the ability to spread) and pathogenicity (the ability to cause disease) of microbes. These mechanisms have developed through some remarkable adaptive and evolutionary processes, in the lines of Darwinian survival and reproduction.

Antibiotic resistance (ABR) is a subgroup of antimicrobial resistance and the group that poses the most clinically significant threat (Davies and Davies 2010). The first antibiotics were introduced in the early 1940s, with warnings of resistance to this exhaustible resource. A revolutionary discovery, antibiotics heralded a transition from an era of high mortality where a paper cut could become infected and kill to significantly improved longevity, health and overall quality of life. Before antibiotics, humanity suffered some of the deadliest pandemics. The Black Death, a plague in the mid-fourteenth century, may have wiped out nearly half of Europe's 450 million population—a horrifying glimpse into a world with no infection control against *Yersinia pestis*, the plague bacterium (Duncan 2005). These days, despite being taken for granted and irrationally prescribed for ailments such as the common cold and flu (both caused by viruses), antibiotics are our sole treatment strategies for

A. Madhav · R. C. Will
Department of Medicine, University of Cambridge, Cambridge, UK

A. Mutreja (✉)
Department of Medicine, University of Cambridge, Cambridge, UK

Translational Health Science and Technology Institute, Faridabad, Haryana, India
e-mail: am872@medschl.cam.ac.uk



S. Thomas (ed.), *Antimicrobial Resistance*,
https://doi.org/10.1007/978-981-15-3658-8_1

bacterial infections. The rapidly rising rate of antibiotic resistance leaves us with a bleak outlook. This issue needs to be addressed urgently before it grows into a public health catastrophe.

The study of infectious diseases is at the crossroads between microbiology and immunology. An immune system is a medium that provides a host with resistance against infections through a network comprising of cells, tissues and molecules (Abbas et al. 2004). The fundamental purpose of an immune system is to defend the host by distinguishing self from non-self. In eukaryotes, the complex, multicellular, multi-organ immune system coordinates efforts to shield the host from foreign invaders. Single-celled prokaryotes, by comparison, do not have the complexity of eukaryotes to support an immune 'system' but can still launch defensive responses akin to those of an immune system. Therefore, the comparison that is proposed here to a mammalian immune system can be used to harness the concepts and hone them to draw parallels between the two, specifically to present similarities in function while addressing distinct mechanistic differences (Fig. 1).

The nature of infectious diseases can be pathogen-mediated, toxin-mediated or a combination (Alberts 2015). An immune response starts when the host detects an invader by recognising non-self-antigens from a foreign source. It then guards the host by limiting transmission of the foreign invader and minimising the damage to the host.

The immune response can be broadly categorised into general 'non-specific' responses and more targeted 'specific' responses tailored to a distinct set of antigens. In humans, the innate immune system, with a combination of physical barriers (e.g. keratin in skin, mucous in the bronchi, acidic urine, etc.) and internal defences (such as the inflammatory response, natural killer cells, antibody production, etc.), acts as a first-line shield that scours for antigenic markers, prepared for any threats. There is evidence that innate immunity has aided positive selection and its ancient evolutionary roots can be traced to prokaryotic bacteria and archaea from around 750 million years ago (Hato and Dagher 2015). To put that into perspective, the earliest remnants of our entire ancestral lineage, the *Homo sapiens*, only date back around 300,000 years (Galway-Witham and Stringer 2018)! This infers that the sentinel innate immune system predates our existence by several millennia, highlighting its integral role in promoting overall host fitness and survival.

Previously, the concept of an innate and adaptive immune system in bacteria was only described in the context of bacteriophages, i.e. viruses that can cause pathogen-mediated infections in host bacteria (Abedon 2012; Bikard and Marraffini 2012; Shabbir et al. 2016). Therefore, if antimicrobials can be viewed as being toxic to microbes, we can explore AMR as the collective result of several microbial defence mechanisms against toxin-mediated infections. It is, however, important to emphasise that the comparison is not only drawn to a single microbe but also to changes that develop within microbial communities through several generations that make them more resilient to the damage caused by antimicrobials.

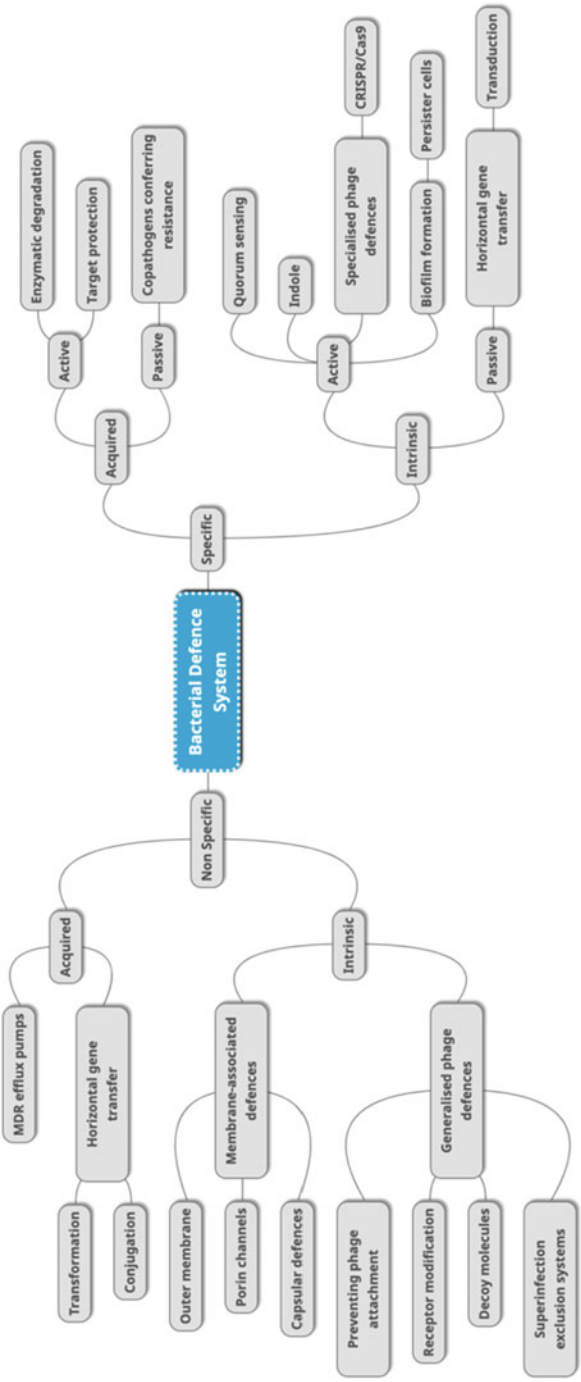


**Fig. 1** Bacterial defence mechanisms can be classified in similar subgroupings as those for mammalian immune responses

## 2 Bacteria

Bacteria are divided into two fundamental groups based on the differential Gram staining result: Gram-positive bacteria retain the purple colour of crystal violet stain in their highly cross-linked and thick peptidoglycan cell wall which leaks out from Gram negatives, due to them having thinner layers of peptidoglycan. These thinner layers get counterstained pink by safranin.

### 2.1 The Non-specific Bacterial Defence System

These mechanisms used by bacteria are not tailored specifically to the invading agent. These can either be:

1. Intrinsic: characteristics that are inherent to the host bacterium and are likely to be highly conserved.
2. Acquired: beneficial traits that have been obtained from other organisms or species that increase their likelihood of survival.

#### 2.1.1 Intrinsic

**Outer Membrane of Gram-Negative Bacteria**

Gram-negative bacteria harbour a characteristic outer membrane (OM) surrounded by a capsule outside their peptidoglycan cell walls. The OM constitutes a matrix of phospholipids, peptides and lipopolysaccharides (LPS) interspersed through the membrane, making this asymmetric bilayer extremely hydrophobic (Fig. 2) (Delcour 2009). LPS is saturated in the OM, and neighbouring negatively charged LPS molecules are linked together through divalent cations of $Mg^{2+}$ and $Ca^{2+}$ (Zavascki et al. 2007; Delcour 2009). LPS is anchored to the cell through lipid A, with branches extending outwards and forming an inner core that is highly conserved among bacteria, more variable outer core and distal O antigen regions (Raetz 1993). The OM is a selectively permeable membrane that regulates the transport of solutes and proteins across it and is also involved in intercellular communication (Olaitan et al. 2014).

There is some evidence indicating that the efficiency of LPS at conferring resistance is related to the length of the chains. Due to this, many bacterial strains have long LPS that carry the O antigen densely packed on the OM, concealing it from several antibiotics. For instance, Gram-negative bacteria are intrinsically resistant to vancomycin, since it needs to penetrate the OM to access its target—the peptidoglycan cell wall. The same reduced outer membrane permeability has been a recurrent observation in *Pseudomonas aeruginosa* isolates, resulting in resistance to some aminoglycoside antibiotics that are frequently used to treat patients with cystic fibrosis (Bryan et al. 1984; Wang et al. 2003). To increase the efficacy of these drugs, they are now combined with a supplemental drug that disrupts the OM resulting in a synergistic effect that improves access to the cell wall.

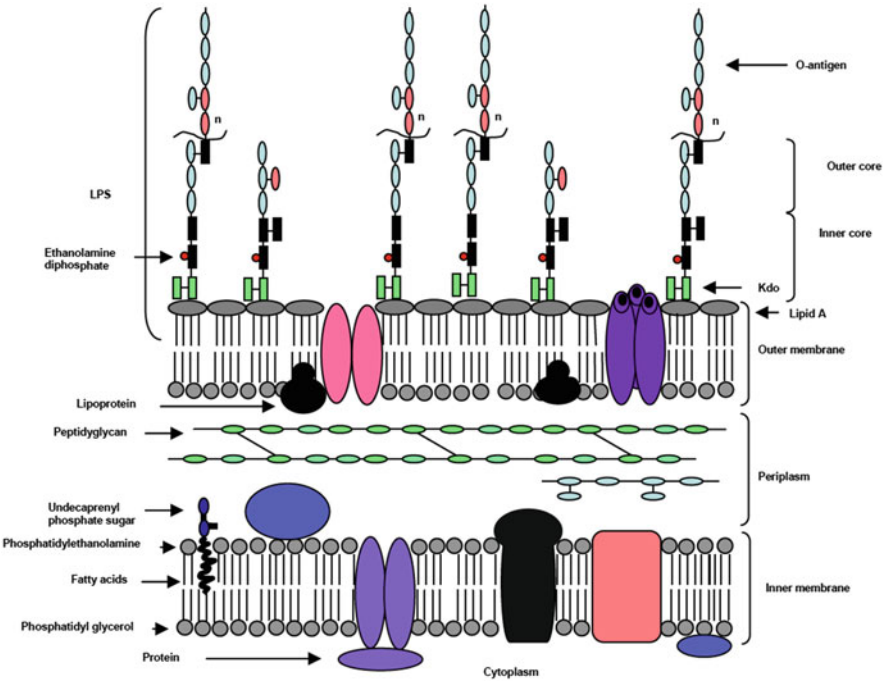


**Fig. 2** Outer membrane of Gram-negative bacteria. (Adapted from Raetz 1993)

The development of a more robust outer membrane would have been evolutionarily advantageous to bacteria. Diverse microbial communities can contain species that naturally produce antibiotic peptides which induce a selection pressure on the population (Miller 2016). The bacterial species that thrive in these communities must adapt rapidly to their changing environments or risk being outcompeted. Hence, OM plays a crucial intermediary role that facilitates communication between a bacterium and its surrounding environment.

### Porin Channels

Porins, first described in *Escherichia coli* (Nakae 1976), are transmembrane channels associated with the OM that selectively transport molecules that are oppositely charged to the overall amino acid charge of the channel (Achouak et al. 2001). They begin as pores on the surface, extending inwards into a channel that transports ions and molecules across the OM. In addition to acting as receptors for phage attachment and immune recognition, they operate as a molecular sieve, obstructing the diffusion of large molecules, like antibiotics into the cytoplasm, contributing in this way to intrinsic resistance (Fernandez and Hancock 2012). Upon infection in vivo, *Salmonella enterica* serovar Typhimurium can alter specific porin channels and regulate OM permeability when exposed to high levels of peroxide during phagocytosis. This reduces its susceptibility to hydrophilic β-lactam

antibiotics that require porin channels to diffuse through the OM (van der Heijden et al. 2016).

### Capsular Defences

The bacterial capsule is one of the most important virulence factors in bacteria but plays a dual role in protecting bacteria from antimicrobial peptides (Campos et al. 2004). It is comparable to the skin and mucosal membranes in innate mammalian immunity and blocks any interaction with receptors on the bacterial surface. The capsule can also cast off some of its surface polysaccharides to neutralise antibiotic molecules in the extracellular space—a mechanism seen in polymyxin resistance. Polymyxin resistance has increased significantly in the recent past, due to the emergence of colistin, an antibiotic within this class, being excessively used in the agriculture, cattle and poultry rearing industries as growth promoters (Watkins et al. 2016). The level of capsule polysaccharide expressed in bacteria is positively correlated to the level of polymyxin resistance, observed in *Klebsiella pneumoniae*. Negatively charged polysaccharides shed from capsule favour binding to positively charged polymyxin molecules in the extracellular space, reducing the amount of antibiotic that reaches the cell (Campos et al. 2004).

### Bacterial Innate Immunity to Bacteriophages

Bacteriophages are viruses that dominate the biosphere, surpassing the collective populations of every other species on the planet (Nabergoj et al. 2018)! Bacteria have co-evolved with bacteriophages, constantly trying to outmanoeuvre one another by developing advantageous traits that promote virulence, pathogenicity and internal defences (Clokie et al. 2011). The role of bacteriophages in treating ABR bacteria is emerging rapidly as a viable solution to address the crisis with minimal collateral damage. Traditional antibiotics annihilate all bacteria without distinction, disrupting the human microbiota and leaving it in a state of dysbiosis, i.e. vulnerable to infection for up to 2 years following treatment in some cases (Jernberg et al. 2007; Jakobsson et al. 2010; Yoon and Yoon 2018). Since bacteriophages are selective in their host range, rarely diverging from a group of related bacterial species, it provides a unique opportunity to develop targeted therapies for specific pathogens.

As we consider this novel approach to treating resistant infections, we must remain cognizant of the ancient evolutionary interplay between these species. Bacteria have evolved several mechanisms to contain phage infections and obstruct its ability to spread or to evade infection altogether. These mechanisms include a combination of non-specific intrinsic features such as defences during phage attachment/entry, defences during replication and defences during host cell lysis. There are also pathogen-driven adaptive responses, involving clustered regularly interspaced short palindromic repeats (CRISPR), which are described later in the chapter.

### Defences During Phage Attachment and Entry

**Preventing phage attachment** This can either be done using a physical barrier that hides the receptors or by modifying the structures of the receptors so the phage fails to recognise and bind to them. The bacterial capsule, like for antibiotics, is the first line of defence against bacteriophages too (Shabbir et al. 2016). Many phages have evolved to infiltrate the bacterial capsule by producing enzymes that can degrade capsule polysaccharides (Abedon 2012). Although this makes bacteria vulnerable to infection, there is some evidence that these phages can confer an advantageous virulence trait to the host bacteria. For example, the capsules of pathogenic streptococci composed of hyaluronic acid can be degraded by hyaluronidase enzymes encoded in a prophage. When a prophage integrates into the host genome, the bacterium can use these degrading enzymes to break down hyaluronan in eukaryotic connective tissue to increase the efficiency of disease transmission (Labrie et al. 2010). This improved fitness is accompanied by the added protection from invasion from other closely related phages.

**Preventing phage entry by receptor modification** The O1 antigen associated with the LPS molecules in *Vibrio cholerae* can be induced to undergo a process called 'temporary phase variation' where the O1 antigen remains genetically encoded but is not phenotypically expressed (Seed et al. 2012). The bacterium avoids being recognised by the phage as a host target but compromises with attenuated virulence (Dy et al. 2014). This type of evasion due to the absence of a receptor is also mirrored in certain human infections. For example, the human CCR5 gene codes for a co-receptor found on T-cells and is essential for HIV invasion. A Δ32 mutation in two alleles of the CCR5 gene gives complete resistance to HIV due to the absence of the co-receptor blocking HIV entry into the cell (Galvani and Slatkin 2003). This mutation is, however, contained within the host genome in humans as opposed to a phenotypic change in *Vibrio cholerae.*

**Deceiving bacteriophages by other mechanisms** Bacteria can secrete decoy molecules such as protein A to occupy the phage receptor and prevent it from attaching to the bacterium. Bacterial protein A secretion rates have been shown to have an inverse relationship with the likelihood of phage adsorption (Nordström and Forsgren 1974). Gram-negative bacteria are also capable of secreting outer membrane vesicles (OMVs) with proteins from the cell membrane that mislead phages away from the host.

**Superinfection exclusion systems** These are membrane-associated proteins that create a barrier to prevent the entry of bacteriophage DNA into the host bacterium, similar to clotting systems that minimise entry of pathogens in humans. The genes for these proteins may have originated from prophages, with a probable role in phage-phage recognition that has adapted to benefit the bacterium by protecting it against co-infection by other closely related phages (Seed 2015). These systems also

neutralise the pathogenicity of the phage, hence protecting the surrounding bacterial population too.

### Defences During Replication

**Cleaving and removing DNA using restriction-modification (RM) systems** These highly sophisticated systems involve a collaborative effort between a methyltransferase (MTase) enzyme that adds a $–CH_3$ methyl group to the host DNA and ignores the phage DNA, allowing its counterpart restriction endonuclease (REase) enzyme to detect and excise the unmethylated phage DNA (Bickle 2003; Vasu and Nagaraja 2013). This is similar to the ability of NK cells to recognise MHC class I receptors on immune cells to classify them as self-cells and stop a cytotoxic response (Orr and Lanier 2010). However, the existence of anti-RM systems such as Ocr of phage T7 which allows it to circumvent host bacterial defences and ArdA and ArdB proteins (widely distributed among bacteria) puts the efficacy of RM systems under scrutiny (McMahon et al. 2009). These anti-RM proteins trick RM systems and seamlessly enter the host to integrate mobile genetic elements carrying foreign DNA, reflected in the frequency of ABR dissemination through horizontal gene transfer.

**Trade-offs during phage infection** Abundant phage infection of a pathogenic bacterium can be beneficial to human immune recognition. MR-5 phage-infected methicillin-resistant *Staphylococcus aureus* (MRSA), a highly antibiotic-resistant bacterial pathogen, shows reduced cytotoxicity towards immune cells and subsequently improved intracellular bactericidal killing by macrophages (Van Belleghem et al. 2018). In these cases, the phage acts as an opsonin that coats the outer surface of the bacterium, similar to complement proteins C3b and C4b that tag antigens in our immune system to be recognised and destroyed by phagocytes (Kaur et al. 2014). Phage therapy administered to mice infected with a lethal dose of *S. aureus* cleared the infection within 4 days of treatment with a survival rate of 97% (Kaźmierczak et al. 2014). As all last-line antibiotics become futile against this pathogen, these findings have important clinical implications as the MR-5 phage has a broad host range and is proven to be effective in both intracellular and extracellular environments.

Similar to MRSA, carbapenem-resistant strains of *Pseudomonas aeruginosa* appear as "critical" in WHO's first ever list of antibiotic-resistant 'priority pathogens'. Being notorious for colonizing lung tissue and producing severe infection, these bacteria are limited in their ability to produce biofilm (discussed later in the chapter) when associated with an abundance of a filamentous Pf phage (Secor et al. 2017). In a murine pneumonia model, Pf phage-associated *P. aeruginosa* adheres to mucin in the airway epithelium and prevents the bacterium from aggressively colonising the host. The controlled inflammatory response that ensues reduces damage to the lung due to lowered neutrophil recruitment and cytokine responses. The phage simultaneously also protects its bacterial host from immune recognition by macrophages, promoting pathogen survival within the host and leading to chronic

infection. This brings up an interesting catch-22, i.e. using targeted phage therapy could ultimately reduce the severity of an infection and improve prognosis; however, the bacterium could manage to persist within the host for a prolonged period and move into a chronic disease state (Secor et al. 2017).

#### Defences During Host Cell Lysis

If all else fails, bacteria initiate programmed cell death using abortive infection systems (Abi) to protect the surrounding population. The bacterium systematically disassembles all cellular components and inactivates any nucleic acids present. This provides a controlled management system instead of the chaotic host cell lysis and dispersal of prophages into the surrounding environment. As intracellular toxins accumulate in a bacterium, transmissible genes encoded by prophages and plasmids are activated to initiate Abi systems (Seed 2015). These systems can directly be compared to apoptosis in the human immune system, a final stress-related response triggered by cell-mediated immunity (Raff 1998). From an evolutionary perspective, this altruistic kin selection of healthy individuals has long been associated with improved inclusive fitness of the overall population.

### 2.1.2 Acquired

#### Multidrug-Resistant (MDR) Efflux Pumps

MDR efflux pumps can be intrinsic or acquired, and their expression is regulated by inducers in their environments. For bacteria, harbouring an MDR phenotype is highly beneficial since it can resist damage from a wide variety of antibiotic substrates with a single resistance mechanism. MDR efflux pumps are highly conserved within bacterial chromosomes of a given species, stressing its evolutionary advantage (Poole 2007). They are categorised into five families based on their structures and substrate specificities, but primarily all efflux pumps flush antibiotics out of the cell before the latter undermines cell integrity. In bacteria, most of these efflux systems exist as a triad of protein complexes consisting of a periplasmic adaptor protein that bridges the domains of the inner and outer membrane transport channels. This assembly is more frequently encountered in Gram-negative bacteria, since the macromolecular system facilitates expulsion of antibiotics across the double membrane (Li and Nikaido 2004). The five families of MDR efflux pumps are (1) the major facilitator superfamily (MFS), (2) the resistance nodulation-division (RND) family, (3) the small multidrug resistance (SMR) family, (4) the multidrug and toxic compound extrusion (MATE) family and (5) the adenosine triphosphate (ATP)-binding cassette (ABC) superfamily (Fig. 3) (Sun et al. 2014). The MFS, RND, SMR and MATE families are examples of secondary active transporters, more specifically antiporters that transport molecules in opposite directions through the cell membrane. In this case, the efflux pumps take advantage of the electrochemical gradient as an energy source to move protons into the cell while simultaneously pumping drug molecules out, combining the processes of facilitated diffusion and active transport (Kumar et al. 2016). The ABC superfamily is the only group that hydrolyses ATP for energy instead (Webber 2003).

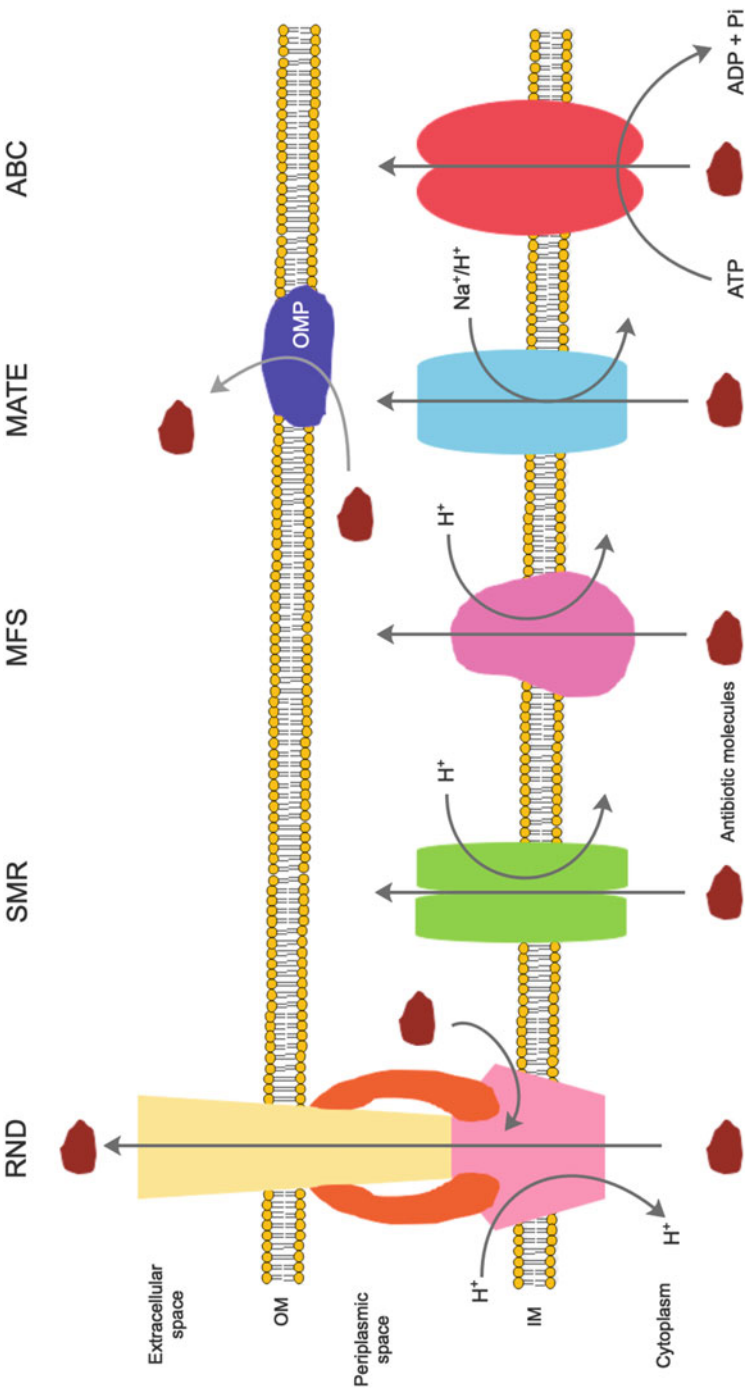


**Fig. 3** Five families of MDR efflux pumps (adapted from Blanco et al. 2016)

RND efflux systems are of great clinical significance due to their versatile substrate specificities. There is also evidence of compensatory RND efflux pump systems that are genetically distinct which act as a reserve and are only activated if the existing systems are inactivated or damaged (Nikaido and Pagès 2012). RND efflux pumps have been associated with promoting the acquisition or expression of new ABR genes that produce resistance-conferring phenotypes. It is possible that RND efflux pumps can respond to environmental stimuli and initiate specific pathways to enhance ABR to individual antibiotic classes. This would create an additive resistance effect to increase the minimum inhibitory concentration of antibiotic (MIC) that would be lethal to the bacterium. This mechanism has been observed in *P. putida* and *P. aeruginosa* (Kim et al. 2017) where indole, a signalling molecule (discussed in detail further in the chapter), upregulates both the expression of RND efflux pumps (also proven in *E. coli* (Hirakawa et al. 2004)) and, in turn, the production of β-lactamase enzymes when exposed to high levels of ampicillin.

### Horizontal Gene Transfer

Bacteria use vectors such as extracellular DNA, plasmids and bacteriophages to exchange genetic material with genetically unrelated species through transformation, conjugation and transduction (discussed later in the chapter), respectively. Collectively, these mechanisms mediate horizontal gene transfer (HGT), which has had huge implications on the worldwide dissemination of ABR since resistance-conferring genotypes can rapidly circulate to other individuals within a community rendering the whole population resistant. The evolution of resistance-conferring phenotypes has been observed as early as 3 years following the introduction of a new antimicrobial (Barlow and Hall 2002). The highly prevalent TEM ESBLs have represented this evolution through its countless allelic variants efficiently circulated through HGT making nearly all β-lactam antibiotics ineffective.

Clinical environments provide a unique set of circumstances favouring both the onset and spread of many resistance determinants through consistent exposure instigating desensitisation to bactericidal substances. These conditions mirror the evolution of bacteria in the environment (albeit significantly accelerated), promoting the survival of those that can thrive under these conditions while providing easy access to vulnerable hosts. A review published in May 2017 assessed the outcomes of 32 extensive studies into hospital water systems as reservoirs for carbapenem-resistant organisms, and their findings implicate sinks, drains and toilet bowls seeded by infected patients as frequent offenders (Kizny Gordon et al. 2017). Additionally, plumbing systems prone to stagnation could provide suitable conditions for a microbiome to proliferate and form biofilms. These studies tracked pathogenic bacterial species through infected patients within the same hospital over a long period and used comparative genomics to determine the mode and route of resistance transmission (Snitkin et al. 2012; Leitner et al. 2015). It was found in 41% of the studies that all water reservoirs were positive for carbapenem-resistant *P. aeruginosa* (Pitten et al. 2001).

### Transformation

Bacteria can take up free floating strands of DNA from their environment and integrate them into their genome, a process known as transformation. *Streptococcus* and *Neisseria* species use transformation as their primary mode of resistance-gene uptake. Both these pathogens have undergone transformational events that added multiple resistance determinants into their genome. In the case of *Neisseria*, some of these determinants have been acquired from commensal organisms under conditions of stress, e.g. during a course of antibiotic treatment. Pathogenic strains of certain species can also exchange DNA with intrinsically resistant organisms present in soil and water in the environment, picking up resistance determinants in the process. Misuse of antibiotics and a lack of a targeted approach to treatment have now led to infections caused by bacteria that have endured the selection pressure and evolved phenotypes to survive under stress.

### Conjugation

Within HGT, conjugation is most frequently used to disseminate ABR. Conjugation uses mobile genetic elements (MGEs) to transport resistance genes to neighbouring bacterial cells. MGEs can be either intracellular transposons, integrons or plasmids that can move between organisms and species. These MGEs have accompanying 'fitness costs' to maintain these features and require compromises from the host such as decreased virulence and reproducibility, hence obstructing the uncontrollable dissemination of resistant pathogens.

Bacteria use MGEs to integrate new DNA into their chromosomes. Integrons are mobile elements that can acquire gene cassettes with various combinations of resistance genes. Although integrons are classified as MGEs, they cannot move independently and require either transposon or plasmid vectors to aid their transport. Transposons are self-contained sequences flanked by insertion sequences on either side which carry integrons between the plasmid and the chromosome. A transposase enzyme helps to incorporate the transposon into a plasmid or chromosome through site-specific homologous recombination. Chromosomally encoded resistance is concerning since it can be vertically transmitted to bacterial progeny; however, plasmid-borne resistance is favoured in conditions of high antibiotic concentrations since it offers increased mobility. Phylogenetic analysis of the OXA β-lactamases highlights two ancient mobilisation events millions of years ago where the resistance gene jumped for the first time from chromosome to plasmid, contradicting the requirement for an antibiotic selection pressure for the event to occur. OXA β-lactamase genes evolved independently and could have provided resistance to naturally occurring antimicrobials or, alternatively, encoded proteins with significantly different roles as opposed to the purpose they serve today.

Plasmids are autonomous double-stranded sections of circular or linear DNA equipped with their own replication and transmission machinery. A troubling consensus of several studies reveals that resistance determinants are now accumulating on plasmids for ease of transmission (Rozwandowicz et al. 2018; Ragupathi et al. 2019). The acquisition of these plasmids could eventually alter the global microbiota

such that many commensal bacterial species can harbour an MDR phenotype in the future, making even combination drug therapy ineffective.

β-lactams and aminoglycosides are two clinically significant antibiotics whose main resistance determinants have been transmitted through HGT. Aminoglycoside resistance mechanisms are mainly due to aminoglycoside-modifying enzymes that are encoded on plasmids. AAC(6′)-Ib-cr confers resistance to both aminoglycosides and fluoroquinolones simultaneously, and its prevalence is higher than *qnr* genes. Random transposon insertion to knock out the resistance gene discovered a novel variant of what was primarily an aminoglycoside acetyltransferase AAC(6′), unlike any of the variants that were previously characterised. Resistance determinants that have risen chromosomally in the antibiotic era are more costly than other plasmid-borne traits that have mutually adapted and circulated with their hosts (Vogwill and MacLean 2015).

## 2.2 The Specific Bacterial Defence System

This section outlines more targeted mechanisms used by bacteria tailored to the invading agent. These mechanisms can either be:

1. Intrinsic active: inherent to the host bacteria and requires active involvement of the host to effectively remove the invader.
2. Intrinsic passive: transfer of intrinsically encoded genetic material by a third-party bacteriophage without any direct interaction between the two bacteria involved (transduction).
3. Acquired active: biochemical processes that lead to resistance due to MGE acquisition and expression.
4. Acquired passive: resistance provided to sensitive bacteria from neighbouring resistant bacterial colonies.

### 2.2.1 Intrinsic Active

#### Quorum Sensing

Within a population, bacteria communicate and respond to stimuli using extracellular signalling molecules called autoinducers through quorum sensing. When the extracellular levels of autoinducers surpass a threshold, it allows each member of the population to ‘sense’ its neighbours in the environment. Different classes of autoinducer peptides are found ubiquitously among Gram-positive and Gram-negative species, and based on these signals, bacteria manipulate gene expression by inducing phenotypic changes such as increased virulence and biofilm formation, which dictate the population’s susceptibility to antibiotics (Rémy et al. 2018). This system creates an elegant equilibrium between cell population density and gene expression. A highly effective strategy to treat resistant *P. aeruginosa* infections relies on breaking down autoinducers which oppress cell-cell communication (also

known as quorum quenching) to restore the population's susceptibility to cytotoxic killing by antibiotics (Mion et al. 2019).

### Indole

Indole is an aromatic signalling molecule whose derivatives are ubiquitous in eukaryotes and prokaryotes alike. In bacteria, this signalling molecule contributes significantly to quorum sensing, and it could act as the interface between the non-specific and specific bacterial defence responses. Indole is also known to upregulate MDR gene expression in response to stress and trigger persister cell formation making it a critical signalling pathway (Kim et al. 2017). This feature is comparable to antigen-presenting cells (APCs) such as macrophages and natural killer (NK) cells from the human innate immune system that trigger a cascade of antigen-specific defence mechanisms.

### CRISPR/Cas9

Most of today's cutting-edge research in the field of molecular biology and genome editing is based on the accidental discovery of the clustered regularly interspaced short palindromic repeats (CRISPR)/Cas9 system in *E. coli* by Japanese scientists Ishino et al. in 1987 (Ishino et al. 1987). The CRISPR locus constitutes multiple regions of palindromic 20–40 repeated bases separated by unrelated, non-repetitive short sequences (spacers). But it was only during the early 2000s that the true potential of CRISPR/Cas9 as a revolutionary genome editing tool was discovered. Seven years later, in 2007, a seminal study by Barrangou et al. established its role in adaptive immunity of *Streptococcus thermophilus* against phage infection (Barrangou et al. 2007; Fineran and Charpentier 2012). This study provided the first evidence that strains of *S. thermophilus* resistant to phage carried spacer sequences that matched the phage DNA (protospacers) (Thurtle-Schmidt and Lo 2018). In addition, researchers also found **C**RISPR-**as**sociated (Cas) genes translate into a protein complex that exhibits helicase and nuclease activity for unwinding and excision repair processes, respectively. This immune system, found to exist in both bacterial and archaeal kingdoms, helps these microbes battle previously encountered pathogens through accessing an immunological 'memory' and initiating a quicker defence response—much like our own T and B memory cells (Abbas et al. 2004). The CRISPR arrays can also be vertically passed on to offspring, making the bacterial adaptive immune response arguably better.

The CRISPR/Cas9 mechanism creates a spacer-acquisition complex with Cas1 and Cas2 proteins using short sequences of phage DNA integrated into the CRISPR loci. Any invading phage DNA is cross-referenced against the existing repertoire of spacer regions to identify the DNA as self or foreign (primed spacer acquisition). In case the bacterium encounters a new pathogen for which the CRISPR loci carry no memory, it will use the spacer-acquisition complex to acquire the new phage DNA and contain it within an unoccupied spacer region for future reference (naïve spacer acquisition). This is similar to vaccination, where an antigen from an attenuated pathogen (bacteria, virus, etc.) is intentionally injected into a human host to trigger

an immune response that will stimulate the production of memory cells and antibodies to prepare the body against infection by a wild-type pathogen.

There are limitations to this system, for example, a mutation in the invading phage DNA that lies in the spacer reference region would allow it to escape bacterial immune recognition by the CRISPR/Cas complex and proceed to infect the cell (Datsenko et al. 2012). Interestingly, it has also been shown that these point mutations can drive spacer acquisition due to the low-affinity binding of protospacers, activating the Cas1-Cas2 spacer-acquisition complex to accommodate new spacer regions. In this way, the bacterium becomes completely resistant by gaining several slightly altered spacer sequences as resistance determinants corresponding to the same phage.

### Biofilm Formation and Persister Cells

Bacteria thrive within intricate networks of several microbial colonies that can adhere to a surface, communicate with each other and proliferate. These complex formations of aggregated bacteria are known as biofilms, characterised by their enclosure within matrices formed by an extracellular polymeric substance (EPS; consisting of polysaccharides, proteins and DNA) (Stewart et al. 2015), regulated expression of specific genes and reduced metabolic activity among the bacterial cells (Donlan 2002). Bacterial communities contained within biofilms are incredibly robust, colonising human surfaces such as the skin and teeth and even hostile environments covered with highly effective antimicrobial surface coatings such as the International Space Station (Sobisch et al. 2019). Biofilm formation offers several advantages to bacteria, from enhancing virulence and antimicrobial resistance up to 1000-fold (Cepas et al. 2019) to protecting microbial communities from predation and host immune responses. In a clinical context, biofilm formation plays a major role in medical device-related infections including ventilator-associated pneumonia and catheter-associated urinary tract infections among others (Percival et al. 2015).

The organisation, metabolic activity and spatial density of cells within a biofilm are mandated by complex intercellular signalling that adapts to environmental cues. Within a biofilm, there can be local differences in growth conditions with altered spatial structure and pH affecting neighbouring cells and creating different microenvironments. Sub-MIC level antibiotic exposure can boost the rate of transfer of resistance determinants within the biofilm by 2–5× (Salcedo et al. 2015). This is a multifactorial phenomenon, affected by the growth conditions of the biofilm, bacterial species contained within and corresponding phenotypes, stage of biofilm formation, etc.

Notably, there is a distinct difference between bacterial cells within a biofilm that are ‘resistant’ to antimicrobials and those that are ‘tolerant’ to them. Resistant cells continue to grow within the biofilm in the presence of environmental antibiotic selection pressure. The truly resilient lot are the tolerant persister cells, which are genotypically identical to their counterparts but are phenotypically dormant and remain metabolically inactive and unaffected by antibiotics (Wood et al. 2013). These cells have been indicted for their roles in chronic infections, surviving waves

of antibiotic treatment, while the remaining population is wiped out and rebuilding the bacterial population when the antibiotics are lowered to sub-MIC levels.

### 2.2.2 Intrinsic Passive

#### Horizontal Gene Transfer

Transduction

Transduction is a form of horizontal gene transfer (HGT) mediated by bacteriophages, and the extent of its contribution to ABR is poorly characterised. Generalised transduction is recurrently observed in both methicillin-resistant and methicillin-sensitive *Staphylococcus aureus* (MRSA/MSSA) hospital-acquired isolates. These strains have successfully transmitted antibiotic-resistance genes, more specifically, tetracycline- and penicillin-resistance genes, to laboratory-grown isolates. A critical observation, however, is that the bacterial strains that are insensitive to phage infection can also obtain resistance determinants through HGT if phage adsorption occurs (Mašlaňová et al. 2016). A recent study emphasised the hypermobility of genetic elements in *S. aureus* through newly discovered lateral transduction when carried by highly abundant prophages and its potentially significant implications on the dissemination of ABR determinants through HGT (Chen et al. 2018).

### 2.2.3 Acquired Active

#### Enzymatic Degradation/Destruction

Bacteria encode resistance genes for enzymes that degrade antibiotics and prevent them from destroying the cell. These enzymatic modifications are seen most frequently among antibiotics that interrupt ribosomal activity such as aminoglycosides, chloramphenicol, streptogramins and lincosamides (Munita and Arias 2016). The enzymes induce either acetylation, phosphorylation or adenylation of the antibiotic molecule, all of which ultimately result in an altered structural conformation that has a lower affinity for the ribosomal target. In a clinical context, aminoglycoside (Ag) resistance is predominantly dispersed by aminoglycoside-modifying enzymes (AMEs) that are divided into three groups based on their mechanism of action: Ag acetyltransferases (AAC), Ag nucleotidyltransferases (ANT) and Ag phosphotransferases (APH) (Labby and Garneau-Tsodikova 2013).

Enzymatic destruction through hydrolysis of the amide bond contained within β-lactam ring is the main mechanism of β-lactam antibiotic resistance, which is mostly encoded by plasmids. The first β-lactamase gene discovered was TEM-1, which hydrolyses ampicillin and was named after Temoneira, the patient it was isolated from. β-lactamases have gradually evolved and have now been surpassed in their spectrum of activity by extended-spectrum β-lactamases (ESBLs). TEM-3 is an ESBL variant of TEM-1 having attained two amino acid substitutions which provides it with the added capacity to hydrolyse third-generation cephalosporins and aztreonam. Since then, there has been evidence of a ‘genetic fusion’ of two

antibiotic-resistance genes which resulted in the evolution of New Delhi metallo-β-lactamase, an ESBL which was first discovered in 2008 (Zarfel et al. 2011). This novel resistance gene would impose less fitness costs than carrying two individual genes and has been a cause of major concern since it confers high-level resistance to nearly all commonly used antibiotics. NDM-1 is now globally distributed, with the largest reservoir in Asia (Khan et al. 2017).

#### Target Protection by Obstruction/Alteration/Mutation

Plasmids and transposons often transport resistant determinants that obstruct or alter antibiotic target sites. In these cases, the target remains functional since the antibiotic fails to bind or binds with lower affinity, reducing its efficacy (Blair et al. 2015). The *ermB* gene uses this mechanism to provide resistance to three different classes of antibiotics: macrolides, lincosamides and streptogramins. These genes methylate the 16s rRNA targets, altering its structure and making it suboptimal for the antibiotic to bind with due to steric hindrance.

Alternatively, bacteria can harbour mutations that prevent antibiotics from recognising the protein. Fluoroquinolone antibiotics are used to disrupt DNA gyrase and topoisomerase IV enzymes in Gram-negative and Gram-positive pathogens, respectively, and are involved in altering the conformation of DNA during DNA replication and repair (Jacoby 2005). Fluoroquinolone resistance is mainly mediated by amino acid substitutions in the *gyrA* and *parC* subunits of these enzymes in their quinolone resistance-determining regions (QRDR), preventing them from being identified as the fluoroquinolone target.

### 2.2.4 Acquired Passive

#### Co-pathogens Conferring Resistance

A bacterial defence mechanism that mimics herd immunity in humans suggests that an established resistant bacterial population can protect an invading susceptible co-pathogen from antibiotic treatment (Chattopadhyay and Jaganandham 2015; Kim et al. 2018). For example, *Bacteroides* spp. release β-lactamase enzymes through OMVs into the surrounding environment and hydrolyse the antibiotic molecules before it reaches a susceptible co-pathogen (e.g. *Salmonella typhimurium*) which, therefore, automatically gains resistance. This phenomenon has also been recorded in resistant *E. coli* which protect susceptible *E. coli* colonies through OMVs.

## 3 Fungi

In comparison to antibiotics, the options for antifungals are very limited (Perlin et al. 2017), and, thus, resistance development to any of the few available classes can be catastrophic (Shor and Perlin 2015). The two mainly used drug classes for treating fungal infections are azoles and echinocandins, and resistance to both has already been demonstrated in several *Candida* species. *Candida* is a commensal fungi in the

human microbiota and an opportunistic pathogen that targets vulnerable hosts in healthcare environments like hospitals (Kullberg and Arendrup 2015; Nobile and Johnson 2015; Perlin et al. 2017; Chowdhary et al. 2017). Indeed, *Candida auris* strains have been demonstrated to be resistant to all four main classes of human antifungal drugs on multiple occasions (Chowdhary et al. 2017). The risk of untreatable superbug fungal infections is looming, and understanding resistance patterns and evolutionary pathways is very important for future infection treatments. This section will discuss two of the main classes of antifungals used to treat many fungal infections and their resistance mechanisms. In addition, alternative mechanisms of resistance including biofilm formation and mycovirus (viruses that infect fungi) resistance will also be discussed.

## 3.1 Azoles

Azoles were—and in some places still are—the main frontline drug for treating fungal infections for many years. They target the lanosterol 14 alpha-demethylase enzyme (encoded by the ERG11 gene), part of the cytochrome P450 family of enzymes used in biosynthesis of steroids and metabolism of organic compounds (Ghannoum and Rice 1999; Koch et al. 2018). Fungi have become resistant to this class through several methods, including increasing the number of drug efflux pumps through transport gene mutation or overexpression (Schubert et al. 2011; Perlin et al. 2017). These efflux pumps work very similarly to those mentioned previously in this chapter (see Sect. 2.1.2), offering a potential parallel to the non-specific acquired immunity in bacteria mentioned previously. The second method of resistance to azoles is a change in the ERG11 target itself (White et al. 2002). This limits the action of the antifungal, as the target has an altered structural conformation (while remaining functional to the fungi). This is analogous to the specific acquired active immunity of bacteria (Sect. 2.2.3).

In 2002, one study analysed the genes encoding for ABC efflux pumps in clinical *C. albicans, mdr1*, *cdr1*, *cdr2* (similar to those mentioned previously in Sect. 2.1.2), and the target encoding gene *erg11* (White et al. 2002). It was found that *cdr1* and *cdr2* were both upregulated in resistant strains and could be co-regulated. *mdr1* upregulation was found sporadically across resistant strains, while point mutations in *erg11* were discovered in many of the samples tested which did not correlate with increased resistance (White et al. 2002). The study showed that azole resistance is only partially understood. In 2011 and 2012, two studies investigated transcription factor mutations in *C. albicans* that could account for mutations and the upregulation of genes (Schubert et al. 2011; Flowers et al. 2012). Some focused on *erg11* mutations rather than the transcription factors and found 31 alleles of the gene, 21 of which had multiple amino acid substitutions, displaying a much higher level of azole resistance than those with single mutations (Flowers et al. 2015). Collectively, it can be said that multiple mutations across several genes bring about cumulative resistance patterns (Schubert et al. 2011; Flowers et al. 2012).

It is believed that resistance to azoles has been built up through the agricultural industry (Denning and Perlin 2011; Perlin et al. 2017; Berger et al. 2017). Azole-based antifungals are often dispersed across large areas of crops, and constant exposure leads to development of resistance in many fungal species (Denning and Perlin 2011; Perlin et al. 2017; Berger et al. 2017). Azole resistance has also been found in *Aspergillus fumigatus*-infected patients that had never been treated with the drug class, implicating an environmental pathway (Denning and Perlin 2011; Perlin et al. 2017; Berger et al. 2017; Brilhante et al. 2019).

## 3.2 Echinocandins

Introduced in the early 2000s, echinocandins were the first novel class of antifungals developed in over 15 years and are now the frontline treatment for many fungal infections, especially candidiasis (Denning 2002; Perlin 2015). The class was licenced in response to several aspergillosis infections where treatment with prior available drugs had failed. The drug targets glucan synthase, a fungi-specific enzyme that is involved in the synthesis of the cell wall polymer β-1,3-glucan (Perlin 2015). While this target is well conserved across many fungal species, mutations in any of the 'hotspot' areas of genes *fks1* and *fks2* (in *Candida*) can lead to resistance (Cowen and Steinbach 2008). Cellular stress pathways are impacted when the organism is under prolonged drug exposure which leads to higher mutation rates during replication and transcription (Perlin 2015). This is again similar to the target protection by obstruction/alteration/mutation section of the bacterial model, found in the specific acquired active immunity (Sect. 2.2.3). Resistance to echinocandins has only been shown to develop during treatment, suggesting that these changes may have no other benefit outside of resistance and are therefore not selected for in wild populations (Cowen and Steinbach 2008; Perlin 2015).

Since resistance is not observed in unexposed fungi, echinocandins should always be effective against a novel infection. However, the rate of echinocandin resistance development in ICU environments is on the rise (Cortegiani et al. 2019). This could be due to a higher rate of infections with prior resistant strains or because the rate of resistance development following first exposure is on the rise. In either case, this is a serious problem for future treatments, as echinocandins are the only current treatment available for multi-resistant strains already resistant to the other classes.

## 3.3 Other Mechanisms of Resistance

Like bacteria, fungi also form biofilms as a buffer to environmental conditions (Nett and Andes 2015). This is another similarity to the specific intrinsic active immunity of the bacterial immune model (Sect. 2.2.1, "Biofilm Formation"). These structures can cluster antifungal compounds away from the fungal cells and act as a barrier preventing drug transmission (Sanglard 2016). It has also been suggested that certain

fungal species can sequester antifungal compounds within their own cell using a similar mechanism, but this is not fully understood (Maebashi et al. 2002). Biofilms can be used by multiple species in parallel, which is of increasing concern as multidrug-resistant species could create biofilms, making any treatment with weaker antifungals impossible (Borghi et al. 2016; Perlin et al. 2017; Sherry et al. 2017). There is, however, development of anti-biofilm compounds, and treatment with both an antifungal and an anti-biofilm compound is a potential avenue that may become the norm (Nett and Andes 2015; Sherry et al. 2017).

Horizontal gene transfer can also occur in fungi as in bacteria (Sect. 2.1.2); however, this has not been linked with transference of antifungal resistance. Instead, the resistance develops in isolation more often (Anderson 2005; Fitzpatrick 2012).

Fungi contain a large group of potentially infectious species and genera, with few antifungal options available. Biofilms make the situation even more challenging, and an equivalent of phage therapy using mycoviruses is still a blue-sky challenge far from medical implementation. While there are still antifungal compounds available for treatment, it may not be long before the worst fungal infections are fully resistant to everything available, or at least resistant enough that a new option will be required.

Much like phage therapy for antibiotic-resistant bacteria (Sect. 2.2.1), mycoviruses (viruses that infect fungi) have been demonstrated to reduce resistance to antifungal pesticides in agricultural environments (Niu et al. 2018; Wang et al. 2019). Although mycoviral infection has not always correlated with decreased resistance and increased susceptibility to antifungal compounds, and while this research area has been mainly constrained within agricultural and food security fields, the results indicate a promising way forward towards countering antifungal-resistant clinical strains (Zoll et al. 2018). A form of mycoviral engineering was put forward in 2016 by Zhang and Nuss, who reported a method of using mycoviruses to attenuate virulence factors within chestnut blight (Zhang and Nuss 2016).

## 4 Viruses

Viruses have the most ‘uniform’ method of developing resistance across all forms, i.e. mutation of the drug targets and pathways (Pizzorno et al. 2011; Bagaglio et al. 2017). Using the examples of influenza and hepatitis C viruses, we discuss how the higher rate of evolutionary change impacts viruses’ resistance to antiviral compounds, and we look at the fitness trade-offs that can arise as a result. Due to viral resistance being so changeable, it is harder to relate to this section to the immune system model we have been using, and it would not be incorrect to suggest that viruses do not fit this model. Viruses that infect other microbes are not discussed here.

## 4.1 Hepatitis C

Hepatitis C affects people all over the world, and it is estimated there were over 170 million cases worldwide between 1989 and 2013 (Messina et al. 2015). This makes resistance to any of the anti-hepatitis C drugs very important, as an untreatable form would impact millions of people across the globe. Thankfully, this is not yet a major problem, as combination therapies have proven to work, although there is now a risk of failure under certain conditions, such as other underlying health conditions (Pawlotsky 2016). Due to the high evolutionary rate, amino acid changes occur rapidly and can lead to the gain and loss of resistance depending on the selection pressures of the viral environment. This gain and loss of resistance due to amino acid substitutions can occur over a short term (a few weeks) or can remain for years, depending on the area of the viral genome in question (Pawlotsky 2016). This makes tracking resistance very difficult, as certain virus strains may remain resistant for several months, while others may gain and lose resistance based on exposure and duration of antiviral treatment (Pawlotsky 2016).

Relying on combination therapy can be risky when resistance develops rapidly. Initiating treatment before ascertaining resistance profiles carries the risk of building up resistance to multiple compounds simultaneously (Bagaglio et al. 2017). It is therefore important for the future that antivirals are also prescribed with resistance in mind. Work by Ottosen et al. in 2015 demonstrated clinical and preclinical resistance to newer anti-hepatitis C compounds and the reduced activity profile of the then-novel antiviral drug miravirsen (Ottosen et al. 2015).

## 4.2 Influenza

Influenza, the causative agent of flu, affects millions of people every year, with one study averaging ~8% incidence among the US population every flu season (Tokars et al. 2018). Vaccines are effective and are recommended in many countries, especially for people with pre-existing health conditions (Demicheli et al. 2018). While there are many treatment options currently available, resistance to the main groups of antivirals has been reported, as has the capability of the virus to retain that resistance between seasons (Pizzorno et al. 2011; Li et al. 2015).

It is important to determine the impact of a resistance mutation on a circulating viral population. A study by Jiang et al. investigated the 'hotspots' of influenza A mutation against one of its treatment options (oseltamivir) and found that while mutations led to resistance, they came with negative fitness costs for the virus (Jiang et al. 2016). This has been shown in other viruses too, where resistance to antivirals has had knock-on effects to decrease overall fitness, with respiratory syncytial virus (a cause of pneumonia and bronchiolitis) shown to have a weaker ability to propagate in vitro compared to the nonresistant wild-type (Battles et al. 2016). Jiang et al. also showed that there was potential for multiple mutations to re-correct this 'weakness', and thus there is an increasing risk of fit resistant strains.

## 5 Discussion and Therapeutic Strategies for the Future

Bacteria are relentless. Their survival strategies for overcoming extremely hostile environments are extraordinary feats of adaptation and natural selection. Several confounding factors affect ABR epidemiology. For example, some resistance genes are more prevalent globally than others, despite producing a similar phenotype. Although studies have indicated fitness costs associated with resistant phenotypes, there is evidence of compensatory evolution caused by mutations that reduce the burden of carrying resistance determinants (Durão et al. 2018). On the other hand, antibiotic-sensitive strains flourish and outcompete resistant strains in the absence of the antibiotic pressure—indicating that a resistance phenotype does not equal a fitter organism. These conflicting findings highlight the current gaps in our understanding of the dynamics of ABR acquisition, transmission and maintenance.

ABR should be approached holistically with a One Health perspective, requiring a major collaboration between the World Health Organization (WHO), the Food and Agriculture Organization (FAO) and the World Organisation for Animal Health (OIE). However, this is challenging. With the threat of a post-antibiotic era, we are faced with an urgent conundrum: to find alternatives to combat bacterial infections because evolutionary resistance is inevitable. Pharmaceutical companies and venture capitalists hesitate to invest in discovering and developing new antibiotics. Between 2003 and 2013, only $1.8 billion out of the total $38 billion venture capital investment was allotted to antimicrobial development (O'Neill 2014). This is due to the enormous time (10+ years) needed for development, high failure rate and relatively low profit margins in this sector.

There are novel therapeutic methods that are more targeted and cause less collateral damage to the microbiota showing promising results. Using bacteriophages to target specific bacteria is a frontrunner among the available options since their effectiveness has been proven in treating pulmonary infections with no evidence of it aggravating the immune system (Rohde et al. 2018). Phage therapy on its own would be used only as a last resort since bacteria can still develop resistance to bacteriophages (Kortright et al. 2019); for routine treatment, they would still need to be used with traditional antibiotics to improve their efficacy. Phages can target MDR efflux pumps on pathogenic species to restore their sensitivity to antibiotic treatment (Saha and Mukherjee 2019). Alternatively, specialised phages can depolymerise the EPS matrix of biofilms, allowing antibiotics to infiltrate the cells contained within.

There are also novel systems for delivering drugs such as nanoemulsion, which use amphiphilic vesicles containing antimicrobial phytochemicals sourced from plants, coated with a surfactant (Krishnamoorthy et al. 2018). Nanoemulsion has been successfully used to deliver eugenol and methyl salicylate to *E. coli* to inhibit biofilm formation and reduce virulence (Prateeksha et al. 2019). This method is especially attractive since there is no selection pressure that could provoke the population to evolve and develop resistance. Their use against MRSA and ESBL-producing *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* has shown encouraging results.

The bacterial CRISPR/Cas9 system has also successfully been used to 're-sensitise' resistant bacterial pathogens by targeting and excising resistance genes (Kim et al. 2016). The ability to distinguish between pathogenic and commensal species through this technique is also very encouraging. However, there still remain major hurdles in the way, especially with regard to mode of delivery, unknown toxicity effects and long-term impacts of genetic manipulation on evolution that demand further investigation (Shabbir et al. 2019).

Exciting new research published in early 2019 has identified *mfd*, a highly conserved DNA translocase protein that is involved in transcription coupled repair, as a driver of bacterial evolution. *mfd* promotes mutagenesis, which drives the development of ABR through accumulating resistance-conferring mutations (Ragheb et al. 2019). Knocking out *mfd* has no detrimental effects on DNA repair, suggesting that its main role is to propagate mutagenesis so bacteria can adapt rapidly to its environment, especially during host invasion. These findings reveal a new concept for 'anti-evolution' drugs that target highly conserved but 'non-essential' proteins that promote ABR. However, this is not unlike the highly controversial topic of using CRISPR for human genome editing, where the downstream effects on the individual genome and its surrounding microbiome remain largely unknown today.

Like with bacteria, resistance is developing fast in both fungi and viruses, with improved treatments being required all around the world. While antiviral resistance mechanisms are more simplistic compared to bacteria and fungi, mutation-driven resistance occurs at a faster rate. While some of these mutations impose fitness costs that prevent a resistant strain from becoming dominant (Jiang et al. 2016; Battles et al. 2016), the risk of compensatory polar mutations is always present. Therefore, tracking resistance transmission in the viral population is of utmost importance, alongside understanding the functional and immunological consequences of these mutations on the wider virus population. This is especially true for combination therapy, as extended exposure to the same drug cocktail could lead to multidrug resistance developing faster.

An intriguing aspect is the use of vaccination to fight AMR. Prophylactic vaccination can reduce the incidence of AMR and irrational use of antimicrobials worldwide. This has been proven in *Haemophilus influenzae* and pneumococcal vaccines since being included in routine immunisation, prior to which pneumococcal β-lactamase resistance was 16.6% among strains collected from various parts of the world. Absurdly, vaccination against viral infections such as influenza can reduce the spread of ABR and antibiotic use since it can reduce the likelihood of acquiring secondary bacterial infections, e.g. pneumonia (Jansen and Anderson 2018). Viral vaccines can also prevent clinicians from incorrectly prescribing antibiotics for viral infections that are symptomatically similar to bacterial pathologies. The net reduction in antibiotic use would also reduce the development of ABR among commensals (Bloom et al. 2018). Additionally, antibiotics, although fast-acting, are only effective for short-term acute bacterial infections, whereas vaccination primes the immune system by creating a 'memory' of the pathogen to be able to tackle repeated infections (Tagliabue and Rappuoli 2018). Vaccine development is currently in its prime and a more favourable solution than the development of new antibiotics, which has been moving at a snail's pace for the past 40 years (Fig. 4).

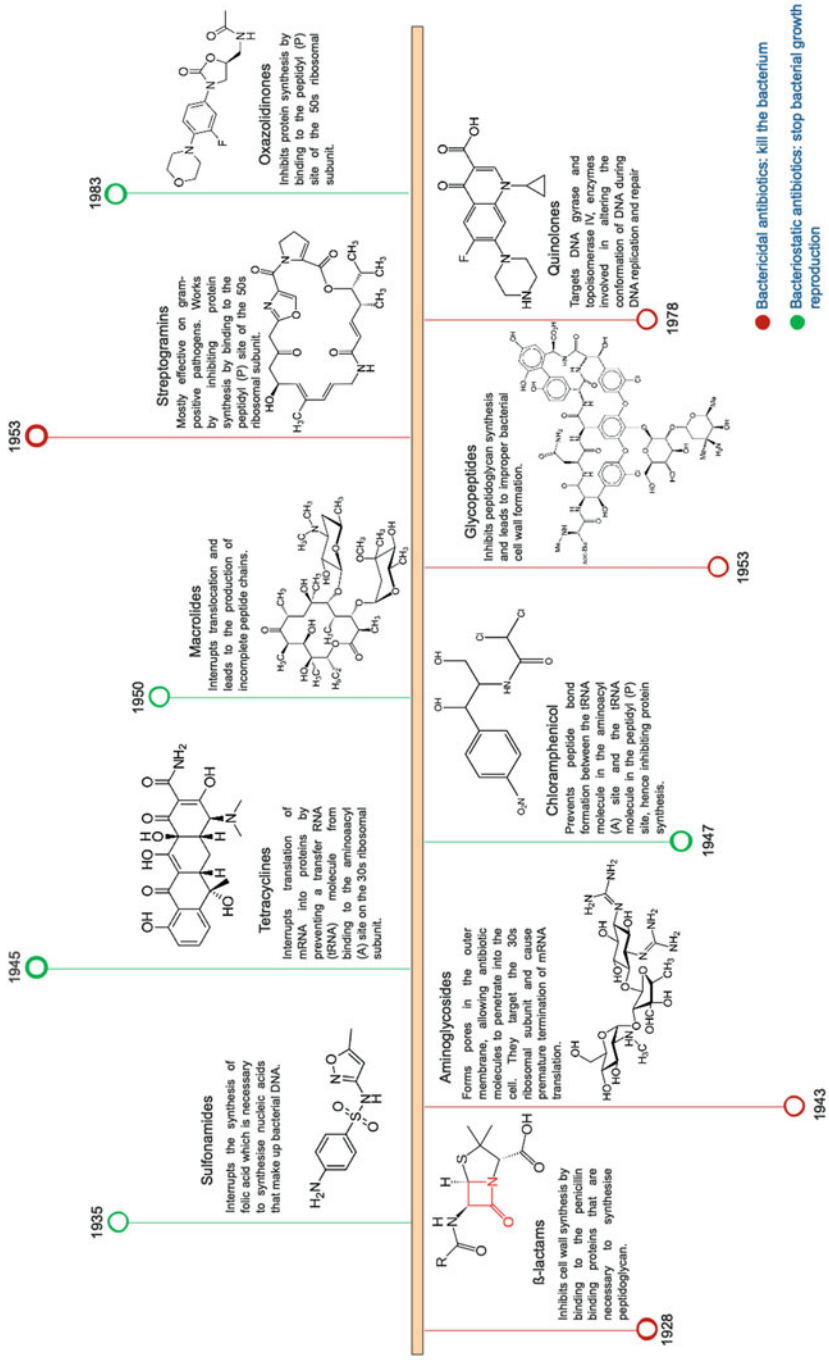


**Fig. 4** Timeline of antibiotic discovery and its mode of action. (Adapter from Farrell et al. 2018)

The intricate mechanisms that bacteria, fungi and viruses have evolved to protect themselves since the dawn of their existence are truly spectacular. Their defence systems have fascinated scientists for decades, and yet a plethora of unanswered questions remain. Society has grossly underestimated the abilities of these microorganisms and taken the only safeguards we currently have against them for granted. Antibiotic obsolescence is on the horizon, and immediate measures need to be taken to preserve their use.

## References


Abbas AK, Lichtman AH, Pillai S, Baker DH (2004) Basic immunology: functions and disorders of the immune system, 2nd edn. Elsevier Saunders, Philadelphia

Abedon ST (2012) Bacterial 'immunity' against bacteriophages. Bacteriophage 2:50–54. https://doi.org/10.4161/bact.18609

Achouak W, Heulin T, Pagès JM (2001) Multiple facets of bacterial porins. FEMS Microbiol Lett 199:1–7. https://doi.org/10.1111/j.1574-6968.2001.tb10642.x

Alberts B (2015) Pathogens and infection. In: Molecular biology of the cell, Sixth edn. Garland Science, Taylor and Francis Group, New York, NY, pp 1263–1275

Anderson JB (2005) Evolution of antifungal-drug resistance: mechanisms and pathogen fitness. Nat Rev Microbiol 3:547–556. https://doi.org/10.1038/nrmicro1179

Bagaglio S, Uberti-Foppa C, Morsica G (2017) Resistance mechanisms in hepatitis C virus: implications for direct-acting antiviral use. Drugs 77:1043–1055. https://doi.org/10.1007/s40265-017-0753-x

Barlow M, Hall BG (2002) Phylogenetic analysis shows that the OXA b-lactamase genes have been on plasmids for millions of years. J Mol Evol 55:314–321. https://doi.org/10.1007/s00239-002-2328-y

Barrangou R, Fremaux C, Deveau H et al (2007) CRISPR provides acquired resistance against viruses in prokaryotes. Science 315:1709–1712. https://doi.org/10.1126/science.1138140

Battles MB, Langedijk JP, Furmanova-Hollenstein P et al (2016) Molecular mechanism of respiratory syncytial virus fusion inhibitors. Nat Chem Biol 12:87–93. https://doi.org/10.1038/nchembio.1982

Berger S, El Chazli Y, Babu AF, Coste AT (2017) Azole resistance in Aspergillus fumigatus: a consequence of antifungal use in agriculture? Front Microbiol 8:1024. https://doi.org/10.3389/fmicb.2017.01024

Bickle TA (2003) Restricting restriction: restriction alleviation. Mol Microbiol 51:3–5. https://doi.org/10.1046/j.1365-2958.2003.03846.x

Bikard D, Marraffini LA (2012) Innate and adaptive immunity in bacteria: mechanisms of programmed genetic variation to fight bacteriophages. Curr Opin Immunol 24:15–20. https://doi.org/10.1016/j.coi.2011.10.005

Blair JMA, Webber MA, Baylay AJ et al (2015) Molecular mechanisms of antibiotic resistance. Nat Rev Microbiol 13:42–51. https://doi.org/10.1038/nrmicro3380

Blanco P, Hernando-Amado S, Reales-Calderon J et al (2016) Bacterial multidrug efflux pumps: much more than antibiotic resistance determinants. Microorganisms 4:14. https://doi.org/10.3390/microorganisms4010014

Bloom DE, Black S, Salisbury D, Rappuoli R (2018) Antimicrobial resistance and the role of vaccines. Proc Natl Acad Sci U S A 115:12868–12871. https://doi.org/10.1073/pnas.1717157115

Borghi E, Borgo F, Morace G (2016) Fungal biofilms: update on resistance. Springer, Cham, pp 37–47

Brilhante RSN, de Alencar LP, Bandeira SP et al (2019) Exposure of Candida parapsilosis complex to agricultural azoles: an overview of the role of environmental determinants for the development of resistance. Sci Total Environ 650:1231–1238. https://doi.org/10.1016/J.SCITOTENV.2018.09.096

Bryan LE, O'Hara K, Wong S (1984) Lipopolysaccharide changes in impermeability-type aminoglycoside resistance in Pseudomonas aeruginosa. Antimicrob Agents Chemother 26:250–255. https://doi.org/10.1128/AAC.26.2.250

Campos MA, Vargas MA, Regueiro V et al (2004) Capsule polysaccharide mediates bacterial resistance to antimicrobial peptides. Infect Immun 72:7107–7114. https://doi.org/10.1128/IAI.72.12.7107-7114.2004

Cepas V, López Y, Muñoz E et al (2019) Relationship between biofilm formation and antimicrobial resistance in gram-negative bacteria. Microb Drug Resist 25:72–79. https://doi.org/10.1089/mdr.2018.0027

Chattopadhyay MK, Jaganandham MV (2015) Vesicles-mediated resistance to antibiotics in bacteria. Front Microbiol 6:758. https://doi.org/10.3389/fmicb.2015.00758

Chen J, Quiles-Puchalt N, Chiang YN et al (2018) Genome hypermobility by lateral transduction. Science 362:207–212. https://doi.org/10.1126/science.aat5867

Chowdhary A, Sharma C, Meis JF (2017) Candida auris: a rapidly emerging cause of hospital-acquired multidrug-resistant fungal infections globally. PLoS Pathog 13:e1006290. https://doi.org/10.1371/journal.ppat.1006290

Clokie MRJ, Millard AD, Letarov AV, Heaphy S (2011) Phages in nature. Bacteriophage 1:31–45. https://doi.org/10.4161/bact.1.1.14942

Cortegiani A, Misseri G, Chowdhary A (2019) What's new on emerging resistant Candida species. Intensive Care Med 45:512–515. https://doi.org/10.1007/s00134-018-5363-x

Cowen LE, Steinbach WJ (2008) Stress, drugs, and evolution: the role of cellular signaling in fungal drug resistance. Eukaryot Cell 7:747–764. https://doi.org/10.1128/EC.00041-08

Datsenko KA, Pougach K, Tikhonov A et al (2012) Molecular memory of prior infections activates the CRISPR/Cas adaptive bacterial immunity system. Nat Commun 3:945. https://doi.org/10.1038/ncomms1937

Davies J, Davies D (2010) Origins and evolution of antibiotic resistance. Microbiol Mol Biol Rev 74:417–433. https://doi.org/10.1128/MMBR.00016-10

Delcour AH (2009) Outer membrane permeability and antibiotic resistance. Biochim Biophys Acta 1794:808–816. https://doi.org/10.1016/j.bbapap.2008.11.005

Demicheli V, Jefferson T, Ferroni E et al (2018) Vaccines for preventing influenza in healthy adults. Cochrane Database Syst Rev 2:CD001269. https://doi.org/10.1002/14651858.CD001269.pub6

Denning DW (2002) Echinocandins: a new class of antifungal. J Antimicrob Chemother 49:889–891. https://doi.org/10.1093/jac/dkf045

Denning DW, Perlin DS (2011) Azole resistance in Aspergillus: a growing public health menace. Future Microbiol 6:1229–1232. https://doi.org/10.2217/FMB.11.118

Donlan RM (2002) Biofilms: microbial life on surfaces. Emerg Infect Dis 8:881–890. https://doi.org/10.3201/eid0809.020063

Duncan CJ (2005) What caused the Black Death? Postgrad Med J 81:315–320. https://doi.org/10.1136/pgmj.2004.024075

Durão P, Balbontín R, Gordo I (2018) Evolutionary mechanisms shaping the maintenance of antibiotic resistance. Trends Microbiol 26:677–691. https://doi.org/10.1016/j.tim.2018.01.005

Dy RL, Richter C, Salmond GPC, Fineran PC (2014) Remarkable mechanisms in microbes to resist phage infections. Annu Rev Virol 1:307–331. https://doi.org/10.1146/annurev-virology-031413-085500

Farrell LJ, Lo R, Wanford JJ et al (2018) Revitalizing the drug pipeline: antibioticDB, an open access database to aid antibacterial research and development. J Antimicrob Chemother 73:2284–2297. https://doi.org/10.1093/jac/dky208

Fernandez L, Hancock REW (2012) Adaptive and mutational resistance: role of porins and efflux pumps in drug resistance. Clin Microbiol Rev 25:661–681. https://doi.org/10.1128/CMR.00043-12

Fineran PC, Charpentier E (2012) Memory of viral infections by CRISPR-Cas adaptive immune systems: acquisition of new information. Virology 434:202–209. https://doi.org/10.1016/j.virol.2012.10.003

Fitzpatrick DA (2012) Horizontal gene transfer in fungi. FEMS Microbiol Lett 329:1–8. https://doi.org/10.1111/j.1574-6968.2011.02465.x

Flowers SA, Barker KS, Berkow EL et al (2012) Gain-of-function mutations in UPC2 are a frequent cause of ERG11 upregulation in azole-resistant clinical isolates of Candida albicans. Eukaryot Cell 11(10):1289–1299. https://doi.org/10.1128/EC.00215-12

Flowers SA, Colón B, Whaley SG et al (2015) Contribution of clinically derived mutations in ERG11 to azole resistance in Candida albicans. Antimicrob Agents Chemother 59(1):450–460. https://doi.org/10.1128/AAC.03470-14

Galvani AP, Slatkin M (2003) Evaluating plague and smallpox as historical selective pressures for the CCR5-32 HIV-resistance allele. Proc Natl Acad Sci 100:15276–15279. https://doi.org/10.1073/pnas.2435085100

Galway-Witham J, Stringer C (2018) How did Homo sapiens evolve? Science 360:1296–1298. https://doi.org/10.1126/science.aat6659

Ghannoum MA, Rice LB (1999) Antifungal agents: mode of action, mechanisms of resistance, and correlation of these mechanisms with bacterial resistance. Clin Microbiol Rev 12:501–517. https://doi.org/10.1128/CMR.12.4.501

Hato T, Dagher PC (2015) How the innate immune system senses trouble and causes trouble. Clin J Am Soc Nephrol 10:1459–1469. https://doi.org/10.2215/CJN.04680514

Hirakawa H, Inazumi Y, Masaki T et al (2004) Indole induces the expression of multidrug exporter genes in Escherichia coli: indole signal induces some drug exporters. Mol Microbiol 55:1113–1126. https://doi.org/10.1111/j.1365-2958.2004.04449.x

Ishino Y, Shinagawa H, Makino K et al (1987) Nucleotide sequence of the iap gene, responsible for alkaline phosphatase isozyme conversion in Escherichia coli, and identification of the gene product. J Bacteriol 169:5429–5433. https://doi.org/10.1128/jb.169.12.5429-5433.1987

Jacoby GA (2005) Mechanisms of resistance to quinolones. Clin Infect Dis 41:S120–S126. https://doi.org/10.1086/428052

Jakobsson HE, Jernberg C, Andersson AF et al (2010) Short-term antibiotic treatment has differing long-term impacts on the human throat and gut microbiome. PLoS One 5:e9836. https://doi.org/10.1371/journal.pone.0009836

Jansen KU, Anderson AS (2018) The role of vaccines in fighting antimicrobial resistance (AMR). Hum Vaccin Immunother 14:2142–2149. https://doi.org/10.1080/21645515.2018.1476814

Jernberg C, Löfmark S, Edlund C, Jansson JK (2007) Long-term ecological impacts of antibiotic administration on the human intestinal microbiota. ISME J 1:56–66. https://doi.org/10.1038/ismej.2007.3

Jiang L, Liu P, Bank C et al (2016) A balance between inhibitor binding and substrate processing confers influenza drug resistance. J Mol Biol 428:538–553. https://doi.org/10.1016/j.jmb.2015.11.027

Kaur S, Harjai K, Chhibber S (2014) Bacteriophage-aided intracellular killing of engulfed methicillin-resistant Staphylococcus aureus (MRSA) by murine macrophages. Appl Microbiol Biotechnol 98:4653–4661. https://doi.org/10.1007/s00253-014-5643-5

Kaźmierczak Z, Górski A, Dąbrowska K (2014) Facing antibiotic resistance: Staphylococcus aureus phages as a medical tool. Viruses 6:2551–2570. https://doi.org/10.3390/v6072551

Khan AU, Maryam L, Zarrilli R (2017) Structure, genetics and worldwide spread of New Delhi Metallo-β-lactamase (NDM): a threat to public health. BMC Microbiol 17:101. https://doi.org/10.1186/s12866-017-1012-8

Kim J-S, Cho D-H, Park M et al (2016) CRISPR/Cas9-mediated re-sensitization of antibiotic-resistant Escherichia coli harboring extended-spectrum β-lactamases. J Microbiol Biotechnol 26:394–401. https://doi.org/10.4014/jmb.1508.08080

Kim J, Shin B, Park C, Park W (2017) Indole-induced activities of β-lactamase and efflux pump confer ampicillin resistance in Pseudomonas putida KT2440. Front Microbiol 8:433. https://doi.org/10.3389/fmicb.2017.00433

Kim SW, Park SB, Im SP et al (2018) Outer membrane vesicles from β-lactam-resistant Escherichia coli enable the survival of β-lactam-susceptible E. coli in the presence of β-lactam antibiotics. Sci Rep 8:5402. https://doi.org/10.1038/s41598-018-23656-0

Kizny Gordon AE, Mathers AJ, Cheong EYL et al (2017) The hospital water environment as a reservoir for carbapenem-resistant organisms causing hospital-acquired infections—a systematic review of the literature. Clin Infect Dis 64:1435–1444. https://doi.org/10.1093/cid/cix132

Koch A, Stein E, Kogel K-H (2018) RNA-based disease control as a complementary measure to fight Fusarium fungi through silencing of the azole target Cytochrome P450 Lanosterol C-14 α-Demethylase. Eur J Plant Pathol 152:1003–1010. https://doi.org/10.1007/s10658-018-1518-4

Kortright KE, Chan BK, Koff JL, Turner PE (2019) Phage therapy: a renewed approach to combat antibiotic-resistant bacteria. Cell Host Microbe 25:219–232. https://doi.org/10.1016/j.chom.2019.01.014

Krishnamoorthy R, Athinarayanan J, Periasamy VS et al (2018) Antimicrobial activity of nanoemulsion on drug-resistant bacterial pathogens. Microb Pathog 120:85–96. https://doi.org/10.1016/j.micpath.2018.04.035

Kullberg BJ, Arendrup MC (2015) Invasive candidiasis. N Engl J Med 373:1445–1456. https://doi.org/10.1056/NEJMra1315399

Kumar S, He G, Kakarla P et al (2016) Bacterial multidrug efflux pumps of the major facilitator superfamily as targets for modulation. Infect Disord Drug Targets 16:28–43. https://doi.org/10.2174/1871526516666160407113848

Labby KJ, Garneau-Tsodikova S (2013) Strategies to overcome the action of aminoglycoside-modifying enzymes for treating resistant bacterial infections. Future Med Chem 5:1285–1309. https://doi.org/10.4155/fmc.13.80

Labrie SJ, Samson JE, Moineau S (2010) Bacteriophage resistance mechanisms. Nat Rev Microbiol 8:317–327. https://doi.org/10.1038/nrmicro2315

Leitner E, Zarfel G, Luxner J et al (2015) Contaminated handwashing sinks as the source of a clonal outbreak of KPC-2-producing Klebsiella oxytoca on a hematology ward. Antimicrob Agents Chemother 59:714–716. https://doi.org/10.1128/AAC.04306-14

Li X-Z, Nikaido H (2004) Efflux-mediated drug resistance in bacteria. Drugs 64:159–204. https://doi.org/10.2165/00003495-200464020-00004

Li T, Chan M, Lee N et al (2015) Clinical implications of antiviral resistance in influenza. Viruses 7:4929–4944. https://doi.org/10.3390/v7092850

Maebashi K, Kudoh M, Nishiyama Y et al (2002) A novel mechanism of fluconazole resistance associated with fluconazole sequestration in *Candida albicans* isolates from a myelofibrosis patient. Microbiol Immunol 46:317–326. https://doi.org/10.1111/j.1348-0421.2002.tb02702.x

Mašlaňová I, Stříbná S, Doškař J, Pantůček R (2016) Efficient plasmid transduction to *Staphylococcus aureus* strains insensitive to the lytic action of transducing phage. FEMS Microbiol Lett 363:fnw211. https://doi.org/10.1093/femsle/fnw211

McMahon SA, Roberts GA, Johnson KA et al (2009) Extensive DNA mimicry by the ArdA anti-restriction protein and its role in the spread of antibiotic resistance. Nucleic Acids Res 37:4887–4897. https://doi.org/10.1093/nar/gkp478

Messina JP, Humphreys I, Flaxman A et al (2015) Global distribution and prevalence of hepatitis C virus genotypes. Hepatology 61:77–87. https://doi.org/10.1002/HEP.27259

Miller SI (2016) Antibiotic resistance and regulation of the gram-negative bacterial outer membrane barrier by host innate immune molecules. mBio 7:e01541–e01516. https://doi.org/10.1128/mBio.01541-16

Mion S, Rémy B, Plener L et al (2019) Quorum quenching lactonase strengthens bacteriophage and antibiotic arsenal against Pseudomonas aeruginosa clinical isolates. Front Microbiol 10:2049. https://doi.org/10.3389/fmicb.2019.02049

Munita JM, Arias CA (2016) Mechanisms of antibiotic resistance. Microbiol Spectr 4. https://doi.org/10.1128/microbiolspec.VMBF-0016-2015

Nabergoj D, Modic P, Podgornik A (2018) Effect of bacterial growth rate on bacteriophage population growth rate. Microbiologyopen 7:e00558. https://doi.org/10.1002/mbo3.558

Nakae T (1976) Identification of the outer membrane protein of E. coli that produces transmembrane channels in reconstituted vesicle membranes. Biochem Biophys Res Commun 71:877–884. https://doi.org/10.1016/0006-291X(76)90913-X

Nett JE, Andes DR (2015) Fungal biofilms: in vivo models for discovery of anti-biofilm drugs. Microbiol Spectr 3:E30. https://doi.org/10.1128/microbiolspec.MB-0008-2014

Nikaido H, Pagès J-M (2012) Broad-specificity efflux pumps and their role in multidrug resistance of Gram-negative bacteria. FEMS Microbiol Rev 36:340–363. https://doi.org/10.1111/j.1574-6976.2011.00290.x

Niu Y, Yuan Y, Mao J et al (2018) Characterization of two novel mycoviruses from Penicillium digitatum and the related fungicide resistance analysis. Sci Rep 8:5513. https://doi.org/10.1038/s41598-018-23807-3

Nobile CJ, Johnson AD (2015) *Candida albicans* biofilms and human disease. Annu Rev Microbiol 69:71–92. https://doi.org/10.1146/annurev-micro-091014-104330

Nordström K, Forsgren A (1974) Effect of protein A on adsorption of bacteriophages to Staphylococcus aureus. J Virol 14:198–202

O'Neill J (2014) Tackling drug-resistant infections globally: final report and recommendations. In: Review on Antimicrobial Resistance. https://amr-review.org/. Accessed 23 Mar 2019

Olaitan AO, Morand S, Rolain J-M (2014) Mechanisms of polymyxin resistance: acquired and intrinsic resistance in bacteria. Front Microbiol 5:643. https://doi.org/10.3389/fmicb.2014.00643

Orr MT, Lanier LL (2010) Natural killer cell education and tolerance. Cell 142:847–856. https://doi.org/10.1016/j.cell.2010.08.031

Ottosen S, Parsley TB, Yang L et al (2015) In vitro antiviral activity and preclinical and clinical resistance profile of miravirsen, a novel anti-hepatitis C virus therapeutic targeting the human factor miR-122. Antimicrob Agents Chemother 59:599–608. https://doi.org/10.1128/AAC.04220-14

Pawlotsky J-M (2016) Hepatitis C virus resistance to direct-acting antiviral drugs in interferon-free regimens. Gastroenterology 151:70–86. https://doi.org/10.1053/J.GASTRO.2016.04.003

Percival SL, Suleman L, Vuotto C, Donelli G (2015) Healthcare-associated infections, medical devices and biofilms: risk, tolerance and control. J Med Microbiol 64:323–334. https://doi.org/10.1099/jmm.0.000032

Perlin DS (2015) Echinocandin resistance in Candida. Clin Infect Dis 61:S612–S617. https://doi.org/10.1093/cid/civ791

Perlin DS, Richardson RR, Izquierdo AA (2017) The global problem of antifungal resistance: prevalence, mechanisms, and management. Lancet Infect Dis 17:e383–e392

Pitten F-A, Panzig B, Schröder G et al (2001) Transmission of a multiresistant Pseudomonas aeruginosa strain at a German University Hospital. J Hosp Infect 47:125–130. https://doi.org/10.1053/jhin.2000.0880

Pizzorno A, Abed Y, Boivin G (2011) Influenza drug resistance. Semin Respir Crit Care Med 32:409–422. https://doi.org/10.1055/s-0031-1283281

Poole K (2007) Efflux pumps as antimicrobial resistance mechanisms. Ann Med 39:162–176. https://doi.org/10.1080/07853890701195262

Prateeksha, Barik SK, Singh BN (2019) Nanoemulsion-loaded hydrogel coatings for inhibition of bacterial virulence and biofilm formation on solid surfaces. Sci Rep 9:6520. https://doi.org/10.1038/s41598-019-43016-w

Raetz CR (1993) Bacterial endotoxins: extraordinary lipids that activate eucaryotic signal transduction. J Bacteriol 175:5745–5753. https://doi.org/10.1128/JB.175.18.5745-5753.1993
Raff M (1998) Cell suicide for beginners. Nature 396:119. https://doi.org/10.1038/24055
Ragheb MN, Thomason MK, Hsu C et al (2019) Inhibiting the evolution of antibiotic resistance. Mol Cell 73:157–165.e5. https://doi.org/10.1016/j.molcel.2018.10.015
Ragupathi ND, Bakthavatchalam Y, Mathur P et al (2019) Plasmid profiles among some ESKAPE pathogens in a tertiary care centre in south India. Indian J Med Res 149:222. https://doi.org/10.4103/ijmr.IJMR_2098_17
Rémy B, Mion S, Plener L et al (2018) Interference in bacterial quorum sensing: a biopharmaceutical perspective. Front Pharmacol 9:203. https://doi.org/10.3389/fphar.2018.00203
Rohde C, Wittmann J, Kutter E (2018) Bacteriophages: a therapy concept against multi-drug-resistant bacteria. Surg Infect (Larchmt) 19:737–744. https://doi.org/10.1089/sur.2018.184
Rozwandowicz M, Brouwer MSM, Fischer J et al (2018) Plasmids carrying antimicrobial resistance genes in Enterobacteriaceae. J Antimicrob Chemother 73:1121–1137. https://doi.org/10.1093/jac/dkx488
Saha D, Mukherjee R (2019) Ameliorating the antimicrobial resistance crisis: phage therapy. IUBMB Life 71:781–790. https://doi.org/10.1002/iub.2010
Salcedo DE, Lee JH, Ha UH, Kim SP (2015) The effects of antibiotics on the biofilm formation and antibiotic resistance gene transfer. Desalin Water Treat 54:3582–3588. https://doi.org/10.1080/19443994.2014.923206
Sanglard D (2016) Emerging threats in antifungal-resistant fungal pathogens. Front Med 3:11. https://doi.org/10.3389/fmed.2016.00011
Schubert S, Barker KS, Znaidi S et al (2011) Regulation of efflux pump expression and drug resistance by the transcription factors Mrr1, Upc2, and Cap1 in Candida albicans. Antimicrob Agents Chemother 55:2212–2223. https://doi.org/10.1128/AAC.01343-10
Secor PR, Michaels LA, Smigiel KS et al (2017) Filamentous bacteriophage produced by Pseudomonas aeruginosa alters the inflammatory response and promotes noninvasive infection *in vivo*. Infect Immun 85:e00648–e00616. https://doi.org/10.1128/IAI.00648-16
Seed KD (2015) Battling phages: how bacteria defend against viral attack. PLoS Pathog 11:e1004847. https://doi.org/10.1371/journal.ppat.1004847
Seed KD, Faruque SM, Mekalanos JJ et al (2012) Phase variable O antigen biosynthetic genes control expression of the major protective antigen and bacteriophage receptor in Vibrio cholerae O1. PLoS Pathog 8:e1002917. https://doi.org/10.1371/journal.ppat.1002917
Shabbir MAB, Hao H, Shabbir MZ et al (2016) Bacteria vs. bacteriophages: parallel evolution of immune arsenals. Front Microbiol 7:1292. https://doi.org/10.3389/fmicb.2016.01292
Shabbir MAB, Shabbir MZ, Wu Q et al (2019) CRISPR-cas system: biological function in microbes and its use to treat antimicrobial resistant pathogens. Ann Clin Microbiol Antimicrob 18:21. https://doi.org/10.1186/s12941-019-0317-x
Sherry L, Ramage G, Kean R et al (2017) Biofilm-forming capability of highly virulent, multidrug-resistant Candida auris. Emerg Infect Dis 23:328–331. https://doi.org/10.3201/eid2302.161320
Shor E, Perlin DS (2015) Coping with stress and the emergence of multidrug resistance in fungi. PLoS Pathog 11:e1004668. https://doi.org/10.1371/journal.ppat.1004668
Snitkin ES, Zelazny AM, Thomas PJ et al (2012) Tracking a hospital outbreak of carbapenem-resistant Klebsiella pneumoniae with whole-genome sequencing. Sci Transl Med 4:148ra116. https://doi.org/10.1126/scitranslmed.3004129
Sobisch L-Y, Rogowski KM, Fuchs J et al (2019) Biofilm forming antibiotic resistant gram-positive pathogens isolated from surfaces on the International Space Station. Front Microbiol 10:543. https://doi.org/10.3389/fmicb.2019.00543
Stewart EJ, Ganesan M, Younger JG, Solomon MJ (2015) Artificial biofilms establish the role of matrix interactions in staphylococcal biofilm assembly and disassembly. Sci Rep 5:13081. https://doi.org/10.1038/srep13081

Sun J, Deng Z, Yan A (2014) Bacterial multidrug efflux pumps: mechanisms, physiology and pharmacological exploitations. Biochem Biophys Res Commun 453:254–267. https://doi.org/10.1016/j.bbrc.2014.05.090

Tagliabue A, Rappuoli R (2018) Changing priorities in vaccinology: antibiotic resistance moving to the top. Front Immunol 9:1068

Thurtle-Schmidt DM, Lo T-W (2018) Molecular biology at the cutting edge: a review on CRISPR/CAS9 gene editing for undergraduates: a review on CRISPR/CAS9 gene editing for undergraduates. Biochem Mol Biol Educ 46:195–205. https://doi.org/10.1002/bmb.21108

Tokars JI, Olsen SJ, Reed C (2018) Seasonal incidence of symptomatic influenza in the United States. Clin Infect Dis 66:1511–1518. https://doi.org/10.1093/cid/cix1060

Van Belleghem J, Dąbrowska K, Vaneechoutte M et al (2018) Interactions between bacteriophage, bacteria, and the mammalian immune system. Viruses 11:10. https://doi.org/10.3390/v11010010

van der Heijden J, Reynolds LA, Deng W et al (2016) *Salmonella* rapidly regulates membrane permeability to survive oxidative stress. mBio 7:e01238–e01216. https://doi.org/10.1128/mBio.01238-16

Vasu K, Nagaraja V (2013) Diverse functions of restriction-modification systems in addition to cellular defense. Microbiol Mol Biol Rev 77:53–72. https://doi.org/10.1128/MMBR.00044-12

Vogwill T, MacLean RC (2015) The genetic basis of the fitness costs of antimicrobial resistance: a meta-analysis approach. Evol Appl 8:284–295. https://doi.org/10.1111/eva.12202

Wang Y, Ha U, Zeng L, Jin S (2003) Regulation of membrane permeability by a two-component regulatory system in Pseudomonas aeruginosa. Antimicrob Agents Chemother 47:95–101. https://doi.org/10.1128/AAC.47.1.95-101.2003

Wang S, Yang Z, Zhang T et al (2019) Molecular characterization of a chrysovirus isolated from the citrus pathogen Penicillium crustosum and related fungicide resistance analysis. Front Cell Infect Microbiol 9:156. https://doi.org/10.3389/fcimb.2019.00156

Watkins RR, Smith TC, Bonomo RA (2016) On the path to untreatable infections: colistin use in agriculture and the end of 'last resort' antibiotics. Expert Rev Anti Infect Ther 14:785–788. https://doi.org/10.1080/14787210.2016.1216314

Webber MA (2003) The importance of efflux pumps in bacterial antibiotic resistance. J Antimicrob Chemother 51:9–11. https://doi.org/10.1093/jac/dkg050

White TC, Holleman S, Dy F et al (2002) Resistance mechanisms in clinical isolates of Candida albicans. Antimicrob Agents Chemother 46:1704–1713. https://doi.org/10.1128/AAC.46.6.1704-1713.2002

Wood TK, Knabel SJ, Kwan BW (2013) Bacterial persister cell formation and dormancy. Appl Environ Microbiol 79:7116–7121. https://doi.org/10.1128/AEM.02636-13

Yoon MY, Yoon SS (2018) Disruption of the gut ecosystem by antibiotics. Yonsei Med J 59:4. https://doi.org/10.3349/ymj.2018.59.1.4

Zarfel G, Hoenigl M, Leitner E et al (2011) Emergence of New Delhi metallo-β-lactamase, Austria. Emerg Infect Dis 17:129–130. https://doi.org/10.3201/eid1701.101331

Zavascki AP, Goldani LZ, Li J, Nation RL (2007) Polymyxin B for the treatment of multidrug-resistant pathogens: a critical review. J Antimicrob Chemother 60:1206–1215. https://doi.org/10.1093/jac/dkm357

Zhang D-X, Nuss DL (2016) Engineering super mycovirus donor strains of chestnut blight fungus by systematic disruption of multilocus vic genes. Proc Natl Acad Sci U S A 113:2062–2067. https://doi.org/10.1073/pnas.1522219113

Zoll J, Verweij PE, Melchers WJG (2018) Discovery and characterization of novel Aspergillus fumigatus mycoviruses. PLoS One 13:e0200511. https://doi.org/10.1371/journal.pone.0200511

# Carbapenem Resistance in Gram-Negative Bacilli: Mechanisms and Challenges

Sarita Mohapatra and Arti Kapil

## 1 Introduction

Antimicrobial resistance (AMR) is an emerging global health problem which requires immediate action. If we see the timeline of bacterial evolution, the first antibiotic was discovered in 1937, and post 2 years, bacteria started developing resistance against this antibiotic. The mechanism of resistance is continuing till date after 70 years of evolution, and probably we are going to revert to pre-antibiotic era in the near future. Bacteria possess various biologic capabilities to resist antibiotics and cause drug-resistant infections in the community.

Lord Jim O'Neill and his team in the year 2014 published an article entitled, "Antimicrobial Resistance: Tackling a Crisis for the Health and Wealth of Nations," where he estimated that by 2050, every year approximately 700,000 people will battle MDR infections and ten million deaths could be caused by AMR (O'Neill 2014). AMR can affect anyone at any age, in any country. Not only is the proportion of infection due to Gram-negative bacilli higher in comparison to Gram-positive cocci, but also the alarming rise of AMR in Gram-negative bacteria is frightening and challenging for the clinician. In the year 2017, the World Health Organization (WHO) prioritized 12 bacteria that pose threat to human health, wherein the majority were GNBs (Eichenberger and Thaden 2019).

Carbapenems are the last resort drug for the treatment of bacterial infections. It is considered to have broad spectrum of activity against both Gram-positive cocci (GPC) and Gram-negative bacilli (GNB). However, recent emergence of multidrug-resistant pathogens seriously threatens this group of drugs. The drastic increase and spread of carbapenem resistance in the absence of any new antimicrobial drug has

The original version of this chapter was revised. A correction to this chapter can be found at https://doi.org/10.1007/978-981-15-3658-8_11

S. Mohapatra (✉) · A. Kapil
Department of Microbiology, All India Institute of Medical Sciences, New Delhi, India



S. Thomas (ed.), *Antimicrobial Resistance*,
https://doi.org/10.1007/978-981-15-3658-8_2

become a major public health concern for the clinicians, worldwide (Luepke et al. 2017). The primary mechanism of resistance to carbapenems is by the production of carbapenemase enzymes. Carbapenem-resistant organisms (CRO) include mostly carbapenem-resistant *Enterobacteriaceae* (CRE), carbapenem-resistant *Pseudomonas aeruginosa* (CRPA), and carbapenem-resistant *Acinetobacter baumannii* (CRAB). Although the discovery of carbapenemase enzymes has been made since 1990, the identification and spread of plasmid-encoded carbapenemases has recently changed the magnitude of the problem in Gram-negative bacteria. CRE is one of the major parts of CROs that cause serious infections and high mortality (Malchione et al. 2019). Hence, CRE is described among the top three global priorities of antibiotic-resistant bacteria (Laxminarayan et al. 2013). Due to the rapid spread of resistance genes via plasmid, this clonal problem disseminates to several parts of the world including Asia, America, and Europe, thus becoming a global problem. Despite their menacing trend, it is important to know how best these drugs can be used and their mechanisms of action with regard to β-lactamase inhibition and to explore new opportunities for drug development.

## 2 Structure of Carbapenems

Carbapenems are the most potent members of the β-lactam group of antibiotics. The unique molecular configuration of carbapenems is due to the presence of carbapenem ring coupled with four-membered β-lactam ring (Fig. 1).

Carbapenems possess broad-spectrum antibacterial activity by virtue of its unique structure. In comparison to penicillin, carbapenems have substitution of carbon for sulfur at C-1 atom and a double bond between C-2 and C-3 atoms (Queenan and Bush 2017; van Dam et al. 2009). The hydroxyethyl side chain of thienamycin has a key role in its action and is important for the activity that confers protection against most of bacteria. It stabilizes carbapenems against a wide spectrum of β-lactamases. Also, the side chain makes them active against Gram-positive cocci including streptococci, enterococci, and staphylococci (methicillin sensitive), anaerobic bacteria, and GNB.

Carbapenems penetrate the bacterial cell wall and bind to the penicillin-binding protein, i.e., carboxypeptidases and transpeptidases. This further inhibits the transpeptidation process during peptidoglycan synthesis. These enzymes aid the cross-linking of peptide covalent bond during the process of peptidoglycan synthesis. Carbapenem inhibits this peptide cross-linking process which weakens the glycan backbone resulting in autolysis and cell death (Laxminarayan et al. 2013). Imipenem, meropenem, ertapenem, doripenem, and faropenem are currently available for clinical use.

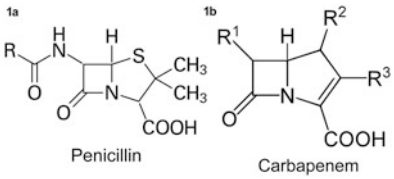


**Fig. 1** Structure of (**a**) penicillin and (**b**) carbapenem

# 3 Mechanism and Epidemiology of Carbapenem Resistance

## 3.1 Carbapenem-Resistant *Enterobacteriaceae* (CRE)

According to the mechanism of action, CRE is broadly divided into two groups: carbapenemase-producing CRE (CP-CRE) and noncarbapenemase-producing CRE (non-CP-CRE). The carbapenem resistance in CRE is primarily due to three mechanisms: (1) the presence of carbapenemases which cause deactivation of the drug, (2) decreased permeability of the drug due to mutation in the porin channels, and (3) upregulation of efflux pumps which leads to release of drug outside the cell (Eichenberger and Thaden 2019). The various combinations of enzymes, their level of expression, upregulation of efflux pumps, and the presence of porin channels determine the minimum inhibitory concentration of a particular drug against that organism.

## 3.2 Carbapenemase-Producing CRE (CP-CRE)

Carbapenemases are a group of beta-lactamases produced either in constitutive or inducible manner. These enzymes can be encoded by chromosome, plasmid, or transposon. As per the classification of beta-lactamases, the enzymes are divided into two groups: Ambler (molecular group) and Bush and Jacoby (functional group) (Diene and Rolain 2014). As per the Ambler classification, carbapenemases are further divided into four groups: A, B, C, and D. Groups A, B, and D contain a large variety of β-lactamases, whereas the clinical relevance of group C in not known (Table 1). Classes A and D contain serine at their active site and are called serine carbapenemases, whereas class B contains zinc ions at their active site and are called metallo-beta-lactamases.

### 3.2.1 Class A Carbapenemases

*Klebsiella pneumoniae* carbapenemase (KPC) is the most clinically relevant carbapenemase enzyme placed in class A group. KPC group of enzymes has the most extensive global distribution among all carbapenemases and is mostly found in *Enterobacteriaceae* family. This was first isolated from a patient admitted to intensive care unit in North Carolina in 1996 (Kelly et al. 2017). Within few years of its discovery, New York/New Jersey area (NY/NJ) had become the global epicenter for CRE. Plasmid-encoded *K. pneumoniae* carbapenemase (KPC) was identified in 2001 and turned out to be the start of major epidemic (Lutgring 2019). This happened primarily due to the emergence of a sequence type of *K. pneumoniae* (ST258) that produces KPC enzymes. Since then, KPC enzymes have disseminated throughout the USA and have become the predominant carbapenemase enzyme in the USA. Later on, it was reported from Colombia, Italy, Greece, Israel, Argentina, Brazil, and China and in many other countries and became the most common carbapenemase enzyme worldwide. Between 2001 and 2010, the incidence of KPC increased from 1 to 12% in 42 states of the USA with 50% mortality rate

**Table 1** Mechanisms of resistance in (a) Cp-CRE and (b) non-CP-CRE

(a) CP-CRE (carbapenemase-producing CRE)

| Ambler class | Bush and Jacoby class | Genes | Gene location | Inhibition | | | Preferred substrates |
|---|---|---|---|---|---|---|---|
| | | | | CLA | EDTA | AZT | |
| A | 2f | bla-GES bla-IMI bla-SME bla-KPC | Plas | + | – | S | Penicillin, cephalosporin, carbapenem |
| | | | Chrom | + | – | R | |
| | | | Chrom | + | – | R | |
| | | | Plas | + | – | R | |
| B | 3 | bla-NDM bla-VIM bla-IMP | Chrom/plas | – | + | S | Most of β-lactam including carbapenem |
| | | | Chrom/plas | – | + | S | |
| | | | Chrom/plas | – | + | S | |
| C | | – | – | – | | | |
| D | 2df | bla-OXA | Chrom/plas | ± | – | S | Carbapenem |

(b) Non-CP-CRE (noncarbapenemase-producing CRE)

| Ambler class | Mechanism | Enzymes |
|---|---|---|
| A | ESBL + alteration in porin/upregulation of efflux pump | CTX-M, SHV, TEM |
| C | AmpC + alteration in porin/upregulation of efflux pump | Inducible AmpC Chromosomal AmpC |
| C | AmpC + alteration in porin/upregulation of efflux pump | Plasmid mediated (CMY, ACT, DHA) |

*CLA* Clavulanic acid, *EDTA* Edetate disodium, *AZT* Aztreonam

(Codjoe and Donkor 2017). A consortium named CRACKLE was federally funded for a prospective multicentric observational study of hospitals in Ohio, Pennsylvania, and Michigan. This study was targeted to detect carbapenem resistance among the *Enterobacteriaceae* such as *E. coli, Klebsiella oxytoca, Enterobacter aerogenes, Enterobacter cloacae, Citrobacter freundii, Proteus mirabilis, Salmonella enterica,* and *Serratia marcescens* (Yigit et al. 2001). Data of this study showed endemicity of KPC-producing CPE primarily *K. pneumoniae* of ST258 in this region. More than 90% of all CP-CRE were due to $bla_{KPC-2}$ and $bla_{KPC-3}$ carbapenemase genes. Among the Mediterranean countries, Italy and Greece have the highest incidence of KPC-producing CP-CRE. This leads to an "endemic situation" for KPC in 2014–2015 in these regions (van Duin et al. 2014). To date, there are 12 variants of KPC, of which $bla_{KPC-2}$ and $bla_{KPC-3}$ are associated with the majority of outbreaks (Miriagou et al. 2010; Okoche et al. 2015; Boutal et al. 2018; Fernandez et al. 2018; Abdallah and Balshi 2018). This group of enzymes hydrolyzes carbapenems, oxymino-cephalosporins, and cephamycin. They show variable inhibition by clavulanic acid or piperacillin-tazobactam. Currently, KPC was reported from the ICUs and wards of various medical and surgical units of hospitals in Greece especially by the *E. coli* harboring KPC-2 gene (Codjoe and Donkor 2017). Other important enzymes in class A are IMI/NMC, SME, and GES. SME enzymes were identified in *Serratia marcescens* in the 1980s. It was

found to be chromosomal mediated and intermittently reported from Europe and the USA (Luepke et al. 2017). Genes for GES enzymes are found in the integrons on transferable plasmids in *P. aeruginosa* and *K. pneumonia* (Queenan and Bush 2017). Strict infection control practices and regular antimicrobial surveillance are the mainstay of containment of these drug-resistant bacteria. Countries like New Zealand and Australia have brought down the prevalence of KPC to as low as 1% due to implementation of strict infection control practices (Codjoe and Donkor 2017).

### 3.2.2 Class B Carbapenemases

These groups of enzymes are also called metallo-β-lactamases as they contain zinc ion at their active site. Initially, these zinc-containing β-lactamase enzymes were considered as clinically irrelevant, occurring only in occasional clinical isolate of *Stenotrophomonas maltophilia* and *Bacillus* spp. that rarely cause human diseases (Albiger et al. 2015). But later on, it was realized that these groups of enzymes are highly prevalent and are one among the major attributes for global carbapenem resistance.

New Delhi metallo-β-lactamase (NDM) was the second most common carbapenemase gene belonging to the metallo-β-lactamases group, i.e., group B. It was first discovered from a Swedish patient travelling back from India and was found encoded by a mobile genetic element. Currently, NDM is endemic in many Asian countries including India, Pakistan, United Kingdom, USA, Kenya, Japan, and Belgium and later on moved to Europe. These metalloenzymes have unique configuration in terms of the presence of zinc ion at the active site. The metallo-beta-lactamases have the potency to hydrolyze carbapenems but have poor ability against monobactams. They are not inhibited by the β-lactamase inhibitors such as clavulanic acid or tazobactam due to presence of $Zn^{+2}$ ion at the active site but are readily inhibited by EDTA dipicolinic acid or 1,10 *o*-phenanthroline. There are a total of ten variants of NDM genes discovered till date. These genes are usually found in the members of *Enterobacteriaceae*, rarely in other GNBs like *P. aeruginosa* and *A. baumannii*. Other carbapenemase enzymes, namely, Verona integron-encoded metallo-β-lactamase (VIM) and imipenemase metallo-β-lactamase (IMP), were reported from many other parts of the world (Bush 2018). VIM carbapenemase was isolated from *Pseudomonas aeruginosa* in an Italian patient at Verona University Hospital. IMP was isolated from a *P. aeruginosa* isolate from Japan in 1991. Since these genes are encoded by plasmids, there was a gradual spread of these genes to other parts of the world. However, the reported outbreaks were limited in terms of geographical area.

### 3.2.3 Class D Carbapenemases

Another group of carbapenemase enzyme was discovered and named as OXA. They have the special ability to hydrolyze oxacillin or cloxacillin. These enzymes belong to the molecular group D. It was first discovered from Turkey and later on was endemic to Middle East, India, and North Africa. There were multiple reports of outbreaks throughout Europe and the USA (Lutgring 2019). These enzymes do

not confer resistance to third- or fourth-generation cephalosporins, and its carbapenemase activity was observed to be weaker. They may or may not be inhibited by clavulanic acid or tazobactam. These enzymes are transmitted both chromosomally and through plasmid. Among these, OXA-23, OXA-24, and OXA-58 are commonly found in *Acinetobacter baumannii*, whereas OXA-48 is found in *Enterobacteriaceae*. The available β-lactamase inhibitors do not have action against this group of enzymes. Moreover, they can mutate easily and expand their spectrum. Hence, these enzymes possess a major concern to the clinician.

## 4 Noncarbapenemase-Producing CRE (Non-CP-CRE)

Apart from carbapenemase enzyme production, *Enterobacteriaceae* family possesses a few alternative mechanisms that lead to carbapenem resistance. These mechanisms are usually paired with themselves or with the carbapenemase production resulting in varied proportions of multidrug resistance (Logan and Weinstein 2017). Unlike the carbapenemase enzymes which deactivate the drug, these mechanisms aim to block the penetration of the antibiotic into the bacterial cell or leakage of the drug from the cell before its action by upregulation of efflux pumps and alteration of porin channels, respectively (Codjoe and Donkor 2017; Bialek-Davenet et al. 2017). AcrAB-TolC RND system and Cus ABC efflux complex are a few common examples found in various bacteria (Masi et al. 2017; Weston et al. 2018; Routh et al. 2011). The resistance genes are usually found in plasmid and can be transmitted from bacteria to bacteria (Courvalin 1994). Alteration in the porin channels is attributed to mutation that results in altered expression. They also produce different types of β-lactamases, such as AmpC type, that do not degrade carbapenems directly. The AmpC enzyme forms a bond with carbapenem molecule, thereby preventing it from accessing its target. These are commonly plasmid-encoded and spread globally. Common example of this type of β-lactamases is AmpC CMY-2. It is frequently found in *Enterobacteriaceae* family, especially in *E. coli*. Chromosomally encoded AmpC is frequently found in *Enterobacter* spp. (Doumith et al. 2009).

### 4.1 Carbapenem-Resistant *Pseudomonas* (CRP)

The resistance in *Pseudomonas* is a combination of many mechanisms. The overexpression of efflux pumps and loss of OprD, the outer membrane porin protein, are the most common mechanisms of carbapenem resistance in *Pseudomonas aeruginosa* (Codjoe and Donkor 2017). It is observed that the resistance to meropenem is mainly due to overexpression of efflux pumps, whereas imipenem is due to alteration in porin channels (Livermore 2001). *Pseudomonas aeruginosa* has also the capability of degranulating the carbapenems by producing drug-inactivating enzymes. These enzymes are either upregulated or acquired from other microorganisms by horizontal transmission. It intrinsically possess AmpC

enzyme. Class A, B, and D group of β-lactamases are transferred horizontally to various species of *Pseudomonas* (Eichenberger and Thaden 2019). This acquisition of β-lactamase enzymes is also seen in the high-risk clones of *Pseudomonas* spp. The three important high-risk clones of *Pseudomonas* that spread globally are ST111, ST175, and ST235, among which ST 235 is most widely spread (Pena et al. 2015; Kos et al. 2015; Correa et al. 2015; Guzvinec et al. 2014). ST 235 contains more than 35 different β-lactamase enzymes from classes A, B, and D. These enzymes are ESBLs PER-1 and GES, VIM-1, and KPC-2.

### 4.2 Carbapenem-Resistant *Acinetobacter baumannii* (CRAB)

Carbapenem-resistant *Acinetobacter baumannii* is in the top of the priority list of antibiotic-resistant pathogen prepared by the WHO. They acquire carbapenem resistance both intrinsically and via horizontal gene transfer. As per CDC reports (2013), 7000 infections and 500 deaths were occurring in the USA due to CRAB every year (Bulens et al. 2018).

Class D group of enzymes are predominantly present in CRAB. OXA-51-like enzyme is one of the oxacillinase enzymes acquired intrinsically and hydrolyze carbapenems at a low level (Corvec et al. 2003; Turton et al. 2006). Other class D enzymes such as OXA-23-, OXA-40-, and OXA-58-like enzymes are also commonly detected, of which OXA-23-like enzymes are the most widespread (Poirel and Nordmann 2006). OXA-23-like enzymes are predominantly reported from Europe and are mostly associated with nosocomial outbreaks. They are typically found to be transmitted by transposons Tn2006 and Tn2007 (Poirel et al. 2010; Corvec et al. 2007; Merino et al. 2014). Other enzymes reported from Europe are OXA-25, OXA-26, and OXA-40 (Merino et al. 2014; Afzal-Shah et al. 2001). OXA-72 is predominantly reported from Asia (Tada et al. 2014). Except few reports, other classes of carbapenemases are extremely rare in CRAB. A series of reports are published from Puerto Rico containing class A KPC-producing *A. baumannii* (Robledo et al. 2010). Few reports are also showing the presence of class B IMP-, VIM-, SIM (Seoul imipenemase)-, and NDM-type enzymes in CRAB isolates which are wildly distributed throughout the world (Potron et al. 2015).

## 5 Current Resistance Status

Carbapenemase-producing organisms are very important epidemiologically because they carry multiple resistance genes. Infection caused by these pathogens resulted in high morbidity and mortality. They easily disseminate the resistant genes to other bacteria causing infection in both community and healthcare settings.

The incidence of CPO is increasing day by day from all the parts of world. This clonal increase in the resistance genes has mostly been attributed to the rapid spread of plasmid-mediated resistance determinants encoding various enzymes. These different carbapenemases are prevalent in different areas of the world. Among

these, KPC has been found to be the most widely spread, whereas NDM is more promiscuous. Later, using the molecular tool like multilocus sequence typing (MLST), pandemicity was observed due to a particular sequence type of *K. pneumoniae*, i.e., ST258 (Codjoe and Donkor 2017). *K. pneumoniae* was found to be the leading cause of infections among all the carbapenem-resistant organisms where treatment remained challenging. They carry plasmids that are invasive in nature and hence disseminate into the community. They also harbor virulent plasmids carrying resistance genes from the nosocomial strains.

In the last few emergences of metallo-beta-lactamases, *Enterobacteriaceae* has become a concern for clinician. NDM was reported in 2009, and to date ten more variants of NDM were discovered from the entire world (Diene and Rolain 2014). As discussed earlier, Oxa-48 is the third most prevalent carbapenemase reported from Europe and North Africa. VIM and IMP are less common in comparison to other enzymes.

Currently, the increasing reports of carriers of carbapenemase genes in the colonizers of gut have become another concern. Gut is a large reservoir of microbes and includes pathogen harboring various antibiotic-resistance genes, especially among the hospitalized patients who are already on antibiotic therapy. During their course, the multidrug-resistant pathogens get selected out and transfer these genes to other commensal flora. Subsequently, these organisms may translocate into the intestinal barrier causing bloodstream infection. They may also contaminate the skin or other body sites (Gargiullo et al. 2019). KPC has become endemic in the areas of America, China, Israel, and South Europe, whereas NDM is predominantly found in South East Asia especially in India, Pakistan, and northern Europe. The wide dissemination of these resistant clones in the community remains undetected until they cause some infection. There is no clear evidence on how many carriers will progress into infection. The patient with carbapenem-resistance carriers should be screened and isolated in the healthcare settings in order to prevent further dissemination of resistant clones to other patients (Tada et al. 2014).

## 6 Antibiotic Resistance Drivers

The important drivers of antibiotic resistance comprise excess use of antibiotics in the animal husbandry, agriculture, and poultry sectors. Unregulated prescription also leads to misuse and irrational usage of antibiotics in the society. So, irrespective of the places where antibiotics are used, they accumulate and develop reservoirs of resistance. These reservoirs subsequently disseminate the resistance genes in the society. The prevalence and rate of increase in the resistance is observed much higher in low- and middle-income countries in comparison to high-income countries. It is mainly due to high population density, poor sanitation, and improper solid waste disposal. These combinedly accelerate dissemination of resistance (Vikesland et al. 2019).

## 7 Usage and Side Effects of Carbapenems

Imipenem was the first carbapenem used to be given with cilastatin as cilastatin inhibits renal metabolism of imipenem and prolongs its half-life. Ertapenem has the maximum in vivo half-life period of 4 h followed by imipenem (1 h), meropenem, and doripenem. Ertapenem is least active against pseudomonal infection in comparison to other carbapenems. Carbapenems are metabolized in the liver, thereby increasing the risk of being hepatotoxic, and sometimes lead to jaundice. These drugs are prone to degradation by an enzyme dyhydropeptidase-1 (DHP-1) located in renal tubules and are usually given with DHP-1 inhibitor such as cilastatin (Codjoe and Donkor 2017).

## 8 Laboratory Detection of Carbapenem-Resistant Organism

In the past few years, carbapenem-resistant organisms have been reported from different parts of the world. Infection caused due to CRP is difficult to treat. Hence, there is an urgent need for a rapid and accurate detection of CRO in the diagnostic laboratories. Several methods are available for the diagnosis of carbapenem resistance. Identification of carbapenemase genes and the types is very useful for the clinician to focus on enzyme-specific therapies as well as infection control practices (Diene and Rolain 2014; http://www.cdc.gov/HAI/pdfs/labSettings/, Lolans et al. 2010). The susceptibility of the different carbapenems varies depending on the presence of different enzymes in addition to a combination of other mechanisms (Aguirre-Quinonero and Martínez-Martínez 2017). Few enzymes hydrolyze a particular carbapenem very efficiently, whereas few others do not. Although molecular methods are considered as the gold standard for detection of these enzymes, the cost and identification of specific genes and the inconvenience make it less popular for routine diagnosis. Currently, different methods are available to detect different enzymes. Broadly the tests are divided into phenotypic and molecular tests. Many of these methods are not able to detect the carbapenemase enzyme as varied mechanisms are involved with each type.

### 8.1 Detection of Carbapenemase Activity Directly from the Clinical Specimen

#### 8.1.1 CDC Screening Criteria

The US Centers for Disease Control and Prevention (CDC) has defined the criteria for screening of CRE from rectal swab or perianal swab (Heritier et al. 2003). The rectal/perianal swab collected from the suspected patient will be inoculated on appropriate media to isolate the CRE, specially the *E. coli* and *K. pneumoniae*. The test is easy to perform with variable sensitivity (65.6–98.8%) and specificity (49.6–86.4%), although the generation of results will be a bit slower when compared to chromogenic method (Potron et al. 2015; Gargiullo et al. 2019).

### 8.1.2 Chromogenic Plates

Chromogenic plates are commercially available to detect the carbapenemase producer within 18–24 h. This method is useful for rapid screening of asymptomatic carriers among the high-risk groups. The sensitivity and specificity of various commercial chromogenic media vary from 53 to 100% (Samra et al. 2008). Bir et al. (2018) compared various phenotypic tests where CHROMagar was found to have highest sensitivity followed by Carba-NP. Their activity was found weaker to detect low hydrolytic carbapenemase enzyme such as Oxa-48. It is rapid and can be done in any laboratory facility, the drawbacks being limited shelf life of the media, difficulty in interpreting, and the risk of reporting false-positive result in case of ESBLs or AmpC enzymes.

Supercarba is a novel screening nonchromogenic media which was able to detect all carbapenemase enzymes including Oxa-48. The sensitivity of this media was found to be 96.5%. However, only the lactose-fermenting colonies could be detected, and the shelf life of the prepared media was observed to be 7–10 days. Recently, modification of Supercarba medium adapting the chromogenic quality has been developed which is called as mSupercarba. mSupercarba was found to be 100% sensitive and specific for the detection of KPC-, Oxa-48-, and MBL-producing CPEs.

## 8.2 Detection of Carbapenemase Activity in the Isolated Bacteria

### 8.2.1 Phenotypic Tests

Broadly phenotypic tests are divided into screening and confirmatory tests.

1. Screening tests
   (a) Disk diffusion
2. Confirmatory test includes:
   (a) MIC determination: microbroth dilution, agar dilution, E test, and automated system
   (b) Inhibitor-based synergy test: combined disk test and double disk synergy test
   (c) Detection based on carbapenem hydrolysis:
      - Modified Hodge test
      - Colorimetric test (Carba-NP, Blue-Carba)
      - Carbapenem inactivation method (mCIM and eCIM)
      - Starch-iodine test
      - Spectrophotometry
      - Electrochemical assay
   (d) Immunochromatography
   (e) Mass spectrometry (MALDI-TOF)

### 8.2.2 Molecular Tests

1. PCR (conventional and real time)
2. LAMP
3. Microarray
4. Whole-genome sequencing using next-generation sequencer

**Screening test** The European Committee on Antimicrobial Susceptibility Testing (EUCAST) or the Clinical and Laboratory Standards Institute (CLSI) has provided break points for screening of carbapenemase activity from the bacterial isolates by disk diffusion test. The interpretative criteria are first screened by the disk diffusion test and further confirmed by any of the confirmatory test. The interpretative criteria are available for imipenem, meropenem, ertapenem, and doripenem. Among the four disks, meropenem provides the best sensitivity and specificity, whereas others suffer from lack of specificity. CLSI breakpoints have been shown to have lower sensitivity in comparison to the EUCAST. Difficulties are observed during the detection of Oxa-48 carbapenemase activity.

**Confirmatory test** Several automated systems are available to detect minimum inhibitory concentration such as Vitek 2 (bioMerieux), Phoenix (BD Diagnostics), and MicroScan (Siemens). The automated systems use interpretative criteria as defined by the specific guidelines. Inoculum size should be determined properly to prevent false susceptibility testing result. Lastly, at least two carbapenems should be included to confirm the result in order to achieve a higher confidence level.

**E strip** Antibiotic gradient diffusion strips are made up of a combination of a beta-lactam substrate and a beta-lactam/beta-lactamase inhibitor. A fixed concentration of EDTA or boronic acid is present which can be specifically used to detect MBL or KPC. A reduction of $\geq$3 twofold dilutions of the carbapenem MIC in the presence of the inhibitor or the presence of a deformed ellipse is interpreted as positive. Meropenem-containing strips have shown to be more efficient than the ones containing imipenem in the detection of MBL producers.

**Inhibitor-based synergy test** These tests are based on the ability to inhibit the activity of carbapenemase enzymes in the presence of inhibitors such as ethylenediaminetetraacetic acid (EDTA), dipicolinic acid (DPA), or phenylboronic acid (PBA). The activity of MBL was found to be best detected in the presence of EDTA and DPA (chelating agents), whereas PBA was observed to inhibit KPC enzymes.

**Combined disk test (CDT)** In this test, the difference in the zone diameter of a carbapenem disk was compared with the zone of the diameter of the disk containing a combination of carbapenem with inhibitor after overnight incubation with the tested strain. They have shown high sensitivities (90–100%) and specificities (96–100%) for detection of class A carbapenemase especially among *Enterobacteriaceae*.

**Double disk synergy test (DDST)** This test is carried out by placing a beta-lactam disk and beta-lactamase inhibitor disk side by side at a distance of 10 mm apart. After the overnight incubation with the test strain, enlarged (synergistic) zone of inhibition is interpreted as positive.

**Detection based on carbapenem hydrolysis** (Fig. 2).

**Modified Hodge test** It is otherwise known as Cloverleaf test. On a lawn of a carbapenem-susceptible organism (*E. coli* ATCC 25922), a disk of either ertapenem or meropenem was put at the center. After overnight incubation with the clinical test isolate, the presence of an indented inhibition zone is interpreted as positive. MHT is less specific and time-consuming. The lack of specificity may be observed for enzymes like AmpC, whereas low sensitivity is observed for NDM group of carbapenemases. It is proven to have the best result against KPC group of enzymes. MHT gives a lot of false-positive result in the presence of noncarbapenemase mechanisms such as porin channel mutation and AmpC production. Due to its false-positive result, it was omitted from CLSI in the year 2018; however, it can still be used as a preliminary screening test to detect carbapenem resistance.

**Colorimetric test (Carba-NP, Blue-Carba)** Colorimetric tests are based on the enzymatic hydrolysis of the beta-lactam ring of a carbapenem due to the presence of carbapenemase activity. This hydrolysis of the beta-lactam ring leads to decrease in the pH and a subsequent shift in color. Change in pH is detected by a pH indicator such as phenol red for Carba-NP bromothymol blue in Blue-Carba test. The biggest

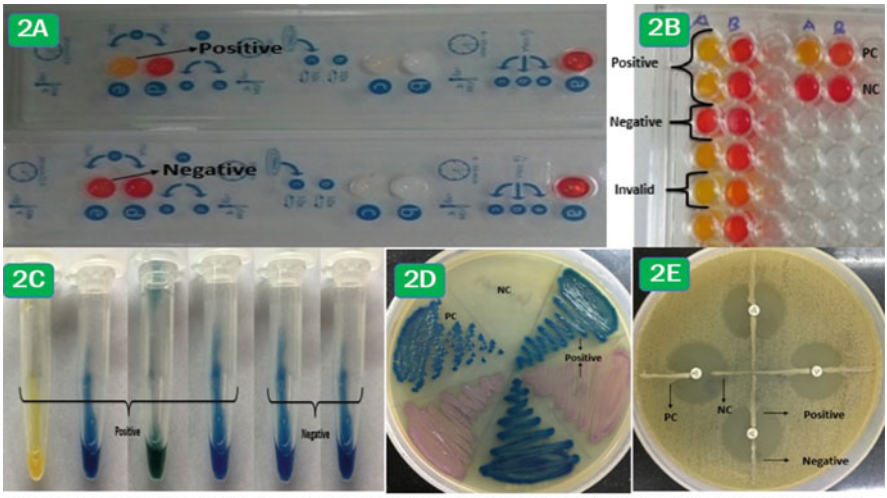


**Fig. 2** Different detection tests based on carbapenem hydrolysis. (**a**) Commercial CarbaNP test, (**b**) In-house CarbaNP test, (**c**) Blue Carba test, (**d**) CHROMagar, (**e**) Modified Hodge test

advantages of these tests are that they are rapid result (in <2 h), simple and easy to perform. There are many kits available commercially by the names RAPIDEC® CARBA-NP (bioMerieux), Rapid CARB Screen, Neo-Rapid CARB, and Rapid CARB Blue kit (Rosco Diagnostica).

**Carba-NP** Nordman described the first Carba-NP test which is highly specific (100%) and highly sensitive (ranging between 90 and 100%) for the detection of carbapenemases in *Enterobacteriaceae*. These are less sensitive to detect carbapenemases with a low hydrolytic activity, i.e., OXA-48 and GES-5 enzymes. False-negative results in Carba-NP tests may be due to capsulated strain or the buffer. KPC requires less than 30 min, NDM and VIM need less than 1 h, and OXA-48 needs more than 1 h.

**Blue-Carba** Blue-CARBA test is a modification of the Carba-NP. This yields similar results as the Carba-NP in terms of sensitivities of 100% and specificities ranging between 91 and 100% in the detection of carbapenemases in *Enterobacteriaceae*. The biggest advantage of Blue-Carba over Carba-NP is that it can be directly tested on bacterial colonies and does not need bacterial extracts as required in the Carba-NP test, thereby reducing the time for result generation.

**Carbapenem inactivation method (mCIM and eCIM)** In this test, a meropenem disk (10 mg) is immersed overnight within a bacterial suspension of a test strain. If the organism produces carbapenemase, it will result in the inactivation of the meropenem disk. After overnight incubation, the disk is checked for its action on an indicator strain. A zone of inhibition greater than 20 mm indicates that the test stain lacks carbapenemase activity. CIM test can detect all the carbapenemase with high and low hydrolytic activity like NDM, KPC, OXA-48, VIM, and IMP, except the strains producing GES-6. The sensitivity is very high 98–100% and the specificity is 100%.

**Starch-iodine test** Strips impregnated with imipenem and starch are used for the test. A color shift from dark to clear is indicative of the presence of carbapenemase. This test shows 100% sensitivity and specificity for KPC.

**Spectrophotometry** Recently, UV rays and mass spectrometry are used for the rapid detection of carbapenem resistance. Mass spectrometry (MALDI-TOF MS) is based on the degradation products of a carbapenem after bacterial hydrolysis (Al-Zahrani 2018). First, the bacterial protein extracts are incubated with a carbapenem substrate. Then, the carbapenem degradation products are detected by the machine. The carbapenemase genes carried by the plasmid form peaks and are identified by the machine. The turnaround time is approximately 4 h. Its sensitivities range between 77 and 100% and specificities from 94 to 100%. False-negative results are found with the organism containing Oxa-48 enzymes.

**Electrochemical assay** It is also called the BYG Carba test (Bogaerts-Yunus-Glupczynski). This is a very rapid novel test that detects the presence of carbapenemases in less than 35 min. It is based on the principle of measuring slight change in pH and in redox activity following hydrolysis of imipenem, and the results are available in real-time curves. Due to the efficient biosensing quality by the electro-active polymer, this test is able to differentiate CPE from non-CPE. BYE Carba test revealed a sensitivity of 95% and a specificity of 100%.

### 8.2.3 Molecular Test

Molecular tests are considered as the gold standards for the identification of carbapenemase genes but require technical expertise, are expensive, and may give false negative if the gene is not fully expressed. A number of PCR methodologies are available to detect carbapenemase genes (Al-Zahrani 2018; Hrabak et al. 2014). Multiplex PCR was useful and time-saving with high levels of sensitivity and specificity for detection. However, the only problem is PCR standardization because of the variable melting points of different genes. A multiplex PCR evaluated in a multicentric study targeting Oxa-48, NDM-1, KPC, VIM, and IMP showed a sensitivity and specificity of 100%. Loop-mediated isothermal amplification was developed and found to be a highly efficient method with 100% sensitivity and specificity for detection of carbapenemase activity.

**Gene Xpert** Currently, Xpert Carba-R assay is used for the detection of $^{bla}KPC$, $^{bla}NDM$, $^{bla}Oxa\text{-}48$, $^{bla}VIM$, and $^{bla}IMP$. It has 100% sensitivity and specificity and is rapid and easy to perform. This is an accurate method for detecting carbapenemase genes from *Enterobacteriaceae*, *P. aeruginosa*, and *A. baumannii* complex (Al-Zahrani 2018).

**Microfluidic chip technology** It allows the rapid detection of pathogens and their resistance genes with high sensitivity and specificity (both >90%). A recently developed microarray-based technique also known as DNA chip is available to identify KPC, OXA, IMP, SPM, VIM, SIM, and GIM carbapenemases. These are based on hybridization technique, and the chips are designed with microscopic DNA spots with a unique DNA sequence. DNA of bacteria is hybridized with complementary base pairs of the chips and is detected by the machine. The biggest advantage of microarray assays over hybridization is that it can detect as many thousand targets in a single run in spite of being a bit expensive (Al-Zahrani 2018).

**Whole-genome sequencing** Whole-genome sequencing using next-generation sequencer (NGS) is used to analyze DNA along the entire genome in a very short time. It gives a complete genetic information and helps in rapid identification of all known resistance mechanisms. Along with the known mechanisms, it helps to identify other mechanisms of resistance such as mutation in the porin channels and plasmid-carrying resistance genes, novel resistance mechanisms, or other virulence determinants that play a role in the pathogenesis. It will be helpful for identification of outbreaks or epidemiological investigations. NGS is still relatively expensive and

requires an expertise in bioinformatics for the data interpretation system. Its use in the routine laboratories in the developing countries will still take time.

## 9 Treatment

The global spread of carbapenem-resistant organism (CRO) infections is becoming a threat for clinicians. The infections due to CRO are difficult to treat due to the limited availability of therapeutic agents causing increased morbidity and mortality. This compels the clinicians for increased usage of older drugs like polymyxin, fosfomycin, tigecycline, and minocycline despite its toxicity, which eventually increases resistance among these drugs (Kaase et al. 2014).

Polymyxin group of drugs are positively charged ions that bind to the negatively charged lipopolysaccharide (LPS), thereby displacing magnesium ions. This subsequently leads to loss of cell wall integrity and cell death. Bacteria may change the LPS, replacing the negatively charged phosphate ion with positive charged L-Ara4N and PetN molecules, thereby preventing further action of colistin on the cell wall. The modification of LPS may be due to chromosomal mechanism which is encoded by the mcr-1 gene or may be plasmid mediated. The plasmid-mediated resistance is observed in *K. pneumoniae* (mgrB, pmr A/B, pho P/Q, crr A/B/C), *P. aeruginosa* (pmrA/B, pho P/Q, par R/S, col R/S, cpr R/S) and *A. baumannii* (pmrA/B, lpx A/C/D, lpsB).

Minocycline has been observed to be useful for the therapeutic management of CRO. It is an older drug belonging to tetracycline group. It is bacteriostatic and inhibits bacterial protein synthesis. Several in vitro studies have reported the significant bactericidal effect of a combination of meropenem and colistin. Minocycline has shown good effect against CRE and CRAB but not against CRPA due to the presence of intrinsic resistance.

Tigecycline is a glycylcycline, used for the treatment of complicated skin and skin structure infections, complicated intra-abdominal infections, and community-acquired pneumonia. It is also a bacteriostatic agent. It has good action against CRE (except *Proteus* spp., *Providentia* spp., and *Morganella morganii*) and CRAB but not against CRPA. Clinical failure was seen in patient treated with tigecycline monotherapy.

Fosfomycin has been approved by FDA for uncomplicated urinary tract infection. It is considered as a broad-spectrum antibiotic and observed to have good action against many deep-seated infections. It is intrinsically resistant against *Acinetobacter baumannii* but has good action against *E. coli* (100%) than *K. pneumoniae* (90.5%) and also against CRE (Apisarnthanarak and Mundy 2012). A combination of doripenem with fosfomycin has been shown to possess 61% clinical cure rate without any microbiological cure rate in patients with ventilator-associated pneumonia due to CRAP (Apisarnthanarak and Mundy 2010, 2012).

Recently, multiple novel beta-lactam/beta-lactamase inhibitors (BL/BLI) including ceftazidime/avibactam, aztreonam/avibactam, meropenem/vaborbactam, imipenem/relebactam, meropenem/nacubactam, and cefepime/zidebactam are

under trial for approval of the clinical use against carbapenem-resistant organisms. Among these drugs, ceftazidime-avibactam is a novel non-beta-lactam/beta-lactam inhibitor and has been approved by the FDA. It is now available in the market. It has action against ESBLs, AmpC, and class A carbapenemases including KPCs and Oxa-lactamases but not against MBLs.

Ceftolozane/tazobactam is another BL/BLI having antipseudomonal activity and has been recently approved by the FDA. It has shown in vitro action against 90% ESBL-producing E. coli and 42–98% *K. pneumoniae* isolates (van Duin and Bonomo 2016; Kullar et al. 2017). Aztreonam/avibactam and cefepime/zidebactam are found effective against ESBL, AmpC, KPC, Oxa, and MBLs. Aztreonam/avibactam has an advantage of being a poor substrate and is less hydrolyzed by class B beta-lactamases. Studies done by the International Network for Optimal Resistance Monitoring (INFORM) observed Czd/Avi has >90% susceptibility against Oxa48-like-producing CREs.

WCK 4282 is another novel high-dose combination of cefepime/tazobactam that has been discovered recently. It is observed 8–16 times more active than piperacillin-tazobactam against ESBLs or any combination of ESBLs with class C β-lactamases. It is also active against class D beta-lactamases like OXA-48/181- and KPC-expressing *Enterobacteriaceae*.

## 10 Role of Infection Control and Antibiotic Stewardship

Antibiotic stewardship plays an important role in the reduction of transmission of these CROs across various geographical regions. The various steps include targeted screening of the high-risk cases, use of rapid diagnostics for detection of CROs, and enzyme-specific targeted intervention. The high-risk cases which need to be under regular surveillance are patients with history of recent hospitalization, recent ICU admission, history of invasive medical devices, antibiotic exposure, and with chronic wounds. Fecal carriage of these patients should be done at regular interval following the CDC protocol and if detected should follow the following bundles. Bundles for NDM and KPC include strict adherence to hand hygiene, contact precaution, cohorting of the positive cases, enhanced environmental sampling, and antibiotic stewardship (Lowe et al. 2013; Fournier et al. 2014; Abdallah et al. 2016).

## 11 Conclusion

Carbapenem-resistant Gram-negative bacteria continue to be an important health problem in hospitals. The growing threat includes the rapid spread to the community accelerated by the presence of transferable carbapenemase genes. Moreover, newer resistant genes and newer mechanisms are evolving because of the frequent recombination and antibiotic pressure. This pressurized usage of older drugs like colistin unfortunately led to the development of colistin-resistant bacteria. Active surveillance to detect CRE during the entry point in a healthcare setting should be the first

step taken to contain this problem. Targeted enzyme-specific therapies are advised for better recovery. An early and aggressive infection control measure and antibiotic stewardship are needed to address this problem.

## References


Abdallah M, Balshi A (2018) First literature review of carbapenem-resistant Providencia. New Microbes New Infect 25:16–23. https://doi.org/10.1016/j.nmni.2018.05.009

Abdallah M, Olafisoye O, Cortes C, Urban C, Landman D, Ghitan M, Collins B, Bratu S, Quale J (2016) Rise and fall of KPC-producing Klebsiella pneumoniae in New York City. J Antimicrob Chemother 71(10):2945–2948. https://doi.org/10.1093/jac/dkw242

Afzal-Shah M, Woodford N, Livermore DM (2001) Characterization of OXA-25, OXA-26, and OXA-27, molecular class D beta-lactamases associated with carbapenem resistance in clinical isolates of *Acinetobacter baumannii*. Antimicrob Agents Chemother 45:583–588. https://doi.org/10.1128/AAC.45.2.583-588.2001

Aguirre-Quinonero A, Martínez-Martínez L (2017) Non-molecular detection of carbapenemases in Enterobacteriaceae clinical isolates. J Infect Chemother 23:1–11. https://doi.org/10.1016/j.jiac.2016.09.008

Albiger B, Glasner C, Struelens MJ, Grundmann H, Monnet DL, European Survey of Carbapenemase-Producing Enterobacteriaceae Working (2015) Carbapenemase-producing Enterobacteriaceae in Europe: assessment by national experts from 38 countries. Euro Surveill 20:D30062

Al-Zahrani IA (2018) Routine detection of carbapenem-resistant Gram-negative bacilli in clinical laboratories: a review of current challenges. Saudi Med J 39(9):861–872. https://doi.org/10.15537/smj.2018.9.22840

Apisarnthanarak A, Mundy LM (2010) Use of high-dose 4-hour infusion of doripenem, in combination with fosfomycin, for treatment of carbapenem-resistant *Pseudomonas aeruginosa* pneumonia. Clin Infect Dis 51:13524. https://doi.org/10.1086/657249

Apisarnthanarak A, Mundy LM (2012) Carbapenem-resistant *Pseudomonas aeruginosa* pneumonia with intermediate minimum inhibitory concentrations to doripenem: combination therapy with high-dose, 4-h infusion of doripenem plus fosfomycin versus intravenous colistin plus fosfomycin. Int J Antimicrob Agents 39:2712. https://doi.org/10.1016/j.ijantimicag.2011.11.012

Bialek-Davenet S, Mayer N, Vergalli J, Duprilot M, Brisse S, Pages JM, Nicolas-Chanoine MH (2017) In-vivo loss of carbapenem resistance by extensively drug-resistant Klebsiella pneumoniae during treatment via porin expression modification. Sci Rep 7:6722. https://doi.org/10.1038/s41598-017-06503-6

Bir R, Mohapatra S, Kumar A, Tyagi S, Sood S, Das BK, Kapil A (2018) Comparative evaluation of In-house Carba NP test with other phenotypic tests for rapid detection of carbapenamase producing carbapenem resistant Enterobacteriaceae. J Clin Lab Anal 33:e22652. https://doi.org/10.1002/jcla.22652

Boutal H, Vogel A, Bernabeu S, Devilliers K, Creton E, Cotellon G, Plaisance M, Oueslati S, Dortet L, Jousset A, Simon S, Naas T, Volland H (2018) A multiplex lateral flow immunoassay for the rapid identification of NDM-1, KPC-, IMP- and VIM-type and OXA-48-like carbapenemase-producing Enterobacteriaceae. J Antimicrob Chemother 73:909–915. https://doi.org/10.1093/jac/dkx521

Bulens SN, Yi SH, Walters MS, Jacob JT, Bower C, Reno J, Wilson L, Vaeth E, Bamberg W, Janelle SJ, Lynfield R, Vagnone PS, Shaw K, Kainer M, Muleta D, Mounsey J, Dumyati G, Concannon C, Beldavs Z, Cassidy PM, Phipps EC, Kenslow N, Hancock EB, Kallen AJ (2018) Carbapenem-nonsusceptible *Acinetobacter baumannii*, 8 US metropolitan areas, 2012–2015. Emerg Infect Dis 24:727–734. https://doi.org/10.3201/eid2404.171461

Bush K (2018) Past and present of carbapenemases on β lactamases. Antimicrob Agents Chemother 62:e01076–e01018. https://doi.org/10.1128/AAC.01076-18

Codjoe FS, Donkor ES (2017) Carbapenem resistance: a review. Med Sci (Basel) 6(1):E1. https://doi.org/10.3390/medsci6010001

Correa A, Del Campo R, Perenguez M, Blanco VM, Rodriguez-Banos M, Perez F, Maya JJ, Rojas L, Canton R, Arias CA, Villegas MV (2015) Dissemination of high-risk clones of extensively drug-resistant *Pseudomonas aeruginosa* in Colombia. Antimicrob Agents Chemother 59:2421–2425. https://doi.org/10.1128/AAC.03926-14

Corvec S, Caro N, Espaze E, Giraudeau C, Drugeon H, Reynaud A (2003) AmpC cephalosporinase hyperproduction in Acinetobacter baumannii clinical strains. J Antimicrob Chemother 52:629–635. https://doi.org/10.1093/jac/dkg407

Corvec S, Poirel L, Naas T, Drugeon H, Nordmann P (2007) Genetics and expression of the carbapenem-hydrolyzing oxacillinase gene blaOXA-23 in *Acinetobacter baumannii*. Antimicrob Agents Chemother 51:1530–1533. https://doi.org/10.1128/AAC.01132-06

Courvalin P (1994) Transfer of antibiotic resistance genes between gram-positive and gram-negative bacteria. Antimicrob Agents Chemother 38:1447–1451. https://doi.org/10.1128/aac.38.7.1447

Diene SM, Rolain JM (2014) Carbapenemase genes and genetic platforms in Gram-negative bacilli: Enterobacteriaceae, Pseudomonas and Acinetobacter species. Clin Microbiol Infect 20:831–838. https://doi.org/10.1111/1469-0691.12655

Doumith M, Ellington MJ, Livermore DM, Woodford N (2009) Molecular mechanisms disrupting porin expression in ertapenem resistant *Klebsiella* and *Enterobacter* spp. clinical isolates from the UK. J Antimicrob Chemother 63(4):659–667. https://doi.org/10.1093/jac/dkp029

Eichenberger EM, Thaden JT (2019) Epidemiology and mechanisms of resistance of extensively drug resistant Gram-negative bacteria. Antibiotics (Basel) 8(2):E37. https://doi.org/10.3390/antibiotics8020037

Fernandez J, Guerra B, Rodicio MR (2018) Resistance to carbapenems in non-typhoidal Salmonella enterica serovars from humans, animals and food. Vet Sci 5:40. https://doi.org/10.3390/vetsci5020040

Fournier S, Monteil C, Lepainteur M, Richard C, Brun-Buisson C, Jarlier V, Ap-Hp Outbreaks Control Group C (2014) Long-term control of carbapenemase-producing Enterobacteriaceae at the scale of a large French multihospital institution: a nine-year experience, France, 2004 to 2012. Euro Surveill 19(19):20802

Gargiullo L, Del Chierico F, D'Argenio P, Putignani L (2019) Gut microbiota modulation for multidrug-resistant organism decolonization: present and future perspectives. Front Microbiol 10:1704. https://doi.org/10.3389/fmicb.2019.01704

Guzvinec M, Izdebski R, Butic I, Jelic M, Abram M, Koscak I, Baraniak A, Hryniewicz W, Gniadkowski M, Tambic Andrasevic A (2014) Sequence types 235, 111, and 132 predominate among multidrug-resistant *Pseudomonas aeruginosa* clinical isolates in Croatia. Antimicrob Agents Chemother 58:6277–6283. https://doi.org/10.1128/AAC.03116-14

Heritier C, Poirel L, Aubert D, Nordmann P (2003) Genetic and functional analysis of the chromosome-encoded carbapenem-hydrolyzing oxacillinase OXA-40 of *Acinetobacter baumannii*. Antimicrob Agents Chemother 47:268–273. https://doi.org/10.1128/AAC.47.1.268-273.2003

Hrabak J, Chud E, Papagiannitsis CC (2014) Detection of carbapenemases in Enterobacteriaceae: a challenge for diagnostic microbiological laboratories. Clin Microbiol Infect 20:839–853. https://doi.org/10.1111/1469-0691.12678

Kaase M, Szabados F, Anders A, Gatermann SG (2014) Fosfomycin susceptibility in carbapenem-resistant Enterobacteriaceae from Germany. J Clin Microbiol 52:1893–1897. https://doi.org/10.1128/JCM.03484-13

Kelly AM, Mathema B, Larson EL (2017) Carbapenem-resistant Enterobacteriaceae in the community: a scoping review. Int J Antimicrob Agents 50:127–134. https://doi.org/10.1016/j.ijantimicag.2017.03.012

Kos VN, Deraspe M, McLaughlin RE, Whiteaker JD, Roy PH, Alm RA, Corbeil J, Gardner H (2015) The resistome of *Pseudomonas aeruginosa* in relationship to phenotypic susceptibility. Antimicrob Agents Chemother 59:427–436. https://doi.org/10.1128/AAC.03954-14

Kullar R, Wagenlehner FM, Popejoy MW, Long J, Yu B, Goldstein EJ (2017) Does moderate renal impairment affect clinical outcomes in complicated intra-abdominal and complicated urinary tract infections? Analysis of two randomized controlled trials with ceftolozane/tazobactam. J Antimicrob Chemother 72:900–905. https://doi.org/10.1093/jac/dkw486

Laxminarayan R, Duse A, Wattal C, Zaidi AK, Wertheim HF, Sumpradit N, Vlieghe E, Hara GL, Gould IM, Goossens H, Greko C, So AD, Bigdeli M, Tomson G, Woodhouse W, Ombaka E, Peralta AQ, Qamar FN, Mir F, Kariuki S, Bhutta ZA, Coates A, Bergstrom R, Wright GD, Brown ED, Cars O (2013) Antibiotic resistance—the need for global solutions. Lancet Infect Dis 13:1057–1098. https://doi.org/10.1016/S1473-3099(13)70318-9

Livermore DM (2001) Of Pseudomonas, porins, pumps and carbapenems. J Antimicrob Chemother 47:247–250. https://doi.org/10.1093/jac/47.3.247

Logan LK, Weinstein RA (2017) The epidemiology of carbapenem-resistant Enterobacteriaceae: the impact and evolution of a global menace. J Infect Dis 215(Suppl 1):S28–S36. https://doi.org/10.1093/infdis/jiw282

Lolans K, Calvert K, Won S, Clark J, Hayden MK (2010) Direct ertapenem disk screening method for identification of KPC-producing *Klebsiella pneumoniae* and *Escherichia coli* in surveillance swab specimens. J Clin Microbiol 48:836e41. https://doi.org/10.1128/JCM.01988-09

Lowe CF, Kus JV, Salt N, Callery S, Louie L, Khan MA, Vearncombe M, Simor AE (2013) Nosocomial transmission of New Delhi metallo-β-lactamase-1-producing *Klebsiella pneumoniae* in Toronto, Canada. Infect Control Hosp Epidemiol 34:49–55

Luepke KH, Suda KJ, Boucher H, Russo RL, Bonney MW, Hunt TD, Mohr JF (2017) Past, present, and future of antibacterial economics: increasing bacterial resistance, limited antibiotic pipeline, and societal implications. Pharmacotherapy 37:71–84. https://doi.org/10.1002/phar.1868

Lutgring JD (2019) Carbapenem-resistant Enterobacteriaceae: an emerging bacterial threat. Semin Diagn Pathol 36:182–186. https://doi.org/10.1053/j.semdp.2019.04.011

Malchione MD, Torres LM, Hartley DM, Koch M, Goodman JL (2019) Carbapenem and colistin resistance in enterobacteriaceae in southeast Asia: review and mapping of emerging and overlapping challenges. Int J Antimicrob Agents 54(4):381–399. https://doi.org/10.1016/j.ijantimicag.2019.07.019

Masi M, Refrigiers M, Pos KM, Pages JM (2017) Mechanisms of envelope permeability and antibiotic influx and efflux in Gram-negative bacteria. Nat Microbiol 2:17001. https://doi.org/10.1038/nmicrobiol.2017.1

Merino M, Poza M, Roca I, Barba MJ, Sousa MD, Vila J, Bou G (2014) Nosocomial outbreak of a multiresistant *Acinetobacter baumannii* expressing OXA-23 carbapenemase in Spain. Microb Drug Resist 20:259–263. https://doi.org/10.1089/mdr.2013.0127

Miriagou V, Cornaglia G, Edelstein M, Galani I, Giske CG, Gniadkowski M, Malamou-Lada E, Martinez-Martinez L, Navarro F, Nordmann P, Peixe L, Pournaras S, Rossolini GM, Tsakris A, Vatopoulos A, Cantón R (2010) Acquired carbapenemases in Gram-negative bacterial pathogens: detection and surveillance issues. Clin Microbiol Infect 16:112–122. https://doi.org/10.1111/j.1469-0691.2009.03116.x

O'Neill J (2014) Review on antimicrobial resistance: tackling a crisis for the health and wealth of nations. Review on Antimicrobial Resistance. https://amr-review.org/sites/default/files/AMR%20Review%20Paper%20-%20Tackling%20a%20crisis%20for%20the%20health%20and%20wealth%20of%20nations_1.pdf

Okoche D, Asiimwe BB, Katabazi FA, Kato L, Najjuka CF (2015) Prevalence and characterization of carbapenem-resistant Enterobacteriaceae isolated from Mulago National Referral Hospital, Uganda. PLoS One 10:e0135745. https://doi.org/10.1371/journal.pone.0135745

Pena C, Cabot G, Gomez-Zorrilla S, Zamorano L, Ocampo-Sosa A, Murillas J, Almirante B, Pomar V, Aguilar M, Granados A, Calbo E, Rodriguez-Baño J, Rodríguez-Lopez F, Tubau F, Martínez-Martínez L, Oliver A, Spanish Network for Research in Infectious Diseases (2015)

Influence of virulence genotype and resistance profile in the mortality of *Pseudomonas aeruginosa* bloodstream infections. Clin Infect Dis 60:539–548. https://doi.org/10.1093/cid/ciu866

Poirel L, Nordmann P (2006) Carbapenem resistance in *Acinetobacter baumannii*: mechanisms and epidemiology. Clin Microbiol Infect 12:826–836. https://doi.org/10.1111/j.1469-0691.2006.01456.x

Poirel L, Naas T, Nordmann P (2010) Diversity, epidemiology, and genetics of class D beta-lactamases. Antimicrob Agents Chemother 54:24–38. https://doi.org/10.1128/AAC.01512-08

Potron A, Poirel L, Nordmann P (2015) Emerging broad-spectrum resistance in *Pseudomonas aeruginosa* and *Acinetobacter baumannii*: mechanisms and epidemiology. Int J Antimicrob Agents 45:568–585. https://doi.org/10.1016/j.ijantimicag.2015.03.001

Queenan AM, Bush K (2017) Carbapenemases: the versatile beta-lactamases. Clin Microbiol Rev 20:440–458. https://doi.org/10.1128/CMR.00001-07

Robledo IE, Aquino EE, Sante MI, Santana JL, Otero DM, Leon CF, Vazquez GJ (2010) Detection of KPC in *Acinetobacter spp*. in Puerto Rico. Antimicrob Agents Chemother 54:1354–1357. https://doi.org/10.1128/AAC.00899-09

Routh MD, Zalucki Y, Su CC, Zhang Q, Shager WM, Yu EW (2011) Efflux pumps of the resistance-nodulation-division family: a perspective of their structure, function, and regulation in Gram-negative bacteria. Adv Enzymol Relat Areas Mol Biol 77:109–146

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*. J Clin Microbiol 46 (9):3110–3311. https://doi.org/10.1128/JCM.00249-08

Tada T, Miyoshi-Akiyama T, Shimada K, Shimojima M, Kirikae T (2014) Dissemination of 16S rRNA methylase ArmA-producing *Acinetobacter baumannii* and emergence of OXA-72 carbapenemase coproducers in Japan. Antimicrob Agents Chemother 58:2916–2920. https://doi.org/10.1128/AAC.01212-13

Turton JF, Woodford N, Glover J, Yarde S, Kaufmann ME, Pitt TL (2006) Identification of *Acinetobacter baumannii* by detection of the blaOXA-51-like carbapenemase gene intrinsic to this species. J Clin Microbiol 44:2974–2976. https://doi.org/10.1128/JCM.01021-06

van Dam V, Olrichs N, Breukink E (2009) Specific labeling of peptidoglycan precursors as a tool for bacterial cell wall studies. Chembiochem 10:617–624. https://doi.org/10.1002/cbic.200800678

van Duin D, Bonomo RA (2016) Ceftazidime/Avibactam and Ceftolozane/Tazobactam: second generation β-lactam/β-lactamase inhibitor combinations. Clin Infect Dis 63:234–241. https://doi.org/10.1093/cid/ciw243

van Duin D, Perez F, Rudin SD, Cober E, Hanrahan J, Ziegler J, Webber R, Fox J, Mason P, Richter SS, Cline M, Hall GS, Kaye KS, Jacobs MR, Kalayjian RC, Salata RA, Segre JA, Conlan S, Evans S, Fowler VG Jr, Bonomo RA (2014) Surveillance of carbapenem-resistant *Klebsiella pneumoniae*: tracking molecular epidemiology and outcomes through a regional network. Antimicrob Agents Chemother 58:4035–4041. https://doi.org/10.1128/AAC.02636-14

Vikesland P, Garner E, Gupta S, Kang S, Maile-Moskowitz A, Zhu N (2019) Differential drivers of antimicrobial resistance across world. Acc Chem Res 52:916–924. https://doi.org/10.1021/acs.accounts.8b00643

Weston N, Sharma P, Ricci V, Piddock LJV (2018) Regulation of the AcrAB-TolC efflux pump in Enterobacteriaceae. Res Microbiol 169:425–431. https://doi.org/10.1016/j.resmic.2017.10.005

Yigit H, Queenan AM, Anderson GJ, Domenech-Sanchez A, Biddle JW, Steward CD, Alberti S, Bush K, Tenover FC (2001) Novel carbapenem-hydrolyzing β-lactamase, KPC-1, from a carbapenem-resistant strain of *Klebsiella pneumoniae*. Antimicrob Agents Chemother 45:1151–1161. https://doi.org/10.1128/AAC.45.4.1151-1161.2001

# Influence of Antimicrobials on the Gut Microbiota

Goutam Chowdhury and T. Ramamurthy

## 1 Introduction

The human gut microbiota constitutes a diverse ecosystem that contains thousands of different microbial species comprising bacteria, archaea, eukaryotes and viruses (Human Microbiome Project Consortium 2012a, b; Thursby and Juge 2017). The healthy gut microbiome is dominated by bacterial species belonging to the Bacteroidetes and Firmicutes phyla. The remaining bacteria included within the four major phyla are Actinobacteria, Fusobacteria, Proteobacteria and Verrucomicrobia and other minor phyla (Rajilić-Stojanović and de Vos 2014; Donaldson et al. 2016). Gut microorganisms carry out several functions such as extraction of energy from the host-indigestible carbohydrates, production of vitamins, promoting immune homeostasis and preventing the colonization of pathogens in the gut (Ha et al. 2014; Bäckhed et al. 2012). Considering these beneficial functions, gut bacteria are the significant contributors in numerous physiological activities in the human, including the regulation of several metabolic pathways, the barrier action against pathogens and improvement of the immune system (Hansen et al. 2012; Jandhyala et al. 2015). The composition of an individual's microbiota is influenced by many factors, specifically the age, geographical location and environment, dietary habits, comorbidities and use of prebiotics, probiotics and antibiotics (Zmora et al. 2019; Yatsunenko et al. 2012; Sommer and Bäckhed 2013). Early attempts to identify the composition of the microbiota relied on culture-based methods, in which 80–90% of the gut microbiota remained anaerobic and unculturable (Furrie 2006; Lozupone et al. 2012). In recent

G. Chowdhury (✉)
Division of Bacteriology, ICMR-National Institute of Cholera and Enteric Diseases, Kolkata, India

T. Ramamurthy
Division of Bacteriology, ICMR-National Institute of Cholera and Enteric Diseases, Kolkata, India

Translational Health Science and Technology Institute, Faridabad, Haryana, India



S. Thomas (ed.), *Antimicrobial Resistance*,
https://doi.org/10.1007/978-981-15-3658-8_3

years, high-throughput sequencing and bioinformatics tools have provided culture-independent approaches to better assess and understand the contribution of the human gut microbiome to health and their potential benefit for therapeutic interventions (Li et al. 2015).

Antibiotic treatment shall eliminate pathogens from the gut/infection site. The antibiotics usually prescribed for treating the infectious diseases or as prophylaxis can also target commensal microbiota because of their wide-spectrum activity (Levy and Marshall 2004; Kim et al. 2017). The use of antibiotics significantly alters community structure or different effects on composition and the richness of the gut microbiome, which is known as 'dysbiosis' (Yoon and Yoon 2018). As a result, the microbiome may not perform actual functions such as the digestion of food, synthesis of vitamins or protection against colonization of pathogens (Dudek-Wicher et al. 2018). Gut microbiomes act as a reservoir for antimicrobial resistance genes (ARGs) and can easily transfer host antibiotic resistance genes to pathogenic microbes (Lange et al. 2016). The horizontal transfer of ARGs between and within gut commensal bacterial communities to opportunistic pathogens in the gut may promote the emergence of multidrug-resistant (MDR) nosocomial pathogens (Jernberg et al. 2010). Hence, the main effects of gut microbiota from post-antibiotic dysbiosis are loss of taxonomic and functional diversity combined with reduced colonization resistance against invading pathogens, which may harbour the ARGs (Lange et al. 2016; Kim et al. 2017). Moreover, some of these antibiotics may exert harmful effects on gut microbiota, resulting in increased pathogenesis and disease severity like *Clostridium difficile* infection, irritable bowel syndrome (IBS), inflammatory bowel disease (IBD), metabolic disorders or liver disease (Lange et al. 2016; Becattini et al. 2016; Iizumi et al. 2017). This review elucidates the composition and role of healthy human gut microbiome to understand the antibiotic-related changes of the gut microbiota and overall consequences on health and disease conditions.

## 2 Human Gut Microbiota and Its Ecosystem

Different molecular techniques have endorsed the diverse composition of host-associated gut microbial communities and the distinctive environmental characteristics along the gastrointestinal tract resulting in the development of specific gut microbial populations (Bik et al. 2006; Booijink et al. 2010; Walter and Ley 2011). The human gastrointestinal (GI) tract contains abundant and diverse microbial communities that are composed of several species of commensal and/or symbiotic microorganisms, including bacteria, archaea, viruses and eukaryotic microbes (Ley et al. 2006; Sommer and Bäckhed 2013). Different environmental factors can affect intestinal microbial stability, which has a close relationship with human health and disease conditions. Collectively, the human gut microbiota has even been considered as an 'essential organ' (O'Hara and Shanahan 2006), carrying about 150 times more genes than are found in the entire human genome (Ursell et al. 2014). The gut microbiota are involved in basic human biological processes, including modulating the metabolic phenotype, regulating epithelial development and

influencing innate immunity (Savage 1977; Ley et al. 2006), but macrobiotic composition and functions may differ according to different geographical locations, ages, sexes, races and diets of the host (Hollister et al. 2014). Gut commensal bacteria colonize the host shortly after birth and progressively develop into a highly diverse ecosystem during growth, reaching a high degree of complexity in adults (Fanaro et al. 2003). The child's delivery mode (vaginally delivered babies or Caesarean section delivery) and nutritional factors are known to strongly impact the composition of gut microbiota (Backhed et al. 2015; Dominguez-Bello et al. 2016; Balmer and Wharton 1989).

During the early stages of development, the microbiota is generally low in diversity and is dominated by two main phyla, Actinobacteria and Proteobacteria (Rodríguez et al. 2015). During the first year of life, the microbial diversity increases, and the microbiota composition converges towards a distinct adult-like microbial profile with temporal patterns that are unique to each infant (Palmer et al. 2007). Around 3 years of age, the composition, diversity and functional abilities of the infant microbiota almost resemble to that of adults (Rodríguez et al. 2015; Koenig et al. 2011). At the taxonomic phyla level, a healthy microbiota in adults is principally composed of Firmicutes, and Bacteroidetes constitute more than 90% of the total microbiota population. Most of the species under the phylum Bacteroidetes belong to the genera of *Bacteroides* and *Prevotella.* The other gut microbial phylum includes Actinobacteria, Proteobacteria, Fusobacteria and Verrucomicrobia. The Firmicutes phylum is composed of over 200 different genera such as *Bacillus, Lactobacillus, Enterococcus*, *Clostridium* and *Ruminococcus*. *Clostridium* spp. alone represents 95% of the Firmicutes phyla. The Actinobacteria phylum is proportionally less abundant and mainly represented by the *Bifidobacterium* spp. (Ley et al. 2006; Tap et al. 2009; Arumugam et al. 2011).

In the gut environment, the microbiota develops elaborate networks through interactions with other bacteria to obtain nutrients required for their survival, colonization and proliferation (Walter and Ley 2011; Dethlefsen et al. 2006). Host-microbial and microbial-microbial interactions establish a balanced state of microbial composition in the intestinal tract (Backhed et al. 2005; Benson et al. 2010). The gut microbiota collectively maintains intestinal relationship by repressing the invasion of other microorganisms from the outside by controlling their growth in the intestinal milieu. Symbiotic gut microbiota metabolizes indigestible compounds, supply essential nutrients, defend against colonization by opportunistic pathogens and contribute to the formation of intestinal architecture (Rinninella et al. 2019).

Many factors influence the gut microbial ecosystem. The density, composition and balance of the gut microbiota are affected by food, prebiotics, probiotics, antibiotics, steroids, alcohol abuse and immunological functions along the gut. Other non-dietary factors, age, sex, stress, gastrointestinal disorders, lifestyle and infection events can also play an essential role in the microbial composition (Gagliardi et al. 2018; Marchesi et al. 2016). The allocation and abundance of gut microbial species diverge significantly in different intestinal regions and depend on gastrointestinal peristalsis, gastric acid secretion and mucosal secretion of IgA, as

well as on the individual's immune characteristics and other environmental influences (Round and Mazmanian 2009; Dominguez-Bello et al. 2019).

## 3 Role of Gut Microbiota in Human Health

The gut microbiota plays a vital role in human health and disease (Cani and Delzenne 2009). Recent studies in genome sequencing technologies, bioinformatics and culture-based technologies have explored the microbiota with reference to their functions at a more detailed level (Kataoka 2016). Several studies suggest that the composition and functional role of microbiota are conserved. Under different health conditions, the dynamic members of gut microbiota vary among infants, elderly, indigenous tribes, etc. (Mohajeri et al. 2018). The gut microbes are very dynamic, as they perform many basic physiological functions in the host such as strengthening the gut integrity, digestion and use energy from food, breakdown of toxins and xenobiotics, production of vitamins and essential amino acids, protection against pathogens of the host and modification of neurological development and behaviour (Sonnenburg and Fischbach 2011; Gensollen et al. 2016). The gut microbiota also play a significant role in the extraction, synthesis and absorption of many nutrients and metabolites, including bile acids, lipids, amino acids, vitamins and short-chain fatty acids (SCFAs) (Stiemsma and Michels 2018). Beneficial microbiota can also modify the virulence factor expression in pathogens by facilitating host barrier function through upregulation of the mucus layer, induction of antimicrobial molecules (RegIII-β and RegIII-γ) and secretion of IgA. Beneficial gut microbiota also protect the host from pathogens and pathobionts by host immunity-mediated resistance like leading gut macrophages promote neutrophil, upregulation of pro-IL-1β, activation of $T_H17$ and $T_H1$ cells and secretion of different cytokines (IL-22).

The specific composition of the gut microbiota is influenced by many factors, including host genotype, age, diet, localized inflammation, antimicrobial use and the presence of pathogenic organisms (Stiemsma and Michels 2018). The gut microbiota maintains a symbiotic relationship with the gut mucosa and regulates substantial metabolic, immunological and gut protective functions in the healthy individual. The gut microbiota helps to protect the body from pathogenic organisms by producing bacteriocins, which have a crucial immune function against pathogenic bacteria colonization by inhibiting their growth (Dobson et al. 2012). These antimicrobial products can influence the constancy and structure of complex microbial populations. For example, *Listeria monocytogenes* are inhibited by *Lactobacillus* (Corr et al. 2007), and enterococci express bacteriocins which can confer competitive advantages in the intestinal tract (Kommineni et al. 2015). The commensal of human origin, *Bacillus thuringiensis*, produces bacteriocins that inhibit spore-forming Gram-positive bacteria, including *Clostridium difficile* without affecting other commensal microbes (Rea et al. 2010). Gut microorganisms prevent pathogenic bacteria colonization by many antagonistic processes like nutrient metabolism, pH modification, antimicrobial peptide secretions and influencing the cell signalling pathways (Keith and Pamer 2019). Gut microbiota also inhibits bacteria invasion by

maintaining the intestinal epithelium integrity and gut peristalsis, as well as supporting the development of the enteric immune system (Canny and McCormick 2008).

The gut microbiota has an important role in human disease aetiology and pathology (Caitriona and Paul 2013). The gut microbiota influences the development of chronic diseases ranging from metabolic disease to gastrointestinal disorders and colorectal cancer. The alterations in gut microbiota have been linked to several important diseases and conditions, including obesity, Crohn's disease, diabetes mellitus and ulcerative colitis (Durack and Lynch 2019). The gut microbiota is actively involved with other organ systems, including the brain, lungs, skin and liver, by influencing their function. The other disorders associated with the gut microbiota dysbiosis include colon cancer, IBD, hypercholesterolemia, non-alcoholic fatty liver disease, etc. (Carding et al. 2015).

Scientific and clinical evidence in support of the functioning of the gut microbiota has led to stimulating developments in therapeutics, such as prebiotics, probiotics, drugs and faecal transplantation leading to improved health (Cho and Chinnapen 2018; Khoruts 2018). Moreover, the gut microbiota functions are highly conserved between individuals, whereas each individual's gut microbiota is characterized by a specific combination of bacterial species due to inter-individual and intra-individual variations throughout human life (Li et al. 2016; Khoruts 2018).

## 4 Metagenomic Analysis of Gut Microbiota

Metagenomic approach helps in understanding the human gut microbiome, including the abundance and diversity of bacterial genera/species, identifying novel genes, microbial networks, ARGs and verifying the functional dysbiosis (Mandal et al. 2015; Weinstock 2012; Song et al. 2018). Metagenomics can also promote an understanding of the functional activity of the human gut microbiome and possibly provide a new strategy for disease diagnosis and treatment (Wang et al. 2015; Heintz-Buschart and Wilmes 2018). Traditional culture methods are less effective as they could identify only 10–20% of gut microbiota (Tannock 2001). However, 16S rDNA sequence-based analysis and metagenomics can identify a large number of human gut microbial communities (Shendure and Ji 2008; Fuller et al. 2009; Human Microbiome Project Consortium 2012a, b). Handelsman et al. (1998) first described the metagenomic approach to investigate the complex gut microbial community. Several comprehensive projects, including the European Metagenomics of the Human Intestinal Tract (MetaHIT) and the American Human Microbiome Project (Human Microbiome Project Consortium 2012a, b), have contributed to the generation of extensive metagenomics data and the reference gene catalogue of the human microbiome (Qin et al. 2010; Human Microbiome Project Consortium 2012a, b). The MetaHIT project results indicated that there are more than three million nonredundant genes in the human gut microbiome that were 150 times larger than the human genome (Qin et al. 2010).

In recent years, many studies have focused on function-based metagenomics of the human gut microbiome in identifying novel genes and their functional pathways (Qin et al. 2010). Metagenomic data provide the gene composition of the whole microbiome, including gene expression. For example, human intestinal microorganisms are producing carbohydrate-active enzymes (CAZymes), which can degrade components of dietary fibre into metabolizable monosaccharides and disaccharides. Tasse et al. (2010) identified a novel gene responsible for CAZymes in human gut microbiota by using functional metagenomics. Gut microbial enzymes also play a critical role in the metabolism of commonly prescribed drugs (Jia et al. 2008). Identification of individual microbial species along with its proteome and metabolome would make it possible to target the microbiota for therapeutic purposes. For example, the bacterial β-glucuronidase enzyme is used as an inhibitor for the successful treatment of chemotherapy-associated diarrhoea (Wallace et al. 2010). Currently, high-throughput sequencing-based metagenomic analysis has been widely used in ARG surveys and comparative human gut-associated resistome (Li et al. 2015). Several ARG reference databases are available in the network, including the Antibiotic Resistance Gene Online (ARGO) database (Scaria et al. 2005), the Antibiotic Resistance Genes Database (ARDB) (Liu and Pop 2009), the Comprehensive Antibiotic Resistance Database (CARD) (McArthur et al. 2013), ARG-ANNOT (Gupta et al. 2014) and PATRIC (Wattam et al. 2014).

ARGs have been reported from the faecal microbial communities of both adults and children from different parts of the world. Prevalence of ARGs in the human gut microbiome was first reported by Seville et al. (2009) through population-level metagenome analysis. This study has shown the distribution of tetracycline and erythromycin resistance genes among human faecal microbiota. Recently, Moore et al. (2013) used functional metagenomics in faecal microbiota from healthy infants and children to identify novel ARGs and other resistomes. Moreover, the exchange of transposable elements and ARGs among gastrointestinal microbes can be easily identified using metagenome analysis (Karami et al. 2007). Recent studies showed that commensal enteric bacteria from the gut of healthy Indians carried transferable ARGs (Bag et al. 2019). Furthermore, Sommer et al. (2009) reported that the ARGs identified in bacteria isolated from faecal samples of individuals in the USA were identical to the known ARGs of human pathogens.

Metagenomics can also determine the dysbiosis and novel changes in the intestinal microbial community and their functions in several disease conditions, including IBD (Bye et al. 2014), obesity (Tarantino 2014), IBS (Carroll et al. 2012), diabetes (de Goffau et al. 2014), colorectal cancer (Ahn et al. 2013), diarrhoea (Pop et al. 2014) and liver diseases (Chen et al. 2011). In the application of metagenomic methods, Wei et al. (2013) found an apparent change in the faecal microbiota of hepatitis B liver cirrhosis patients and healthy controls. The application of metagenomics has huge potential in disclosing the mechanisms and correlations between the human intestinal microbiome and diseases (Wang et al. 2015; Feng et al. 2018).

## 5 Influence of Antibiotics on Normal Gut Microbiota

The biological balance between the human gut and associated microorganisms can be disturbed by several factors, including antimicrobial agents (Lange et al. 2016). The effect of antibiotics on the human gut microbiota depends on both drug-related and host-related factors. Antimicrobial agents can influence the gut microbiota in different ways (Dethlefsen et al. 2006). The level of the antibiotic-induced alterations in the microbiota depends on several components like class, pharmacokinetics, pharmacodynamics, range of action, dosage, duration of treatment, administration route and the bacterial target (Munita and Arias 2016). Most of the commercially available antibiotics have a broad spectrum of action, and they influence not only on harmful bacteria but also on beneficial gut microbiota that may lead to antibiotic-associated microbial dysbiosis (Carding et al. 2015).

Many studies have revealed that broad-spectrum antibiotics could cause microbial dysbiosis, affecting the abundance of 30–40% of bacteria in the gut community. This results in a significant drop in the bacterial richness, diversity and decreased colonization resistance and leads to varying states of disease as well as the emergence of AMR (Carding et al. 2015; Yoon and Yoon 2018). Numerous studies described about short- and long-term impacts of antibiotic exposure on the healthy gut microbiota including depletion of bacterial diversity, loss of potential competitors, low expression of antibacterial and IgG and decreased neutrophil-mediated killing of exogenous pathogens (Jakobsson et al. 2010; Jernberg et al. 2010) (Fig. 1). Dysbiosis helps in the release of antimicrobial molecules, which intrudes the process of colonization resistance and allows the pathogens to invade the intestinal epithelium by extraluminal translocation. With the help of MexAB-OprM efflux pump systems, MDR *Pseudomonas* spp. develop quorum-sensing machinery to recognize host stress and express multiple virulence determinants (Hirakata et al. 2002).

Several broad-spectrum antibiotics are precisely active against anaerobic bacteria, which are naturally abundant and play an important role in maintaining a healthy gut. Antibiotic treatment for specific groups of anaerobic bacteria may have a substantial consequence on the functional stability of the gut microbiota (Bartlett 2002). Lincosamides, mainly clindamycin, are the broad-spectrum antibiotics that target anaerobic bacteria. Clindamycin is one of the severe antibiotic risk factors on the intestinal microbiota as evidenced by colonization and consequent progression of *C. difficile* infection (Hull and Beck 2004). The abundance of *C. difficile* is generally low in healthy individuals. Due to the use of clindamycin, there may be an increase in numbers of *C. difficile* and reduction in normal anaerobic microbiota. Due to this loss of stability in the intestinal flora, patients may encounter severe diarrhoea, gastritis, fulminant colitis, toxic megacolon, bowel perforation and sepsis (Banawas 2018) (Table 1). The impacts of amoxicillin or amoxicillin with clavulanic acid are mild to moderate on the gut microbiota. However, an increase of resistant enterobacteria and a decrease in aerobic Gram-positive cocci in response to amoxicillin have been reported (Sullivan et al. 2001). In addition, the microbial functions such as biochemical profile, vitamin A and E levels and lipid metabolism were also affected due to short-term exposure to amoxicillin (Marker et al. 2017).

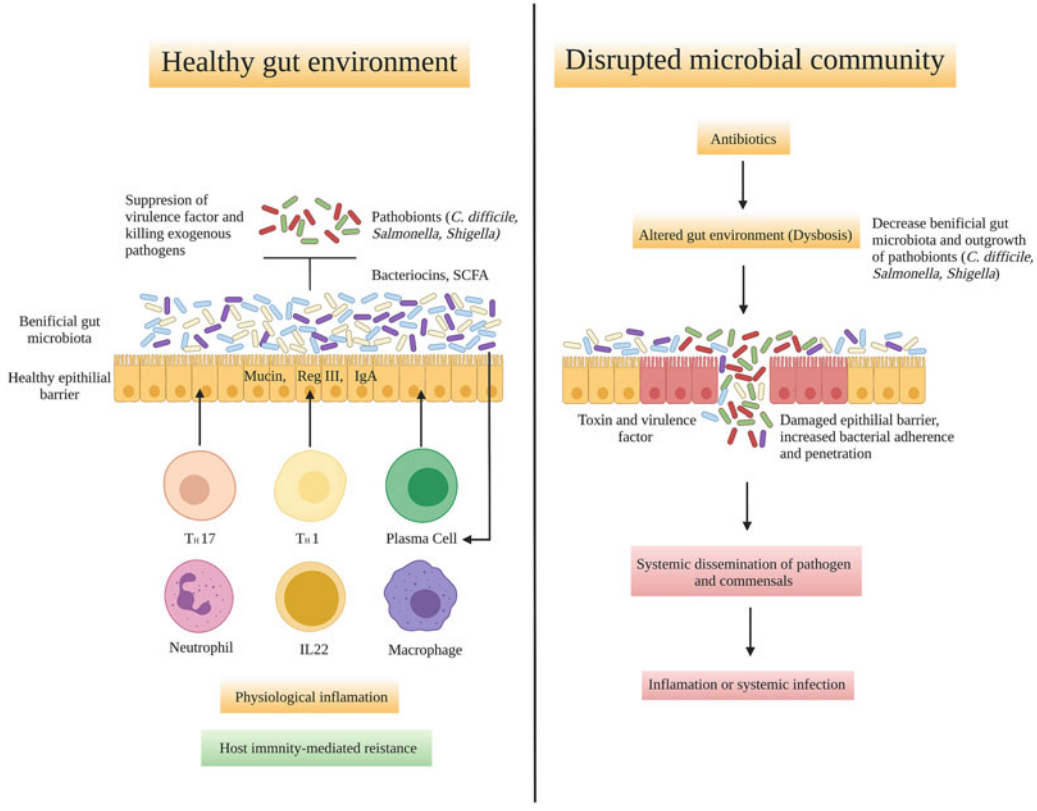


**Fig. 1** The role of commensal microbiota in gut homeostasis and prevention of exogenous pathogens and pathobiont infection. Antibiotic administration disrupts complex beneficial microbial community, causing loss of mucus due to the diminishment of microbiota and resistance to colonization by pathogens (*C. difficile, Salmonella, Shigella*) that have the potential to disseminate systemically and induce inflammation and systemic infection (Kamada et al. 2013; Keith and Pamer 2019). This image was created using the tool 'BioRender.com'

**Table 1** Effect of different antibiotics on the human gut microbiota related to health and diseases

| Antibiotics class | Effect on gut microbiota | | Clinical relevance | References |
|---|---|---|---|---|
| | Decrease | Increase | | |
| Clindamycin | *Lactobacilli*, *Bifidobacteria*, Enterococci, Streptococci, *Bacteroides* | *Clostridium* | High risk of pseudomembranous colitis due to *Clostridium difficile* overgrowth, gastritis, diarrhoea and intestinal pain. Reduced resistance to colonization by pathogens | Jernberg et al. (2010) Rashid et al. (2015) |
| Amoxicillin and clavulanic acid | *Lactobacilli*, *Bacteroides*, *Clostridium*, *Bifidobacteria* | Proteobacteria *Enterobacteriaceae* | Increased risk of opportunistic infections caused by *Escherichia* spp. or *Klebsiella* spp. | Pérez-Cobas et al. (2013) |
| Penicillin | *Bifidobacteria*, Eubacteria, *Lactobacilli*, *Streptococcus*, *Arthromitus*, *Allobaculum*, *Turicibacter* | Bacteroidetes/Firmicutes ratio *Staphylococcus*, *Roseburia*, *Escherichia*, *Shigella* | Suppression of segmented filamentous bacteria (SFB) and development of gastrointestinal tract (GI) diseases like Crohn's, ulcerative colitis, IBS and IBD | Nord et al. (1993) Leclercq et al. (2017) Jin et al. (2017) |
| Ampicillin | Total bacterial diversity, *Bifidobacteria*, *Enterobacter*, Firmicutes | Bacteroidetes, Proteobacteria, enterococci | Reduced gut microbiota metabolic functions with corresponding pathological consequences of decreased ecological stability and increased susceptibility to infections. Development obesity-related disorders like metabolic imbalance in gut | Anukam (2017) |
| Ciprofloxacin | *Bifidobacterium*, Actinobacteria, *Alistipes*, *Faecalibacterium*, *Oscillospira*, *Ruminococcus*, *Bifidobacterium* | Bacteroidetes/Firmicutes ratio Enterococci, *Helicobacter* sp., *Blautia schinkii* | Influenced the abundance of about a third of the bacterial taxa in the gut and decreased taxonomic richness of gut microbiota within days of initial exposure | Stewardson et al. (2015) Rashid et al. (2015) Panda et al. (2014) |

(continued)

**Table 1** (continued)

| Antibiotics class | Effect on gut microbiota | | Clinical relevance | References |
|---|---|---|---|---|
| | Decrease | Increase | | |
| Clarithromycin | Actinobacteria,<br>Firmicutes,<br>*Escherichia coli* | *Bacteroides*,<br>Proteobacteria,<br>Enterococci, *Enterobacter*,<br>*Citrobacter* | Development of GI diseases like IBS, NAFLD and NASH. Increases in the *ermB* resistance gene, which encodes the macrolide target-modifying RNA methylase | Jakobsson et al. (2010) |
| Meropenem | Total beneficial bacteria<br>Enterobacteria, streptococci,<br>*Clostridium*,<br>*Bacteroides*,<br>*Bifidobacterium*,<br>*Eubacterium* | Enterococci | Overgrowth of enterococci and *C. difficile* which may produce systemic infections in immunocompromised patients and may lead to diarrhoea or colitis and development of antimicrobial resistance among the gut microbiota | Bergan et al. (1991)<br>Del Chierico et al. (2014) |
| Azithromycin | Total bacteria diversity<br>Actinobacteria,<br>*Christensenella*, *Gordonibacter*<br>*Lactobacillus* | *Bacteroides*<br>Proteobacteria<br>Firmicutes/Bacteroidetes ratio | Increased adipogenesis and altered gut microbiota composition, SCFA production and bile acid metabolism might be one risk factor for childhood obesity | Korpela et al. (2016)<br>Li et al. (2017) |
| Vancomycin | Firmicutes,<br>*Clostridium*,<br>*Bifidobacteria Bacteroides* | Enterococci, *Enterobacteriaceae*<br>Proteobacteria | Depleted the most intestinal microbiota genera and operational taxonomic units (OTUs) and vast expansion of genera associated with infections, including *Klebsiella* and *Escherichia/Shigella* spp. | Vrieze et al. (2014)<br>Isaac et al. (2017) |
| Metronidazole | Bacteroidetes<br>*Clostridium*<br>*Lactobacillus* | *Enterobacteriaceae*,<br>*Eubacterium*, *Porphyromonas*,<br>*Helicobacter* spp. | Increased risk of opportunistic infections caused by *Escherichia* spp. or *Klebsiella* spp. | Zhang et al. (2014) |

| | | | | |
|---|---|---|---|---|
| Tetracycline | Enterococci,<br>*E. coli*,<br>*Lactobacilli*,<br>*Bifidobacteria* | Enterobacteria,<br>Proteobacteria | Disrupt the balance in the microbiota composition of the human GI tract by inducing resistant microbial strains, which allows for overgrowth of pathogenic, opportunistic or resistant microbial strains | Jung et al. (2018) |
| Ceftriaxone | Firmicutes *Enterobacteriaceae*<br>*Lactobacilli*<br>*E. coli*<br>*Bifidobacteria* | Enterococci<br>Proteobacteria,<br>*Clostridium* | Induced disruption of colonization resistance and alteration of mucosal homeostasis facilitate increased intestinal abundance of a limited number of commensals along with enterococci | Chakraborty et al. (2018)<br>Burdet et al. (2019) |

The impact of β-lactam combination, including ampicillin and third-generation cephalosporins like ceftriaxone, includes a decrease in Firmicutes and an increase in Bacteroidetes, Proteobacteria, enterococci, *Candida* spp. and *C. difficile* (Knecht et al. 2014). Intrapartum antibiotic prophylaxis with penicillin, ampicillin or ampicillin along with erythromycin led to decrease in the levels of Actinobacteria and Bacteroidetes and an increase of Proteobacteria and Firmicutes group (Nogacka et al. 2017). Macrolides seem to make long-term changes in the gut microbiota, particularly reduction of Actinobacteria and Firmicutes, total bacterial diversity and increase in the relative abundance of Bacteroidetes and Proteobacteria (Parker et al. 2017). The gut microbiota is also affected after treatment with fluoroquinolones such as ciprofloxacin, levofloxacin and moxifloxacin. Recently, fluoroquinolones are shown to increase the Bacteroidetes/Firmicutes ratio but decrease total bacterial diversity in the gut (Panda et al. 2014). In contrast, vancomycin, a glycopeptide antibiotic that interferes with bacterial cell wall synthesis, significantly reduces Firmicutes, *Enterococcus* spp., *Bifidobacteria* and *Bacteroides* spp. and promotes the growth of less sensitive enterococci and pathogenic *Enterobacteriaceae* (Isaac et al. 2017) (Table 1).

The long-term use of antibiotics could reduce the total microbial diversity and increase the susceptibility to infections caused by opportunistic and nosocomial pathogens (Kim et al. 2017). The gut microbiota can also contribute to a variety of diseases through different mechanisms, including the domination of particular bacterial community and the production of harmful catabolites, to induce inflammation or enhance bacterial virulence (Bäumler and Sperandio 2016). Antibiotic-associated diarrhoea due to nosocomial pathogens such as *C. difficile* can cause recurrent infections and long-term persistence of pseudomembranous colitis (Bien et al. 2013). Due to the irrational use of antibiotics, many of the MDR enteric bacteria such as *Escherichia coli*, *Klebsiella pneumoniae*, *Enterococcus faecalis* and *Enterococcus faecium* can cause recurrent infections, most of which are nosocomial infections (Exner et al. 2017; Kumar et al. 2017).

Gut dysbiosis is strongly correlated with many diseases such as IBD, colorectal cancer (CRC), obesity, non-alcoholic fatty liver disease (NAFLD) and non-alcoholic steatohepatitis (NASH) (Keeney et al. 2014). A common feature is that many of these diseases are linked to microbial dysbiosis with a significant reduction in bacterial diversity (Lepage et al. 2011). Moreover, alteration in the proportion of the two most abundant phyla, Firmicutes and *Bacteroides*, seems to be an important cause in the development of these diseases (Sokol et al. 2009). Changes in these two dominant phyla, coupled with an increase in abundance of members of the Proteobacteria phylum, are known to play a crucial role in IBD (Hold et al. 2014) (Table 1). Under the dysbiotic condition, the IBD metagenome contains 25% fewer functional genes than the individuals with a healthy gut (Zuo and Ng 2018). Many microbiome studies explored to find the relationship between dysbiosis and CRC. The abundance or loss of certain taxa after antibiotic treatment may lead to cancerogenesis by the interaction of the dysbiotic microbiota with mucosal immune cells and epithelial cells (Sobhani et al. 2013). Other contributing factors comprise genetic susceptibility and chronic colonization of *B. fragilis* which harbour unique

virulence traits that can induce CRC due to epithelial cell DNA damage (Arthur and Jobin 2011).

Turnbaugh et al. (2006) demonstrated a possible relationship between the gut microbiome and obesity. An increase in the relative abundance of Firmicutes and a proportional decrease in Bacteroidetes are found to be associated with obesity in both animal models and human volunteer studies. However, in humans, the microbiome is exposed primarily to different environmental influences, including diet and host hormonal factors (Davis 2016). A close link between the gut and the liver suggests that the microbiome can affect liver function at multiple levels and contribute to the pathogenesis of various chronic liver and metabolic diseases (Acharya and Bajaj 2017). It has been identified that dysbiosis can lead to the development of obesity-related NAFLD and NASH (Zhao et al. 2019) (Table 1). The gut dysbiosis may also cause abnormal accumulation of bacterial products in the portal circulation and drive hepatic inflammation (Chassaing et al. 2014).

## 6 Human Gut Resistome

The emergence and spread of AMR are currently one of the greatest threats to human health (Levy and Marshall 2004; Zaman et al. 2017). The human gut microbiota comprises a very active microbial ecosystem with many network systems among different species. This association offers sufficient opportunity for the horizontal transfer of genetic materials (Francino 2016). The human gut resistome is defined as the collection of functional genes that potentially encode for resistance to antibiotics (Van Schaik 2015). The gut resistome is influenced by many factors including antibiotic usage, food habits, medical history and genetic factors (Xia et al. 2019). The diversity of gut microbiota and its resistome depend on the use of a specific antibiotic class, potency, spectrum and duration of therapy.

Long-term usage of antibiotic is the main concern in the spread of AMR bacteria and transmission of ARGs within the human gut (Forslund et al. 2013; Gibson et al. 2015). Several studies have shown the presence of macrolide resistance in *Staphylococcus epidermidis* and enterococci in patients who used clarithromycin for several years (Jernberg et al. 2010). Another study has shown the possibility of the transfer of ARGs among bacterial populations within the gut, but the exact mechanisms are not fully elucidated (Lester et al. 2006). The gut is an ideal milieu for effective transmission of ARGs with nutritional and/or other favourable factors with higher numbers of bacterial cells (Francino 2016).

The gut resistome has been detected more in both adults and children who have undergone antibiotic treatment (Zhang et al. 2011; Gosalbes et al. 2016). ARGs against aminoglycosides and β-lactam antibiotics were found from the gut microbiome of 6-month-old infants without any prior exposure to antibiotics (Gibson et al. 2016). Commensal bacterial populations seem to be at high risk of antibiotic pressure. Karami et al. (2007) demonstrated the transfer of a plasmid carrying a β-lactamase gene from a resistant *E. coli* to a susceptible strain from a child who was treated with ampicillin. Mobilization and horizontal transfer of ARGs

through conjugation and transduction was found to be common among gut microbiota (Van Schaik 2015). ARGs may also stem from an accumulation of gene mutations, enzymatic inactivation of the antibiotic, modification of the antibiotic target or the prevention of the accumulation of lethal intracellular concentrations of the antibiotic through efflux pumps (Zankari et al. 2012).

The use of antibiotics exceeding the permissible dosage or inadequate dose during the treatment course may select MDR bacteria in the gut environment. As a consequence, ARGs can spread between different/same members of the opportunistic pathogens and gut commensal (Davies and Davies 2010; Martinez 2014). Exchange of ARGs has been reported in the human gut between strains carrying vancomycin and sulfonamide resistance genes in *E. coli* (Trobos et al. 2009). The human gut can be considered as one of the major reservoirs of ARGs and also conducive milieu for the transfer and spread of ARGs within different bacterial species.

## 7 Infant Gut Microbiota and Antibiotics

The typical developmental progression of the infant gut microbiota is patterned, so far extremely dynamic and individual-specific, and is shaped by many factors, including host physiology, genetics, diet and environment (Gibson et al. 2016; Nogacka et al. 2018). Antibiotics are the most commonly used drugs in neonatal and paediatric populations in the developing countries (Gibson et al. 2015; Korpela et al. 2016). Treating bacterial infections with antibiotics can cause long-term changes to the commensal microbiota, which is at its most susceptible and impressionable in preterm infants and young children. Antibiotic perturbation of the actively developing infant gut microbiota likely has profound impacts on human health and disease like inflammatory bowel disease, overweight and asthma throughout life, as alteration of the gut microbiota during this timeframe may disrupt metabolic and immune development (Browne 2016). Equally important is the potential enrichment of the reservoir of antibiotic resistance genes ('resistome') available for transfer to pathogens (Gibson et al. 2015), compromising the treatment of infections in vulnerable populations. Recent studies have combined sequence-based approaches with in vitro phenotyping to investigate the effects of treating infants with antibiotics and have characterized variations in microbial community composition and the acquisition of antibiotic resistance genes in the gut microbiota. Antibiotic treatment can also target specific phylogenetic subgroups of the infant gut microbiota (Coker et al. 2020). Treatment of preterm infants with a variety of antibiotics has been found to increase the percentage of potentially pathogenic *Enterobacteriaceae* while lowering the relative percentage of microbial taxa linked to a healthy microbiota such as *Bifidobacteriaceae*, Bacilli and Lactobacillales spp. (Tanaka et al. 2009; Arboleya et al. 2015). The intestinal microbiota of children that were recently exposed to macrolides showed high levels of resistance to this antibiotic, as shown by metagenomic sequencing. Furthermore, children who had been treated with macrolides in the first 2 years of life were more likely to have developed asthma

and to be overweight than children who had not been treated with antibiotics, which might be caused by disruption to microbial homeostasis during this critical developmental window (Trasande et al. 2013; Korpela et al. 2016).

Many studies have shown that antibiotic resistance genes in the infant gut microbiota are likely established within the first week of life, even in the absence of antibiotic exposure (Zhang et al. 2011; Mitsou et al. 2010). However, several studies have found long-term microbial disruption in infant gut microbiota due to being exposed to antibiotics. Children exposed to antibiotics in the first 6 months of life were found to have a statistically significant increase in body mass. On the other hand, children treated with other medications or antibiotics after 6 months of life showed no such correlation (Trasande et al. 2013). In another study, antibiotic exposure during the first year of life was found to be associated with being overweight at age 12 (Rashid et al. 2015). Similarly, antibiotics have been found to play a role in the induction of hypersensitivity pneumonitis (Russell et al. 2015). The phylogenetic and resistome composition of the infant gut microbiota is likely connected, yet dynamic, with the gut environment and antibiotic pressure increasing opportunities for horizontal gene transfer (Smillie et al. 2011; Stecher et al. 2012). Opportunistic pathogens, such as *E. coli*, *Enterobacter cloacae* and *Klebsiella pneumoniae*, were prevalent across all preterm infants that were associated with a higher number of antibiotic resistance genes than other species. The transfer of antibiotic resistance genes can be influenced by many factors, including antibiotic spectrum, duration and delivery route, as well as microbial community composition and antibiotic susceptibility (Nobel et al. 2015). Notably, the route of antibiotic administration can strongly impact the emergence of resistant populations in the infant gut microbiota. Significant alterations in the composition of the developing infant gut microbiota in response to antibiotic treatment can cause a similar transformation in functional capacity, the most clinically relevant example being antibiotic resistance (Gibson et al. 2015).

In neonates, the early development of the microbiota differs between antibiotic-treated and non-treated infants. However, nothing is currently known about the long-term associations between lifetime antibiotic use and gut microbiota in infants. So, it is particularly critical to understand the short-and long-term effects of antibiotic treatments on the gut microbiota.

## 8 Therapeutic Interventions of Post-antibiotic Gut Microbiome Reconstitution

Reconstitution of the human microbiome after antibiotic treatment is often slow and incomplete and, in many cases, may take months to years to revert to the naive configuration (Lankelma et al. 2017). Faecal microbiota transplantation (FMT), prebiotics and probiotics are the several approaches that have been proposed to constitute an effective preventive treatment for antibiotic-induced dysbiosis and associated adverse effects in healthy human gut microbiome (Olek et al. 2017; Allen et al. 2013).

Faecal microbiota transplantation (FMT) refers to the transplantation of functional bacteria in the faeces of healthy donors into the gastrointestinal tract of the patient to restore the stability of the intestinal microecology, which subsequently treats diseases associated with disorders of intestinal microorganisms (Rossen et al. 2015). Now, FMT has been used experimentally to treat GI diseases including colitis, constipation, IBD, chronic fatigue syndrome, Parkinson's disease and multiple sclerosis by the production of antimicrobials and activation of the immune system of the mucous layer (Zhang et al. 2012). FMT was first reported as a novel treatment and was found to be effective against pseudomembranous enterocolitis and *C. difficile* infection (CDI) in 1958. The success rate of FMT on CDI patients was found to be over 90% of cases, compared with cure rates of 26–30% with the previous standard of care to antibiotic treatment (Eiseman et al. 1958). CDI is typically induced by exposure to different antibiotics, which reduce the indigenous gut flora. Disruption of the host's gut microbiota allows opportunistic and resistant microbes better access to intestinal nutrients. Depletion of microbial diversity also reduces the host's natural defences against pathogenic bacteria, including antimicrobial peptides and bile acids, both of which effectively control *C. difficile* expansion and spore formation. Recent studies on CDI patients showed profound dysbiosis commonly characterized by the complete disappearance of Bacteroidetes with a marked reduction in Firmicutes and massive increases in the relative abundances of Proteobacteria (Bagdasarian et al. 2015; Weingarden et al. 2016). FMT increased the abundance of the family *Bacteroidaceae* and phylum Firmicutes and reduced the population of Proteobacteria in CDI patients. In dysbiosis of normal microbiota in CDI, the presence of primary bile salts such as taurocholate, cholate and chenodeoxycholic acid stimulates the germination of *C. difficile* spore. In contrast, post-FMT like bactericidal peptides such as thuricin CD, produced by *Bacillus thuringiensis*, and nisin, produced by Gram-positive bacteria, both have high potency against certain Gram-positive microbes and inhibit spore germination as well as vegetative growth of *C. difficile* (Weingarden et al. 2014; Bakken et al. 2011). FMT can also increase the microbial diversity of the intestines, maintain the intestinal microecological balance and rebuild the function of the immune system by restoring Toll-like receptor (TLR) signalling. FMT also restores the metabolism of secondary bile acids in the intestines, which makes the metabolism of secondary bile acids in the gastrointestinal tract of patients similar to that of donors and improves insulin resistance (Borody and Khoruts 2011). FMT is a highly effective therapy for recurring and refractory CDI. However, the use of FMT requires strict principles regarding the enhanced composition of intestinal microbiota, based on the composition and functions of microorganisms that fit the causative disease to be treated, the age of the recipient and the stage of disease progression (Kelly et al. 1994).

While probiotics introduce beneficial bacteria into the gut, prebiotics act as a fertilizer for the beneficial gut bacteria that is already there. Prebiotics can also be defined as a class of compounds or food ingredient which beneficially affects the host by selectively stimulating the growth and/or activity of one or a limited number of beneficial bacteria in the gut and therefore improving host health (Gibson and Roberfroid 1995; Davani-Davari et al. 2019). Several evidences demonstrate that the

direction of different prebiotics (inulin, fructooligosaccharides) modulates the gut microbiota by stimulating beneficial indigenous flora while inhibiting the growth of pathogenic bacteria therein. The most efficient prebiotics may also reduce or suppress the numbers and activities of pathogenic organisms. Prebiotics naturally exist in many foods (fruits and vegetables), which people may consume regularly, basically, foods that are high in fibre (Gibson et al. 2017). Two of the best-known prebiotics are inulin and trans-galactooligosaccharides (TOS) that occur naturally in foods such as garlic, onions, leeks, shallots, asparagus, spinach, Jerusalem artichokes, chicory, peas, beans, lentils, oats, apples and bananas (de Cossio et al. 2017; Akram et al. 2019). Different in vitro data supporting the presence of resistant fibres or starches in the diet have been shown to benefit or increase the numbers of *Lactobacillus* and *Bifidobacterium* genera in the intestinal tract (Radke et al. 2017). Different scientific studies expose that most targets for prebiotic use are *Lactobacilli* and *Bifidobacteria* and it is mostly based on their success in the probiotic area (Charbonneau et al. 2016). Prebiotics will then be a sole tool to create colonic microflora with controlled compositions that will then be correlated with specific physiological conditions (Cremon et al. 2018; Sanders et al. 2019).

Probiotics are commensal or nonpathogenic 'live microorganisms that, when administered in adequate amounts, confer a health benefit to the host' (FAO/WHO 2002). Numerous probiotic strains have been investigated for clinical efficiency, including multiple bacteria (*Lactobacillus*, *Bifidobacteria*, *Enterococcus* and streptococci) and fungi (*Saccharomyces boulardii* and *S. cerevisiae*). But common commercially available probiotics are *Bifidobacterium, Lactobacillus* and *Streptococcus* genera as well as yeasts of the *Saccharomyces* genus either singly or in combinations (Ni et al. 2019). The action of probiotics is ascribed through several mechanisms such as competitive exclusion, antibacterial effects, competition with pathogens for nutrients and receptor binding, fortification of the mucosal barrier, promotion of innate and adaptive immune responses of the host, production of bacteriocins and signalling molecules (Sanders et al. 2019). Probiotics have been used with success as preventive to reduce the incidence of diarrhoea in the community as well as hospital settings. Probiotics have also been used successfully to reduce the severity and duration of diarrhoea, especially in children with rotavirus infection. *Lactobacillus* GG and *S. boulardii* are the probiotic organisms used in over 13 controlled trails whose therapeutic efficacy was most marked against rotavirus diarrhoea as shown by Szajewska et al. (2016). A recent meta-analysis also showed that *Lactobacillus* GG is associated with moderate clinical benefit in the treatment of acute diarrhoea in children (Szajewska et al. 2006). A recent meta-analysis has confirmed the efficiency of probiotics in the prevention of antibiotic-associated diarrhoea (AAD) (Guo et al. 2019). Prevention of *C. difficile* infection has been successfully achieved with the administration of *S. boulardii* and *Lactobacillus* GG probiotics which were safe and had significant efficacy in the prevention of AAD (Azad et al. 2018).

## 9 Conclusion

The human gut microbiome maintains homeostasis and symbiotic relationship with the host. Antibiotics have a tremendous impact on alteration of the gut microbiota composition and function, i.e. dysbiosis, which may lead to several diseases, including colon cancer, IBD, obesity, NAFLD, etc. Increasing resistance of gut microbiota to several antibiotics is becoming a major threat to human health and also a global challenge for infection control. Recently, metagenomic analysis has been widely used for rapid identification of the composition of the gut microbiota and new and unknown functional ARGs in pathogenic as well as commensal bacteria. Metagenomic data are useful in identifying the human gut microbiome carrying ARGs and studying the dynamics of their acquisition/exchange in the human microbiome. Further investigations are necessary to identify synthesized molecules of gut microbiota and their association with symbiosis, dysbiosis and diseases.

## References

Acharya C, Bajaj JS (2017) Gut microbiota and complications of liver disease. Gastroenterol Clin North Am 46:155–169. https://doi.org/10.1016/j.gtc.2016.09.013

Ahn J, Sinha R, Pei Z et al (2013) Human gut microbiome and risk for colorectal cancer. J Natl Cancer Inst 105:1907–1911. https://doi.org/10.1093/jnci/djt300

Akram W, Garud N, Joshi R (2019) Role of inulin as prebiotics on inflammatory bowel disease. Drug Discov Ther 13:1–8. https://doi.org/10.5582/ddt.2019.01000

Allen SJ, Wareham K, Wang D et al (2013) Lactobacilli and bifidobacteria in the prevention of antibiotic-associated diarrhoea and Clostridium difficile diarrhoea in older inpatients (PLACIDE): a randomised, double-blind, placebo-controlled, multicentre trial. Lancet 382:1249–1257. https://doi.org/10.1016/S0140-6736(13)61218-0

Anukam K (2017) Effects of ampicillin on the gut microbiome of an adult male as determined by 16S rRNA V4 metagenomics sequencing and green genes bioinformatics suite. J Adv Microbiol 7:1–18. https://doi.org/10.9734/JAMB/2017/38867

Arboleya S, Sánchez B, Milani C et al (2015) Intestinal microbiota development in preterm neonates and effect of perinatal antibiotics. J Pediatr 166:538–544. https://doi.org/10.1016/j.jpeds.2014.09.041

Arthur J, Jobin C (2011) The struggle within: microbial influences on colorectal cancer. Inflamm Bowel Dis 17:396–409. https://doi.org/10.1002/ibd.21354

Arumugam M, Raes J, Pelletier E et al (2011) Enterotypes of the human gut microbiome. Nature 473:174–180. https://doi.org/10.1038/nature09944

Azad MAK, Sarker M, Li T, Yin J (2018) Probiotic species in the modulation of gut microbiota: an overview. Biomed Res Int 2018:9478630. https://doi.org/10.1155/2018/9478630

Backhed F, Ley RE, Sonnenburg JL (2005) Host-bacterial mutualism in the human intestine. Science 307:1915–1920. https://doi.org/10.1126/science.1104816

Bäckhed F, Fraser CM, Ringel Y et al (2012) Defining a healthy human gut microbiome: current concepts, future directions, and clinical applications. Cell Host Microbe 12:611–622. https://doi.org/10.1016/j.chom.2012.10.012

Backhed F, Roswall J, Peng Y et al (2015) Dynamics and stabilization of the human gut microbiome during the first year of life. Cell Host Microbe 17:690–703. https://doi.org/10.1016/j.chom.2015.04.004

Bag S, Ghosh TS, Banerjee S et al (2019) Molecular insights into antimicrobial resistance traits of commensal human gut microbiota. Microb Ecol 77:546–557. https://doi.org/10.1007/s00248-018-1228-7

Bagdasarian N, Rao K, Malani PN (2015) Diagnosis and treatment of *Clostridium difficile* in adults: a systematic review. JAMA 313:398–408. https://doi.org/10.1001/jama.2014.17103

Bakken JS, Borody T, Brandt LJ et al (2011) Treating Clostridium difficile infection with fecal microbiota transplantation. Clin Gastroenterol Hepatol 9:1044–1049

Balmer SE, Wharton BA (1989) Diet and faecal flora in the newborn: breast milk and infant formula. Arch Dis Child 64:1672–1677. https://doi.org/10.1136/adc.64.12.1672

Banawas SS (2018) Clostridium difficile infections: a global overview of drug sensitivity and resistance mechanisms. Biomed Res Int 21:8414257. https://doi.org/10.1155/2018/8414257

Bartlett JG (2002) Clinical practice. Antibiotic-associated diarrhea. N Engl J Med 346:334–339. https://doi.org/10.1056/NEJMcp011603

Bäumler AJ, Sperandio V (2016) Interactions between the microbiota and pathogenic bacteria in the gut. Nature 535(7610):85–93

Becattini S, Taur Y, Pamer EG (2016) Antibiotic-induced changes in the intestinal microbiota and disease. Trends Mol Med 22:458–478. https://doi.org/10.1016/j.molmed.2016.04.003

Benson AK, Kelly SA, Legge R et al (2010) Individuality in gut microbiota composition is a complex polygenic trait shaped by multiple environmental and host genetic factors. Proc Natl Acad Sci U S A 107:18933–18938. https://doi.org/10.1073/pnas.1007028107

Bergan T, Nord CE, Thorsteinsson SB (1991) Effect of meropenem on the intestinal microflora. Eur J Clin Microbiol Infect Dis 10:524–527. https://doi.org/10.1007/bf01963943

Bien J, Palagani V, Bozko P (2013) The intestinal microbiota dysbiosis and Clostridium difficile infection: is there a relationship with inflammatory bowel disease? Ther Adv Gastroenterol 6:53–68. https://doi.org/10.1177/1756283X12454590

Bik EM, Eckburg PB, Gill SR et al (2006) Molecular analysis of the bacterial microbiota in the human stomach. Proc Natl Acad Sci U S A 103:732–737. https://doi.org/10.1073/pnas.0506655103

Booijink CC, El-Aidy S, Rajilić-Stojanović M et al (2010) High temporal and inter-individual variation detected in the human ileal microbiota. Environ Microbiol 12:3213–3227. https://doi.org/10.1111/j.1462-2920.2010.02294

Borody TJ, Khoruts A (2011) Fecal microbiota transplantation and emerging applications. Nat Rev Gastroenterol Hepatol 9:88–96. https://doi.org/10.1038/nrgastro.2011.244

Browne H (2016) Antibiotics, gut bugs and the young. Nat Rev Microbiol 14:336. https://doi.org/10.1038/nrmicro.2016.73

Burdet C, Grall N, Linard M et al (2019) Ceftriaxone and cefotaxime have similar effects on the intestinal microbiota in human volunteers treated by standard-dose regimens. Antimicrob Agents Chemother 24;63(6). pii: e02244-18. https://doi.org/10.1128/AAC.02244-18

Bye W, Ishaq N, Bolin TD et al (2014) Overgrowth of the indigenous gut microbiome and irritable bowel syndrome. World J Gastroenterol 20:2449–2455. https://doi.org/10.3748/wjg.v20.i10.244

Caitriona MG, Paul DC (2013) Role of the gut microbiota in health and chronic gastrointestinal disease: understanding a hidden metabolic organ. Ther Adv Gastroenterol 6:295–308. https://doi.org/10.1177/1756283X13482996

Cani PD, Delzenne NM (2009) The role of the gut microbiota in energy metabolism and metabolic disease. Curr Pharm Des 15:1546–1558. https://doi.org/10.2174/138161209788168164

Canny GO, McCormick BA (2008) Bacteria in the intestine, helpful residents or enemies from within? Infect Immun 76:3360–3373. https://doi.org/10.1128/IAI.00187-08

Carding S, Verbeke K, Vipond DT et al (2015) Dysbiosis of the gut microbiota in disease. Microb Ecol Health Dis 26:26191. https://doi.org/10.3402/mehd.v26.26191

Carroll IM, Ringel-Kulka T, Siddle JP (2012) Alterations in composition and diversity of the intestinal microbiota in patients with diarrhea-predominant irritable bowel syndrome. Neurogastroenterol Motil 24:521–530. https://doi.org/10.1111/j.1365-2982.2012.01891.x

Chakraborty R, Lam V, Kommineni S (2018) Ceftriaxone administration disrupts intestinal homeostasis, mediating noninflammatory proliferation and dissemination of commensal enterococci. Infect Immun 86:00674–00618. https://doi.org/10.1128/IAI.00674-18

Charbonneau MR, O'Donnell D, Blanton LV et al (2016) Sialylated milk oligosaccharides promote microbiota-dependent growth in models of infant undernutrition. Cell 164:859–871. https://doi.org/10.1016/j.cell.2016.01.024

Chassaing B, Etienne-Mesmin L, Gewirtz AT (2014) Microbiota-liver axis in hepatic disease. Hepatology 59:328–339. https://doi.org/10.1002/hep.26494

Chen Y, Yang F, Lu H et al (2011) Characterization of fecal microbial communities in patients with liver cirrhosis. Hepatology 54:562–572. https://doi.org/10.1002/hep.24423

Cho JA, Chinnapen DJF (2018) Targeting friend and foe: emerging therapeutics in the age of gut microbiome and disease. J Microbiol 56:183–188. https://doi.org/10.1007/s12275-018-8037-z

Coker MO, Hoen AG, Dade E et al (2020) Specific class of intrapartum antibiotics relates to maturation of the infant gut microbiota: a prospective cohort study. BJOG 127(2):217–227. https://doi.org/10.1111/1471-0528.15799

Corr SC, Gahan CG, Hill C (2007) Impact of selected Lactobacillus and Bifidobacterium species on Listeria monocytogenes infection and the mucosal immune response. FEMS Immunol Med Microbiol 50:380–388. https://doi.org/10.1111/j.1574-695X.2007.00264.x

Cremon C, Barbaro MR, Ventura M, Barbara G (2018) Pre- and probiotic overview. Curr Opin Pharmacol 43:87–92. https://doi.org/10.1016/j.coph.2018.08.010

Davani-Davari D, Negahdaripour M, Karimzadeh I et al (2019) Prebiotics: definition, types, sources, mechanisms, and clinical applications. Foods 8:E92. https://doi.org/10.3390/foods8030092

Davies J, Davies D (2010) Origins and evolution of antibiotic resistance. Microbiol Mol Biol Rev 74:417–433. https://doi.org/10.1128/MMBR.00016-10

Davis CD (2016) The gut microbiome and its role in obesity. Nutr Today 51:167–174. https://doi.org/10.1097/NT.0000000000000167

de Cossio LF, Fourrier C, Sauvant J et al (2017) Impact of prebiotics on metabolic and behavioral alterations in a mouse model of metabolic syndrome. Brain Behav Immun 64:33–49. https://doi.org/10.1016/j.bbi.2016.12.022

de Goffau MC, Fuentes S, van den Bogert B et al (2014) Aberrant gut microbiota composition at the onset of type 1 diabetes in young children. Diabetologia 57:1569–1577. https://doi.org/10.1007/s00125-014-3274-0

Del Chierico F, Vernocchi P, Dallapiccola B, Putignani L (2014) Mediterranean diet and health: food effects on gut microbiota and disease control. Int J Mol Sci 15:11678–11699. https://doi.org/10.3390/ijms150711678

Dethlefsen L, Eckburg PB, Bik EM, Relman DA (2006) Assembly of the human intestinal microbiota. Trends Ecol Evol 21:517–523. https://doi.org/10.1016/j.tree.2006.06.013

Dobson A, Cotter PD, Ross RP, Hill C (2012) Bacteriocin production: a probiotic trait? Appl Environ Microbiol 78:1–6. https://doi.org/10.1128/AEM.05576-11

Dominguez-Bello MG, De Jesus-Laboy KM, Shen N et al (2016) Partial restoration of the microbiota of caesarean-born infants via vaginal microbial transfer. Nat Med 22:250–253. https://doi.org/10.1038/nm.4039

Dominguez-Bello MG, Godoy-Vitorino F, Knight R, Blaser MJ (2019) Role of the microbiome in human development. Gut 68:1108–1114. https://doi.org/10.1136/gutjnl-2018-317503

Donaldson GP, Lee SM, Mazmanian SK (2016) Gut biogeography of the bacterial microbiota. Nat Rev Microbiol 14:20–32. https://doi.org/10.1038/nrmicro3552

Dudek-Wicher RK, Junka A, Bartoszewicz M (2018) The influence of antibiotics and dietary components on gut microbiota. Prz Gastroenterol 13:85–92. https://doi.org/10.5114/pg.2018.76005

Durack J, Lynch SV (2019) The gut microbiome: relationships with disease and opportunities for therapy. J Exp Med 216:20–40. https://doi.org/10.1084/jem.20180448

Eiseman Á, Silen W, Bascom G, Kauvar A (1958) Fecal enema as an adjunct in the treatment of pseudomembranous enterocolitis. Surgery 44:854–859

Exner M, Bhattacharya S, Christiansen B et al (2017) Antibiotic resistance: what is so special about multidrug-resistant Gram-negative bacteria? GMS Hyg Infect Control 10:12. https://doi.org/10.3205/dgkh000290

Fanaro S, Chierici R, Guerrini P, Vigi V (2003) Intestinal microflora in early infancy: composition and development. Acta Paediatr Suppl 91:48–55

FAO/WHO (2002) Guidelines for the evaluation of probiotics in food. https://www.who.int/foodsafety/fs_management/en/probiotic_guidelines.pdf

Feng J, Li B, Jiang X et al (2018) Antibiotic resistome in a large-scale healthy human gut microbiota deciphered by metagenomic and network analyses. Environ Microbiol 20:355–368. https://doi.org/10.1111/1462-2920.14009

Forslund K, Sunagawa S, Kultima JR (2013) Country-specific antibiotic use practices impact the human gut resistome. Genome Res 23:1163–1169. https://doi.org/10.1101/gr.155465.113

Francino MP (2016) Antibiotics and the human gut microbiome: dysbioses and accumulation of resistances. Front Microbiol 6:1543. https://doi.org/10.3389/fmicb.2015.01543

Fuller CW, Middendorf LR, Benner SA et al (2009) The challenges of sequencing by synthesis. Nat Biotechnol 27:1013–1023. https://doi.org/10.1038/nbt.1585

Furrie E (2006) A molecular revolution in the study of intestinal microflora. Gut 55:141–143. https://doi.org/10.1136/gut.2005.081695

Gagliardi A, Totino V, Cacciotti F et al (2018) Rebuilding the gut microbiota ecosystem. Int J Environ Res Public Health 7(15):E1679. https://doi.org/10.3390/ijerph15081679

Gensollen T, Iyer SS, Kasper DL, Blumberg RS (2016) How colonization by microbiota in early life shapes the immune system. Science 352:539–544. https://doi.org/10.1126/science.aad9378

Gibson GR, Roberfroid MB (1995) Dietary modulation of the human colonic microbiota: introducing the concept of prebiotics. J Nutr 125:1401–1412. https://doi.org/10.1093/jn/125.6.1401

Gibson MK, Crofts TS, Dantas G (2015) Antibiotics and the developing infant gut microbiota and resistome. Curr Opin Microbiol 27:51–56. https://doi.org/10.1016/j.mib.2015.07.007

Gibson MK, Wang B, Ahmadi S et al (2016) Developmental dynamics of the preterm infant gut microbiota and antibiotic resistome. Nat Microbiol 1:16024. https://doi.org/10.1038/nmicrobiol.2016.24

Gibson GR, Hutkins R, Sanders ME et al (2017) Expert consensus document: the International Scientific Association for Probiotics and Prebiotics (ISAPP) consensus statement on the definition and scope of prebiotics. Nat Rev Gastroenterol Hepatol 14:491–502. https://doi.org/10.1038/nrgastro.2017.75

Gosalbes MJ, Vallès Y, Jiménez-Hernández N (2016) High frequencies of antibiotic resistance genes in infants' meconium and early fecal samples. J Dev Orig Health Dis 7:35–44. https://doi.org/10.1017/S2040174415001506

Guo Q, Goldenberg JZ, Humphrey C (2019) Probiotics for the prevention of pediatric antibiotic-associated diarrhea. Cochrane Database Syst Rev 30:CD004827. https://doi.org/10.1002/14651858.CD004827.pub5

Gupta SK, Padmanabhan BR, Diene SM (2014) ARG-ANNOT, a new bioinformatic tool to discover antibiotic resistance genes in bacterial genomes. Antimicrob Agents Chemother 58:212–220. https://doi.org/10.1128/AAC.01310-13

Ha CW, Lam YY, Holmes AJ (2014) Mechanistic links between gut microbial community dynamics, microbial functions and metabolic health. World J Gastroenterol 20:16498–16517. https://doi.org/10.3748/wjg.v20.i44.16498

Handelsman J, Rondon MR, Brady SF et al (1998) Molecular biological access to the chemistry of unknown soil microbes: a new frontier for natural products. Chem Biol 5:245–249. https://doi.org/10.1016/s1074-5521(98)90108-9

Hansen CH, Nielsen DS, Kverka M et al (2012) Patterns of early gut colonization shape future immune responses of the host. PLoS One 7:e34043. https://doi.org/10.1371/journal.pone.0034043

Heintz-Buschart A, Wilmes P (2018) Human gut microbiome: function matters. Trends Microbiol 26:563–574. https://doi.org/10.1016/j.tim.2017.11.002

Hirakata Y, Srikumar R, Poole K (2002) Multidrug efflux systems play an important role in the invasiveness of Pseudomonas aeruginosa. J Exp Med 196:109–118. https://doi.org/10.1084/jem.20020005

Hold GL, Smith M, Grange C, Watt ER et al (2014) Role of the gut microbiota in inflammatory bowel disease pathogenesis: what have we learnt in the past 10 years? World J Gastroenterol 20:1192–1210. https://doi.org/10.3748/wjg.v20.i5.1192

Hull MW, Beck PL (2004) Clostridium difficile-associated colitis. Can Fam Physician 50:1536−40, 1543−5. Review. Erratum in: Can Fam Physician. 2005 Feb;51:192

Hollister EB, Gao C, Versalovic J (2014) Compositional and functional features of the gastrointestinal microbiome and their effects on human health. Gastroenterology 146:1449–1458. https://doi.org/10.1053/j.gastro.2014.01.052

Human Microbiome Project Consortium (2012a) A framework for human microbiome research. Nature 486:215–221. https://doi.org/10.1038/nature11209

Human Microbiome Project Consortium (2012b) Structure, function and diversity of the healthy human microbiome. Nature 486:207–214. https://doi.org/10.1038/nature11234

Iizumi T, Battaglia T, Ruiz V, Perez Perez GI (2017) Gut microbiome and antibiotics. Arch Med Res 48:727–734. https://doi.org/10.1016/j.arcmed.2017.11.004

Isaac S, Scher JU, Djukovic A et al (2017) Short- and long-term effects of oral vancomycin on the human intestinal microbiota. J Antimicrob Chemother 72:128–136. https://doi.org/10.1093/jac/dkw383

Jakobsson HE, Jernberg C, Andersson AF et al (2010) Short-term antibiotic treatment has differing long-term impacts on the human throat and gut microbiome. PLoS One 5:e9836. https://doi.org/10.1371/journal.pone.0009836

Jandhyala SM, Talukdar R, Subramanyam C et al (2015) Role of the normal gut microbiota. World J Gastroenterol 21:8787–8803. https://doi.org/10.3748/wjg.v21.i29.8787

Jernberg C, Löfmark S, Edlund C, Jansson JK (2010) Long-term impacts of antibiotic exposure on the human intestinal microbiota. Microbiology 156:3216–3223. https://doi.org/10.1099/mic.0.040618-0

Jia W, Li H, Zhao L, Nicholson JK (2008) Gut microbiota: a potential new territory for drug targeting. Nat Rev Drug Discov 7:123–129. https://doi.org/10.1038/nrd2505

Jin S, Zhao D, Cai C et al (2017) Low-dose penicillin exposure in early life decreases Th17 and the susceptibility to DSS colitis in mice through gut microbiota modification. Sci Rep 7:43662. https://doi.org/10.1038/srep43662

Jung JY, Ahn Y, Khare S, Gokulan K (2018) An in vitro study to assess the impact of tetracycline on the human intestinal microbiome. Anaerobe 49:85–94. https://doi.org/10.1016/j.anaerobe.2017.12.011

Kamada N, Chen GY, Inohara N, Núñez G (2013) Control of pathogens and pathobionts by the gut microbiota. Nat Immunol 14:685–690. https://doi.org/10.1038/ni.2608

Karami N, Martner A, Enne VI et al (2007) Transfer of an ampicillin resistance gene between two Escherichia coli strains in the bowel microbiota of an infant treated with antibiotics. J Antimicrob Chemother 60:1142–1145. https://doi.org/10.1093/jac/dkm327

Kataoka K (2016) The intestinal microbiota and its role in human health and disease. J Med Invest 63:27–37. https://doi.org/10.2152/jmi.63.27

Keeney KM, Yurist-Doutsch S, Arrieta MC, Finlay BB (2014) Effects of antibiotics on human microbiota and subsequent disease. Annu Rev Microbiol 68:217–235. https://doi.org/10.1146/annurev-micro-091313-103456

Keith JW, Pamer EG (2019) Enlisting commensal microbes to resist antibiotic-resistant pathogens. J Exp Med 216:10–19. https://doi.org/10.1084/jem.20180399

Kelly CP, Pothoulakis C, LaMont JT (1994) Clostridium difficile colitis. N Engl J Med 330:257–262. https://doi.org/10.1056/NEJM199401273300406
Khoruts A (2018) Targeting the microbiome: from probiotics to fecal microbiota transplantation. Genome Med 10:80. https://doi.org/10.1186/s13073-018-0592-8
Kim S, Covington A, Pamer EG (2017) The intestinal microbiota: antibiotics, colonization resistance, and enteric pathogens. Immunol Rev 279:90–105. https://doi.org/10.1111/imr.12563
Knecht H, Neulinger SC, Heinsen FA et al (2014) Effects of β-lactam antibiotics and fluoroquinolones on human gut microbiota in relation to Clostridium difficile associated diarrhea. PLoS One 9:89417. https://doi.org/10.1371/journal.pone.0089417
Koenig JE, Spor A, Scalfone N et al (2011) Succession of microbial consortia in the developing infant gut microbiome. Proc Natl Acad Sci U S A 108:4578–4585. https://doi.org/10.1073/pnas.1000081107
Kommineni S, Bretl DJ, Lam V et al (2015) Bacteriocin production augments niche competition by enterococci in the mammalian gastrointestinal tract. Nature 526:719–722. https://doi.org/10.1038/nature15524
Korpela K, Salonen A, Virta LJ et al (2016) Intestinal microbiome is related to lifetime antibiotic use in Finnish pre-school children. Nat Commun 7:10410. https://doi.org/10.1038/ncomms10410
Kumar P, Bag S, Ghosh TS et al (2017) Molecular insights into antimicrobial resistance traits of multidrug resistant enteric pathogens isolated from India. Sci Rep 7:14468. https://doi.org/10.1038/s41598-017-14791-1
Lange K, Buerger M, Stallmach A, Bruns T (2016) Effects of antibiotics on gut microbiota. Dig Dis 34:260–268. https://doi.org/10.1159/000443360
Lankelma JM, Cranendonk DR, Belzer C (2017) Antibiotic-induced gut microbiota disruption during human endotoxemia: a randomised controlled study. Gut 66:1623–1630. https://doi.org/10.1136/gutjnl-2016-312132
Leclercq S, Mian FM, Stanisz AM et al (2017) Low-dose penicillin in early life induces long-term changes in murine gut microbiota, brain cytokines and behavior. Nat Commun 8:15062. https://doi.org/10.1038/ncomms15062
Lepage P, Häsler R, Spehlmann ME et al (2011) Twin study indicates loss of interaction between microbiota and mucosa of patients with ulcerative colitis. Gastroenterology 141:227–236. https://doi.org/10.1053/j.gastro.2011.04.011
Lester CH, Frimodt-Møller N, Sørensen TL et al (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. Antimicrob Agents Chemother 50:596–599. https://doi.org/10.1128/AAC.50.2.596-599.2006
Levy SB, Marshall B (2004) Antibacterial resistance worldwide: causes, challenges and responses. Nat Med 10:122–129. https://doi.org/10.1038/nm1145
Ley RE, Peterson DA, Gordon JI (2006) Ecological and evolutionary forces shaping microbial diversity in the human intestine. Cell 124:837–848. https://doi.org/10.1016/j.cell.2006.02.017
Li B, Yang Y, Ma L et al (2015) Metagenomic and network analysis reveal wide distribution and co-occurrence of environmental antibiotic resistance genes. ISME J 9:2490–2502. https://doi.org/10.1038/ismej.2015.59
Li D, Wang P, Wang P et al (2016) The gut microbiota: a treasure for human health. Biotechnol Adv 34:1210–1224. https://doi.org/10.1016/j.biotechadv.2016.08.003
Li R, Wang H, Shi Q et al (2017) Effects of oral florfenicol and azithromycin on gut microbiota and adipogenesis in mice. PLoS One 12:e0181690. https://doi.org/10.1371/journal.pone.0181690
Liu B, Pop M (2009) ARDB—antibiotic resistance genes database. Nucleic Acids Res 37:443–447. https://doi.org/10.1093/nar/gkn656
Lozupone CA, Stombaugh JI, Gordon JI et al (2012) Diversity, stability and resilience of the human gut microbiota. Nature 489:220–230. https://doi.org/10.1038/nature11550
Mandal RS, Saha S, Das S (2015) Metagenomic surveys of gut microbiota. Genomics Proteomics Bioinformatics 13:148–158. https://doi.org/10.1016/j.gpb.2015.02.005

Marchesi JR, Adams DH, Fava F et al (2016) The gut microbiota and host health: a new clinical frontier. Gut 65:330–339. https://doi.org/10.1136/gutjnl-2015-309990

Marker LM, Hammer AS, Andresen L et al (2017) Short-term effect of oral amoxicillin treatment on the gut microbial community composition in farm mink. FEMS Microbiol Ecol 1:93. https://doi.org/10.1093/femsec/fix092

Martinez JL (2014) General principles of antibiotic resistance in bacteria. Drug Discov Today Technol 11:33–39. https://doi.org/10.1016/j.ddtec.2014.02.001

McArthur AG, Waglechner N, Nizam F et al (2013) The comprehensive antibiotic resistance database. Antimicrob Agents Chemother 57:3348–3357. https://doi.org/10.1128/AAC.00419-13

Mitsou EK, Kirtzalidou E, Pramateftaki P, Kyriacou A (2010) Antibiotic resistance in faecal microbiota of Greek healthy infants. Benef Microbes 1:297–306. https://doi.org/10.3920/BM2010.0007

Mohajeri MH, Brummer RJM, Rastall RA et al (2018) The role of the microbiome for human health: from basic science to clinical applications. Eur J Nutr 57:1–14. https://doi.org/10.1007/s00394-018-1703-4

Moore AM, Patel S, Forsberg KJ et al (2013) Pediatric fecal microbiota harbor diverse and novel antibiotic resistance genes. PLoS One 8:e78822. https://doi.org/10.1371/journal.pone.0078822

Munita JM, Arias CA (2016) Mechanisms of antibiotic resistance. Microbiol Spectr 4(2). https://doi.org/10.1128/microbiolspec.VMBF-0016-2015

Ni Y, Yang X, Zheng L et al (2019) Lactobacillus and bifidobacterium improves physiological function and cognitive ability in aged mice by the regulation of gut microbiota. Mol Nutr Food Res 21:1900603. https://doi.org/10.1002/mnfr.201900603

Nobel YR, Cox LM, Kirigin FF et al (2015) Metabolic and metagenomic outcomes from early-life pulsed antibiotic treatment. Nat Commun 6:7486. https://doi.org/10.1038/ncomms8486

Nogacka A, Salazar N, Suárez M et al (2017) Impact of intrapartum antimicrobial prophylaxis upon the intestinal microbiota and the prevalence of antibiotic resistance genes in vaginally delivered full-term neonates. Microbiome 5:93. https://doi.org/10.1186/s40168-017-0313-3

Nogacka AM, Salazar N, Arboleya S, Suárez M et al (2018) Early microbiota, antibiotics and health. Cell Mol Life Sci 75:83–91. https://doi.org/10.1007/s00018-017-2670-2

Nord CE, Brismar B, Kasholm-Tengve B, Tunevall G (1993) Effect of piperacillin/tazobactam treatment on human bowel microflora. J Antimicrob Chemother 31:61–65. https://doi.org/10.1093/jac/31.suppl_a.61

O'Hara AM, Shanahan F (2006) The gut flora as a forgotten organ. EMBO Rep 7:688–693. https://doi.org/10.1038/sj.embor.7400731

Olek A, Woynarowski M, Ahrén IL (2017) Efficacy and safety of Lactobacillus plantarum DSM 9843 (LP299V) in the prevention of antibiotic-associated gastrointestinal symptoms in children-randomized, double-blind, placebo-controlled study. J Pediatr 186:82–86. https://doi.org/10.1016/j.jpeds.2017.03.047

Palmer C, Bik EM, DiGiulio DB et al (2007) Development of the human infant intestinal microbiota. PLoS Biol 5:177. https://doi.org/10.1371/journal.pbio.0050177

Panda S, El khader I, Casellas F et al (2014) Short-term effect of antibiotics on human gut microbiota. PLoS One 9:e95476. https://doi.org/10.1371/journal.pone.0095476

Parker EPK, Praharaj I, John J (2017) Changes in the intestinal microbiota following the administration of azithromycin in a randomised placebo-controlled trial among infants in south India. Sci Rep 7:9168. https://doi.org/10.1038/s41598-017-06862-0

Pérez-Cobas AE, Artacho A, Knecht H et al (2013) Differential effects of antibiotic therapy on the structure and function of human gut microbiota. PLoS One 8:e80201. https://doi.org/10.1371/journal.pone.0080201

Pop M, Walker AW, Paulson J et al (2014) Diarrhea in young children from low-income countries leads to large-scale alterations in intestinal microbiota composition. Genome Biol 15:76. https://doi.org/10.1186/gb-2014-15-6-r76

Qin J, Li R, Raes J et al (2010) A human gut microbial gene catalogue established by metagenomics sequencing. Nature 464:59–65. https://doi.org/10.1038/nature0882

Radke M, Picaud JC, Loui A et al (2017) Starter formula enriched in prebiotics and probiotics ensures normal growth of infants and promotes gut health: a randomized clinical trial. Pediatr Res 81:622–631. https://doi.org/10.1038/pr.2016.270

Rajilić-Stojanović M, de Vos WM (2014) The first 1000 cultured species of the human gastrointestinal microbiota. FEMS Microbiol Rev 38:996–1047. https://doi.org/10.1111/1574-6976.12075

Rashid MU, Zaura E, Buijs MJ et al (2015) Determining the long-term effect of antibiotic administration on the human normal intestinal microbiota using culture and pyrosequencing methods. Clin Infect Dis 15:60. https://doi.org/10.1093/cid/civ137

Rea MC, Sit CS, Clayton E et al (2010) Thuricin CD, a posttranslationally modified bacteriocin with a narrow spectrum of activity against Clostridium difficile. Proc Natl Acad Sci U S A 107:9352–9357. https://doi.org/10.1073/pnas.0913554107

Rinninella E, Raoul P, Cintoni M et al (2019) What is the healthy gut microbiota composition? A changing ecosystem across age, environment, diet, and diseases. Microorganisms 7:E14. https://doi.org/10.3390/microorganisms7010014

Rodríguez JM, Murphy K, Stanton C et al (2015) The composition of the gut microbiota throughout life, with an emphasis on early life. Microb Ecol Health Dis 26:26050. https://doi.org/10.3402/mehd.v26.26050

Rossen NG, MacDonald JK, de Vries EM et al (2015) Fecal microbiota transplantation as novel therapy in gastroenterology: a systematic review. World J Gastroenterol 21:5359–5371. https://doi.org/10.3748/wjg.v21.i17.5359

Round JL, Mazmanian SK (2009) The gut microbiota shapes intestinal immune responses during health and disease. Nat Rev Immunol 9:313–323. https://doi.org/10.1038/nri2515

Russell SL, Gold MJ, Reynolds LA et al (2015) Perinatal antibiotic-induced shifts in gut microbiota have differential effects on inflammatory lung diseases. J Allergy Clin Immunol 135:100–109. https://doi.org/10.1016/j.jaci.2014.06.027

Sanders ME, Merenstein DJ, Reid G et al (2019) Probiotics and prebiotics in intestinal health and disease: from biology to the clinic. Nat Rev Gastroenterol Hepatol 16:605–616. https://doi.org/10.1038/s41575-019-0173-3

Savage DC (1977) Microbial ecology of the gastrointestinal tract. Annu Rev Microbiol 31:107–133. https://doi.org/10.1146/annurev.mi.31.100177.000543

Scaria J, Chandramouli U, Verma SK (2005) Antibiotic Resistance Genes Online (ARGO): a Database on vancomycin and beta-lactam resistance genes. Bioinformation 1:5–7. https://doi.org/10.6026/97320630001005

Seville LA, Patterson AJ, Scott KP et al (2009) Distribution of tetracycline and erythromycin resistance genes among human oral and fecal metagenomic DNA. Microb Drug Resist 15:159–166. https://doi.org/10.1089/mdr.2009.0916

Shendure J, Ji H (2008) Next-generation DNA sequencing. Nat Biotechnol 26:1135–1145. https://doi.org/10.1038/nbt1486

Smillie CS, Smith MB, Friedman J et al (2011) Ecology drives a global network of gene exchange connecting the human microbiome. Nature 480:241–244. https://doi.org/10.1038/nature10571

Sobhani I, Amiot A, Le Baleur Y et al (2013) Microbial dysbiosis and colon carcinogenesis: could colon cancer be considered a bacteria-related disease? Ther Adv Gastroenterol 26:215–229. https://doi.org/10.1177/1756283X12473674

Sokol H, Seksik P, Furet JP, Firmesse O, Nion-Larmurier I et al (2009) Low counts of Faecalibacterium prausnitzii in colitis microbiota. Inflamm Bowel Dis 15:1183–1189. https://doi.org/10.1002/ibd.20903

Sommer F, Bäckhed F (2013) The gut microbiota—masters of host development and physiology. Nat Rev Microbiol 11:227–238. https://doi.org/10.1038/nrmicro2974

Sommer MOA, Dantas G, Church GM (2009) Functional characterization of the antibiotic resistance reservoir in the human microflora. Science 325:1128–1131. https://doi.org/10.1126/science.1176950

Song EJ, Lee ES, Nam YD (2018) Progress of analytical tools and techniques for human gut microbiome research. J Microbiol 56:693–705. https://doi.org/10.1007/s12275-018-8238-5

Sonnenburg JL, Fischbach MA (2011) Community health care: therapeutic opportunities in the human microbiome. Sci Transl Med 3:78. https://doi.org/10.1126/scitranslmed.3001626

Stecher B, Denzler R, Maier L et al (2012) Gut inflammation can boost horizontal gene transfer between pathogenic and commensal Enterobacteriaceae. Proc Natl Acad Sci U S A 109:1269–1274. https://doi.org/10.1073/pnas.1113246109

Stewardson AJ, Gaïa N, François P et al (2015) Collateral damage from oral ciprofloxacin versus nitrofurantoin in outpatients with urinary tract infections: a culture-free analysis of gut microbiota. Clin Microbiol Infect 21:344. https://doi.org/10.1016/j.cmi.2014.11.016

Stiemsma LT, Michels KB (2018) The role of the microbiome in the developmental origins of health and disease. Pediatrics 141:e20172437. https://doi.org/10.1542/peds.2017-2437

Sullivan A, Edlund C, Nord CE (2001) Effect of antimicrobial agents on the ecological balance of human microflora. Lancet Infect Dis 1:101–114. https://doi.org/10.1016/S1473-3099(01)00066-4

Szajewska H, Ruszczyński M, Radzikowski A (2006) Probiotics in the prevention of antibiotic-associated diarrhea in children: a meta-analysis of randomized controlled trials. J Pediatr 149:367–372. https://doi.org/10.1016/j.jpeds.2006.04.053

Szajewska H, Konarska Z, Kołodziej M (2016) Probiotic bacterial and fungal strains: claims with evidence. Dig Dis 34:251–259. https://doi.org/10.1159/000443359

Tanaka S, Kobayashi T, Songjinda P et al (2009) Influence of antibiotic exposure in the early postnatal period on the development of intestinal microbiota. FEMS Immunol Med Microbiol 56:80–87. https://doi.org/10.1111/j.1574-695X.2009.00553.x

Tannock GW (2001) Molecular assessment of intestinal microflora. Am J Clin Nutr 73:410–414. https://doi.org/10.1093/ajcn/73.2.410s

Tap J, Mondot S, Levenez F et al (2009) Towards the human intestinal microbiota phylogenetic core. Environ Microbiol 11:2574–2584. https://doi.org/10.1111/j.1462-2920.2009.01982.x

Tarantino G (2014) Gut microbiome, obesity-related comorbidities, and low-grade chronic inflammation. J Clin Endocrinol Metab 99:2343–2346. https://doi.org/10.1210/jc.2014-2074

Tasse L, Bercovici J, Pizzut-Serin S et al (2010) Functional metagenomics to mine the human gut microbiome for dietary fiber catabolic enzymes. Genome Res 20:1605–1612. https://doi.org/10.1101/gr.108332.110

Thursby E, Juge N (2017) Introduction to the human gut microbiota. Biochem J 474:1823–1836. https://doi.org/10.1042/BCJ20160510

Trasande L, Blustein J, Liu M et al (2013) Infant antibiotic exposures and early-life body mass. Int J Obes (Lond) 37:16–23. https://doi.org/10.1038/ijo.2012.132

Trobos M, Lester CH, Olsen JE, Frimodt-Møller N et al (2009) Natural transfer of sulphonamide and ampicillin resistance between Escherichia coli residing in the human intestine. J Antimicrob Chemother 63:80–86. https://doi.org/10.1093/jac/dkn437

Turnbaugh PJ, Ley RE, Mahowald MA et al (2006) An obesity-associated gut microbiome with increased capacity for energy harvest. Nature 444:1027–1031. https://doi.org/10.1038/nature05414

Ursell LK, Haiser HJ, Van Treuren W et al (2014) The intestinal metabolome: an intersection between microbiota and host. Gastroenterology 146:1470–1476. https://doi.org/10.1053/j.gastro.2014.03.001

Van Schaik W (2015) The human gut resistome. Philos Trans R Soc Lond B Biol Sci 370:20140087. https://doi.org/10.1098/rstb.2014.0087

Vrieze A, Out C, Fuentes S et al (2014) Impact of oral vancomycin on gut microbiota, bile acid metabolism, and insulin sensitivity. J Hepatol 60:824–831. https://doi.org/10.1016/j.jhep.2013.11.034

Wallace BD, Wang H, Lane KT et al (2010) Alleviating cancer drug toxicity by inhibiting a bacterial enzyme. Science 330:831–835. https://doi.org/10.1126/science.1191175

Walter J, Ley R (2011) The human gut microbiome: ecology and recent evolutionary changes. Annu Rev Microbiol 65:411–429. https://doi.org/10.1146/annurev-micro-090110-102830
Wang WL, Xu SY, Ren ZG et al (2015) Application of metagenomics in the human gut microbiome. World J Gastroenterol 21:803–814. https://doi.org/10.3748/wjg.v21.i3.803
Wattam AR, Abraham D, Dalay O (2014) PATRIC, the bacterial bioinformatics database and analysis resource. Nucleic Acids Res 42:581–591. https://doi.org/10.1093/nar/gkt1099
Wei X, Yan X, Zou D et al (2013) Abnormal fecal microbiota community and functions in patients with hepatitis B liver cirrhosis as revealed by a metagenomic approach. BMC Gastroenterol 13:175. https://doi.org/10.1186/1471-230X-13-175
Weingarden AR, Chen C, Bobr A, Yao D, Lu Y, Nelson VM et al (2014) Microbiota transplantation restores normal fecal bile acid composition in recurrent *Clostridium difficile* infection. Am J Physiol Gastrointest Liver Physiol 306:310–319. https://doi.org/10.1152/ajpgi.00282.2013
Weingarden AR, Chen C, Zhang N et al (2016) Ursodeoxycholic acid inhibits *Clostridium difficile* spore germination and vegetative growth, and prevents the recurrence of ileal pouchitis associated with the infection. J Clin Gastroenterol 50:624–630. https://doi.org/10.1097/MCG.0000000000000427
Weinstock GM (2012) Genomic approaches to studying the human microbiota. Nature 13 (489):250–256. https://doi.org/10.1038/nature11553
Xia Y, Zhu Y, Li Q, Lu J (2019) Human gut resistome can be country-specific. PeerJ 7:e6389. https://doi.org/10.7717/peerj.6389
Yatsunenko T, Rey FE, Manary MJ et al (2012) Human gut microbiome viewed across age and geography. Nature 486:222–227. https://doi.org/10.1038/nature11053
Yoon MY, Yoon SS (2018) Disruption of the gut ecosystem by antibiotics. Yonsei Med J 59:4–12. https://doi.org/10.3349/ymj.2018.59.1.4
Zaman SB, Hussain MA, Nye R et al (2017) A review on antibiotic resistance: alarm bells are ringing. Cureus 9:e1403. https://doi.org/10.7759/cureus.1403
Zankari E, Hasman H, Cosentino S et al (2012) Identification of acquired antimicrobial resistance genes. J Antimicrob Chemother 67:2640–2644. https://doi.org/10.1093/jac/dks261
Zhang L, Kinkelaar D, Huang Y (2011) Acquired antibiotic resistance: are we born with it? Appl Environ Microbiol 77:7134–7141. https://doi.org/10.1128/AEM.05087-11
Zhang F, Luo W, Shi Y, Fan Z, Ji G (2012) Should we standardize the 1,700-year-old fecal microbiota transplantation? Am J Gastroenterol 107:1755. https://doi.org/10.1038/ajg.2012.251
Zhang Y, Limaye PB, Renaud HJ, Klaassen CD (2014) Effect of various antibiotics on modulation of intestinal microbiota and bile acid profile in mice. Toxicol Appl Pharmacol 277:138–145. https://doi.org/10.1016/j.taap.2014.03.009
Zhao ZH, Lai JK, Qiao L, Fan JG (2019) Role of gut microbial metabolites in nonalcoholic fatty liver disease. J Dig Dis 20:181–188. https://doi.org/10.1111/1751-2980.12709
Zmora N, Suez J, Elinav E (2019) You are what you eat: diet, health and the gut microbiota. Nat Rev Gastroenterol Hepatol 16:35–56. https://doi.org/10.1038/s41575-018-0061-2
Zuo T, Ng SC (2018) The gut microbiota in the pathogenesis and therapeutics of inflammatory bowel disease. Front Microbiol 9:2247. https://doi.org/10.3389/fmicb.2018.02247

# Influence of Abiotic Factors in the Emergence of Antibiotic Resistance

Sanjucta Dutta and T. Ramamurthy

## 1 Introduction

The number of bacterial species living in all biospheres may exceed one million, and majority of them are harmless or beneficial to humans. A larger number of species are opportunistic pathogens, which only in certain conditions cause human diseases; and several more are just a part of the normal human flora, not linked to diseases. With the aim of treating infectious diseases and limiting microbial proliferation, antimicrobial agents are being developed. Many organisms produce their own antimicrobials for self-defense that protect them from other microbes concurrently existing in the same milieu. For the treatment of different infectious diseases, many of these innate antimicrobial agents are characterized, studied, and modified extensively to enhance the activity (Munita and Arias 2016; Aslam et al. 2018). Some of the antimicrobial agents are very specific to pathogens or have broad-spectrum activity affecting a wide range of microbes.

The discovery and use of antimicrobial agents have brought a big transformation in the infection management, but simultaneously generated resistance as an adaptive response in microbes (Cleveland et al. 2012; Alexander et al. 2013; Vale-Silva and Sanglard 2015; Alareqi et al. 2016; Lima et al. 2016; Brinkac et al. 2017). This mechanism is often due to acquired resistance from the same or other bacterial species, whereas in some, innate resistance plays a considerable role in antimicrobial resistance (AMR).

AMR dates centuries back, as footprint of tetracycline has been described from the skeletons of Sudanese Nubia (350–500 AD) (Bassett et al. 1980). Several antimicrobial resistance genes (ARGs) have been testified from microbiota of an eleventh-century mummy (Santiago-Rodriguez et al. 2015), millions of years-old

S. Dutta (✉)
Beth Israel Deaconess Medical Center, Boston, MA, USA

T. Ramamurthy
Translational Health Science and Technology Institute, Faridabad, Haryana, India



S. Thomas (ed.), *Antimicrobial Resistance*,
https://doi.org/10.1007/978-981-15-3658-8_4

caves, and ancient permafrost sediment (D'Costa et al. 2011; Bhullar et al. 2012). AMR is considered as one of the most important public health concerns of the twenty-first century by the World Health Organization (WHO) and has been a cause of huge economic burden globally (Munita and Arias 2016; Aslam et al. 2018). AMR bacteria infect about two million people, causing 23,000 deaths each year in the United States (Li and Webster 2018). In Europe, the fatal number is approximately 25,000 due to antibiotic resistance (Brinkac et al. 2017). Misuse or uncontrolled use of antibiotics in clinical settings is linked to rise in AMR in microbes.

AMR is common in the microbial community and has evolved with different defense and adaptive mechanisms with time (Martinez 2009; Davies and Davies 2010; Leisner et al. 2016). Several species of bacteria have remarkable genomic plasticity that helps them to survive and compete in response to antimicrobial molecules produced by the other microbes. Due to genomic plasticity, bacteria are flexible enough to use several different genetic mechanisms like mutation, horizontal gene transfer (HGT) to adopt or survive the action of antimicrobials (Fletcher 2015), and/or a wide range of adverse environmental factors. The presence of more than one ARG coupled with other factors leads to multidrug resistance (MDR).

Recently, many studies have reported the role of the environment as an important reservoir and in transmission of resistance (Martínez 2008; Wright 2010; Ashbolt et al. 2013; Bondarczuk et al. 2016; Hiltunen et al. 2017; Bengtsson-Palme et al. 2018). An increase in the level of antimicrobial-resistant bacteria (ARB) carrying ARGs, especially in water and wastewater, has been reported in several studies (Rizzo et al. 2013; Devarajan et al. 2015; Sharma et al. 2016; Li et al. 2017). The increasing concentration of ARB in different environmental niches like soil and industrial and farming wastewater is a potential threat to the ecosystems (Devarajan et al. 2015), which eventually enters the human food chain through different vectors.

Some of the ARGs found in pathogens are thought to have originated from bacteria mostly present in the external environment that exist as a result of conjugation, where genetic materials are transferred from cell to cell or acquiring genetic material from the virus by transduction (Martínez 2012; Wellington et al. 2013). After the gene-acquiring process, a microbe can transform itself by incorporating exogenous DNA from the environment into its own genome. Mobile genetic elements (MGEs) that often contain ARGs can spread very effectively between unrelated species. All these events are initiated to a certain extent due to selective forces exerted by antibiotics both in clinical and natural environmental sources like infection site, soil, water, and manure used in agriculture, which in turn results in maintenance and spread of antibiotic resistance (Huang et al. 2012; Ashbolt et al. 2013; Berendonk et al. 2015; He et al. 2016). Many findings suggest chemicals other than antibiotics also act as selective factors in stimulating AMR, which include heavy metals (Baker-Austin et al. 2006; Seiler and Berendonk 2012), pesticides (Rangasamy et al. 2018; Ramakrishnan et al. 2019), nano-materials (Qiu et al. 2012; Ding et al. 2016), disinfectants (Guo et al. 2015; Zhang et al. 2017), cosmetics (Orús et al. 2015), and disinfection derivatives (Lv et al. 2015; Li et al. 2016). Heavy metals are naturally present in the environment, and studies have proved that anthropogenic activities accelerate the release and deposition of metals in various

environments (Seiler and Berendonk 2012; Rodríguez Martín et al. 2015; Wang et al. 2015; Zhang et al. 2015; Zhang et al. 2018).

The decrease in the antibiotic use directly prevents the spread and maintenance of antibiotic resistance in clinical and in environmental settings (Salyers and Amábile-Cuevas 1997). The other factors like deposition of heavy metals from manure, anthropogenic chemicals, and micro−/nanoplastic molecules should be taken into consideration and managed accordingly. This chapter reviews different environmental factors that support in the increase of AMR.

## 2 Environmental Factors

It is well documented that antibiotic resistance is a serious health concern (Pruden et al. 2006; Peak et al. 2007; Munir et al. 2011), and an increase in the spread of the ARGs is directly responsible for the emergence of resistant bacteria. The upsurge and dissemination of the ARGs are directly associated with the constant antibiotic pressure. AMR bacteria are stimulated and signaled to transfer ARGs to other bacteria with the help of MGEs (Allen et al. 2010; Rizzo et al. 2013). With advancement of time, researchers have detected considerable rise in the level of ARGs even in the absence of antibiotic pressure (Alonso et al. 2001; Ji et al. 2012). Studies have also been conducted to identify other chemicals responsible for selecting or supporting AMR in several bacterial populations. In many of these studies, it was established that substances other than antibiotics, such as heavy metals, pesticides, detergents, microplastics, and cosmetics, could also support the spread of ARGs (Alonso et al. 2001; Baker-Austin et al. 2006; Ji et al. 2012). Several studies have successfully established the relationships between heavy metals and ARG abundance in diverse environmental compartments like soil (Zhao et al. 2019), different types of animal manure, and water (Zhao et al. 2018; Sui et al. 2019). Different factors and anthropogenic activities that drive antimicrobial resistance are discussed below.

### 2.1 Heavy Metals

In the environment, heavy metals are naturally present in abundance or deposited by various human activities. Some of these heavy metals are required in trace amounts for several cellular functions. Metals are divided into four groups based on our health requirements (Kochare and Tamir 2015): (1) Essential metals, also known as the micronutrients, are required for cellular functions; and they are the components for DNA and RNA polymerase, e.g., copper (Cu), zinc (Zn), cobalt (Co), chromium (Cr), manganese (Mn), and iron (Fe). (2) Nonessential metals, e.g., barium (Ba), aluminum (Al), and lithium (Li); (3) less toxic metals, e.g., tin (Sn) and aluminum (Al); and (4) highly toxic metals, e.g., mercury (Hg), cadmium (Cd), and arsenic (As), are considered as cellular toxins as they can form harmful complexes. Heavy

**Table 1** Similar resistance mechanisms exhibited by microbes in response to metals or antibiotics

| Resistance Mechanism | Antibiotics | Metals | References |
|---|---|---|---|
| Reduction of membrane permeability | Cip, Tet, β-lactams | Cu, Zn, Mn, Co | Ruiz et al. (2003); Knapp et al. (2017) |
| Inactivation of the antibiotic molecule | β-lactams, Chlor | As, Hg | Wright (2005) |
| Rapid efflux of the antibiotic | Chlor, Tet, β-lactams | Cu, Co, Zn, Ni | Nies (2003); Ma et al. (2019) |
| Mutation of cellular targets | Cip, β-lactams, trim | Hg, Zn, Cu | Levy (2002); Baker-Austin et al. (2006) |

metals from the environment interact with microorganisms and induce the development of resistance.

The mechanisms underlying microbial resistance to antimicrobials are for self-defense or expressed in response to other antibiotics/chemicals which are broadly classified under four different categories (Krulwich et al. 2005; Baker-Austin et al. 2006): (1) reduction of membrane permeability, (2) inactivation of the antibiotic molecules, (3) rapid efflux of the antibiotic, and/or (4) mutation of cellular targets. Microbes adopt almost similar resistance mechanisms for heavy metals as well as antimicrobials (Table 1). It is well known that different MGEs such as plasmids or transposons help the bacteria to get converted to AMR phenotype. One widely reported mechanism is integron, which is actively associated with gene transfer in the presence of metal ions. The stress exerted by heterogeneous metal ions leads to the selection of resistant bacteria. Heavy metals have long-term selection pressure on ARGs compared to antibiotics because of their nonbiodegradable nature (Stepanauskas et al. 2005). Recent studies have shown that an increase of ARGs is related to the presence of Cu and Ni in soil samples (Hu et al. 2016, 2017). Oxides of Cu, Zn, and Cd at higher concentrations have shown to increase the rate of horizontal gene transfer (HGT) but decrease conjugational transfer (Martinez et al. 2006; Suzuki et al. 2012). Few other studies have shown the increase of conjugational transfer and HGT even at sublethal concentrations, but with a different mechanism (Jutkina et al. 2018; Zhang et al. 2018).

Several reports emphasize the importance of co-occurrence of AMR and resistance to metals in the same bacteria. When a metabolic pathway is activated as a defense mechanism in response to two different antimicrobials, it is termed as a cross-reaction (Baker-Austin et al. 2006; Zhu et al. 2013; Poole 2017; Ding et al. 2019) (Fig. 1). An example of a cross-reaction is an efflux pump, in which several MDR efflux pumps help microbes sustain exposure to both antibiotics and heavy metals. In the co-resistance category, the ARGs and metal resistance genes (MRGs) are positioned on the same genetic element like integrons, plasmids, or transposons (Fig. 2). Co-resistance has been reported in *Salmonella enterica* serotype abortus equi that showed resistance to ampicillin, arsenic, chromium, cadmium, and mercury positioned in a plasmid (Ghosh et al. 2000). Removal of this plasmid makes the strain susceptible to ampicillin and all the metals (Ghosh et al. 2000).

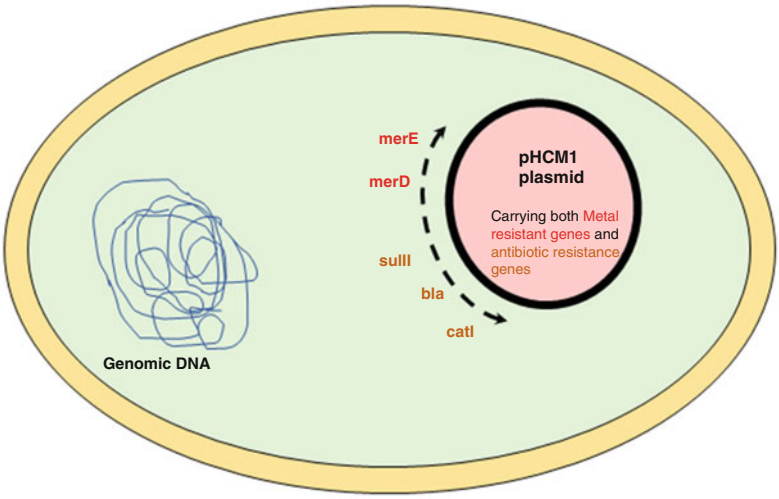


**Fig. 1** Co-selection of metal and antibiotic by (i) co-resistance: when resistance-conferring genes to both antibiotics and metals are located in the same genetic element. Due to this physical linkage, when one is expressed, it co-selects the other. pHCM1 plasmid is one such example where mercury resistance is linked to other antibiotic resistance genes

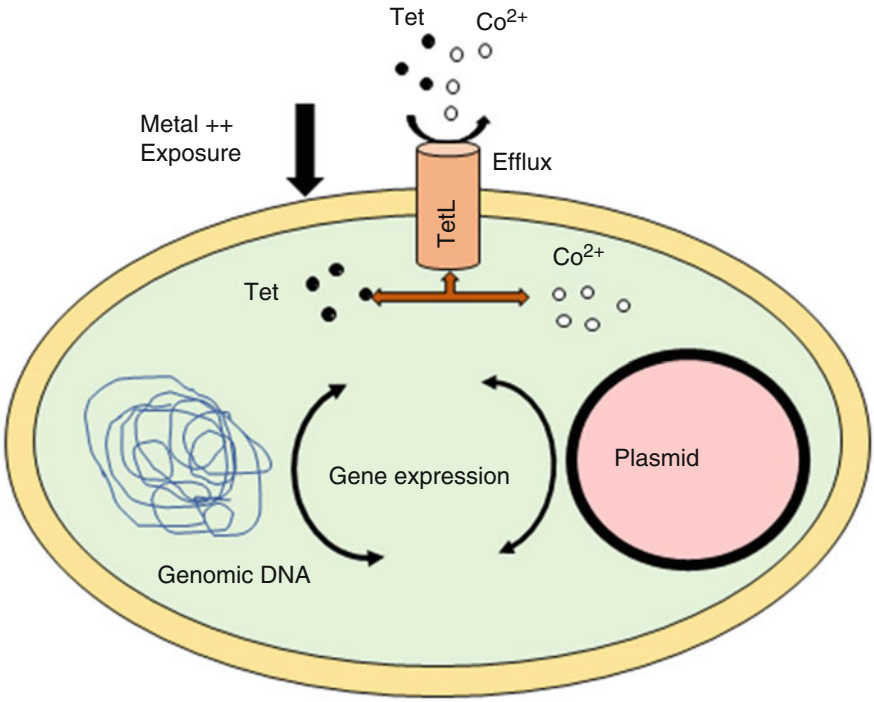


**Fig. 2** Co-selection of metal and antibiotic by (ii) cross-regulation: when the same pathway is activated as a defense mechanism in response to metals or antibiotics, for example, TetA(L) efflux pump which pumps out tetracycline and heavy metal cobalt conferring cross-resistance to both metals (cobalt) and antibiotics (tetracycline)

Heavy metals are present in all the environmental niches. Metal atoms form cations by losing electrons making them more bioavailable and express antimicrobial activity. This process is greatly influenced by several environmental conditions modified by pH, oxygen level, or concentration of organic matter. Generally, microbial toxicity of heavy metals is due to their chemical affinity for thiol groups and macro-biomolecules and also depends on the solubility of the metal compounds under physiological conditions (Lemire et al. 2013). In response to the exposure of different toxic metals, bacterial self-defense has developed a certain resistance mechanism to avoid cellular toxicity. This mechanism has been divided into four groups. (1) First is complex formation or sequestration of toxic metals (Silver and Phung 1996). By selectively binding to macromolecules as a biochemical response, the concentration of the free toxic ions in the cytoplasm is reduced. The addition of either metals or antibiotics to planktonic cells can stimulate the production of extracellular polymeric substances (EPSs), which leads to cell adhesion and ultimately the formation of a biofilm as a survival mechanism (Teitzel and Parsek 2003). Bacterial cells within biofilm can withstand a higher concentration of antimicrobial agents. The EPS matrix and the polysaccharides contained in biofilm bind toxic metals (Teitzel and Parsek 2003). As a result, tolerance of bacteria to heavy metals and antibiotics is enhanced. (2) The second mechanism is by detoxification through reduction of intracellular ions (Nies 2003). An example is mercury reductase, encoded by the *merA* gene. The MerA protein reduces $Hg^{2+}$ ions to less toxic Hg (Schiering et al. 1991). (3) Third is excretion of toxic ions by efflux systems (Nies and Silver 1995). In this, the cation/proton antiporter Czc has been identified in *Alcaligenes eutrophus* that mediates resistance to the metal ions $Cd^{2+}$, $Zn^{2+}$, and $Co^{2+}$ by removal of metals though the inner and outer membranes from the cytoplasm (Silver and Phung 1996). Genes responsible for efflux mechanism to metal resistance also confer resistance to antibiotics. (4) Fourth is change in morphology and/or pigment production. To overcome environmental change due to toxic metal contamination, a bacterial cell can undergo morphological changes and even secretes pigments. On exposure to toxic lead, *Pseudomonas aeruginosa* show pyoverdine and pyochelin pigment production (Naik et al. 2013).

In the environment, there is a continuous inflow of heavy metals due to anthropogenic, industrial, agricultural, or other human activities. Use of heavy metals such as Cd, Hg, Cu, and Zn in feeds, organic and inorganic fertilizers, and aquaculture substances has immensely contributed to metal contamination. Along with heavy metals, antibiotics are also used in different forms to increase agricultural or farm produce. Many studies have proved genetic exchange of resistance genes between environmental antibiotic-resistant bacteria and human pathogens sustained by the heavy metals in various environmental niches.

## 2.2 Pesticides

Pesticides are widely used to increase agricultural products. Improper application and storage of pesticides often contaminate plant tissues and environments that remain for a very long time. Pesticide contamination may cause changes at all levels of biological organization directly or indirectly. Terrestrial and aquatic ecosystems are polluted with pesticides due to leach-out from the agricultural fields. The presence of pesticides in these environments can induce resistance or persistence, even to degradation by the microbes. The pesticide-resistant microbes can use biofilm formation, efflux pumps, enzymes, membrane transport systems, and genetic makeup with plasmid- and chromosome-encoded catabolic genes for degradation. These pesticide degraders may also develop antimicrobial resistance as an extra functional trait (Ramakrishnan et al. 2019). Similar to heavy metal resistance genes, pesticide resistance genes are also transferred as gene clusters along with the genes crucial for antibiotic resistance. The consumption of pesticide-contaminated food products and the use of antibiotics by humans and in livestock animals have helped the development of antibiotic- and pesticide-resistant bacterial communities. Pesticide-antibiotic cross-resistance and the subsequent expansion of MDR bacteria are detailed in several reviews (Rangasamy et al. 2018; Ramakrishnan et al. 2019).

In a study, it was shown that the presence of monocrotophos insecticides in the agricultural soil led to the development of multidrug resistance among bacteria (Rangasamy et al. 2017). In the agricultural soil, *Bacillus* isolates were resistant against monocrotophos as well as antibiotics such as chloramphenicol, ampicillin, cefotaxime, streptomycin, and tetracycline. Involvement of plasmid in drug as well as insecticide resistance was confirmed through plasmid curing of resistant bacterial strains (Rangasamy et al. 2017). Several other studies also have proved that the MDR has been increased in phyto- and human pathogenic bacteria due to the presence of pesticides (Kurenbach et al. 2015; Patyka et al. 2016).

## 2.3 Anthropogenic Substances

Different anthropogenic activities lead to accumulation of substances in the environment that causes a potential rise and spread of resistant bacteria. The mode of action involved in the selection of resistant bacteria employs one or more of the mechanisms discussed before, i.e., "co-selection." Polyaromatic hydrocarbons (PAHs) enter into the environment primarily through activities like coal pyrolysis, liquid fossil fuels, and biomass combustion (Bosch et al. 2015). Some PAHs are considered hazardous to humans with carcinogenic, mutagenic, and genotoxic effects. When exposed to PAH, metabolic activation of bacterial molecules with cytochrome P450 leads to the formation of highly reactive electrophilic species binding with DNA (Binková and Srám 2004). PAH was also responsible for causing a major shift in the composition of the soil microbial community (Yang et al. 2014; Gorovtsov et al. 2018). *Proteobacteria* are prevalent in a PAH-contaminated soil along with the PAH-degrading *Streptomyces* group. This same genus of bacteria is

reported to carry ARGs (D'costa et al. 2006). Few findings indicate decreased HGT rate in the presence of PAH due to the formation of covalent bonding to the plasmids (Kang et al. 2015; Chen et al. 2017). Even when the HGT is interrupted in certain cases, the copy number of ARG can still increase with the growth of PAH-degrading bacteria (Gorovtsov et al. 2018).

## 2.4 Microplastics

Microplastics of $\geq$5.0 mm in size exist as contaminants in the environment and possess a very long half-life up to centuries (Kazmiruk et al. 2018). They are decisively made for a specific purpose (primary microplastics) or formed through natural degradation of plastics (secondary microplastics) (Rummel et al. 2017; Imran et al. 2019). Millions of tons of particles are discharged into the marine environment as industrial by-products, degraded plastic wastes, municipal wastes, fishing nets, etc. (Cole et al. 2011; Law and Thompson 2014; Keswani et al. 2016; Arias-Andres et al. 2018). Plastics causing marine pollution are mostly polyethylene (PE), polystyrene (PS), polyethylene terephthalate (PET), polyvinyl chloride (PVC), polyurethane (PUR), and polypropylene (PP) (Hidalgo-Ruz et al. 2012; Fok et al. 2017; Revel et al. 2018). Due to their very small size, microplastics are accumulated by oysters and crabs (Watts et al. 2016), get ingested by fishes, and eventually spread in the aquatic ecosystem (Nelms et al. 2018; Revel et al. 2018). Microplastics are considered as one of the most difficult pollutants to control with a long-lasting reactive surface. They can adhere to or adsorb organic matter and chemical substances like antibiotics, pesticides, heavy metals, and other xenobiotics (Hirai et al. 2011). These microplastics support colonization by different microbial species (Zettler et al. 2013; Oberbeckmann et al. 2016; Kettner et al. 2017), and these communities are collectively termed as "the plastisphere" (Keswani et al. 2016). They are also capable of changing the structure and composition of other microbial communities present in the surrounding environment (Reisser et al. 2013; Harrison et al. 2014; Oberbeckmann et al. 2014; Bryant et al. 2016).

It has been well documented that microplastics can adsorb antibiotics (sulfadiazine, ciprofloxacin, amoxicillin, trimethoprim, and tetracycline) on their surfaces and its low density often helps them get dispersed into the food chains and humans and animals (Li et al. 2018). Microplastics can vary in their physicochemical properties like specific surface area, crystallinity, and pore size distribution, which determine the adsorption capacity of each type (Li et al. 2018). It has been reported that tetracycline-containing microplastics in the soil can stimulate bacterial phage-mediated ARG dispersion (Sun et al. 2018). Increased proportion of HGT is reported among phylogenetically diverse bacteria on the microplastic surface compared to the free-living aquatic bacterial population (Arias-Andres et al. 2018). This microsurface acts as a perfect platform for selection of AMR in the presence of metal ions, antibiotics, and other pollutants.

## 2.5 Biocides

Biocides are used to control microbial growth; and these agents are used most commonly in antiseptics, disinfectants, and preservatives. Biocides functionally differ from antibiotics. Unlike antibiotics, which are very precise in action, biocides are formulated to act on multiple cellular targets and hence mostly used in higher concentrations compared to antibiotics. Biocides mostly disturb the bacterial membrane and react with the cell protein molecule or genetic material. Biocidal agents like alcohol target the bacterial membrane, whereas aldehydes and anionic surfactants affect the cell wall. In the case of hydrogen peroxide, acridine dyes, and chlorine compounds, it is the nucleic acid that gets affected (Davin-Regli and Pagès 2012).

The activity of biocides depends on the duration of contact time, pH, and temperature, the presence of organic matter or other interfering or enhancing materials/compounds, and the target microorganism. The most common adaptation mechanism expressed by microbes in response to biocide exposure is the activation of efflux pump, but some studies have observed an adaptive mechanism due to changes in protein synthesis only when biocide is present in low concentrations (Mutoh et al. 1999). Resistance to formaldehyde among members of *Enterobacteriaceae* is plasmid mediated and in *P. aeruginosa* is due to chromosomal elements. Both these resistant forms are quite stable to even grow at a concentration much higher than the lethal dose (Meyer and Cookson 2010). Biocides in certain isolates can upregulate efflux pumps by activating different biochemical pathways, which is reversed in the absence of biocides. Hence, the defense mechanisms expressed by the microbes to biocides raise the concern of simultaneous development of antimicrobial resistance as a co-selection mechanism as both the antibacterial molecules at certain extent use the similar resistance mechanisms (Pal et al. 2017; Jutkina et al. 2018; Wales and Davies 2015) (Table 2). One of the most commonly used biocides, triclosan, has received great attention in relation to the rise of resistant bugs. Triclosan is most commonly and widely used in disinfectants, preservatives, toothpastes, baby toys, and many other daily products (Westfall et al. 2019). It is bacteriostatic at lower concentrations, but is bactericidal at higher concentrations and causes cell death by affecting the plasma membrane. Just like antibiotics but unlike other biocides, triclosan at low concentrations acts on a specific cellular target. The most studied and mapped target site for triclosan is enoyl-acyl carrier protein reductase (FabI), an essential enzyme in bacterial fatty acid synthesis. Researchers have shown that mutations in FabI and its overexpression decrease bacterial susceptibility to triclosan (Heath et al. 1999; Slater-Radosti et al. 2001). Along with defective fatty acid synthesis, accumulation of the alarmone guanosine tetraphosphate (ppGpp) gives rise to persistent bacterial cells which show high resistance to antibiotics (Westfall et al. 2019). Another aspect that raises concern in biocide use is that some antibiotics share same target site FabI-like triclosan. The selective pressure created by its use may co-regulate resistance to both biocides and antibiotics (Ciusa et al. 2012).

**Table 2** Reduced antibiotic sensitivity on exposure to low-level biocide

| Species | Biocide | Reduced Sensitivity to Antibiotics | References |
|---|---|---|---|
| *E. coli* | Benzalkonium chloride, triclosan | Cefotaxime, ampicillin, tetracycline, ciprofloxacin, trimethoprim, vancomycin, gentamicin | Soumet et al. (2012) |
| *Campylobacter jejuni*, *Campylobacter coli* | Didecyldimethyl ammonium chloride and benzalkonium chloride | Ampicillin, tetracycline, gentamicin, streptomycin, erythromycin, and enrofloxacin | Donaghy et al. (2019) |
| *Pseudomonas aeruginosa* | Benzalkonium chloride | Ciprofloxacin, gentamycin, amikacin, imipenem, polymyxin B | Mc Cay et al. (2010) |
| *Salmonella typhimurium* | Benzalkonium chloride, aldehydes, quaternary ammonium compounds | Chloramphenicol, ciprofloxacin, clindamycin, erythromycin, gentamicin, tetracycline, nalidixic acid, kanamycin | Donaghy et al. (2019); Braoudaki and Hilton (2004) |
| *Enterobacter* spp. | Chlorhexidine digluconate | Cefotaxime, ceftazidime, imipenem, sulfamethoxazole | Gadea et al. (2017) |
| *Salmonella* spp. | Triclosan | Piperacillin, ceftiofur, amikacin, Gentamicin, kanamycin, nalidixic acid, cefoxitin | Condell et al. (2012) |

## 2.6 Cosmetic Products

The development of AMR microorganisms is due to the selective pressure from preservatives, which are added in the cosmetic formulations mainly to inhibit the growth of microorganisms. Cosmetic products could also be a risk for the emergence and spread of AMR bacteria. Tolerance to formaldehyde donors such as diazolidinyl urea, imidazolidinyl urea, quaternium-15, sodium hydroxymethylglycinate, and DMDM hydantoin by *Enterobacter gergoviae*, *Pseudomonas putida*, and *Burkholderia cepacia* isolated from cosmetics products has shown cross-resistance to antibiotics (Orús et al. 2015). Reduced susceptibility to formaldehyde donors was detected in isolates along with increasing resistance to β-lactams, quinolones, rifampicin, and tetracycline. The outer membrane protein modifications and efflux mechanism activities were found to be responsible for the resistance traits (Orús et al. 2015). Chlorhexidine/chlorhexidine gluconate (CHG) has been used in disinfectants, oral care, and cosmetics. In vitro exposure of *Staphylococcus aureus* to sublethal concentrations of chlorhexidine showed cross-resistance to amikacin, cefepime, and tetracycline (Wu et al. 2016). Also, studies on *Klebsiella pneumoniae* had shown that bacterial exposure to CHG induced cross-resistance to colistin, which is used in the treatment of MDR pathogens (Wand et al. 2016).

## 3 Transmission of Antibiotic Resistance from the Environment to Humans

The environment is continuously exposed to different substances favoring the emergence and spread of antimicrobial-resistant bacteria. Microbes work as a reservoir for ARGs in the environment as well as the human body. Depending on the environmental conditions and other stress factors, different bacterial species remain viable and act as potential carriers. Genetic elements of the dead bacterial cells also act as probable sources of HGTs. Many human activities exert a high level of AMR transmission (Fletcher 2015). Several abiotic stress conditions directly support the emergence of AMR mechanism, which will be sustained through several genetic mechanisms including HGTs. The human microbiota is under constant pressure with several stimulating factors of AMR. Microplastics harboring AMR bacteria and/or substances that initiate different resistant mechanisms in microbes also reach the food chain.

## 4 Mitigation Policies

With the rise of the AMR issue globally, many strategies have been proposed based on several research to clearly understand the mechanism of AMR selection and transmission. One of the risk mitigation strategies commonly and widely focused is on minimizing the uptake of antibiotics and reducing the spread of AMR from the key environmental reservoirs like sewage, wastewater, manure, farms, and antibiotic manufacture waste. The most common mitigation method is restricting the use and release of antibiotics into the environment. The principle behind this is decreasing the antibiotic load, thereby lowering the selection pressure for ARGs which eventually controls the risk of AMR (Bengtsson-Palme and Larsson 2016). With the rise in risk of AMR globally, the number of research on the same aspect is numerous. A large number of data are also available which could be translated to administer a mitigation process (Vikesland et al. 2017).

There are uses of similar antibacterial approaches in treating human and/or animal infections, and the rise of a resistant form of bacteria can spread from one to another and eventually to the environment. Each plays an important role in the global spread of AMR. With the aim of bridging the multidisciplinary knowledge gap between humans, animals, and the environment to deal with AMR risk, a very comprehensive and multisectoral approach is "One Health," where humans, animals, and the environment are considered as one. Under this initiative, the WHO, Food and Agriculture Organization (FAO) of the United Nations, World Organisation for Animal Health (OIE), Centers for Disease Control and Prevention (CDC), United States Department of Agriculture (USDA), National Oceanic and Atmospheric Administration (NOAA), and US National Environmental Health Association (NEHA) all work together in minimizing the risk of rise and spread of AMR. But it is to be remembered that there are also other selection pressures simultaneously

present in the environment responsible for ARG and AMR pool, proper understanding of which is also required.

## 5 Conclusion

The rise of antibiotic-resistant bacteria has been a major global issue for years which is facing challenges for control due to several processes and factors. Most importantly, the microbial community acts as a reservoir, and their rapid adaptive mechanisms to withstand environmental challenges are very high. As a result, long-lasting solutions for the AMR control will be a challenging issue. The resistant phenotypes will continue to occur until the establishment of a new metabolic pathway that restricts AMR acquirement and dissemination. Limiting the use of fertilizers and pesticides and controlling the effluents may reduce the ARGs in the microbial community. Replacing AMR-promoting substances in different products that causes less stress to the microbial community may limit the rise of AMR. Understanding the role of ecological factors in AMR transfer could help us devise strategies to control this fundamental evolutionary process. The most important aspect is human awareness. For this, results generated from various environmental studies should be compiled for the proper implementation of control measures.

## References

Alareqi LMQ, Mahdy MAK, Lau YL, Fong MY, Abdul-Ghani R, Mahmud R (2016) Molecular markers associated with resistance to commonly used antimalarial drugs among *Plasmodium falciparum* isolates from a malaria-endemic area in Taiz governorate-Yemen during the transmission season. Acta Trop 162:174–179. https://doi.org/10.1016/j.actatropica.2016.06.016

Alexander BD, Johnson MD, Pfeiffer CD, Jiménez-Ortigosa C, Catania J, Booker R, Castanheira M, Messer SA, Perlin DS, Pfaller MA (2013) Increasing echinocandin resistance in *Candida glabrata*: clinical failure correlates with presence of FKS mutations and elevated minimum inhibitory concentrations. Clin Infect Dis 56(12):1724–1732. https://doi.org/10.1093/cid/cit136

Allen HK, Donato J, Wang HH, Cloud-Hansen KA, Davies J, Handelsman J (2010) Call of the wild: antibiotic resistance genes in natural environments. Nat Rev Microbiol 8(4):251–259. https://doi.org/10.1038/nrmicro2312

Alonso A, Sánchez P, Martínez JL (2001) Environmental selection of antibiotic resistance genes. Environ Microbiol 3(1):1–9. https://doi.org/10.1046/j.1462-2920.2001.00161

Arias-Andres M, Klümper U, Rojas-Jimenez K, Grossart HP (2018) Microplastic pollution increases gene exchange in aquatic ecosystems. Environ Pollut 237:253–261. https://doi.org/10.1016/j.envpol.2018.02.058

Ashbolt NJ, Amézquita A, Backhaus T, Borriello P, Brandt KK, Collignon P, Coors A, Finley R, Gaze WH, Heberer T, Lawrence JR, Joakim Larsson DG, McEwen SA, Ryan JJ, Schönfeld J, Silley P, Snape JR, Van den Eede C, Topp E (2013) Human health risk assessment (HHRA) for environmental development and transfer of antibiotic resistance. Environ Health Perspect 121(9):993–1001. https://doi.org/10.1289/ehp.1206316

Aslam B, Wang W, Arshad MI, Khurshid M, Muzammil S, Rasool MH, Nisar MA, Alvi RF, Aslam MA, Qamar MU, Salamat MKF, Baloch Z (2018) Antibiotic resistance: a rundown of a global crisis. Infect Drug Resist 11:1645–1658. https://doi.org/10.2147/IDR.S173867

Baker-Austin C, Wright MS, Stepanauskas R, McArthur JV (2006) Co-selection of antibiotic and metal resistance. Trends Microbiol 14(4):176–182. https://doi.org/10.1016/j.tim.2006.02.006

Bassett EJ, Keith MS, Armelagos GJ, Martin DL, Villanueva AR (1980) Tetracycline-labeled human bone from ancient Sudanese Nubia (A.D. 350). Science 209(4464):1532–1534. https://doi.org/10.1126/science.7001623

Bengtsson-Palme J, Kristiansson E, Larsson DGJ (2018) Environmental factors influencing the development and spread of antibiotic resistance. FEMS Microbiol Rev 42(1). https://doi.org/10.1093/femsre/fux053

Bengtsson-Palme J, Larsson DG (2016) Concentrations of antibiotics predicted to select for resistant bacteria: proposed limits for environmental regulation. Environ Int 86:140–149. https://doi.org/10.1016/j.envint.2015.10.015

Berendonk TU, Manaia CM, 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 JL (2015) Tackling antibiotic resistance: the environmental framework. Nat Rev Microbiol 13(5):310–317. https://doi.org/10.1038/nrmicro3439

Bhullar K, Waglechner N, Pawlowski A, Koteva K, Banks ED, Johnston MD, Barton HA, Wright GD (2012) Antibiotic resistance is prevalent in an isolated cave microbiome. PLoS One 7(4):e34953. https://doi.org/10.1371/journal.pone.0034953

Binková B, Srám RJ (2004) The genotoxic effect of carcinogenic PAHs, their artificial and environmental mixtures (EOM) on human diploid lung fibroblasts. Mutat Res 547 (1-2):109–121. https://doi.org/10.1016/j.mrfmmm.2003.12.006

Bondarczuk K, Markowicz A, Piotrowska-Seget Z (2016) The urgent need for risk assessment on the antibiotic resistance spread via sewage sludge land application. Environ Int 87:49–55. https://doi.org/10.1016/j.envint.2015.11.011

Bosch C, Andersson A, Kruså M, Bandh C, Hovorková I, Klánová J, Knowles TD, Pancost RD, Evershed RP, Gustafsson Ö (2015) Source apportionment of polycyclic aromatic hydrocarbons in central European soils with compound-specific triple isotopes (δ(13)C, Δ(14)C, and δ(2)H). Environ Sci Technol 49(13):7657–7665. https://doi.org/10.1021/acs.est.5b01190

Braoudaki M, Hilton AC (2004) Adaptive resistance to biocides in salmonella enterica and *Escherichia coli* O157 and cross-resistance to antimicrobial agents. J Clin Microbiol 42 (1):73–78. https://doi.org/10.1128/JCM.42.1.73-78.2004

Brinkac L, Voorhies A, Gomez A, Nelson KE (2017) The threat of antimicrobial resistance on the human microbiome. Microb Ecol 74(4):1001–1008. https://doi.org/10.1007/s00248-017-0985-z

Bryant JA, Clemente TM, Viviani DA, Fong AA, Thomas KA, Kemp P, Karl DM, White AE, DeLong EF (2016) Diversity and activity of communities inhabiting plastic debris in the North Pacific gyre. mSystems 1(3):e00024–e00016. https://doi.org/10.1128/mSystems.00024-16

Chen B, He R, Yuan K, Chen E, Lin L, Chen X, Sha S, Zhong J, Lin L, Yang L, Yang Y, Wang X, Zou S, Luan T (2017) Polycyclic aromatic hydrocarbons (PAHs) enriching antibiotic resistance genes (ARGs) in the soils. Environ Pollut 220(Pt B):1005–1013. https://doi.org/10.1016/j.envpol.2016.11.047

Ciusa ML, Furi L, Knight D, Decorosi F, Fondi M, Raggi C, Coelho JR, Aragones L, Moce L, Visa P, Freitas AT, Baldassarri L, Fani R, Viti C, Orefici G, Martinez JL, Morrissey I, Oggioni MR (2012) A novel resistance mechanism to triclosan that suggests horizontal gene transfer and demonstrates a potential selective pressure for reduced biocide susceptibility in clinical strains of *Staphylococcus aureus*. Int J Antimicrob Agents 40(3):210–220. https://doi.org/10.1016/j.ijantimicag.2012.04.021

Cleveland AA, Farley MM, Harrison LH, Stein B, Hollick R, Lockhart SR, Magill SS, Derado G, Park BJ, Chiller TM (2012) Changes in incidence and antifungal drug resistance in candidemia: results from population-based laboratory surveillance in Atlanta and Baltimore, 2008-2011. Clin Infect Dis 55(10):1352–1361. https://doi.org/10.1093/cid/cis697

Cole M, Lindeque P, Halsband C, Galloway TS (2011) Microplastics as contaminants in the marine environment: a review. Mar Pollut Bull 62(12):2588–2597. https://doi.org/10.1016/j.marpolbul.2011.09.025

Condell O, Iversen C, Cooney S, Power KA, Walsh C, Burgess C, Fanning S (2012) Efficacy of biocides used in the modern food industry to control salmonella enterica, and links between biocide tolerance and resistance to clinically relevant antimicrobial compounds. Appl Environ Microbiol 78(9):3087–3097. https://doi.org/10.1128/AEM.07534-11

Davies J, Davies D (2010) Origins and evolution of antibiotic resistance. Microbiol Mol Biol Rev 74(3):417–433. https://doi.org/10.1128/MMBR.00016-10

Davin-Regli A, Pagès JM (2012) Cross-resistance between biocides and antimicrobials: an emerging question. Rev Sci Tech 31(1):89–104. Review. PMID: 22849270

D'Costa VM, King CE, Kalan L, Morar M, Sung WW, Schwarz C, Froese D, Zazula G, Calmels F, Debruyne R, Golding GB, Poinar HN, Wright GD (2011) Antibiotic resistance is ancient. Nature 477(7365):457–461. https://doi.org/10.1038/nature10388

D'Costa VM, McGrann KM, Hughes DW, Wright GD (2006) Sampling the antibiotic resistome. Science 311(5759):374–377. https://doi.org/10.1126/science.1120800

Devarajan N, Laffite A, Graham ND, Meijer M, Prabakar K, Mubedi JI, Elongo V, Mpiana PT, Ibelings BW, Wildi W, Poté J (2015) Accumulation of clinically relevant antibiotic-resistance genes, bacterial load, and metals in freshwater lake sediments in Central Europe. Environ Sci Technol 49(11):6528–6537. https://doi.org/10.1021/acs.est.5b01031

Ding C, Pan J, Jin M, Yang D, Shen Z, Wang J, Zhang B, Liu W, Fu J, Guo X, Wang D, Chen Z, Yin J, Qiu Z, Li J (2016) Enhanced uptake of antibiotic resistance genes in the presence of nanoalumina. Nanotoxicology 10(8):1051–1060. https://doi.org/10.3109/17435390.2016.1161856

Ding J, An XL, Lassen SB, Wang HT, Zhu D, Ke X (2019) Heavy metal-induced co-selection of antibiotic resistance genes in the gut microbiota of collembolans. Sci Total Environ 683:210–215. https://doi.org/10.1016/j.scitotenv.2019.05.302

Donaghy JA, Jagadeesan B, Goodburn K, Grunwald L, Jensen ON, Jespers AD, Kanagachandran K, Lafforgue H, Seefelder W, Quentin MC (2019) Relationship of sanitizers, disinfectants, and cleaning agents with antimicrobial resistance. J Food Prot 82(5):889–902. https://doi.org/10.4315/0362-028X.JFP-18-373

Fletcher S (2015) Understanding the contribution of environmental factors in the spread of antimicrobial resistance. Environ Health Prev Med 20(4):243–252. https://doi.org/10.1007/s12199-015-0468-0

Fok L, Cheung PK, Tang G, Li WC (2017) Size distribution of stranded small plastic debris on the coast of Guangdong, South China. Environ Pollut 220(PtA):407–412. https://doi.org/10.1016/j.envpol.2016.09.079

Gadea R, Glibota N, Pérez Pulido R, Gálvez A, Ortega E (2017) Adaptation to biocides cetrimide and chlorhexidine in bacteria from organic foods: association with tolerance to other antimicrobials and physical stresses. J Agric Food Chem 65(8):1758–1770. https://doi.org/10.1021/acs.jafc.6b04650

Ghosh A, Singh A, Ramteke PW, Singh VP (2000) Characterization of large plasmids encoding resistance to toxic heavy metals in Salmonella abortus equi. Biochem Biophys Res Commun 272(1):6–11. https://doi.org/10.1006/bbrc.2000.2727

Gorovtsov AV, Sazykin IS, Sazykina MA (2018) The influence of heavy metals, polyaromatic hydrocarbons, and polychlorinated biphenyls pollution on the development of antibiotic resistance in soils. Environ Sci Pollut Res Int 25(10):9283–9292. https://doi.org/10.1007/s11356-018-1465-9

Guo MT, Yuan QB, Yang J (2015) Distinguishing effects of ultraviolet exposure and chlorination on the horizontal transfer of antibiotic resistance genes in municipal wastewater. Environ Sci Technol 49(9):5771–5778. https://doi.org/10.1021/acs.est.5b00644

Harrison JP, Schratzberger M, Sapp M, Osborn AM (2014) Rapid bacterial colonization of low-density polyethylene microplastics in coastal sediment microcosms. BMC Microbiol 14:232. https://doi.org/10.1186/s12866-014-0232-4

He LY, Ying GG, Liu YS, Su HC, Chen J, Liu SS, Zhao JL (2016) Discharge of swine wastes risks water quality and food safety: antibiotics and antibiotic resistance genes from swine sources to the receiving environments. Environ Int 92-93:210–219. https://doi.org/10.1016/j.envint.2016.03.023

Heath RJ, Rubin JR, Holland DR, Zhang E, Snow ME, Rock CO (1999) Mechanism of triclosan inhibition of bacterial fatty acid synthesis. J Biol Chem 274(16):11110–11114. https://doi.org/10.1074/jbc.274.16.11110

Hidalgo-Ruz V, Gutow L, Thompson RC, Thiel M (2012) Microplastics in the marine environment: a review of the methods used for identification and quantification. Environ Sci Technol 46 (6):3060–3075. https://doi.org/10.1021/es2031505

Hiltunen T, Virta M, Laine AL (2017) Antibiotic resistance in the wild: an eco-evolutionary perspective. Philos Trans R Soc Lond Ser B Biol Sci 372(1712). https://doi.org/10.1098/rstb.2016.0039

Hirai H, Takada H, Ogata Y, Yamashita R, Mizukawa K, Saha M, Kwan C, Moore C, Gray H, Laursen D, Zettler ER, Farrington JW, Reddy CM, Peacock EE, Ward MW (2011) Organic micropollutants in marine plastics debris from the open ocean and remote and urban beaches. Mar Pollut Bull 62(8):1683–1692. https://doi.org/10.1016/j.marpolbul.2011.06.004

Hu HW, Wang JT, Li J, Li JJ, Ma YB, Chen D, He JZ (2016) Field-based evidence for copper contamination induced changes of antibiotic resistance in agricultural soils. Environ Microbiol 18(11):3896–3909. https://doi.org/10.1111/1462-2920.13370

Hu HW, Wang JT, Li J, Shi XZ, Ma YB, Chen D, He JZ (2017) Long-term nickel contamination increases the occurrence of antibiotic resistance genes in agricultural soils. Environ Sci Technol 51(2):790–800. https://doi.org/10.1021/acs.est.6b03383

Huang J-J, Hu H-Y, Lu S-Q, Li Y, Tang F, Lu Y, Wei B (2012) Monitoring and evaluation of antibiotic-resistant bacteria at a municipal wastewater treatment plant in China. Environ Int 42:31–36. https://doi.org/10.1016/j.envint.2011.03.001

Imran M, Das KR, Naik MM (2019) Co-selection of multi-antibiotic resistance in bacterial pathogens in metal and microplastic contaminated environments: An emerging health threat. Chemosphere 215:846–857. https://doi.org/10.1016/j.chemosphere.2018.10.114

Ji X, Shen Q, Liu F, Ma J, Xu G, Wang Y, Wu M (2012) Antibiotic resistance gene abundances associated with antibiotics and heavy metals in animal manures and agricultural soils adjacent to feedlots in Shanghai; China. J Hazard Mater 235–236:178–185. https://doi.org/10.1016/j.jhazmat.2012.07.040

Jutkina J, Marathe NP, Flach CF, Larsson DGJ (2018) Antibiotics and common antibacterial biocides stimulate horizontal transfer of resistance at low concentrations. Sci Total Environ 616-617:172–178. https://doi.org/10.1016/j.scitotenv.2017.10.312

Kang F, Hu X, Liu J, Gao Y (2015) Noncovalent binding of polycyclic aromatic hydrocarbons with genetic bases reducing the in vitro lateral transfer of antibiotic resistant genes. Environ Sci Technol 49(17):10340–10348. https://doi.org/10.1021/acs.est.5b02293

Kazmiruk TN, Kazmiruk VD, Bendell LI (2018) Abundance and distribution of microplastics within surface sediments of a key shellfish growing region of Canada. PLoS One 13(5): e0196005. https://doi.org/10.1371/journal.pone.0196005

Keswani A, Oliver DM, Gutierrez T, Quilliam RS (2016) Microbial hitchhikers on marine plastic debris: human exposure risks at bathing waters and beach environments. Mar Environ Res 118:10–19. https://doi.org/10.1016/j.marenvres.2016.04.006

Kettner MT, Rojas-Jimenez K, Oberbeckmann S, Labrenz M, Grossart HP (2017) Microplastics alter composition of fungal communities in aquatic ecosystems. Environ Microbiol 19 (11):4447–4459. https://doi.org/10.1111/1462-2920.13891

Knapp CW, Callan AC, Aitken B, Shearn R, Koenders A, Hinwood A (2017) Relationship between antibiotic resistance genes and metals in residential soil samples from Western Australia. Environ Sci Pollut Res Int 24(3):2484–2494. https://doi.org/10.1007/s11356-016-7997-y

Kochare T, Tamir B (2015) Assessment of dairy feeds for heavy metals. Am Sci Res J Eng Technol Sci 11:20–31

Krulwich TA, Lewinson O, Padan E, Bibi E (2005) Do physiological roles foster persistence of drug/multidrug-efflux transporters? A case study. Nat Rev Microbiol 3(7):566–572. https://doi.org/10.1038/nrmicro1181

Kurenbach B, Marjoshi D, Amabile-Cuevas CF, Ferguson GC, Godsoe W, Gibson P, Heinemann JA (2015) Sublethal exposure to commercial formulations of the herbicides dicamba, 2,4-dichlorophenoxyacetic acid, and glyphosate cause changes in antibiotic susceptibility in *Escherichia coli* and *Salmonella enterica* serovar Typhimurium. MBio 6(2):e00009–e00015. https://doi.org/10.1128/mBio.00009-15

Law KL, Thompson RC (2014) Microplastics in the seas. Science 345(6193):144–145. https://doi.org/10.1126/science.1254065

Leisner JJ, Jørgensen NO, Middelboe M (2016) Predation and selection for antibiotic resistance in natural environments. Evol Appl 9(3):427–434. https://doi.org/10.1111/eva.12353

Lemire JA, Harrison JJ, Turner RJ (2013) Antimicrobial activity of metals: mechanisms, molecular targets and applications. Nat Rev Microbiol 11(6):371–384. https://doi.org/10.1038/nrmicro3028

Levy SB (2002) Active efflux, a common mechanism for biocide and antibiotic resistance. J Appl Microbiol 92(Suppl):65S–71S. https://doi.org/10.1046/j.1365-2672.92.5s1.4.x

Li B, Webster TJ (2018) Bacteria antibiotic resistance: new challenges and opportunities for implant-associated orthopedic infections. J Orthop Res 36(1):22–32. https://doi.org/10.1002/jor.23656

Li D, Zeng S, He M, Gu AZ (2016) Water disinfection byproducts induce antibiotic resistance-role of environmental pollutants in resistance phenomena. Environ Sci Technol 50(6):3193–3201. https://doi.org/10.1021/acs.est.5b00644

Li H, Duan M, Gu J, Zhang Y, Qian X, Ma J, Zhang R, Wang X (2017) Effects of bamboo charcoal on antibiotic resistance genes during chicken manure composting. Ecotoxicol Environ Saf 140:1–6. https://doi.org/10.1016/j.ecoenv.2017.01.007

Li J, Zhang K, Zhang H (2018) Adsorption of antibiotics on microplastics. Environ Pollut 237:460–467. https://doi.org/10.1016/j.envpol.2018.02.050

Lima YA, Cardoso LP, Reis MN, Stefani MM (2016) Incident and long-term HIV-1 infection among pregnant women in Brazil: transmitted drug resistance and mother-to-child transmission. J Med Virol 88(11):1936–1943. https://doi.org/10.1002/jmv.24540

Lv L, Yu X, Xu Q, Ye C (2015) Induction of bacterial antibiotic resistance by mutagenic halogenated nitrogenous disinfection byproducts. Environ Pollut 205:291–298. https://doi.org/10.1016/j.envpol.2015.06.026

Ma X, Guo N, Ren S, Wang S, Wang Y (2019) Response of antibiotic resistance to the co-existence of chloramphenicol and copper during bio-electrochemical treatment of antibiotic-containing wastewater. Environ Int 126:127–133. https://doi.org/10.1016/j.envint.2019.02.002

Martínez JL (2008) Antibiotics and antibiotic resistance genes in natural environments. Science 321(5887):365–367. https://doi.org/10.1126/science.1159483

Martinez JL (2009) The role of natural environments in the evolution of resistance traits in pathogenic bacteria. Proc Biol Sci 276(1667):2521–2530. https://doi.org/10.1098/rspb.2009.0320

Martínez JL (2012) Bottlenecks in the transferability of antibiotic resistance from natural ecosystems to human bacterial pathogens. Front Microbiol 2:265. https://doi.org/10.3389/fmicb.2011.00265

Martinez RJ, Wang Y, Raimondo MA, Coombs JM, Barkay T, Sobecky PA (2006) Horizontal gene transfer of PIB-type ATPases among bacteria isolated from radionuclide- and metal-contaminated subsurface soils. Appl Environ Microbiol 72(5):3111–3118. https://doi.org/10.1128/AEM.72.5.3111-3118.2006

Mc Cay PH, Ocampo-Sosa AA, Fleming GT (2010) Effect of subinhibitory concentrations of benzalkonium chloride on the competitiveness of *Pseudomonas aeruginosa* grown in continuous culture. Microbiology 156(Pt 1):30–38. https://doi.org/10.1099/mic0.029751-0

Meyer B, Cookson B (2010) Does microbial resistance or adaptation to biocides create a hazard in infection prevention and control? J Hosp Infect 76(3):200–205. https://doi.org/10.1016/j.jhin.2010.05.020

Munir M, Wong K, Xagoraraki I (2011) Release of antibiotic resistant bacteria and genes in the effluent and biosolids of five wastewater utilities in Michigan. Water Res 45(2):681–693. https://doi.org/10.1016/j.watres.2010.08.033

Munita JM, Arias CA (2016) Mechanisms of antibiotic resistance. Microbiol Spectr 4(2). https://doi.org/10.1128/microbiolspec.VMBF-0016-2015

Mutoh N, Nakagawa CW, Yamada K (1999) The role of catalase in hydrogen peroxideresistance in fission yeast Schizosaccharomyces pombe. Can J Microbiol 45(2):125–129. https://doi.org/10.1139/w98-216

Naik M, Dubey S, Khanolkar D, D'Costa B (2013) P-type ATPase and MdrL efflux pump-mediated lead and multi-drug resistance in estuarine bacterial isolates. Curr Sci 105(10):1366–1372. www.jstor.org/stable/24098901

Nelms SE, Galloway TS, Godley BJ, Jarvis DS, Lindeque PK (2018) Investigating microplastic trophic transfer in marine top predators. Environ Pollut 238:999–1007. https://doi.org/10.1016/j.envpol.2018.02.016

Nies DH (2003) Efflux-mediated heavy metal resistance in prokaryotes. FEMS Microbiol Rev 27 (2-3):313–339. https://doi.org/10.1016/S0168-6445(03)00048-2

Nies DH, Silver S (1995) Ion efflux systems involved in bacterial metal resistances. J Ind Microbiol 14(2):186–199. https://doi.org/10.1007/BF01569902

Oberbeckmann S, Loeder MG, Gerdts G, Osborn AM (2014) Spatial and seasonal variation in diversity and structure of microbial biofilms on marine plastics in northern European waters. FEMS Microbiol Ecol 90(2):478–492. https://doi.org/10.1111/1574-6941.12409

Oberbeckmann S, Mark Osborn A, Duhaime MB, Carter DA (2016) Microbes on a bottle: substrate, season and geography influence community composition of microbes colonizing marine plastic debris. PLoS ONE 11(8):e0159289. https://doi.org/10.1371/journal.pone.0159289

Orús P, Gomez-Perez L, Leranoz S, Berlanga M (2015) Increasing antibiotic resistance in preservative-tolerant bacterial strains isolated from cosmetic products. Int Microbiol 18 (1):51–59. https://doi.org/10.2436/20.1501.01.234

Pal C, Asiani K, Arya S, Rensing C, Stekel DJ, Larsson DGJ, Hobman JL (2017) Metal resistance and its association with antibiotic resistance. Adv Microb Physiol 70:261–313. https://doi.org/10.1016/bs.ampbs.2017.02.001

Patyka V, Buletsa N, Pasichnyk L, Zhitkevich N, Kalinichenko A, Gnatiuk T, Butsenko L (2016) Specifics of pesticides effects on the phytopathogenic bacteria. Ecol Chem Eng 23(2):311–331. https://doi.org/10.1515/eces-2016-0022

Peak N, Knapp CW, Yang RK, Hanfelt MM, Smith MS, Aga DS, Graham DW (2007) Abundance of six tetracycline resistance genes in wastewater lagoons at cattle feedlots with different antibiotic use strategies. Environ Microbiol 9(1):143–151. https://doi.org/10.1111/j.1462-2920.2006.01123.x

Poole K (2017) At the nexus of antibiotics and metals: the impact of cu and Zn on antibiotic activity and resistance. Trends Microbiol 25(10):820–832. https://doi.org/10.1016/j.tim.2017.04.010

Pruden A, Pei R, Storteboom H, Carlson KH (2006) Antibiotic resistance genes as emerging contaminants: studies in northern Colorado. Environ Sci Technol 40(23):7445–7450. https://doi.org/10.1021/es060413l

Qiu Z, Yu Y, Chen Z, Jin M, Yang D, Zhao Z, Wang J, Shen Z, Wang X, Qian D, Huang A, Zhang B, Li J-W (2012) Nanoalumina promotes the horizontal transfer of multiresistance genes mediated by plasmids across genera. Proc Natl Acad Sci 109(13):4944–4949. https://doi.org/10.1073/pnas.1107254109

Ramakrishnan B, Venkateswarlu K, Sethunathan N, Megharaj M (2019) Local applications but global implications: can pesticides drive microorganisms to develop antimicrobial resistance? Sci Total Environ 654:177–189. https://doi.org/10.1016/j.scitotenv.2018.11.041

Rangasamy K, Athiappan M, Devarajan N, Parray JA (2017) Emergence of multi drug resistance among soil bacteria exposing to insecticides. Microb Pathog 105:153–165. https://doi.org/10.1016/j.micpath.2017.02.011

Rangasamy K, Athiappan M, Devarajan N, Samykannu G, Parray JA, Aruljothi KN, Shameem N, Alqarawi AA, Hashem A, Abd Allah EF (2018) Pesticide degrading natural multidrug resistance bacterial flora. Microb Pathog 114:304–310. https://doi.org/10.1016/j.micpath.2017.12.013

Reisser J, Shaw J, Wilcox C, Hardesty BD, Proietti M, Thums M, Pattiaratchi C (2013) Marine plastic pollution in waters around Australia: characteristics, concentrations, and pathways. PLoS One 8(11):e80466. https://doi.org/10.1371/journal.pone.0080466

Revel M, Châtel A, Mouneyrac C (2018) Micro(nano)plastics: a threat to human health? Curr Opin Environ Sci Health 1:17–23. https://doi.org/10.1016/j.coesh.2017.10.003

Rizzo L, Manaia C, Merlin C, Schwartz T, Dagot C, Ploy MC, Michael I, Fatta-Kassinos D (2013) Urban wastewater treatment plants as hotspots for antibiotic resistant bacteria and genes spread into the environment: a review. Sci Total Environ 447:345–360. https://doi.org/10.1016/j.scitotenv.2013.01.032

Rodríguez Martín JA, De Arana C, Ramos-Miras JJ, Gil C, Boluda R (2015) Impact of 70 years urban growth associated with heavy metal pollution. Environ Pollut 196:156–163. https://doi.org/10.1016/j.envpol.2014.10.014

Ruiz N, Montero T, Hernandez-Borrell J, Viñas M (2003) The role of Serratia marcescens porins in antibiotic resistance. Microb Drug Resist 9(3):257–264. https://doi.org/10.1089/107662903322286463

Rummel CD, Jahnke A, Gorokhova E, Kühnel D, Schmitt-Jansen M (2017) The impacts of biofilm formation on the fate and potential effects of microplastic in the aquatic environment. Environ Sci Technol Lett 4(7):258–267. https://doi.org/10.1021/acs.estlett.7b00164

Salyers AA, Amábile-Cuevas CF (1997) Why are antibiotic resistance genes so resistant to elimination? Antimicrob Agents Chemother 41(11):2321–2325

Santiago-Rodriguez TM, Fornaciari G, Luciani S, Dowd SE, Toranzos GA, Marota I, Cano RJ (2015) Gut microbiome of an 11th century A.D. pre-Columbian Andean mummy. PLoS One 10(9):e0138135. https://doi.org/10.1371/journal.pone.0138135

Schiering N, Kabsch W, Moore MJ, Distefano MD, Walsh CT, Pai EF (1991) Structure of the detoxification catalyst mercuric ion reductase from Bacillus sp. strain RC607. Nature 352(6331):168–172. https://doi.org/10.1038/352168a0

Seiler C, Berendonk TU (2012) Heavy metal driven co-selection of antibiotic resistance in soil and water bodies impacted by agriculture and aquaculture. Front Microbiol 3:399. https://doi.org/10.3389/fmicb.2012.00399

Sharma VK, Johnson N, Cizmas L, McDonald TJ, Kim H (2016) A review of the influence of treatment strategies on antibiotic resistant bacteria and antibiotic resistance genes. Chemosphere 150:702–714. https://doi.org/10.1016/j.chemosphere.2015.12.084

Silver S, Phung LT (1996) Bacterial heavy metal resistance: new surprises. Annu Rev Microbiol 50:753–789. https://doi.org/10.1146/annurev.micro.50.1.753

Slater-Radosti C, Van Aller G, Greenwood R, Nicholas R, Keller PM, DeWolf WE Jr, Fan F, Payne DJ, Jaworski DD (2001) Biochemical and genetic characterization of theaction of triclosan on *Staphylococcus aureus*. J Antimicrob Chemother 48(1):1–6. https://doi.org/10.1093/jac/48.1.1

Soumet C, Sheath E, Legrandois P, Maris P (2012) Resistance to phenicol compounds following adaptation to quaternary ammonium compounds in *Escherichia coli*. Vet Microbiol 158(1–2):147–152. https://doi.org/10.1016/j.vetmic.2012.01.030

Stepanauskas R, Glenn TC, Jagoe CH, Tuckfield RC, Lindell AH, McArthur JV (2005) Elevated microbial tolerance to metals and antibiotics in metal-contaminated industrial environments. Environ Sci Technol 39(10):3671–3678. https://doi.org/10.1021/es048468f

Sui Q, Zhang J, Chen M, Wang R, Wang Y, Wei Y (2019) Fate of microbial pollutants and evolution of antibiotic resistance in three types of soil amended with swine slurry. Environ Pollut 245:353–362. https://doi.org/10.1016/j.envpol.2018.11.003

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-comediated ARGs in microplastic-contaminated greenhouse soil facilitated by sophorolipid. J Hazard Mater 345:131–139. https://doi.org/10.1016/j.jhazmat.2017.11.036

Suzuki S, Kimura M, Agusa T, Rahman HM (2012) Vanadium accelerates horizontal transfer of tet (M) gene from marine Photobacterium to *Escherichia coli*. FEMS Microbiol Lett 336(1):52–56. https://doi.org/10.1111/j.1574-6968.2012.02653.x

Teitzel GM, Parsek MR (2003) Heavy metal resistance of biofilm and planktonic *Pseudomonas aeruginosa*. Appl Environ Microbiol 69(4):2313–2320. https://doi.org/10.1128/AEM.69.4.2313-2320.2003

Vale-Silva LA, Sanglard D (2015) Tipping the balance both ways: drug resistance and virulence in *Candida glabrata*. FEMS Yeast Res 15(4):fov025. https://doi.org/10.1093/femsyr/fov025

Vikesland PJ, Pruden A, Alvarez PJJ, Aga D, Bürgmann H, Li XD, Manaia CM, Nambi I, Wigginton K, Zhang T, Zhu YG (2017) Toward a comprehensive strategy to mitigate dissemination of environmental sources of antibiotic resistance. Environ Sci Technol 51 (22):13061–13069. https://doi.org/10.1021/acs.est.7b03623

Wales AD, Davies RH (2015) Co-selection of resistance to antibiotics, biocides and heavy metals, and its relevance to foodborne pathogens. Antibiotics (Basel) 4(4):567–604. https://doi.org/10.3390/antibiotics4040567

Wand ME, Bock LJ, Bonney LC, Sutton JM (2016) Mechanisms of increased resistance to chlorhexidine and cross-resistance to colistin following exposure of *Klebsiella pneumoniae* clinical isolates to chlorhexidine. Antimicrob Agents Chemother 61(1):e01162–e01116. https://doi.org/10.1128/AAC.01162-16

Wang R, Zhou X, Shi H (2015) Triple functional DNA-protein conjugates: signal probes for Pb(2+) using evanescent wave-induced emission. Biosens Bioelectron 74:78–84. https://doi.org/10.1016/j.bios.2015.06.003

Watts AJ, Urbina MA, Goodhead R, Moger J, Lewis C, Galloway TS (2016) Effect of microplastic on the gills of the shore crab *Carcinus maenas*. Environ Sci Technol 50(10):5364–5369. https://doi.org/10.1021/acs.est.6b01187

Wellington EM, Boxall AB, Cross P, Feil EJ, Gaze WH, Hawkey PM, Johnson-Rollings AS, Jones DL, Lee NM, Otten W, Thomas CM, Williams AP (2013) The role of the natural environment in the emergence of antibiotic resistance in Gram-negative bacteria. Lancet Infect Dis 13 (2):155–165. https://doi.org/10.1016/S1473-3099(12)70317-1

Westfall C, Flores-Mireles AL, Robinson JI, Lynch AJL, Hultgren S, Henderson JP, Levin PA (2019) The widely used antimicrobial triclosan induces high levels of antibiotic tolerance in vitro and reduces antibiotic efficacy up to 100-fold in vivo. Antimicrob Agents Chemother 63(5):e02312–e02318. https://doi.org/10.1128/AAC.02312-18

Wright GD (2005) Bacterial resistance to antibiotics: enzymatic degradation and modification. Adv Drug Deliv Rev 57(10):1451–1470. https://doi.org/10.1016/j.addr.2005.04.002

Wright GD (2010) Antibiotic resistance in the environment: a link to the clinic? Curr Opin Microbiol 13(5):589–594. https://doi.org/10.1016/j.mib.2010.08.005

Wu D, Lu R, Chen Y, Qiu J, Deng C, Tan Q (2016) Study of cross-resistance mediated by antibiotics, chlorhexidine and Rhizoma coptidis in *Staphylococcus aureus*. J Glob Antimicrob Resist 7:61–66. https://doi.org/10.1016/j.jgar.2016.07.011

Yang S, Wen X, Zhao L, Shi Y, Jin H (2014) Crude oil treatment leads to shift of bacterial communities in soils from the deep active layer and upper permafrost along the China-Russia crude oil pipeline route. PLoS One 9(5):e96552. https://doi.org/10.1371/journal.pone.0096552

Zettler ER, Mincer TJ, Amaral-Zettler LA (2013) Life in the "plastisphere":microbial communities on plastic marine debris. Environ Sci Technol 47(13):7137–7146. https://doi.org/10.1021/es401288x

Zhang LP, Xing YP, Liu C, Zhou XH, Shi HC (2015) Label-free colorimetricdetection of Cu2þ, on the basis of Fenton reaction-assisted signal amplificationwith unmodified gold nanoparticles as indicator. Sens Actuators B Chem 215:561–567. https://doi.org/10.1016/j.snb.2015.03.062

Zhang Y, Gu AZ, He M, Li D, Chen J (2017) Subinhibitory concentrations of disinfectants promote the horizontal transfer of multidrug resistance genes within and across Genera. Environ Sci Technol 51(1):570–580. https://doi.org/10.1021/acs.est.6b03132

Zhang Y, Gu AZ, Cen T, Li X, He M, Li D, Chen J (2018) Sub-inhibitory concentrations of heavy metals facilitate the horizontal transfer of plasmid-mediated antibiotic resistance genes in water environment. Environ Pollut 237:74–82. https://doi.org/10.1016/j.envpol.2018.01.032

Zhao Y, Cocerva T, Cox S, Tardif S, Su JQ, Zhu YG, Brandt KK (2019) Evidence for co-selection of antibiotic resistance genes and mobile genetic elements in metal polluted urban soils. Sci Total Environ 656:512–520. https://doi.org/10.1016/j.scitotenv.2018.11.372

Zhao Y, Su JQ, An XL, Huang FY, Rensing C, Brandt KK, Zhu YG (2018) Feed additives shift gut microbiota and enrich antibiotic resistance in swine gut. Sci Total Environ 621:1224–1232. https://doi.org/10.1016/j.scitotenv.2017.10.106

Zhu YG, Johnson TA, Su JQ, Qiao M, Guo GX, Stedtfeld RD, Hashsham SA, Tiedje JM (2013) Diverse and abundant antibiotic resistance genes in Chinese swine farms. Proc Natl Acad Sci U S A 110(9):3435–3440. https://doi.org/10.1073/pnas.1222743110

# Polluted Coastal and Estuarine Environments: A Potential Reservoir for AMR Determinants in Various Pathogenic Bacteria

Mohamed Hatha A. A., Divya Puthenkandathil Sukumaran, Reshma Silvester, and Nifty John

## 1 Introduction

Antimicrobial resistance is a growing and significant threat to global public health, requiring better understanding of the sources and mechanisms involved in its emergence and spread. The World Health Organization (WHO) has described antibiotic resistance as one of the greatest threats to human health. Inappropriate and indiscriminate use of antibiotics is a known cause for the spread of antibiotic resistance in the environment (Kummerer 2003). Antibiotics and antibiotic-resistant bacteria (ARB) reach the environment through treated or untreated wastewater discharges, livestock operations, aquaculture farms, and pharmaceutical and food industries, which may exert a selection pressure that eventually promotes the emergence, maintenance, and spread of antibiotic resistance in environmental bacteria (Cabello et al. 2013; Berendonk et al. 2015; Martinez 2009).

Prophylactic and therapeutic use of antibiotics in aquaculture has resulted in the emergence of antibiotic-resistant bacteria in aquatic systems, and thus the aquatic environment has become a potential source of transmission of these bacteria to other systems (Patil et al. 2016; Hafez et al. 2018). It is estimated that an increase in antimicrobial-resistant bacteria will be problematic in the treatment of bacterial infections, and this may lead to ten million deaths each year by 2050 (Li et al. 2019). This scenario has resulted in a crucial need for the surveillance of genes and mutations responsible for the development of antibiotic resistance globally.

There is a rapid rise in antimicrobial resistance in many bacterial genera due to the excessive use of antibiotics in healthcare, agriculture, and aquaculture systems (Silvester et al. 2015). Almost 90% of antibiotics used in humans and animals are not completely degraded within the intestine. The antibiotic residues and antibiotic-

M. H. A. A. (✉) · D. P. Sukumaran · R. Silvester · N. John
Department of Marine Biology, Microbiology and Biochemistry, School of Marine Sciences, Cochin University of Science and Technology, Kochi, Kerala, India



S. Thomas (ed.), *Antimicrobial Resistance*,
https://doi.org/10.1007/978-981-15-3658-8_5

resistant bacteria (ARB) present in the guts of humans and animals enter the natural environment through urine and fecal discharges (Gao et al. 2012). This makes the natural waters a potential source of ARB in the environment. Antibiotic-resistant bacteria are easily transmitted via humans, animals, plants, water, soil, and air, among which water is the main route for the transfer of resistance. Natural water bodies are potential reservoirs for horizontal gene transfer (HGT) of antibiotic resistance genes (ARGs) between environmental bacteria and the human and animal pathogens (Igbinosa 2014). The natural environment is a major route of transmission of antibiotic-resistant bacteria and ARGs (Woolhouse et al. 2015). Anthropogenic activities including daily living as well as agricultural practices and animal rearing systems result in the release of a large quantum of wastes into the natural waters. This waste usually carries varying concentrations of antibiotics, antibiotic-resistant bacteria, and ARGs (Hassard et al. 2016). The anthropogenic activities are changing the environmental reservoirs of antibiotic resistance genes (D'Costa et al. 2006). This will increase the possibility of transfer of ARGs into clinically relevant pathogens. Freshwater and coastal ecosystems act as a source of antibiotic-resistant bacteria and play a pivotal role in the transmission of antibiotic resistance genes (ARGs) among the bacteria (Lupo et al. 2012).

## 2 Polluted Natural Waters: An Ideal Setting for Selection and Spread of Diverse Antibiotic-Resistant Bacteria

Uncontrolled population growth of humans across the globe has resulted in considerable pressure on infrastructure, which has resulted in the release of a large quantum of untreated and partially treated wastewater into natural waters (Borja et al. 2010; Rodgers et al. 2019). Release of sewage causes entry of a large number of potentially pathogenic bacteria including several antibiotic-resistant bacteria from various sources into the natural environment. There are point and nonpoint sources of antibiotic pollution in the environment. Point sources are defined as "any single identifiable source of pollution from which pollutants are discharged" (Armon and Starosvetsky 2015). Industries, household units, hospitals, slaughterhouses, and wastewater treatment plants (WWTPs) are the major identified point sources for antibiotic pollution. Nonpoint sources include the ballast water, septic systems, animal wastes, agriculture, and surface runoff from land.

### 2.1 Wastewater Treatment Plants as a Source of Antibiotic-Resistant Bacteria and Antibiotic Resistance Genes (ARGs)

Despite recent advances in water quality and wastewater treatments, waterborne diseases still continue to be a major challenge to public health worldwide (Zhou and Smith 2002). Wastewater treatment plants (WWTPs) and untreated hospital effluents are major "hot spots" for the selection and transmission of drug-resistant bacteria (Rizzo et al. 2013). The wastewater treatment plants (WWTPs), drug manufacturing

units, and agricultural effluents release huge quantities of antibiotic residues and drug-resistant bacteria into the environment (Knapp et al. 2011). The receiving waterbodies downstream the wastewater treatment plants/hospitals and surface waters in and around animal feeding activities are the most vulnerable areas contaminated with antibiotic-resistant bacteria (Silbergeld et al. 2008).

WWTPs act as one of the potential sources of antibiotic-resistant bacteria in the environment. In WWTPs, wastewater from various sources, including municipalities, hospitals, and industries, is mixed and treated in a multistep purification process. Even though sewage treatment reduces the total number of bacteria in wastewater, the effluent released from the plants usually carries a large number of both resistant and susceptible bacteria (Siddiqui et al. 2016). Conventional wastewater treatment processes, such as activated sludge and anaerobic digestion, provide an ideal setting for horizontal gene transfer, which is responsible, although not exclusively, for increasing the incidence and prevalence of antibiotic-resistant infections (Warnes et al. 2012). Various factors contribute to the spread of antibiotic-resistant bacteria in wastewater. It includes high levels of antibiotics and biocides, increased concentrations of resistant bacteria, and the abundance of organic and inorganic substrates (Gullberg et al. 2014).

From India, only limited data is available about the presence of antibiotic-resistant bacteria associated with WWTPs. Marathe et al. (2013) studied a wastewater treatment plant (WWTP) in Patancheru, near Hyderabad, receiving wastewater from bulk drug production facilities. The plant was observed to have high levels of multidrug-resistant (MDR) organisms and thus could act as a breeding ground for transfer of ARGs. Akiba et al. (2016) reported the presence of extended-spectrum cephalosporin-resistant and carbapenem-resistant *E. coli* isolates from sewage treatment plants (STPs) in India (Bihar, Goa, Karnataka, Tamil Nadu, and Telangana). Lamba and Ahammad (2017) studied the route of proliferation of carbapenem (KPC)-resistant and extended-spectrum β-lactam (ESBL)-resistant bacteria and selected resistant genes in the samples collected from 12 sewage treatment plants in New Delhi and reported presence of high levels of antibiotic resistance (ARB and ARGs) in the STP effluents. Among the different ARGs, β-lactam, sulfonamide, and tetracycline resistance genes are frequently found in wastewater (Kim et al. 2008). In another study published in *Lancet*, the authors highlighted the widespread dissemination of the New Delhi metallo-β-lactamase-1 (NDM-1) genes among many bacterial species in various water sources in India including the chlorinated municipal drinking water samples (Walsh et al. 2011). High levels of beta-lactam and fluoroquinolone resistance genes were detected in phage DNA isolated from hospital and urban treated effluents (Marti et al. 2013).

Various classes of antibiotics are present in treated effluents and sewage sludge which range in concentrations from nanogam/liter to microgram/liter (Kummerer 2009). The presence of such low concentration of antibiotics in the environment leads to selection of drug-resistant mutants. Long-term exposure of environmental bacteria to antibiotics may speed up the evolution of resistance and accelerate the ARG exchange between bacteria (Gillings and Stokes 2012). The co-location of antibiotics and ARGs in WWTPs can result in the selection of novel combinations of

antimicrobial resistance that can be transferred between microorganisms through horizontal gene transfer (HGT), thereby increasing the prevalence and combination of multidrug resistance in the environmental microbial flora. Micropollutants such as antibiotics and antibiotic-resistant bacteria and genes are not completely removed by current wastewater treatment technologies, and they enter the environment through the effluent discharge into the water bodies (Sabri et al. 2018). Hence, improved wastewater treatment plants that can fully remove all kinds of antibiotic residues are highly recommended.

## 2.2 Hospital Wastewater as a Source of Antibiotic-Resistant Bacteria in Natural Waters

Untreated effluents from hospital settings, especially intensive care units (ICUs), are another potential reservoir for harmful infectious pathogens and pose risk of spreading antimicrobial resistance genes in the environment (Siddiqui et al. 2016). High rate of antibiotic consumption occurs in hospital settings which facilitate emergence of ARB and spread of ARGs. Discharge of low concentration of unmetabolized antibiotics into the environments from hospitals may also lead to the selection of antibiotic-resistant mutants among natural microflora. The impact of hospital size on the transmission of ARGs was studied by Kouyos et al. (2011), and findings revealed that small-sized hospitals lead to considerably lower resistance levels when compared to larger hospitals. A recent study reported that widespread presence of antibiotics and ARGs in hospital wastewater and the effluents even after treatment may contribute to antibiotic pollution and resistance in the aquatic environment (Rodriguez-Mozaz et al. 2015). In a study by Mutiyar and Mittal (2014), alarmingly high levels of sulfonamide, fluoroquinolone, and tinidazole residues were detected from the hospital effluents in India. A research highlighted that the amount of antibiotics disposed from Indian hospitals were sufficient to cause genotoxic alterations and mutations in the bacterial strains (Diwan et al. 2010).

## 2.3 Pharma Industry Effluents: A Right Concoction for Selection of AMR Bacteria and ARGs

From the onset of industrial revolution, humans have been disposing organic and inorganic toxins, metals, disinfectants, biocides, and antibiotic residues into the natural water bodies (Davies and Davies 2010). India, being a leading producer of pharmaceuticals, contributes a significant part to the global rise of AMR. There was an alarming report from Hyderabad, India, recently on the irresponsible disposal of a large amount of ciprofloxacin (50 kg/day) by the pharmaceutical manufacturing units into the nearby rivers (Fick et al. 2009). In another study, antibiotic residues were detected in sewers near drug manufacturing industries in Hyderabad (Lubbert et al. 2017). This antibiotic residue-laden wastewater is a storehouse for drug-resistant mutants which when released into the natural waters results in the exchange

of ARGs within them with autochthonous microflora of the receiving waters further aggravating the problem.

### 2.4 Slaughterhouse Wastewater and Waste Discharges from Animal Production Systems in the Spread of ARB and ARGs in Natural Waters

India is a major producer of food animals (meat, meat products, and farmed seafood) for export to the global market. Antibiotics are widely used in the Indian animal and aquaculture farms for prophylaxis, disease prevention, and growth promotion, thus aiming to increase the overall productivity. India is one of the largest consumers of antimicrobials in food animals. Antibiotic use in animals is not limited to disease treatment and prevention. It is used as a growth promoter to boost feed efficiency and increase weight gain. Drugs such as avilamycin, bambermycin, efrotomycin and ionophores, quinolones, macrolides, and aminoglycosides are widely used as growth promoters in feeds (Mahalmani et al. 2019; McEwen and Fedorka-Cray 2000). In order to curb the issue of uncontrolled consumption of antibiotics in the Indian domestic sector, certain regulations have been framed by government authorities. The FSSAI recently issued a regulation to ban the use of colistin in food. Accordingly, colistin will be included in the list of antibiotics and veterinary drugs that are prohibited for use in processing meat and meat products, poultry and eggs, and seafood including shrimps, prawns, or fish and fishery products. The WHO has marked colistin as a "highest priority critically important antimicrobial" for humans. The main objective of the Indian National Action Plan on AMR (2017–2021) is to slowly ban the use of critically important antibiotics in animal production.

Even though there are strict regulations on the use of antibiotics in animals meant for export, no such guidelines are currently formulated for food animals meant for domestic use in India. The indiscriminate use of antimicrobials in aquaculture and agricultural systems can act as an emerging source of environmental antimicrobial resistance. Antibiotic-resistant bacteria of animal origin have been frequently found in the natural water systems surrounding the farming units. There are reports stating the presence of antibiotic residues in poultry, meat, and milk from India (Chaudhry and Tomar 2017; Sahu and Saxena 2014). The excessive use of antibiotics in the aquaculture industry has led to the emergence of antimicrobial resistance in the seafood bacterial pathogens. These unsustainable practices have taken the industry on a rollercoaster ride, bringing it intermittently close to collapse.

## 3 The Possible Drivers for the Resistance

AMR is a complex problem with multiple and interconnected drivers, which may include changing dynamics in travel, trade, climate change, and populations (Hendriksen et al. 2019). According to Vikesland et al. (2019), antimicrobial resistance is a cross-boundary challenge that is driven by clinical, biological, social,

political, economic, and environmental drivers and affects not only humans but also domestic and non-domestic animals and ecosystems. The increased use and misuse of antibiotics in hospital settings is the main cause of escalating antibiotic resistance. A study by Fischer and Bild (2019) demonstrated a high correlation between antibiotic use in the hospital sector and population-level rates of infections with antibiotic-resistant bacteria. Batard et al. (2013) studied the relationship between hospital antibiotic use and quinolone resistance in *E. coli* and reported that the level of clinical usage of quinolones influences the incidence of quinolone resistance in *E. coli* hospital isolates. The consumption of two other classes of antibiotics, cephalosporins and tetracyclines, is also associated with quinolone resistance.

Antibiotics are used for the treatment of bacterial infections in domestic animals and animals raised as food. Antibiotics are also used as growth promoters in food-producing animals such as pig, cow, and poultry. Antibiotics are also used in aquaculture/fish farming to treat bacterial infections caused by *Aeromonas*, *Flavobacterium*, etc., in addition to being used as fish feed. In aquaculture, antibiotics such as tetracycline and sulfonamides are commonly used.

Antibiotic resistance can also be widely disseminated by birds; the Arctic tern travels to up to six continents, migrates to Antarctica for winter, and is a potent driver of antibiotic resistance (Sjolund et al. 2008). They reported that *Escherichia coli* isolates originating from Arctic birds carry antimicrobial drug resistance determinants. Dolejska et al. (2007) suggested that young black-headed gulls are an important host reservoir of resistant *E. coli* strains, probably reflecting the presence of such strains in their sources of food and/or water. Dolejska et al. (2009) also reported that black-headed gulls were identified as important reservoirs of antibiotic-resistant *Salmonella* and *E. coli*, including extended-spectrum beta-lactamase-producing isolates.

## 4 AMR and Climate Change

MacFadden et al. (2018) suggested that the spread of antibiotic resistance may be modified and potentially accelerated by regional temperature and future climate change. Recently, the association of AMR with climate gained widespread attention, since resistance increased with increasing local temperatures in the USA (ESCMID 2019). A study conducted by researchers found significant associations of carbapenem-resistant *Pseudomonas aeruginosa* (CRPA), carbapenem-resistant *Klebsiella pneumoniae* (CRKP), multiresistant *Escherichia coli* (MREC), and methicillin-resistant *Staphylococcus aureus* (MRSA) with the warm-season mean temperature, which had a higher contribution to MRSA variance than outpatient antimicrobial drug use. They suggested that climatic factors significantly contribute to the prediction of AMR in different types of healthcare systems and societies, while climate change might increase AMR transmission, in particular that of carbapenem resistance.

## 5 Antibiotic Resistance-Enhancing Mechanisms

The increased dissemination of antibiotic-resistant bacteria is a serious problem worldwide. According to Berendonk et al. (2015), the rapid dissemination of antibiotic-resistant bacteria in environmental settings is caused by horizontal gene transfer of ARGs, genetic mutation, and recombination. These three mechanisms can occur in combination. Besides this, there are so many different factors responsible for triggering the evolution of antibiotic-resistant bacteria, e.g., selective pressure created by antibiotics, biocides, and/or heavy metals and biotic and abiotic factors. Antibiotic resistance determinants, which are associated with mobile genetic elements (MGEs), including integrons, transposons, and plasmids, are more prone to be transferred via horizontal gene transfer. Due to the availability of many of the above factors, polluted natural waters provide a congenial habitat for the selection and spread of AMR bacteria and ARGs in the environment and food chain.

### 5.1 Integrons

Integrons are genetic elements of variable length that contain a 5′ conserved integrase gene (*int*), gene cassettes with other antibiotic resistance genes, and an integration site for the gene cassette, *attI* (Babic et al. 2006). Integrons are among the main types of mobile elements currently known to be involved in the capture, mobilization, and spread of antibiotic resistance genes found in gram-negative bacteria. They are genetic platforms that are responsible for integration and rearrangements of resistance determinants called gene cassettes (Mazel 2006). According to Fluit and Schmitz (2004), integrons can be classified into two major groups: the resistance integrons (RIs) and the superintegrons (SIs). RIs carry mostly gene cassettes that encode resistance against antibiotics and can be located either on the chromosome or on plasmids. To date, four classes of resistance integrons (classes 1, 2, 3, and 4) have been found to be associated with resistance gene cassettes. *Class 1 integrons* are most frequently found among multiresistant gram-negative bacteria (Fluit and Schmitz 2004). Class 1 integrase *intI1* could carry diverse antibiotic resistance genes (ARGs) and conduct horizontal gene transfer among bacteria (Ma et al. 2017). Sulfonamide-resistant genes (*sul1*, *sul2*), tetracycline resistance genes (*tetA*, *tetB*, *tetC*, *tetM*), beta-lactam resistance genes (*VEB-1*, *VEB-6*), aminoglycoside resistance genes (*aadA7*), and trimethoprim resistance genes (*dhfrA12*, *dhfrA17*) are usually associated with class 1 integrons (Ma et al. 2017). Several investigators observed a significant correlation between the presence of class 1 integrons and multiresistance in gram-negative isolates (Leverstein-van Hall et al. 2003; Hansson et al. 2002; Bass et al. 1999; Martinez-Freijo et al. 1998).

## 5.2 Plasmids

Plasmids are extrachromosomal genetic elements that replicate independently of chromosomes (Lerminiaux and Cameron 2019). Plasmids often carry genes essential for initiation and control of replication and accessory genes such as antimicrobial resistance or virulence genes (Amabile-Cuevas and Chicurel 1992; Bergstrom et al. 2000; Thomas 1973). Antibiotic resistance determinants such as quinolone resistance genes, beta-lactamase-encoding genes ($bla_{CMY-1}$, $bla_{OXA-48}$, $bla_{KPC}$), and extended-spectrum beta-lactamase resistance genes ($bla_{CTX-M-1}$, $bla_{CTX-M-14}$) are circulated on plasmids (Blair et al. 2015). Martinez-Martinez et al. (1998) reported the first plasmid-mediated quinolone resistance mechanism, the qnr gene, which encodes a protein that protects type II topoisomerases from quinolones. Plasmid-mediated resistance to quinolones is mediated by *qnrA*, *qnrB*, *qnrC*, *qnrD*, *qnrS*, *aac (6′)-Ib-cr*, and *qepA*. Quinolone resistance genes usually associate with β-lactam resistance on multidrug resistance plasmids (Niero et al. 2018). Several studies have reported the horizontal transfer of quinolone resistance genes by conjugation (Kim et al. 2011; Yousfi et al. 2016).

## 5.3 Conjugative Transposons

Conjugative transposons are important for the dissemination of antibiotic resistance genes as conjugative plasmids. Also, conjugative transposons are involved in horizontal gene transfer events and contribute to long-term bacterial evolution and short-term adaptation, enabling rapid responses to environmental changes (Scott 2002). Stokes et al. (2007) identified aTn*1403*, a multiple-antibiotic resistance transposon made up of three distinct transposons. For quinolone resistance gene *aac(6')-Ib*-cr, seven alleles were reported around the world, and these alleles are spreading in two successful genetic platforms, *class 1 integrons* and Tn*3*-derivative transposons (Andres et al. 2013; Quiroga et al. 2007, 2015). Tetracycline-resistant strains possess a transposon of theTn*916-1545* family, as they carry the gene *int* coding for the integrase typical of these mobile elements (Clewell et al. 1995). The association of the *tet(M)* gene with Tn*916-1545* of transposon was confirmed by PCR (Rizzotti et al. 2009). Tn*5393* is a 6,705-bp transposon made up of a transposition module and a module carrying the *strA* and *strB* genes with an insertion sequence (Chiou and Jones 1993).

## 5.4 Single Nucleotide Polymorphisms

Whole-genome sequencing (WGS) has recently emerged as a reliable diagnostic tool for predicting antibacterial resistance in bacterial pathogens. It gives a complete view of the genotype of a bacterial isolate (Lauener et al. 2019). Detection of single nucleotide polymorphisms (SNPs) in the antibacterial resistance genes within same bacterial species is possible by this method (Mehla and Ramana 2015; Ramanathan et al. 2017).

Mutations in bacterial genome can cause antibacterial resistance by several mechanisms such as inactivation of the antibiotics and alteration or modification of the antibiotics or by triggering alteration of membrane permeability and efflux pumps (Alekshun and Levy 2007; Mehla and Ramana 2015). Mutations generally occur in the genes encoding central regulatory pathways or the primary drug target such as *gyrA*, *parC*, *23S rRNA*, *rpsL*, *rplC*, *rplD*, *gidB*, *rpoB*, and *walKR*. Resistance to major classes of antibiotics such as beta-lactams, aminoglycosides, quinolones, glycopeptides, macrolides, rifamycins, sulfonamides, polymyxins, and lipopeptides can all occur via mutations (Guerillot et al. 2018). SNPs in bacterial genomes have been reported both in efflux pump genes associated with increased antibiotic resistance (Kanji et al. 2017) and in the *23S rRNA*, *gyrA*, and *rpoB* genes exhibiting increased resistance to clarithromycin, levofloxacin, and rifampicin (Ramanathan et al. 2017). Lauener et al. (2019) observed nucleotide deletions and mutations resulting in the generation of stop codons in the antibiotic-resistant *Pseudomonas* isolates compared to susceptible ones. Stepwise acquisition of mutations (beginning with the gyrA mutation, followed by additional point mutations in topoisomerase IV) imparting greater antibiotic resistance in *Aeromonas* has been reported (Beka et al. 2018). Very low concentrations of antibiotics exerting a selection pressure on ciprofloxacin resistance were observed by them.

Genome variations (copy number variations and structure variations) can cause antibiotic resistance in *Aeromonas hydrophila* instead of single nucleotide polymorphism (Zhang et al. 2018).

## 6 AMR and ARGs Among Pathogens of Significance in Coastal and Estuarine Waters

### 6.1 *Vibrio* spp.

Vibrios are gram-negative halophilic bacteria occurring naturally in aquatic biotopes ranging from shallow coastal waters to the deepest parts of the ocean (Silvester et al. 2015). They are abundantly found in aquatic environments, including estuaries, marine coastal waters and sediments, and aquaculture settings (Denner et al. 2002). There are more than 100 recognized species under the genus *Vibrio* (Okada et al. 2010), among which 12 species are reported to be pathogenic to humans. It includes *Vibrio alginolyticus*, *V. cholerae*, *V. cincinnatiensis*, *Photobacterium damselae* (earlier *V. damselae*), *V. harveyi*, *Grimontia hollisae* (*earlier V. hollisae*), *V. fluvialis*, *V. furnissii*, *V. metschnikovii*, *V. mimicus*, *V. parahaemolyticus*, and *V. vulnificus* (Oliver et al. 2013).

During the past few decades, there has been a rapid emergence of antimicrobial resistance in many bacterial genera due to the excessive use of antibiotics in humans and agriculture and aquaculture systems (Cabello 2006). So far, research has been mainly focused on antibiotic-resistant bacteria in clinical environments. But recently, the rapid increase in community-acquired infections due to resistant bacteria has driven the interest in antibiotic resistance in natural environments (Forsberg

et al. 2012). The antibiotics recommended for treatment of *Vibrio* infections are fluoroquinolones, tetracyclines, third-generation cephalosporins, aminoglycosides, and folate pathway inhibitors (Daniels and Shafaie 2000). The excessive use of antibiotics has led to the emergence of multidrug-resistant strains that exhibit resistance to a single or a combination of antibiotics. Multiple-drug resistance among pathogenic microorganisms like *Vibrio* spp. in estuarine/marine environments will cause serious implications for those who consume seafood contaminated with these pathogenic vibrios and also for the recreational and commercial users of the aquatic environments (Shaw et al. 2014). There are numerous studies on the prevalence of multidrug-resistant *Vibrio* in natural waters and food sources from India. Table 1 gives a summary of the studies on antibiotic resistance among *Vibrio* from various coastal water environments in India.

## 6.2 *Aeromonas* spp.

Coexistence of different species of *Aeromonas* such as *A. salmonicida*, *A. bestiarum*, *A. encheleia*, *A. media*, and *A. rivipollensis* in the oligohaline area of the Seine estuary has been reported (Chaix et al. 2017). The quality of water in the estuary was found to be poor owing to wastewater treatment plants and was found to be contaminated by trace metals, such as cadmium and lead, and organic compounds such as polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), and pesticides (Carpentier et al. 2002). Multidrug-resistant *Aeromonas* isolates in water and sediment samples of estuarine environments were also reported (Henriques et al. 2006; Silva et al. 2014).

Exposure of bacteria to chronically low levels of biocides and metals can increase antibiotic resistance in them. This can be due to co-selection of genes that confer resistance to chemical hazards, biocides, antibiotics, and metals (Singer et al. 2016). The influence of metals such as mercury and arsenic exerting a selection pressure for antibiotic resistance in *Aeromonas* from environmental samples (Huddleston et al. 2006) and the presence of antibiotic-resistant *Aeromonas* in highly polluted river water receiving urban effluents (Goñi-Urriza et al. 2000) have been reported. Blasco, Esteve, and Alcaide (2008) observed that drug-resistant bacteria can occur in natural waters even in the absence of any selective pressure.

Adaptive antibiotic resistance is the ability of bacteria to survive antibiotic stress by altering the expression of some genes or proteins wherein global regulatory pathways, certain porins, and efflux pumps play important roles. An increase in translation-related proteins and a decrease in central metabolic-related proteins under oxytetracycline stress in *A. hydrophila* is observed by Yao et al. (2016). They have further observed that different exogenous metabolites such as malic acid, serine, methionine, etc. when compounded with oxytetracycline significantly decreased the survival capabilities of *A. hydrophila.* These findings are generating increasing interest as they may provide new strategies to the treatment of antibiotic-resistant bacterial infections. Summary of studies on AMR Aeromonas spp. from aquatic environments in Table 2.

**Table 1** Summary of studies on antibiotic-resistant *Vibrio* spp. from coastal environments

| Organism | Source of isolation | Antibiotic resistance | References |
|---|---|---|---|
| *V. parahaemolyticus* | Finfish samples from fish landing sites (Kasimedu, Thiruvanmiyur, Ennore) in coastal areas of Chennai | 100% of isolates resistant to cefazolin and gentamycin | Velappan and Munusamy (2019) |
| *V. parahaemolyticus* | Water samples from shrimp farms on the southwest coast of India | High-level resistance toward ampicillin, polymyxin B, and furazolidone | Devi et al. (2009) |
| *Vibrio* spp. | Water samples from shrimp culture ponds and hatcheries on the east coast of India | Highly resistant to chlortetracycline, oxytetracycline, neomycin, kanamycin, nalidixic acid, ampicillin | Vaseeharan et al. (2005) |
| *V. cholerae* | Water from Palk Bay | High level resistance towards β-lactam, vancomycin, nitrofurantoin, gentamycin, azithromycin, oxytetracycline, tetracycline and chloramphenicol | Sneha et al. (2016) |
| *V. parahaemolyticus* | Water samples from various stations in Cochin estuary | High resistance to amoxicillin, furazolidone, ampicillin, carbenicillin, nitrofurantoin, sulfamethoxazole, and enrofloxacin | Silvester et al. (2015) |
| *V. cholera* | Seafood harvested from Cochin waters | Resistant to cefpodoxime, ticarcillin, augmentin, and colistin | Kumar and Lalitha (2013) |
| *V. cholerae non-O1/non-O139* | Surface waters in Kolkata | Resistance to ampicillin, furazolidone, neomycin, streptomycin, tetracycline, and ciprofloxacin was observed among these isolates | Bag et al. (2008) |
| *V. cholerae non-O1/non-O139* | Aquatic locations in Alleppey district, Kerala | Resistance to cefotaxime, nalidixic acid, streptomycin and tetracycline, trimethoprim, co-trimoxazole, furazolidone, neomycin and ofloxacin, ciprofloxacin, norfloxacin, spectinomycin, gentamicin, and chloramphenicol | Jagadeeshan et al. (2009) |
| *Vibrio* spp. | Water samples from Narmada river | More than 50% of the isolates were resistant toward ampicillin, ceftazidime, erythromycin, chloramphenicol, cefuroxime | Sharma et al. (2009) |

(continued)

**Table 1** (continued)

| Organism | Source of isolation | Antibiotic resistance | References |
|---|---|---|---|
| *V. cholerae* | Stream water samples from Wayanad, Kerala | 100% resistance was observed to all antibiotics under study except for doxycycline | Mathews et al. (2018) |
| *V. harveyi* | Water suspended sediment samples from shrimp ponds and from the sea coasts, in the east coast of the Bay of Bengal, India | 100% isolates resistant to amikacin, ampicillin, meropenem, azithromycin, cefuroxime, ciprofloxacin, imipenem, erythromycin, gentamycin, nalidixic acid, norfloxacin, penicillin, ofloxacin, rifampicin, streptomycin, and vancomycin | Stalin and Srinivasan (2016) |
| *Vibrio* spp. | Water samples collected from two brackish water shrimp farms and nine coastal landing sites of Kerala | Highest incidence of antibiotic resistance was evident against amoxicillin, ampicillin, carbenicillin, and cefuroxime followed by rifampicin and streptomycin | Manjusha et al. (2005) |
| *Vibrio cholerae non-01* | Parangipettai coastal environment, southeast of India | Higher resistance to oxytetracycline, streptomycin, sulfadiazine, tetracycline | Sathiyamurthy et al. (1997) |
| *V. parahaemolyticus* | Seawater from Ponnani | Highest incidence of antibiotic resistance was recorded against cephalothin and nitrofurantoin | Reyhanath and Kutty (2014) |
| *Vibrio* spp. | Seafood collected from Cochin market | High incidence of resistance toward ampicillin, colistin, cephalothin, amoxicillin, carbenicillin, and ceftazidime | Sudha et al. (2014) |
| *Vibrio* spp. | Hooghly river, West Bengal, India | The isolates showed resistance to β-lactam derivatives and furazolidone | Mookerjee et al. (2015) |
| *Vibrio harveyi* | *P. monodon* hatchery along the southeast coast of India | All (100%) of the strains were resistant to bacitracin, streptomycin, methicillin, kanamycin, penicillin, amoxicillin, erythromycin, cephalothin, cephalexin, and azithromycin | Parvathi et al. (2011) |
| *Vibrio* spp. | Water and sediment samples from shrimp culture ponds and water from source seawater from Thulukenkulam, Chennai (Tamil Nadu), and Nellore (Andhra Pradesh) | All the isolates were 100% resistant to ampicillin, cloxacillin, oxacillin, erythromycin, vancomycin, penicillin G, and furazolidone | Srinivasan and Ramasamy (2009) |

| | | | |
|---|---|---|---|
| *V. parahaemolyticus*, *V. alginolyticus*, *V. anguillarum*, *V. vulnificus* | Shrimp farm samples from Uran, Maharashtra | *V. parahaemolyticus* and *V. anguillarum* were resistant to oxytetracycline and polymyxin B. *V. alginolyticus* and *V. vulnificus* resistant to ampicillin | Thakur et al. (2003) |
| *V. cholerae* | Aquatic environs in Calcutta | Resistant to furazolidone, ampicillin, neomycin, sulfonamide, trimethoprim, nalidixic acid, ciprofloxacin, streptomycin, chloramphenicol, tetracycline, and gentamicin | Thungapathra et al. (2002) |
| *Vibrio* spp. | Shrimp from inland ponds in Thailand | Resistant to ampicillin and oxytetracycline and nalidixic acid | Yano et al. (2014) |
| *Vibrio* spp. | Shrimp farms in Malaysia | Isolates were resistant to ampicillin, amikacin, kanamycin, and gentamicin and third-generation cephalosporins | Letchumanan et al. (2015) |
| *V. parahaemolyticus* | Cockle harvesting water in Malaysia | Resistant to streptomycin, tobramycin, carbenicillin, teicoplanin, cephalothin, clindamycin, rifampicin, sulfamethoxazole, and ofloxacin | Lesley et al. (2011) |
| *V. parahaemolyticus* | Shrimp farms in Italy | Resistant to amoxicillin and ampicillin, cefalexin, colistin, erythromycin, cefalothin, and streptomycin | Ottaviani et al. (2013) |
| *V. parahaemolyticus* | Louisiana Gulf in Mexico | Resistant to ampicillin, oxytetracycline, and tetracycline | Han et al. (2007) |
| *V. parahaemolyticus* | Coastal seawater in peninsular Malaysia | Resistance was observed to penicillin, ampicillin, carbenicillin, erythromycin, bacitracin, cephalothin, moxalactam, kanamycin, tetracycline, nalidixic acid, and gentamicin | Tanil et al. (2005) |
| *V. parahaemolyticus and V. vulnificus* | Recreational and commercial areas of Chesapeake Bay and Maryland Coastal Bays | Resistant to chloramphenicol and ampicillin | Shaw et al. (2014) |

**Table 2** Summary of studies on AMR *Aeromonas* spp. from aquatic environments

| Organism | Source of isolation | Antibiotic resistance | References |
|---|---|---|---|
| *Aeromonas veronii biovar veronii, A. veronii biovar sobria, A. hydrophila, A. caviae, A. enteropelogenes,* and *A. dhakensis* | Zebrafish (Danio rerio) | Amoxicillin, nalidixic acid, oxytetracycline, ampicillin, tetracycline, rifampicin, and imipenem | Hossain et al. (2019) |
| *Aeromonas veronii* | Channel catfish in China | Ciprofloxacin, levofloxacin, and norfloxacin | Yang et al. (2017) |
| *Aeromonas* spp. | Treated wastewater and samples of river water collected upstream and downstream from the effluent discharge point | Beta-lactams, tetracyclines, and aminoglycosides | Harnisz and Korzeniewska 2018 |
| *Aeromonas* spp. | Raw fish samples collected from retail markets of Chhattisgarh state in India | Extended spectrum β-lactamases (ESBL) ampicillin, cephalexin, and oxytetracycline | Khan et al. (2019) |
| *Aeromonas hydrophila* | Fish samples, Brazil | Oxacillin, tetracycline, and nalidixic acid | Freitas et al. (2018) |
| *Aeromonas hydrophila* and *A. veronii* | Lakes of Udaipur (Rajasthan), India | *A. veronii* were resistant to penicillin, vancomycin, kanamycin, polymyxin B, rifampicin, erythromycin, and streptomycin *A. hydrophila* were resistant to penicillin, vancomycin, kanamycin, polymyxin B, rifampicin, erythromycin, streptomycin, amikacin, and trimethoprim | Rawal et al. (2016) |
| *Aeromonas hydrophila* | From frozen fish marketed in Egypt | Cloxacillin, erythromycin, streptomycin, cefotaxime, sulfamethoxazole, cephalothin, chloramphenicol, and oxytetracycline | Hafez et al. (2018) |
| *Aeromonas* | Contents of healthy fish | Amoxicillin–clavulanic acid, streptomycin, aztreonam, cefotaxime, tetracycline, ceftazidime, and imipenem | Hammad et al. (2018) |
| *Aeromonas* species | Different aquatic sources in Melaka, Malaysia | Novobiocin, sulfamethoxazole, and trimethoprim | Odeyemi and Ahmad 2017 |

| | | | |
|---|---|---|---|
| *Aeromonas* | Water from natural water reservoirs and industrial cooling towers in Spain | Ticarcillin, erythromycin, and amoxicillin/clavulanic acid | Blasco et al. (2008) |
| Motile aeromonads | Rainbow Trout Farms in Denmark | Oxolinic acid, sulfadiazine-trimethoprim, and oxytetracycline | Schmidt et al. (2000) |
| Motile aeromonads | Ornamental fish and aquarium water samples, South India | Amoxycillin, carbenicillin, cephalothin, nalidixic acid, streptomycin, tetracycline, and trimethoprim | John and Hatha 2013 |
| *Aeromonas* spp | Tropical seafood, aquafarms, and mangroves in South India | Vancomycin, nalidixic acid, tetracycline, co-trimoxazole, and rifampicin | Joseph et al. (2013) |
| Motile aeromonads | Ornamental fish samples, South India | Amoxycillin, carbenicillin, cephalothin, nalidixic acid, streptomycin, and tetracycline | John and Hatha 2012 |
| *Aeromonas* | Ulcers of EUS-affected fish in India | Erythromycin, sulfadiazine, novobiocin, rifampin, and chloramphenicol | Das et al. (2009) |
| *Aeromonas hydrophila* | Marketed fish and prawn of South India | Methicillin, rifampicin, bacitracin, and novobiocin | Vivekanandhan et al. (2002) |
| *Aeromonas hydrophila*, *A. sobria,* and *A. caviae* | Farm-raised freshwater fish | Oxytetracycline, amoxycillin, novobiocin, and polymyxin-B | Hatha et al. (2005) |
| *Aeromonas hydrophila* | Retail seafood outlets of Coimbatore | Bacitracin, rifampicin, polymyxin, novobiocin, trimethoprim, and tetracycline | Thayumanavan et al. (2007) |

## 6.3 *Escherichia coli*

*E. coli*, a member of the *Enterobacteriaceae* family, is a common inhabitant of the human and animal gut. It is the most common cause of gram-negative nosocomial and community-acquired infections. Lately, *E. coli* resistant to at least two classes of antibiotic agents has become an ordinary finding in human and veterinary medicine and has an increasing impact on the available therapeutic options. *E. coli* strains can be classified as follows: (1) commensal, (2) intestinal pathogenic (enteric/diarrheagenic), or (3) extraintestinal pathogenic *E. coli* (ExPEC) (Russo and Johnson 2000). Intestinal pathogenic *E. coli* can be classified into enteropathogenic *E. coli* (EPEC), enterohemorrhagic *E. coli* (EHEC), enterotoxigenic *E. coli* (ETEC), enteroaggregative *E. coli* (EAEC), enteroinvasive *E. coli* (EIEC), and diffusely adherent *E. coli* (DAEC) (Nataro and Kaper 1998).

Water testing often uses the presence of coliforms including *E. coli* as an indicator of contamination. *E. coli* is widely distributed in all warm-blooded animals including humans, domestic livestock, pets, wild animals, and birds and is of interest in the study of antibiotic resistance since they are a common carrier of resistance genes and are capable of transferring those genes to other bacteria and bacterial species. Most municipalities in developed countries treat human sewage to reduce the bacterial load before releasing it into surrounding lakes, rivers, and oceans or spreading it on land. However, studies indicate that treated sewage and the water into which it is circulated remain heavily contaminated with antibiotic-resistant *E. coli* from humans (Coleman et al. 2013) and animals (Mariano et al. 2009; Krumperman 1983). However, in India due to population pressure and insufficient infrastructure to treat the waste, untreated or partially treated sewage and wastewater are directly released into the aquatic environment. Estuaries and coastal water bodies, which are the major sources of seafood in India, are often contaminated by the activities of adjoining populations and by the release of partially treated or untreated sewage from the townships into these water bodies. The fish harvested from such areas often contain human pathogenic microorganisms. In addition, poor sanitation and cross-contamination at landing centers and the open fish markets exacerbate the situation (Kumar et al. 2005). Summary of studies on antibiotic-resistant *Escherichia coli* from various aquatic environments in India is given in Table 3.

## 6.4 *Enterococci* and *Salmonella*

Enterococci are gram-positive bacteria and belong to the natural microflora of the intestinal tract of humans and animals (Facklam et al. 2002). They are ubiquitous in the feces of warm-blooded animals and have higher survival rate and resistance level in the environment. Hence, enterococci have been commonly used as excellent indicators of fecal pollution of environmental waters (Lleo et al. 2005). *E. faecalis* and *E. faecium* are the major *Enterococcus* species found in water systems. The infections caused by the enterococci include urinary tract infections (UTIs), wound infections, and bacteremia. The drug of choice for the treatment of enterococcal

**Table 3** Summary of studies on antibiotic-resistant *Escherichia coli* from various aquatic environments

| Organism | Source of isolation | Antibiotic resistance | References |
|---|---|---|---|
| *E. coli* | Hospital wastewater from Madhya Pradesh, India | Carbapenemase resistance | Chandran et al. (2014) |
| *E. coli* | Drinking water and seepage samples in New Delhi | NDM-1 β-lactamase | Walsh et al. (2011) |
| *E. coli* | Sewage treatment plants (STPs) in South India | Third-generation cephalosporin resistance | Akiba et al. (2015) |
| *E. coli* | Yamuna River in India | 100% resistance toward cefotaxime, ceftazidime, cephalothin, ciprofloxacin, imipenem, meropenem, and nitrofurantoin | Bhardwaj et al. (2015), Ahammad et al. (2014) |
| *E. coli* | River Ganga water | Multidrug resistance to amoxicillin, cephalothin, piperacillin, tetracycline, and ciprofloxacin | Biswas et al. (2015), Ram et al. (2007) |
| *E. coli* | Gomti river water, Lucknow | Multidrug resistance | Akhter et al. (2014) |
| *E. coli* | River Cauvery | 96% multidrug resistance | Skariyachan et al. (2015) |
| *E. coli* | Kazipally lake, Hyderabad in India | Quinolone resistance | Bengtsson-Palme et al. (2014) |
| *E. coli* | Mula-Mutha River at Pune City and downstream of the city | Ceftazidime and ciprofloxacin resistance | Dhawde et al. (2018) |
| *E. coli* | Kshipra River in Central India | Extended-spectrum beta-lactamase | Diwan et al. (2018) |
| *E.coli* | Natural sources of water in east Sikkim | Multidrug resistance | Poonia et al. (2014) |
| *E. coli* | Cooum estuary, Chennai Port, and Kasimedu fishing harbor in Chennai | 60% multidrug resistance | Vignesh et al. (2012) |
| *E. coli* | Girgaon beach in the southwest, Dadar and Mahim beach in central, and Juhu and Versova beach in the northwest, Mumbai | Multidrug resistance | Maloo et al. (2018) |
| *E. coli* | Water samples of Cochin estuary | Ampicillin, tetracycline, nalidixic acid, cefoxitin, co-trimoxazole, trimethoprim, and streptomycin | Divya and Hatha (2019) |
| *E. coli* | Seafood from India | Cephalothin, penicillin, and vancomycin | Kumar et al. (2005) |
| *E. coli* | Pelagic fishes in the Bay of Bengal at Digha coast, West Bengal, India | Ampicillin and streptomycin | Ghosh and Mandal (2010) |
| *E. coli* | Fresh seafood retail markets of Western Mumbai | Extended-spectrum β-lactamase | Singh et al. (2017) |

(continued)

**Table 3** (continued)

| Organism | Source of isolation | Antibiotic resistance | References |
|---|---|---|---|
| *E. coli* | Tagus estuary in Portugal | Streptomycin, tetracycline, beta-lactams, and sulfonamides | Pereira et al. (2013) |
| *E. coli* | Seine estuary in France | Tetracycline, amoxicillin, ticarcillin, chloramphenicol | Laroche et al. (2009) |
| *E. coli* | River Mur, Austria | Ampicillin, piperacillin, cefuroxime, cefotaxime, cefepime, ceftazidime, trimethoprim, and tetracycline | Zarfel et al. (2017) |
| *E. coli* | Surface water of Taihu Lake Basin, China | Multidrug resistance | Zhang et al. (2015) |
| *E. coli* | Coastal surface waters in England and Wales | Third-generation cephalosporins | Leonard et al. (2015) |
| *E. coli* | Aquaculture sites of Singapore | Beta-lactamase resistance | Ng et al. (2018) |
| *E. coli* | Dutch recreational waters | ESBL resistance | Blaak et al. (2014) |
| *E. coli* | River sources in Durban, South Africa | Streptomycin, tetracycline, ampicillin, novobiocin | Olaniran et al. (2009) |
| *E. coli* | River, Fayetteville, USA | Trimethoprim/ sulfamethoxazole resistance | Suhartono et al. (2016) |
| *E. coli* | River Danube, Vienna | ESBL and/or carbapenemase resistance | Kittinger et al. (2016) |

infections in humans is ampicillin, and vancomycin is used as an alternative (Hadi et al. 2010). The imprudent use of vancomycin has resulted in the emergence of vancomycin-resistant enterococci (VRE). *E. faecium* and *E. faecalis* are known as major reservoirs of acquired glycopeptide and multidrug resistance (Van and Willems 2010). Enterococci from nonhuman reservoirs are known to have a critical role in the acquisition and spread of antibiotic resistance determinants (Daniel et al. 2015). They are prone to acquire a wide range of antimicrobial resistance factors through horizontal gene transfer. Enterococci have multiple intrinsic drug resistance mechanisms, thus conferring resistance to antibiotics such as penicillin, monobactam, and low levels of aminoglycosides (Huycke et al. 1998). Multidrug-resistant enterococci can be transmitted to humans *via* various routes including contaminated food and water (Leclercq 2009). Enterococci can tolerate a high salt concentration which helps them to survive longer in marine environments (Harwood et al. 2000). A study by Carvalho et al. (2014) pointed out the existence of a relationship between the quantum of sewage disposed into the marine environment and the presence of multidrug-resistant enterococci in the same environment. The presence of MDR *Enterococcus* spp. in coastal and estuarine waters may pose health risk to humans exposed to such waters during recreational purposes. There are various studies reporting the presence of multiple antibiotic-resistant *Enterococcus* in the estuarine and coastal waters (Alipour et al. 2014; Belding and Boopathy 2018;

Carvalho et al. 2014; Lata et al. 2009; Moore et al. 2008; Vignaroli et al. 2018). In most of the developing countries, water systems act as a dumping site for household and industries. The vancomycin-resistant enterococci harboring the *vanA* gene were found to be prevalent in surface waters of the river Ganga (Lata et al. 2009). Moreover, pathogenic vancomycin-resistant *E. faecalis* was reported in surface waters of the river Gomti (Lata et al. 2016).

*Salmonella* from the gastrointestinal tract of humans and animals are released with feces or exudates into the environment and enter the surface waters through rainfall and surface runoff (Wiedemann et al. 2015). The CDC ranked antibiotic-resistant *Salmonella* as a "serious threat" based on the hazard level (Nair 2019). *Salmonella* persists even after antibiotic treatment and is known to cause brain tissue damage in mouse models (Chaudhuri et al. 2018). The antibiotic resistance mechanism in typhoid l *Salmonella* is mainly through chromosomal mutation, plasmid or transposon (Phan and Wain 2008). A previous report on the study of fecal sludge highlights that 70% of surface water in India is polluted and contaminated (WaterAid report 2016). These water systems have a major role in the dissemination of multidrug-resistant *Salmonella* (Nair 2019). *S. typhi*, the causative agent of typhoid, is often transmitted from humans to humans *via* contaminated waters. In India, the antibiotic treatment of typhoid fever is becoming increasingly ineffective due to the rapid emergence of ciprofloxacin resistance. Ciprofloxacin is the main drug of choice for treatment of infections caused by *Salmonella enterica* serovar Typhi and *S. enterica* serovar Paratyphi A (Dahiya et al. 2017). Drug-resistant *Salmonella* has been isolated from various water sources and sewage systems worldwide (Guillaume et al. 2000; Hassan et al. 2015; Liu et al. 2018; Mahmud et al. 2019). Carvalho et al. (2013) isolated antibiotic-resistant *Salmonella* from shrimp-harvesting waters in Brazil. Three isolated strains exhibited multiple resistance to ampicillin, tetracycline, oxytetracycline, and nitrofurantoin. *Salmonella* spp. from waters off the Veraval coast along India showed maximum resistance against macrolides and polypeptides (Maloo et al. 2014).

## 7 Status of AMR from Pristine Ecosystems like the Arctic and Antarctica

The Svalbard region (79° 58′ N) is considered as a relatively pristine environment. It has an exceedingly small human population with no agriculture or industry. This region never freezes due to Gulf currents, potentially receiving year-round allochthonous inputs (McCann et al. 2019). The most plausible source of allochthonous ARGs in this region is bird and other wildlife guano, disseminated either by local human wastes or via direct carriage and deposition (McCann et al. 2019). Hatha et al. (2013) reported the presence of antibiotic-resistant *E. coli* isolates in the droppings of migratory bird *Branta leucopis*, widely known as Barnacle goose, a prominent bird population in the Svalbard region which could act as a means of dissemination of potentially pathogenic bacteria into this environment. Hatha et al. (2015) reported relatively high antibiotic resistance among heterotrophic

bacteria from Arctic fjord sediments than water, indicating a better selection pressure for drug resistance mutants in the fjord sediments. Neethu et al. (2015) reported a positive correlation between antibiotic and metal resistance in gram-negative bacteria isolated from Kongsfjord, Arctic.

McCann et al. (2019) reported accumulation of apparent allochthonous ARGs and MGEs in the Kongsfjorden region on Svalbard. Antimicrobial resistance determinants have been observed in *Escherichia coli* isolates originating from Arctic birds (Sjolund et al. 2008). Arctic foxes prey upon these nesting birds and scavenge human rubbish around the Kongsfjorden settlement. Further, seabirds congregate near the wastewater outfall from the research station into the bay, presenting another possible local AR dispersal pathway (McCann et al. 2019).

Antarctic waters are considered relatively more pristine than the other oceanic waters experiencing anthropogenic influences only over larger timescales from the Northern Hemisphere (Bonner 1984). Wang et al. (2016) reported that soil near Antarctic research stations is an important environmental reservoir of antibiotic resistance genes (ARGs), which are increasingly recognized as environmental contaminants in Antarctica. The presence of ARGs in Antarctic soils is correlated with the proximity to the research stations. Miller et al. (2009) reported the prevalence of antibiotic resistance bacteria isolated from seawater and penguin fecal samples collected near Palmer Station, Antarctica. Lagana et al. (2019) reported that plastics can serve as vectors for the spread of multiple resistances to antibiotics across Antarctic marine environments. The bacteria isolated from plastic showed resistance against cefuroxime and cefazolin (cephalosporins), cinoxacin (quinolones) and ampicillin, amoxicillin + clavulanic acid, and carbenicillin and mezlocillin (beta-lactams).

## 8 "One Health Policy" and Global Action Plans [WHO (2015) and Government of India (2017)]

"One Health policy" is the coordinated surveillance systems of human AMR with animal surveillance systems and AMR in environmental settings. Antimicrobials used to treat various infectious diseases in animals may be similar to those used for humans. Resistant bacteria arising in humans, animals, or the environment may spread from one to the other and from one country to another. AMR does not recognize geographic or human-animal borders. The WHO, Food and Agriculture Organization (FAO) of the United Nations, and World Organisation for Animal Health (OIE) speak with one voice and take collective action to minimize the emergence and spread of AMR. Their aim is to ensure that antimicrobial agents continue to be effective and useful for curing diseases in humans and animals, promote prudent and responsible use of antimicrobial agents, and ensure global access to medicines of good quality.

In 2015, the WHO launched the Global Antimicrobial Resistance Surveillance System (GLASS), the first global collaborative effort to standardize AMR surveillance. GLASS supports the strategic objective of WHO's Global Action Plan on

AMR (GAP-AMR) to strengthen the AMR evidence base. GLASS provides a standardized approach to the collection, analysis, and sharing of AMR data by countries and seeks to document the status of existing or newly developed national AMR surveillance systems. GLASS is supported by WHO collaborating centers, involving strong commitment from participating countries and close collaborations with AMR regional networks.

India's National Action Plan (NAP) on AMR was released in April 2017 by the Union Ministry of Health and Family Welfare. The objectives of the NAP include improving awareness, enhancing surveillance measures, strengthening infection prevention and control, research and development, promoting investments, and collaborative activities to control AMR. On the basis of the NAP, various states have begun the process of initiating their State Action Plans.

## 9 Remedial Measures to Be Taken to Control AMR in Coastal and Estuarine Environments

The rising spread of AMR bacteria in estuaries and coastal environments is a reflection of discharges from municipal, industrial, and hospital wastewater. The problem is exacerbated by agriculture, aquaculture, and poultry farms upstream (Ghaderpour et al. 2015). Therefore, remedial measures to reduce AMR in estuaries and coastal environments should focus primarily on the reduction/prudent use of antibiotics in humans and animals. Increasing awareness must be generated among all stakeholders on the risks of irresponsible use of antibiotics, leading to antibiotic resistance, with its consequences on public health. Everybody must strictly observe the policies governing prescriptions of antibiotics.

Proper management of domestic ARB- and ARG-containing wastes from hospitals, industries, and agricultural sectors is another important aspect. Take-back programs to return unused antibiotics to pharmacies are suggested by the World Health Organization. Proper disposal of unused drugs must be implemented in countries where such programs are not operational at large scale. Onsite treatment plants equipped to deactivate antibiotics and resistant bacteria (OSTP-Zero ARB) must be taken into concern (Lundborg and Tamhankar 2017). Finally, the use of health-promoting compounds such as probiotics must be promoted.

## 10 Conclusion

One of the characteristic features of the coastal and estuarine environments is the constant pollution of these natural waters. Burgeoning population of coastal cities across the world exerts huge pressure on infrastructure to treat the wastes resulting in the release of an enormous quantity of organic wastes including sewage into these water bodies. With regard to the emergence and dissemination of drug-resistant mutants of pathogenic bacteria across the globe, the polluted natural waters play a crucial role by offering the congenial habitat for coexistence of opportunists,

pathogens, bacteriophages, stressed hosts, and mechanisms that enhance horizontal gene transfer. While several pathogens, both known and hitherto unknown, continue to make their presence felt, antibiotic-resistant strains of pathogenic vibrios, motile aeromonads, and diarrhegenic *E. coli* still pose a serious threat. Coastal water bodies being highly productive support excellent fish and shellfish resources. The above pathogens also find their way to human hosts through contaminated fish and shellfish and those who use polluted aquatic biotopes for recreation and bathing. Emergence of strains that are resistant to last-resort drugs such as colistin by virtue of resistance genes on mobile genetic elements is a matter of grave concern. Concerted efforts should be made to highlight the consequences of excessive and inappropriate use of antibiotics to the public. Right policy decisions to control/treat and release organic wastes and sewage to natural waters must be implemented to curb the threat of rising AMR in bacterial pathogens.

## References

Ahammad ZS et al (2014) Increased waterborne blaNDM-1 resistance gene abundances associated with seasonal human pilgrimages to the Upper Ganges River. Environ Sci Technol 48:3014–3020. https://doi.org/10.1021/es405348h

Akhter A, Imran M, Akhter F (2014) Antimicrobial resistant coliform bacteria in the Gomti river water and determination of their tolerance level. Bioinformation 10(4):167–174. https://doi.org/10.6026/97320630010167

Akiba M, Senba H, Otagiri H, Prabhasankar VP, Taniyasu S, Yamashita N, Lee K, Yamamoto T, Tsutsui T, Ian Joshua D, Balakrishna K, Bairy I, Iwata T, Kusumoto M, Kannan K, Guruge KS (2015) Impact of wastewater from different sources on the prevalence of antimicrobial-resistant *Escherichia coli* in sewage treatment plants in South India. Ecotoxicol Environ Saf 115:203–208. https://doi.org/10.1016/j.ecoenv.2015.02.018

Akiba M, Sekizuka T, Yamashita A, Kuroda M, Fujii Y, Murata M, Lee K, Joshua DI, Balakrishna K, Bairy I, Subramanian K, Krishnan P, Munuswamy N, Sinha RK, Iwata T, Kusumoto M, Guruge KS (2016) Distribution and relationships of antimicrobial resistance determinants among extended-spectrum-cephalosporin-resistant or carbapenem-resistant *Escherichia coli* isolates from rivers and sewage treatment plants in India. Antimicrob Agents Chemother 60:2972–2980. https://doi.org/10.1128/AAC.01950-15

Alekshun MN, Levy SB (2007) Molecular mechanisms of antibacterial multidrug resistance. Cell 128:1037–1050. https://doi.org/10.1016/j.cell.2007.03.004

Alipour M, Hajiesmaili R, Talebjannat M, Yahyapour Y (2014) Identification and antimicrobial resistance of *Enterococcus* spp. isolated from the river and coastal waters in northern Iran. Sci World J 2014:287458. https://doi.org/10.1155/2014/287458

Amabile-Cuevas CF, Chicurel ME (1992) Bacterial plasmids and gene flux. Cell 70:189–199. https://doi.org/10.1016/0092-8674(92)90095-T

Andres P, Lucero C, Soler-Bistué A, Guerriero L, Albornoz E, Tran T, Zorreguieta A, PMQR Group, Galas M, Corso A, Tolmasky ME, Petroni A (2013) Differential distribution of plasmid-mediated quinolone resistance genes in clinical enterobacteria with unusual phenotypes of quinolone susceptibility from Argentina. Antimicrob Agents Chemother 57:2467–2475. https://doi.org/10.1128/AAC.01615-12

Armon RH, Starosvetsky J (2015) Point source pollution indicators. In: Armon RH, Hänninen O (eds) Environmental Indicators. Springer, New York, NY, pp 495–499

Babic M, Hujer AM, Bonomo RA (2006) What's new in antibiotic resistance? Focus on beta-lactamases. Drug Resist Update 9:142–156. https://doi.org/10.1016/j.drup.2006.05.005

Bag PK, Bhowmik P, Hajra TK, Ramamurthy T, Sarkar P, Majumder M, Chowdhury G, Das SC (2008) Putative virulence traits and pathogenicity of *Vibrio cholerae* non-O1, non-O139 isolates from surface waters in Kolkata, India. App Environ Microbiol 74(18):5635–5644. https://doi.org/10.1128/AEM.00029-08

Bass L, Liebert CA, Lee MD, Summers AO, White DG, Thayer SG, Maurer JJ (1999) Incidence and characterization of integrons, genetic elements mediating multiple-drug resistance, in avian *Escherichia coli*. Antimicrob Agents Chemother 43:2925–2929

Batard E, Ollivier F, Boutoille D, Hardouin JB, Montassier E, Caillon J, Ballereau F (2013) Relationship between hospital antibiotic use and quinolone resistance in *Escherichia coli*. Int J Infect Dis 17:e254–e258. https://doi.org/10.1016/j.ijid.2012.10.005

Beka L, Fullmer MS, Colston SM, Nelson MC, Talagrand-Reboul E, Walker P, Ford B, Whitaker IS, Lamy B, Gogarten JP, Graf J (2018) Low-level antimicrobials in the medicinal leech select for resistant pathogens that spread to patients. MBio 9:1–17. https://doi.org/10.1128/mBio.01328-18

Belding C, Boopathy R (2018) Presence of antibiotic-resistant bacteria and antibiotic resistance genes in coastal recreational waters of southeast Louisiana, USA. J Water Supply Res Technol Aqua 67(8):800–809. https://doi.org/10.2166/aqua.2018.076

Bengtsson-Palme J, Boulund F, Fick J, Kristiansson E, Larsson DG (2014) Shotgun metagenomics reveals a wide array of antibiotic resistance genes and mobile elements in a polluted lake in India. Front Microbiol 5:1–14. https://doi.org/10.3389/fmicb.2014.00648

Berendonk TU, Manaia CM, Merlin C, Fatta-Kassinos D, Cytryn E, Walsh F, Bürgmann H, Sørum H, Norström M, Pons MN, Kreuzinger N, Huovinen P, Stefani S, Schwartz T, Kisand V, Baquero F, Martinez JL (2015) Tackling antibiotic resistance: the environmental framework. Nat Rev Microbiol 13:310–317. https://doi.org/10.1038/nrmicro3439

Bergstrom CT, Lipsitch M, Levin BR (2000) Natural selection, infectious transfer and the existence conditions for bacterial plasmids. Genetics 155:1505–1519

Bhardwaj R, Gupta A, Garg JK (2015) Prevalence of multidrug resistance in *Escherichia coli* strains isolated from river Yamuna, Delhi stretch. Int J Bioassay. https://doi.org/10.21746/ijbio.2015.11.0010

Biswas K, Paul D, Sinha SN (2015) Prevalence of multiple antibiotic-resistant coliform bacteria in the water of River Ganga. Sci Publ Gr 1:44. https://doi.org/10.11648/j.fem.20150103.12

Blaak H, de Kruijf P, Hamidjaja RA, van Hoek AH, de Roda Husman AM, Schets FM (2014) Prevalence and characteristics of ESBL-producing *E. coli* in Dutch recreational waters influenced by wastewater treatment plants. Vet Microbiol 171:448–459. https://doi.org/10.1016/j.vetmic.2014.03.007

Blair JM, Webber MA, Baylay AJ, Ogbolu DO, Piddock LJ (2015) Molecular mechanisms of antibiotic resistance. Nat Rev Microbiol 13:42–51. https://doi.org/10.1038/nrmicro3380

Blasco MD, Esteve C, Alcaide E (2008) Multiresistant waterborne pathogens isolated from water reservoirs and cooling systems. J Appl Microbiol 105:469–475. https://doi.org/10.1111/j.1365-2672.2008.03765.x

Bonner WN (1984) Conservation and the Antarctic. In: Laws RM (ed) Antarctic ecology, vol 2. Academic, London, pp 821–850

Borja A, Dauer DM, Elliott M, Simenstad CA (2010) Medium-and long-term recovery of estuarine and coastal ecosystems: patterns, rates and restoration effectiveness. Estuar Coasts 33:1249–1260. https://doi.org/10.1007/s12237-010-9347-5

Cabello FC (2006) Heavy use of prophylactic antibiotics in aquaculture: a growing problem for human and animal health and for the environment. Environ Microbiol 8:1137–1144

Cabello FC, Godfrey HP, Tomova A, Ivanova L, Dölz H, Millanao A, Buschmann AH (2013) Antimicrobial use in aquaculture re-examined: its relevance to antimicrobial resistance and to animal and human health. Environ Microbiol 15:1917–1942. https://doi.org/10.1111/1462-2920.12134

Carpentier S, Moilleron R, Beltran C, Hervé D, Thévenot D (2002) Quality of dredged material in the river Seine basin (France). II. Micropollutants. Sci Total Environ 299:57–72

Carvalho EM, Costa RA, Araujo AJ, Carvalho FCT, Pereira SP, Sousa OV, Vieira RH (2014) Multiple antibiotic-resistance of Enterococcus isolated from coastal water near an outfall in Brazil. AJMR 8(17):1825–1831. https://doi.org/10.5897/AJMR2014.6672

Chaix G, Roger F, Berthe T, Lamy B, Jumas-Bilak E, Lafite R, Forget-Leray J, Petit F (2017) Distinct Aeromonas populations in water column and associated with copepods from estuarine environment (Seine, France). Front Microbiol 8:1–13. https://doi.org/10.3389/fmicb.2017.01259

Chandran SP, Diwan V, Tamhankar AJ, Joseph BV (2014) Detection of carbapenem resistance genes and cephalosporin, and quinolone resistance genes along with oqxAB gene in Escherichia coli in hospital wastewater: a matter of concern. J Appl Microbiol 117(4):984–995. https://doi.org/10.1111/jam.12591

Chaudhry D, Tomar P (2017) Antimicrobial resistance: the next big pandemic. Int J Community Med Public Health 4:2632–2636. https://doi.org/10.18203/2394-6040.ijcmph20173306

Chaudhuri D, Chowdhury AR, Biswas B, Chakravortty D (2018) *Salmonella typhimurium* infection leads to colonization of the mouse brain and is not completely cured with antibiotics. Front. Microbiol 9:1632. https://doi.org/10.3389/fmicb.2018.01632

Chiou CS, Jones AL (1993) Nucleotide sequence analysis of a transposon (Tn*5393*) carrying streptomycin resistance genes in *Erwinia amylovora* and other Gram-negative bacteria. J Bacteriol 175:732–740. https://doi.org/10.1128/jb.175.3.732-740.1993

Clewell DB, Flannagan SE, Jaworski DD (1995) Unconstrained bacterial promiscuity: the Tn916-Tn1545 family of conjugative transposons. Trends Microbiol 3:229–236. https://doi.org/10.1016/S0966-842X(00)88930-1

Coleman BL, Louie M, Salvadori MI, McEwen SA, Neumann N, Sibley K, Irwin RJ, Jamieson FB, Daignault D, Majury A, Braithwaite S, Crago B, McGeer AJ (2013) Contamination of Canadian private drinking water sources with antimicrobial resistant *Escherichia coli*. Water Res 47:3026–3036. https://doi.org/10.1016/j.watres.2013.03.008

D'Costa VM, McGrann KM, Hughes DW, Wright GD (2006) Sampling the antibiotic resistome. Science 311:374–377. https://doi.org/10.1126/science.1120800

Dahiya S, Sharma P, Kumari B, Pandey S, Malik R, Manral N, Veeraraghavan B, Pragasam AK, Ray P, Gautam V, Sistla S, Parija SC, Walia K, Ohri V, Das BK, Sood S, Kapil A (2017) Characterisation of antimicrobial resistance in Salmonellae during 2014-2015 from four centres across India: an ICMR antimicrobial resistance surveillance network report. Indian J Med Microbiol 35:61. https://doi.org/10.4103/ijmm.IJMM_16_382

Daniel DS, Lee SM, Dykes GA, Rahman S (2015) Public health risks of multiple-drug-resistant Enterococcus spp. in Southeast Asia. Appl Environ Microbiol 81(18):6090–6097. https://doi.org/10.1128/AEM.01741-15

Daniels NA, Shafaie AA (2000) Review of pathogenic vibrio infections for clinicians. Infect Med 17:665–685

Das A, Saha D, Pal J (2009) Antimicrobial resistance and in vitro gene transfer in bacteria isolated from the ulcers of EUS-affected fish in India. Lett Appl Microbiol 49:497–502. https://doi.org/10.1111/j.1472-765X.2009.02700.x

Davies J, Davies D (2010) Origins and evolution of antibiotic resistance. Microbio Mol Bio Rev 74 (3):417–433. https://doi.org/10.1128/MMBR.00016-10

Denner EBM, Vybiral D, Fischer UR, Velimirov B, Busse HJ (2002) *Vibrio calviensis* sp. nov., a halophilic, facultatively oligotrophic 0.2 micron-filterable marine bacterium. Int J Syst Evol Microbiol 1(52):549–553. https://doi.org/10.1099/00207713-52-2-549

Devi R, Surendran PK, Chakraborty K (2009) Antibiotic resistance and plasmid profiling of Vibrio parahaemolyticus isolated from shrimp farms along the coast of India. World J Microbiol Biotechnol 25:2005–2012

Dhawde R, Macaden R, Saranath D, Nilgiriwala K, Ghadge A, Birdi T (2018) Antibiotic resistance characterization of environmental *E. coli* isolated from river Mula-Mutha, Pune District, India. Int J Environ Res Public Health 15:1–15. https://doi.org/10.3390/ijerph15061247

Divya SP, Hatha AAM (2019) Screening of tropical estuarine water in south-west coast of India reveals emergence of ARGs-harboring hypervirulent *Escherichia coli* of global significance. Int J Hyg Environ Health 222:235–248. https://doi.org/10.1016/j.ijheh.2018.11.002

Diwan V, Tamhankar AJ, Khandal RK, Sen S, Aggarwal M, Marothi Y, Iyer RV, Sundblad-Tonderski K, Stålsby-Lundborg C (2010) Antibiotics and antibiotic-resistant bacteria in waters associated with a hospital in Ujjain, India. BMC Public Health 10:414. https://doi.org/10.1186/1471-2458-10-414

Diwan V, Hanna N, Purohit M, Chandran S, Riggi E, Parashar V, Tamhankar AJ, Stålsby Lundborg C (2018) Seasonal variations in water-quality, antibiotic residues, resistant bacteria and antibiotic resistance genes of *Escherichia coli* isolates from water and sediments of the Kshipra River in Central India. Int J Environ Res Public Health 15(6):1281. https://doi.org/10.3390/ijerph15061281

Dolejska M, Cizek A, Literak I (2007) High prevalence of antimicrobial-resistant genes and integrons in *Escherichia coli* isolates from Black-headed Gulls in the Czech Republic. J Appl Microbiol 103:11–19. https://doi.org/10.1111/j.1365-2672.2006.03241.x

Dolejska M, Bierosova B, Kohoutova L, Literak I, Cizek A (2009) Antibiotic-resistant Salmonella and *Escherichia coli* isolates with integrons and extended-spectrum beta-lactamases in surface water and sympatric black-headed gulls. J Appl Microbiol 106:1941–1950. https://doi.org/10.1111/j.1365-2672.2009.04155.x

ESCMID (2019) Ecological study identifies potential association between antimicrobial resistance and climate change. European Society of Clinical Microbiology and Infectious Diseases. https://www.sciencedaily.com/releases/2019/04/190414111454.htm. Accessed 7 Nov 2019

Facklam RR, Carvalho MS, Teixeira LM (2002) History, taxonomy, biochemical characteristics, and antibiotic susceptibility testing of enterococci. In: Gilmore MS (ed) The *Enterococci:* pathogenesis, molecular biology, and antibiotic resistance. ASM Press, Washington, DC, pp 1–54

Fick J, Soderstrom H, Lindberg RH, Phan C, Tysklind M, Larsson DG (2009) Contamination of surface, ground, and drinking water from pharmaceutical production. Environ Toxicol Chem 28:2522–2527. https://doi.org/10.1897/09-073.1

Fischer MM, Bild M (2019) Hospital use of antibiotics as the main driver of infections with antibiotic-resistant bacteria—a reanalysis of recent data from the European Union. bioRxiv:553537. https://doi.org/10.1101/553537

Fluit AC, Schmitz FJ (2004) Resistance integrons and super-integrons. Clin. Microbiol. Infect. 10:272–288. https://doi.org/10.1111/j.1198-743X.2004.00858.x

Forsberg KJ, Reyes A, Wang B, Selleck EM, Sommer MOA, Dantas G (2012) The shared antibiotic resistome of soil bacteria and human pathogens. Science 337:1107–1111

Freitas MR, Freire NB, Peixoto LJS et al (2018) The presence of plasmids in *Aeromonas hydrophila* and its relationship with antimicrobial and heavy metal-resistance profiles. Ciencia Rural 48:9. https://doi.org/10.1590/0103-8478cr20170813

Gao P, Munir M, Xagoraraki I (2012) Correlation of tetracycline and sulfonamide antibiotics with corresponding resistance genes and resistant bacteria in a conventional municipal wastewater treatment plant. Sci Tot Environ 421:173–183. https://doi.org/10.1016/j.scitotenv.2012.01.061

Ghaderpour A, Ho WS, Chew LL, Bong CW, Chong VC, Thong KL, Chai LC (2015) Diverse and abundant multi-drug resistant *E. coli* in Matang mangrove estuaries, Malaysia. Front Microbiol 6:977. https://doi.org/10.3389/fmicb.2015.00977

Ghosh K, Mandal S (2010) Antibiotic resistant bacteria in consumable fishes from Digha coast, West Bengal, India. Proc Zool Soc 63(1):13–20

Gillings MR, Stokes HW (2012) Are humans increasing bacterial evolvability? Trends Ecol Evol 6:346–352. https://doi.org/10.1016/j.tree.2012.02.006

Goñi-Urriza M, Pineau P, Capdepuy M, Roques C, Caumette P, Quentin C (2000) Antimicrobial resistance of mesophilic *Aeromonas* spp. isolated from two European rivers. J Antimicrob Chemother 46:297–301. https://doi.org/10.1093/jac/46.2.297

Guerillot R, Li L, Baines S, Howden B, Schultz MB, Seemann T, Howden BP (2018) Comprehensive antibiotic-linked mutation assessment by resistance mutation sequencing (RM-seq). Genome Med 10:1–15. https://doi.org/10.1186/s13073-018-0572-z

Guillaume G, Verbrugge D, Chasseur-Libotte ML, Moens W, Collard JM (2000) PCR typing of tetracycline resistance determinants (Tet A–E) in *Salmonella enterica* serotype Hadar and in the microbial community of activated sledges from hospital and urban wastewater treatment facilities in Belgium. FEMS MicrobEcol 32:77–85. https://doi.org/10.1111/j.1574-6941.2000.tb00701.x

Gullberg E, Albrecht LM, Karlsson C, Sandegren L, Andersson DI, Baquero F (2014) Selection of a multidrug resistance plasmid by sublethal levels of antibiotics and heavy metals. MBio 5(5).

Hadi U, van den Broek P, Kolopaking EP, Zairina N, Gardjito W, Gyssens IC (2010) Study Group Antimicrobial Resistance in Indonesia: prevalence and prevention (AMRIN). Cross-sectional study of availability and pharmaceutical quality of antibiotics requested with or without prescription (over the counter) in Surabaya, Indonesia. BMC Infect Dis 10:203. https://doi.org/10.1186/1471-2334-10-203

Hafez AEE, Darwish WS, Elbayomi RM et al (2018) Prevalence, antibiogram and molecular characterization of *Aeromonas hydrophila* isolated from frozen fish marketed in Egypt. Slov Vet Res 55:445–454. https://doi.org/10.26873/SVR-671-2018

Hammad AM, Moustafa AH, Mansour MM, Fahmy BM, Hamada MG, Shimamoto T, Shimamoto T (2018) Molecular and phenotypic analysis of hemolytic *Aeromonas* strains isolated from food in Egypt revealed clinically important multidrug resistance. Clini J food Prot 81:1015–1021. https://doi.org/10.4315/0362-028x.jfp-17-360

Han F, Walker RD, Janes ME, Prinyawiwatkul W, Ge B (2007) Antimicrobial susceptibilities of *Vibrio parahaemolyticus* and *Vibrio vulnificus* isolates from Louisiana Gulf and retail raw oysters. Appl Environ Microbiol 73:7096–7098. https://doi.org/10.1128/AEM.01116-07

Hansson K, Sundstrom L, Pelletier A, Roy PH (2002) IntI2 integron integrase in Tn7. J Bacteriol 184(6):1712–1721. https://doi.org/10.1128/JB.184.6.1712-1721.2002

Harnisz M, Korzeniewska E (2018) The prevalence of multidrug-resistant *Aeromonas* spp. in the municipal wastewater system and their dissemination in the environment. Sci Total Environ 626:377–383. https://doi.org/10.1016/J.SCITOTENV.2018.01.100

Harwood VJ, Whitlock J, Withington V (2000) Classification of antibiotic resistance patterns of indicator bacteria by discriminant analysis: use in predicting the source of fecal contamination in subtropical waters. Appl Environ Microbiol 66(9):3698–3704. https://doi.org/10.1128/aem.66.9.3698-3704.2000

Hassan MM, Ahaduzzaman M, Alam M, Bari MS, Amin KB, Faruq AA (2015) Antimicrobial resistance pattern against *E. coli* and *Salmonella* spp. in environmental effluents. Int J Nat Sci 5 (2):52–58. https://doi.org/10.3329/ijns.v5i2.28612

Hassard F, Gwyther CL, Farkas K, Andrews A, Jones V, Cox B, Brett H, Jones DL, McDonald JE, Malham SK (2016) Abundance and distribution of enteric bacteria and viruses in coastal and estuarine sediments—a review. Front Microbiol 7:1692. https://doi.org/10.3389/fmicb.2016.01692

Hatha AAM, Vivekanandhan G, Julie Joice G, Christol (2005) Antibiotic resistance pattern of motile aeromonads from farm raised fresh water fish. Int J Food Microbiol 98:131–134. https://doi.org/10.1016/j.ijfoodmicro.2004.05.017

Hatha AAM, Divya PS, Saramma AV et al (2013) Migratory bird, Branta leucopis (Barnacle goose), a potential carrier of diverse Escherichia coli serotypes into pristine arctic environment. Curr Sci 104:1078–1080

Hatha AAM, Neethu CS, Nikhil SM, Mujeeb Rahiman KM, Krishnan KP, Saramma AV (2015) Relatively high antibiotic resistance among heterotrophic bacteria from arctic fjord sediments than water—evidence towards better selection pressure in the fjord sediments. Polar Sci 9:382–388. https://doi.org/10.1016/j.polar.2015.10.002

Hendriksen RS, Munk P, Njage P, van Bunnik B, McNally L, Lukjancenko O, Röder T, Nieuwenhuijse D, Pedersen SK, Kjeldgaard J, Kaas RS, Clausen PTLC, Vogt JK, Leekitcharoenphon P, van de Schans MGM, Zuidema T, de Roda Husman AM, Rasmussen S, Petersen B et al (2019) Global monitoring of antimicrobial resistance based on

metagenomics analyses of urban sewage. Nat Commun 10:112. https://doi.org/10.1038/s41467-019-08853-3

Henriques IS, Fonseca F, Alves A, Saavedra MJ, Correia A (2006) Occurrence and diversity of integrons and β-lactamase genes among ampicillin-resistant isolates from estuarine waters. Res Microbiol 157:938–947. https://doi.org/10.1016/J.RESMIC.2006.09.003

Hossain S, Dahanayake PS, De Silva BCJ, Wickramanayake MVKS, Wimalasena SHMP, Heo GJ (2019) Multidrug resistant *Aeromonas* spp. isolated from zebrafish ( *Danio rerio*): antibiogram, antimicrobial resistance genes and class 1 integron gene cassettes. Lett Appl Microbiol 68:370–377. https://doi.org/10.1111/lam.13138

Huddleston JR, Zak JC, Jeter RM (2006) Antimicrobial susceptibilities of *Aeromonas* spp. isolated from environmental sources. Appl Environ Microbiol 72:7036–7042. https://doi.org/10.1128/AEM.00774-06

Huycke MM, Sahm DF, Gilmore MS (1998) Multiple-drug resistant enterococci: the nature of the problem and an agenda for future. Emerg Infect Dis *4*:239–249. https://doi.org/10.3201/eid0402.980211

Igbinosa IH (2014) Antibiogram profiling and pathogenic status of *Aeromonas* species recovered from Chicken. Saudi J Biol Sci 21:481–485. https://doi.org/10.1016/j.sjbs.2014.06.003

Jagadeeshan S, Kumar P, Abraham WP, Thomas S (2009) Multi-resistant *Vibrio cholerae* non-O1/non-O139 from waters in South India: resistance patterns and virulence-associated gene profiles. J Basic Microbiol 49(6):538–544. https://doi.org/10.1002/jobm.200900085

John N, Hatha AAM (2012) Prevalence, distribution and drug resistance of motile aeromonads in freshwater ornamental fishes. Indian J Fish 59:161–164

John N, Hatha AAM (2013) Distribution, extracellular virulence factors and drug resistance of motile aeromonads in fresh water ornamental fishes and associated carriage water. Int J Aquac 3:92–100. https://doi.org/10.5376/ija.2013

Joseph AV, Sasidharan RS, Nair HP, Bhat SG (2013) Occurrence of potential pathogenic *Aeromonas* species in tropical seafood, aquafarms and mangroves off Cochin coast in South India. Vet World 6:300–306. https://doi.org/10.5455/vetworld.2013.300-306

Kanji A, Hasan R, Ali A, Zaver A, Zhang Y, Imtiaz K, Hasan Z (2017) Single nucleotide polymorphisms in efflux pumps genes in extensively drug resistant *Mycobacterium tuberculosis* isolates from Pakistan. Tuberculosis 107:20–30. https://doi.org/10.1016/j.tube.2017.07.012

Khan R, Shakya S, Chandrakar C, Pandey AK, Hattimare DD, Patyal A, Ali SL (2019) Isolation, identification and antimicrobial susceptibility pattern of extended-spectrum-β-lactamase *Aeromonas* spp. from fish samples of Chhattisgarh State, India. Int J Curr Microbiol Appl Sci 8:1011–1015

Kim J, Kang HY, Lee Y (2008) The identification of CTX-M-14, TEM-52, and CMY-1 enzymes in *Escherichia coli* isolated from the Han River in Korea. J Microbiol 46:478–481. https://doi.org/10.1007/s12275-008-0037-y

Kim KY, Park JH, Kwak HS, Woo GJ (2011) Characterization of the quinolone resistance mechanism in foodborne Salmonella isolates with high nalidixic acid resistance. Int J Food Microbiol 146:52–56. https://doi.org/10.1016/j.ijfoodmicro.2011.01.037

Kittinger C, Lipp M, Folli B, Kirschner A, Baumert R, Galler H, Grisold AJ, Luxner J, Weissenbacher M, Farnleitner AH, Zarfel G (2016) Enterobacteriaceae isolated from the River Danube: antibiotic resistances, with a focus on the presence of ESBL and carbapenemases. PLoS One 11:e0165820. https://doi.org/10.1371/journal.pone.0165820

Knapp CW, McCluskey SM, Singh BK, Campbell CD, Hudson G, Graham DW (2011) Basal antibiotic resistance gene abundances correlate with metal and Geochemical Conditions in Archived Scottish Soils. PLoS One 6(11):e27300. https://doi.org/10.1371/journal.pone.0027300

Kouyos RD, Abel Zur Wiesch P, Bonhoeffer S (2011) On being the right size: the impact of population size and stochastic effects on the evolution of drug resistance in hospitals and the community. PLoS Pathog 7:e1001334. https://doi.org/10.1371/journal.ppat.1001334

Krumperman PH (1983) Multiple antibiotic resistance indexing of *Escherichia coli* to identify high-risk source of fecal contamination of foods. Appl Environ Microbiol 46(1):165–170

Kumar HS, Parvathi A, Karunasagar I (2005) Prevalence and antibiotic resistance of Escherichia coli in tropical seafood. World J Microbiol Biotechnol 21:619–623. https://doi.org/10.1007/s11274-004-3555-8

Kumar R, Lalitha KV (2013) Prevalence and molecular characterization of *Vibrio cholerae* O1, non-O1 and non-O139 in tropical seafood in Cochin, India. Foodborne Pathog Dis 10 (3):278–283. https://doi.org/10.1089/fpd.2012.1310

Kummerer K (2003) Significance of antibiotics in the environment. J Antimicrob Chemother 52:5–7. https://doi.org/10.1093/jac/dkg293

Kummerer K (2009) Antibiotics in the aquatic environment-a review—part I. Chemosphere. 75:417–434. https://doi.org/10.1016/j.chemosphere.2008.11.086

Laganà P, Caruso G, Corsi I, Bergami E, Venuti V, Majolino D, La Ferla R, Azzaro M, Cappello S (2019) Do plastics serve as a possible vector for the spread of antibiotic resistance? First insights from bacteria associated to a polystyrene piece from King George Island (Antarctica). Int J Hyg Environ Health 222:89–100. https://doi.org/10.1016/j.ijheh.2018.08.009

Lamba M, Ahammad SZ (2017) Sewage treatment effluents in Delhi: a key contributor of β-lactam resistant bacteria and genes to the environment. Chemosphere 188:249–256. https://doi.org/10.1016/j.chemosphere.2017.08.133

Laroche E, Pawlak B, Berthe T, Skurnik D, Petit F (2009) Occurrence of antibiotic resistance and class 1, 2 and 3 integrons in Escherichia coli isolated from a densely populated estuary (Seine, France). FEMS Microbiol Ecol 68:118–130. https://doi.org/10.1111/j.1574-6941.2009.00655.x

Lata P, Ram S, Agrawal M, Shanker R (2009) Real time PCR for the rapid detection of vanA gene in surface waters and aquatic macrophyte by molecular beacon probe. Environ Sci Technol 43 (9):3343–3348. https://doi.org/10.1021/es803635y

Lata P, Ram S, Shanker R (2016) Multiplex PCR based genotypic characterization of pathogenic vancomycin resistant *Enterococcus faecalis* recovered from an indian river along a city landscape. Springerplus 5:1199. https://doi.org/10.1186/s40064-016-2870-5

Lauener FN, Imkamp F, Lehours P, Buissonnière A, Benejat L, Zbinden R, Keller PM, Wagner K (2019) Genetic determinants and prediction of antibiotic resistance phenotypes in *Helicobacter pylori*. J Clin Med 8:53. https://doi.org/10.3390/jcm8010053

Leclercq R (2009) Epidemiological and resistance issues in multi-drug resistant staphylococci and enterococci. Clin Microbiol Infect 15:224–231. https://doi.org/10.1111/j.1469-0691.2009.02739.x

Leonard AFC, Zhang L, Balfour AJ et al (2015) Human recreational exposure to antibiotic resistant bacteria in coastal bathing waters. Environ Int 82:92–100. https://doi.org/10.1016/j.envint.2015.02.013

Lerminiaux NA, Cameron ADS (2019) Horizontal transfer of antibiotic resistance genes in clinical environments. Can J Microbiol 65:34–44. https://doi.org/10.1139/cjm-2018-0275

Lesley M, Velnetti L, Cheah Y, Son R, Kasing A, Samuel L, Micky V, Nishibuchi M (2011) Antibiotic resistance and plasmid profiling of *Vibrio parahaemolyticus* isolated from cockles (Anadaragranosa) at TanjungKarang, Kuala Selangor. Int Food Res J 18:1183–1188

Letchumanan V, Yin WF, Lee LH, Chan KG (2015) Prevalence and antimicrobial susceptibility of Vibrio parahaemolyticus isolated from retail shrimps in Malaysia. Front Microbiol 6:33. https://doi.org/10.3389/fmicb.2015.00033

Leverstein-van Hall MA, MHE B, Rogier A, Donders T, Paauw A, Fluit AC, Verhoef J (2003) Multidrug resistance among *Enterobacteriaceae* is strongly associated with the presence of integrons and is independent of species or isolate origin. J Infect Dis 187:251–259. https://doi.org/10.1086/345880

Li R, Jay JA, Stenstrom MK (2019) Fate of antibiotic resistance genes and antibiotic-resistant bacteria in water resource recovery facilities. Water Environ Res 91:5–20. https://doi.org/10.1002/wer.1008

Lleo MDM, Bonato B, Benedetti D, Canepari P (2005) Survival of enterococcal species in aquatic environments. FEMS Microbiol Ecol 54:189–196. https://doi.org/10.1016/j.femsec.2005.03.016

Liu H, Whitehouse CA, Li B (2018) Presence and persistence of Salmonella in water: the impact on microbial quality of water and food safety. Front Public Health 6:1–13. https://doi.org/10.3389/fpubh.2018.00159

Lubbert C, Baars C, Dayakar A, Lippmann N, Rodloff AC, Kinzig M, Sorgel F (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 45:479–491

Lundborg CS, Tamhankar AJ (2017) Antibiotic residues in the environment of South East Asia. BMJ 358:42–45. https://doi.org/10.1136/bmj.j2440

Lupo A, Coyne S, Ulrich Berendonk T (2012) Origin and evolution of antibiotic resistance: the common mechanisms of emergence and spread in water bodies. Front Microbiol 3:18. https://doi.org/10.3389/fmicb.2012.00018

Ma L, Li AD, Yin XL, Zhang T (2017) The prevalence of integrons as the carrier of antibiotic resistance genes in natural and man-made environments. Environ Sci Technol 51:5721–5728. https://doi.org/10.1021/acs.est.6b05887

MacFadden DR, McGough SF, Fisman D, Santillana M, Brownstein JS (2018) Antibiotic resistance increases with local temperature. Nat Clim Chang 8:510–514. https://doi.org/10.1038/s41558-018-0161-6

Mahalmani VM, Sarma P, Prakash A, Medhi B (2019) Positive list of antibiotics and food products: current perspective in India and across the globe. Indian J Pharmacol 51:231–235. https://doi.org/10.4103/ijp.IJP_548_19

Mahmud AT, Tanim MT, Chowdhury MT, Rahaman MM, Rahman SR, Rahman MM (2019) Genetic diversity of *Salmonella enterica* strains isolated from sewage samples of different hospitals in Bangladesh. Bangladesh J Microbiol 35(1):57–60. https://doi.org/10.3329/bjm.v35i1.39804

Maloo A et al (2014) Occurrence and distribution of multiple antibiotic-resistant bacteria of Enterobacteriaceae family in waters of Veraval coast, India. Environ Exp Biol 12:43–50

Maloo A et al (2014) Occurrence and distribution of multiple antibiotic-resistant bacteria of Enterobacteriaceae family in waters of Veraval coast, India. Environ Exp Biol 12:43–50

Maloo A, Fulke AB, Mulani N, Sukumaran S, Ram A (2018) Pathogenic multiple antimicrobial resistant *Escherichia coli* serotypes in recreational waters of Mumbai, India: a potential public health risk. Environ Sci Pollut Res 24:11504–11517. https://doi.org/10.1007/s11356-017-8760-8

Manjusha S, Sarita GB, Elyas KK, Chandrasekaran M (2005) Multiple antibiotic resistances of Vibrio isolates from coastal and brackish water areas. Am J Biochem Biotechnol 1(4):201–206. https://doi.org/10.3844/ajbbsp.2005.193.198

Marathe NP, Regina VR, Walujkar SA et al (2013) A treatment plant receiving waste water from multiple bulk drug manufacturers is a reservoir for highly multi-drug resistant integron-bearing bacteria. PLoS One 8:2–11. https://doi.org/10.1371/journal.pone.0077310

Mariano V, McCrindle CME, Cenci-Goga B, Picard JA (2009) Case-control study to determine whether river water can spread tetracycline resistance to unexposed impala (*Aepyceros melampus)* in Kruger National Park (South Africa). Appl Environ Microbiol 75(1):113–118. https://doi.org/10.1128/AEM.01808-08

Marti E, Jofre J, Balcazar JL (2013) Prevalence of antibiotic resistance genes and bacterial community composition in a river influenced by a wastewater treatment plant. PLoS One 8 (10):e78906. https://doi.org/10.1371/journal.pone.0078906

Martinez JL (2009) The role of natural environments in the evolution of resistance traits in pathogenic bacteria. Proc R Soc B 276:2521–2530. https://doi.org/10.1098/rspb.2009.0320

Martinez-Freijo P, Fluit AC, Schmitz FJ, Grek VS, Verhoef J, Jones ME (1998) Class I integrons in Gram-negative isolates from different European hospitals and association with decreased

susceptibility to multiple antibiotic compounds. J Antimicrob Chemother 42:689–696. https://doi.org/10.1093/jac/42.6.689

Martinez-Martinez L, Pascual A, Jacoby GA (1998) Quinolone resistance from a transferable plasmid. Lancet 351:797–799. https://doi.org/10.1016/S0140-6736(97)07322-4

Mathews EB, Sunil B, Prejit N, Sunanda C, Vinod VK, Vergis J, Asha K, Sunitha R (2018) Occurrence and antibiotic susceptibility testing of *Vibrio cholerae* from district Wayanad, Kerala, India. Proc Natl Acad Sci India Sect B Biol Sci 88(2):673–678

Mazel D (2006) Integrons: agents of bacterial evolution. Nat Rev Microbiol 4:608–620. https://doi.org/10.1038/nrmicro1462

McCann CM, Christgen B, Roberts JA, Su JQ, Arnold KE, Gray ND, Zhu YG, Graham DW (2019) Understanding drivers of antibiotic resistance genes in High Arctic soil ecosystems. Environ Int 125:497–504. https://doi.org/10.1016/j.envint.2019.01.034

McEwen SA, Fedorka-Cray PJ (2000) Antimicrobial use and resistance in animals. Clin Infect Dis 34:S93–S106. https://doi.org/10.1086/340246

Mehla K, Ramana J (2015) DBDiaSNP: an open-source knowledgebase of genetic polymorphisms and resistance genes related to diarrheal pathogens. Omi A J Integr Biol 19:354–360. https://doi.org/10.1089/omi.2015.0030

Miller RV, Gammon K, Day MJ (2009) Antibiotic resistance among bacteria isolated from seawater and penguin fecal samples collected near Palmer Station, Antarctica. Can J Microbiol 55:37–45. https://doi.org/10.1139/W08-119

Mohamed Hatha AA, Divya PS, Saramma A V., et al. (2013) Migratory bird, Branta leucopis (Barnacle goose), a potential carrier of diverse Escherichia coli serotypes into pristine arctic environment. Curr Sci 104:1078–1080

Mookerjee S, Batabyal P, Sarkar MH, Palit A (2015) Seasonal prevalence of enteropathogenic Vibrio and their phages in the riverine estuarine ecosystem of South Bengal. PLoS One 10(9): e0137338. https://doi.org/10.1371/journal.pone.0137338

Moore DF, Guzman JA, McGee C (2008) Species distribution and antimicrobial resistance of enterococci isolated from surface and ocean water. J Appl Microbiol 105:1017–1025. https://doi.org/10.1111/j.1365-2672.2008.03828.x

Mutiyar PK, Mittal AK (2014) Risk assessment of antibiotic residues in different water matrices in India: key issues and challenges. Environ Sci Pollut Res 21:7723–7736. https://doi.org/10.1007/s11356-014-2702-5

Nair A (2019) Strengthening strategies to tackle salmonellosis, antimicrobial resistance and sanitation crisis. Curr Sci 116:9

Nataro JP, Kaper JB (1998) Diarrheagenic *Escherichia coli*. Clin Microbiol Rev 11:142–201

Neethu CS, Rahiman KMM, Saramma AV, Hatha AAM (2015) Heavy-metal resistance in gram-negative bacteria isolated from Kongsfjord, Arctic. Can J Microbiol 61:429–435. https://doi.org/10.1139/cjm-2014-0803

Ng C, Chen H, Goh SG, Haller L, Wu Z, Charles FR, Trottet A, Gin K (2018) Microbial water quality and the detection of multidrug resistant *E. coli* and antibiotic resistance genes in aquaculture sites of Singapore. Mar Pollut Bull 135:475–480. https://doi.org/10.1016/j.marpolbul.2018.07.055

Niero G, Bortolaia V, Vanni M, Intorre L, Guardabassi L, Piccirillo A (2018) High diversity of genes and plasmids encoding resistance to third-generation cephalosporins and quinolones in clinical *Escherichia coli* from commercial poultry flocks in Italy. Vet Microbiol 216:93–98. https://doi.org/10.1016/j.vetmic.2018.02.012

Odeyemi OA, Ahmad A (2017) Antibiotic resistance profiling and phenotyping of *Aeromonas* species isolated from aquatic sources. Saudi J Biol Sci 24:65–70. https://doi.org/10.1016/j.sjbs.2015.09.016

Okada N, Matsuda S, Matsuyama J et al (2010) Presence of genes for type III secretion system 2 in *Vibrio mimicus* strains. BMC Microbiol 10:302. https://doi.org/10.1186/1471-2180-10-302

Olaniran AO, Naicker K, Pillay B (2009) Antibiotic resistance profiles of *Escherichia coli* isolates from river sources in Durban, South Africa. World J Microbiol Biotechnol 25:1743–1749. https://doi.org/10.1007/s11274-009-0071-x

Oliver JD, Pruzzo C, Vezzulli L, Kaper JB (2013) Vibrio species. In: Doyle MP, Buchanan RL (eds) Food microbiology: fundamentals and frontiers, 4th edn. ASM, Washington, pp 401–440

Ottaviani D, Leoni F, Talevi G, Masini L, Santarelli S, Rocchegiani E, Susini F, Montagna C, Monno R, D'Annibale L, Manso E, Oliva M, Pazzani C (2013) Extensive investigation of antimicrobial resistance in *Vibrio parahaemolyticus* from shellfish and clinical sources, Italy. Int J Antimicrob Agents 42:191–193. https://doi.org/10.1016/j.ijantimicag.2013.05.003

Parvathi A, Mendez D, Anto C (2011) Distribution of putative virulence genes and antimicrobial drug resistance in *Vibrio harveyi*. Ind J Microbiol 51(3):332–337. https://doi.org/10.1007/s12088-011-0128-z

Patil HJ, 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. Front Microbiol 7:1875. https://doi.org/10.3389/fmicb.2016.01875

Pereira A, Santos A, Tacao M, Alves A, Henriques I, Correia A (2013) Genetic diversity and antimicrobial resistance of *Escherichia coli* from Tagus estuary (Portugal). Sci Total Environ 461–462:65–71. https://doi.org/10.1016/j.scitotenv.2013.04.067

Phan MD, Wain JJ (2008) IncHI plasmids, a dynamic link between resistance and pathogenicity. Infect Dev Countr 2(4):272–278. https://doi.org/10.3855/jidc.221

Poonia S, Singh TS, Tsering DC (2014) Antibiotic susceptibility profile of bacteria isolated from natural sources of water from rural areas of East Sikkim. Indian J Community Med Indian Assoc Prev Soc Med 39(3):156

Quiroga MP, Andres P, Petroni A, Soler Bistue AJ, Guerriero L, Vargas LJ, Zorreguieta A, Tokumoto M, Quiroga C, Tolmasky ME, Galas M, Centron D (2007) Complex class 1 integrons with diverse variable regions, including *aac(6')-Ib-cr*, and a novel allele, *qnrB10*, associated with IS*CR1* in clinical enterobacterial isolates from Argentina. Antimicrob Agents Chemother 51:4466–4470. https://doi.org/10.1128/AAC.00726-07

Quiroga MP, Orman B, Errecalde L, Kaufman S, Centron D (2015) Characterization of Tn6238 with a new allele of aac(6′)-Ib-cr. Antimicrob Agents Chemother 59:2893–2897. https://doi.org/10.1128/AAC.03213-14

Ram S, Vajpayee P, Shanker R (2007) Prevalence of multi-antimicrobial-agent resistant shiga toxin and enterotoxin producing *Escherichia coli* in surface waters of river Ganga. Environ Sci Technol 41:7383–7388. https://doi.org/10.1021/es0712266

Ramanathan B, Jindal HM, Le CF, Gudimella R, Anwar A, Razali R, Sekaran SD (2017) Next generation sequencing reveals the antibiotic resistant variants in the genome of *Pseudomonas aeruginosa*. PLoS One 12:1–15. https://doi.org/10.1371/journal.pone.0182524

Rawal I, Joshi H, Chaudhary BL (2016) Isolation, identification, and antibiotics resistance of *Aeromonas* spp. from lakes of Udaipur (Rajasthan), India. Asian J Pharm 10:132–136. https://doi.org/10.22377/ajp.v10i2.612

Reyhanath PV, Kutty R (2014) Incidence of multidrug resistant Vibrio parahaemolyticus isolated from Ponnani, South India. Iran J Microbiol 6(2):60–67. https://www.ncbi.nlm.nih.gov/pubmed/25705353

Rizzo L, Manaia C, Merlin C, Schwartz T, Dagot C, Ploy MC, Michael I, Fatta-Kassinos D (2013) Urban wastewater treatment plants as hotspots for antibiotic resistant bacteria and genes spread into the environment: a review. Sci Total Environ 447:345–360

Rizzotti L, La Gioia F, Dellaglio F, Torriani S (2009) Molecular diversity and transferability of the tetracycline resistance gene tet(M), carried on Tn916-1545 family transposons, in enterococci from a total food chain. Antonie van Leeuwenhoek 96:43–52. https://doi.org/10.1007/s10482-009-9334-7

Rodgers K, McLellan I, Peshkur T, Williams R, Tonner R, Hursthouse AS, Knapp CW, Henriquez FL (2019) Can the legacy of industrial pollution influence antimicrobial resistance in estuarine sediments? Environ Chem Lett 17:595–607. https://doi.org/10.1007/s10311-018-0791-y

Rodriguez-Mozaz S, Chamorro S, Marti E, Huerta B, Gros M, Sànchez-Melsió A, Borrego CM, Barceló D, Jose Balcazar JL (2015) Occurrence of antibiotics and antibiotic resistance genes in hospital and urban wastewaters and their impact on the receiving river. Water Res 69:234–242. https://doi.org/10.1016/j.watres.2014.11.021

Russo TA, Johnson JR (2000) Proposal for a new inclusive designation for extraintestinal pathogenic isolates of *Escherichia coli*: ExPEC. J Infect Dis 181:1753–1754. https://doi.org/10.1086/315418

Sabri NA, Schmitt H, Van der Zaan B, Gerritsen HW, Zuidema T, Rijnaarts HHM, Langenhoff AAM (2018) Prevalence of antibiotics and antibiotic resistance genes in a wastewater effluent-receiving river in the Netherlands. J Environ Chem Eng. https://doi.org/10.1016/j.jece.2018.03.004

Sahu R, Saxena P. Antibiotics in chicken meat (2014) PML/PR-48/2014. New Delhi, India: Centre for Science and Environment, Centre for Science and Environment, India. Available from https://cdn.cseindia.org/userfiles/Antibiotics%20in%20Chicken_Lab%20Report_Final%2029%20July.pdf.

Sathiyamurthy K, Purushothaman A, Ramaiyan V (1997) Antibiotic-resistant in Parangipettai coastal environs, South East India. Microb Drug Resist 3(3):267–270

Sathiyamurthy K, Purushothaman A, Ramaiyan V (1997) Antibiotic-resistant *Vibrio cholerae* in parangipettai coastal environs, South East India. Microb Drug Resist 3(3):267(70). https://doi.org/10.1089/mdr.1997.3.267

Schmidt AS, Bruun MS, Dalsgaard I, Pedersen K, Larsen JL (2000) Occurrence of antimicrobial resistance in fish-pathogenic and environmental bacteria associated with four Danish rainbow trout farms. Appl Environ Microbiol 66:4908–4915. https://doi.org/10.1128/aem.66.11.4908-4915.2000

Scott KP (2002) The role of conjugative transposons in spreading antibiotic resistance between bacteria that inhabit the gastrointestinal tract. Cell Mol Life Sci 59:2071–2082. https://doi.org/10.1007/s000180200007

Sharma A, Bora CR, Chaurasia RK, Sahu V (2009) Antibiotic susceptibility and genetic analysis of *Vibrio* species isolated from riverine environment. Curr Res Bacteriol 2:36–49

Shaw KS, Rosenberg Goldstein RE, He X, Jacobs JM, Crump BC, Sapkota AR (2014) Antimicrobial susceptibility of *Vibrio vulnificus* and *Vibrio parahaemolyticus* recovered from recreational and commercial areas of Cheaspeake Bay and Maryland coastal bay. PLoS One 9:e89616. https://doi.org/10.1371/journal.pone.0089616

Siddiqui Md K, Khatoon N, Roy PC (2016) Untreated liquid hospital waste: potential source of multidrug resistant bacteria. Bangladesh J Microbiol 32(1–2):21–24

Silbergeld EK, Graham J, Price LB (2008) Industrial food animal production, antimicrobial resistance, and human health. Annu Rev Public Health 29:151–169. https://doi.org/10.1146/annurev.publhealth.29.020907.090904

Silva CM, Evangelista-Barreto NS, RHSDF V, Mendonça KV, Sousa OV (2014) Population dynamics and antimicrobial susceptibility of *Aeromonas* spp. along a salinity gradient in an urban estuary in Northeastern Brazil. Mar Pollut Bull 89:96–101. https://doi.org/10.1016/J.MARPOLBUL.2014.10.031

Silvester R, Alexander D, Hatha AAM (2015) Prevalence, antibiotic resistance, virulence and plasmid profiles of *Vibrio parahaemolyticus* from a tropical estuary and adjoining traditional prawn farms along the south west coast of India. Ann Microbiol 65:2141–2149. https://doi.org/10.1007/s13213-015-1053-x

Singer AC, Shaw H, Rhodes V, Hart A (2016) Review of antimicrobial resistance in the environment and its relevance to environmental regulators. Front Microbiol 7:1–22. https://doi.org/10.3389/fmicb.2016.01728

Singh AS, Lekshmi M, Prakasan S, Nayak BB (2017) Multiple antibiotic-resistant, extended Spectrum-β-Lactamase (ESBL)-producing Enterobacteria in fresh seafood. Microorganisms 5:53. https://doi.org/10.3390/microorganisms5030053

Sjolund M, Bonnedahl J, Hernandez J, Bengtsson S, Cederbrant G, Pinhassi J, Kahlmeter G, Olsen B (2008) Dissemination of multidrug-resistant bacteria into the Arctic. Emerg Infect Dis 14:70–72. https://doi.org/10.3201/eid1401.070704

Skariyachan S, Mahajanakatti AB, Grandhi NJ, Prasanna A, Sen B, Sharma N, Vasist KS, Narayanappa R (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. Environ Monit Assess 187:279. https://doi.org/10.1007/s10661-015-4488-4

Sneha KG, Anas A, Jayalakshmy KV, Jasmin C, Vipin Das PV, Pai SS, Pappu S, Nair M, Muraleedharan KR, Sudheesh K, Nair S (2016) Distribution of multiple antibiotic resistant Vibrio spp across Palk Bay. Reg Stud Mar Sci 3:242–250. https://doi.org/10.1016/j.rsma.2015.11.004

Ramasamy P, Srinivasan P (2009) Occurrence, distribution and antibiotic resistance patterns of Vibrio species associated with viral diseased shrimp of South Indian aquaculture environment. Int J Agric Sci 1(2):1–10

Stalin N, Srinivasan P (2016) Molecular characterization of antibiotic resistant *Vibrio harveyi* isolated from shrimp aquaculture environment in the south east coast of India. Microb Pathog 97:110–118. https://doi.org/10.1016/j.micpath.2016.05.021

Stokes HW, Elbourne LD, Hall RM (2007) Tn1403, a multiple-antibiotic resistance transposon made up of three distinct transposons. Antimicrob Agents Chemother 51:1827–1829. https://doi.org/10.1128/AAC.01279-06

Sudha S, Mridhula C, Silvester R, Hatha AAM (2014) Prevalence and antibiotic resistance of pathogenic Vibrios in shellfishes from Cochin market. Indian J Geo Marine Sci 43(5):815–824

Suhartono S, Savin M, Gbur EE (2016) Genetic redundancy and persistence of plasmid-mediated trimethoprim/sulfamethoxazole resistant effluent and stream water *Escherichia coli*. Water Res 103:197–204. https://doi.org/10.1016/j.watres.2016.07.035

Tanil GB, Radu S, Nishibuchi M, Rahim RA (2005) Characterization of *Vibrio parahaemolyticus* isolated from coastal seawater in peninsular Malaysia. Southeast Asian J Trop Med Public Health 36(4):940

Thakur AB, Vaidya RB, Suryawanshi SA (2003) Pathogenicity and antibiotic susceptibility of *Vibrio* species isolated from moribund shrimps. IJMS 32(1):71–75

Thayumanavan SR, Vivekanandhan G, Savithamani K, Lakshmanaperumalsamy P (2007) Haemolytic and multidrug resistant *Aeromonas hydrophila* cross contamination in retail seafood outlets of Coimbatore, South India. Am J Food Technol:87–94. https://doi.org/10.3923/ajft.2007.87.94

Thomas CM (1973) Paradigms of plasmid organization. Mol Microbiol 74:335–337

Thungapathra M, Amita SKK, Chaudhuri SR, Garg P, Ramamurthy T, Nair GB, Ghosh A (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. Antimicrob Agents Chemother 46(9):2948–2955. https://doi.org/10.1128/AAC.46.9.2948-2955.2002

Van SW, Willems RJ (2010) Genome-based insights into the evolution of Enterococci. Clin Microbiol Infect 16:527–532. https://doi.org/10.1111/j.1469-0691.2010.03201.x

Vaseeharan B, Ramasamy P, Murugan T, Chen JC (2005) In vitro susceptibility of antibiotics against Vibrio spp. and Aeromonas spp. isolated from Penaeus monodon hatcheries and ponds. Int J Antimicrob Agents 26:285–291. https://doi.org/10.1016/j.ijantimicag.2005.07.005

Velappan M, Munusamy D (2019) In vitro susceptibility of commonly used antibiotics against *Vibrio parahaemolyticus*, isolated from Finfishes, Chennai, India. J Infec Dis Treat 5(1):1

Vignaroli C, Pasquaroli S, Citterio B, Di Cesare A, Mangiaterra G, Fattorini D, Biavasco F (2018) Antibiotic and heavy metal resistance in Enterococci from coastal marine sediment. Environ Pollut 237:406–413. https://doi.org/10.1016/j.envpol.2018.02.073

Vignesh S, Muthukumar K, James RA (2012) Antibiotic resistant pathogens versus human impacts: a study from three eco-regions of the Chennai coast, southern India. Mar Pollut Bull 64:790–800. https://doi.org/10.1016/j.marpolbul.2012.01.015

Vikesland P, Garner E, Gupta S, Kang S, Maile-Moskowitz A, Zhu N (2019) Differential drivers of antimicrobial resistance across the World. Acc Chem Res 52:916–924. https://doi.org/10.1021/acs.accounts.8b00643

Vivekanandhan G, Savithamani K, Hatha AAM, Lakshmanaperumalsamy P (2002) Antibiotic resistance of *Aeromonas hydrophila* isolated from marketed fish and prawn of South India. Int J Food Microbiol 76:165–168. https://doi.org/10.1016/S0168-1605(02)00009-0

Walsh TR, Weeks J, Livermore DM, Toleman MA (2011) Dissemination of NDM-1 positive bacteria in the New Delhi environment and its implications for human health: an environmental point prevalence study. Lancet Infect Dis 11:355–362

Wang F, Stedtfeld RD, Kim OS et al (2016) Influence of soil characteristics and proximity to antarctic research stations on abundance of antibiotic resistance genes in soils. Environ Sci Technol 50(23):12621–12629

Warnes SL, Highmore CJ, Keevil CW (2012) Horizontal transfer of antibiotic resistance genes on abiotic touch surfaces: implications for public health. mBio 3:e00489-12. https://doi.org/10.1128/mBio.00489-12

WaterAid India (2016) An assessment of faecal sludge management policies and programmes at the national and select states level. WaterAid Report. http://wateraidindia.in/wpcontent/uploads/2016/01/Faecal-SludgeManagement-Report.pdf

WHO (World Health Organization) (2015) One health policy. WHO, Geneva. http://www.euro.who.int/en/health-topics/disease-prevention/antimicrobial-resistance/about-amr/one-health

WHO (World Health Organization) (2017) Global antimicrobial resistance surveillance system (GLASS) report early implementation. WHO, Geneva

Wiedemann A, Virlogeux-Payant I, Chausse AM, Schikora A, Velge P (2015) Interactions of Salmonella with animals and plants. Front Microbiol 5:791. https://doi.org/10.3389/fmicb.2014.00791

Woolhouse M, Ward M, Van Bunnik B, Farrar J (2015) Antimicrobial resistance in humans, livestock and the wider environment. Philos Trans R Soc Lond B Biol Sci 370 (1670):20140083. https://doi.org/10.1098/rstb.2014.0083

Yang Q, Zhao M, Wang KY, Wang J, He Y, Wang EL, Liu T, Chen DF, Lai W (2017) Multidrug-resistant *Aeromonas veronii* recovered from Channel Catfish (*Ictalurus punctatus* ) in China: prevalence and mechanisms of fluoroquinolone resistance. Microb Drug Resist 23:473–479. https://doi.org/10.1089/mdr.2015.0296

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 38:30–45. https://doi.org/10.1016/j.foodcont.2013.09.019

Yao Z, Li W, Lin Y, Wu Q, Yu F, Lin W, Lin X (2016) Proteomic analysis reveals that metabolic flows affect the susceptibility of *Aeromonas hydrophila* to antibiotics. Sci Rep 6:1–10. https://doi.org/10.1038/srep39413

Yousfi M, Mairi A, Touati A, Hassissene L, Brasme L, Guillard T, De Champs C (2016) Extended spectrum β -lactamase and plasmid mediated quinolone resistance in *Escherichia coli* fecal isolates from healthy companion animals in Algeria. J Infect Chemother 22:431–435. https://doi.org/10.1016/j.jiac.2016.03.005

Zarfel G, Lipp M, Gürtl E et al (2017) Troubled water under the bridge: screening of River Mur water reveals dominance of CTX-M harboring *Escherichia coli* and for the first time an environmental VIM-1 producer in Austria. Sci Total Environ 593–594:399–405. https://doi.org/10.1016/j.scitotenv.2017.03.138

Zhang SH, Lv X, Han B et al (2015) Prevalence of antibiotic resistance genes in antibiotic-resistant *Escherichia coli* isolates in surface water of Taihu Lake Basin, China. Environ Sci Pollut Res 22:11412–11421. https://doi.org/10.1007/s11356-015-4371-4

Zhang G, Zhang Y, Wang H, Xu L, Lv L (2018) Comparative genomic analysis of five high drug-resistance *Aeromonas hydrophila* strains induced by doxycycline in laboratory and nine reference strains in Genbank. Aquac Res 49:2553–2559. https://doi.org/10.1111/are.13717

Zhou H, Smith DW (2002) Advanced technologies in water and wastewater treatment. J Environ Engin Sci 1:247–264. https://doi.org/10.1139/s02-020

# AMR in Animal Health: Issues and One Health Solutions for LMICs

Robin J. Paul and Digna Varghese

## 1 Introduction

Antimicrobial resistance (AMR), according to the World Health Organization (WHO), is one of 2019's top ten threats to global health (WHO 2019) along with others such as climate change, air pollution, noncommunicable diseases (NCDs), global influenza pandemic, and Ebola. Also, AMR is going to be an overwhelming burden for low- and middle-income countries (LMICs) due to issues such as excessive antibiotic usage in humans and animals, poor infection prevention in hospitals, lack of urban sanitation along with chronic malnutrition, privatization of healthcare, and lack of meaningful regulations on animal antibiotic usage. However, global agenda setting for AMR control in animal health is dominated by high-income countries (HICs) and may not reflect priorities and needs of LMICs, where the production systems, levels of resistance, and also the types of disease caused by bacteria in farmed animals and poultry are different. The global plans for controls are also hampered due to the lack of data pertaining to AMR arising from animal health in LMICs, thereby posing a major challenge in formulating appropriate policies to address the issue, which has to be ultimately driven by robust data.

There has been significant progress in reducing antibiotic use in agriculture, particularly in HICs, but there is a long way to go in LMICs. There has been progress in raising awareness on avoiding unnecessary use of antibiotics, but questions remain about its impact and effectiveness in changing behavior. Proposals and regulatory controls to restrict over-the-counter sales of antibiotics have foundered in the face of the realities of living conditions and access to healthcare in LMICs (Clift 2019).

R. J. Paul (✉)
State Laboratory, Department of Animal Husbandry, Govt. of Kerala, Ernakulam, Kerala, India

D. Varghese
P.G. and Research Department of Chemistry, Christ College (Autonomous), Irinjalakuda, Kerala, India



S. Thomas (ed.), *Antimicrobial Resistance*,
https://doi.org/10.1007/978-981-15-3658-8_6

A recent study on global trends in antimicrobial resistance in animals in LMICs indicates that the highest levels of AMR in animals are currently found in large LMICs like China and India with massive livestock and poultry populations and recommends that these countries should take immediate actions to preserve antimicrobials that are essential in human medicine by restricting their use in animal production. This study further indicates that in some middle-income countries, particularly South America, the AMR surveillance must be scaled up to match that of low-income African countries that are currently outperforming them despite more limited resources (Van Boeckel et al. 2019). Though the study is an aggregation of point prevalence surveys, it seems to be imperfect in the absence of systematic surveillance. However, the maps provided with this study should be useful to guide policy interventions in many LMICs.

The greatest burden of zoonotic diseases lies within poor, marginalized, rural communities that live in close proximity with livestock and may have reduced access to safe food and healthcare (OIE 2017). The major issue with antibiotic resistance is that the resistant clones of several major zoonotic animal pathogens, like *Salmonella*, *Campylobacter*, and enterohemorrhagic *E. coli* (EHEC), have been increasingly isolated from the food supply, including food animals, poultry, retail meat products, fresh produce, and seafood. All major resistance determinants, including those that confer resistance to β-lactams, extended-spectrum β-lactams, fluoroquinolones, aminoglycosides, tetracyclines, and chloramphenicol, have been identified in various *Salmonella* serovars isolated from the food supply. It has become increasingly clear that antibiotic resistance in food animals will remain a significant hurdle to tackle in the near future (Nair et al. 2018).

A study by Sivalingam et al. (2019) on environmental prevalence of carbapenem-resistant *Enterobacteriaceae* (CRE) in tropical ecosystems in India underscores the urgent need for systematic surveillance studies concerning AMR bacteria in vegetables, seafood, and fish that are in direct contact with contaminated urban effluents, soil, and irrigation water at various stages, such as the harvesting and postharvest levels. This study recommends the implementation of strict national policies to curb the indiscriminate use of antibiotics in aquaculture, agriculture, veterinary, livestock farms, poultry, food animals, and antibiotic stewardship.

There are many ways in which antibiotics in animals can affect humans, wherein the direct contact between animals and humans is the primary cause of disease due to resistant bacteria. The liberal use of antibiotics in pig farms, including beta-lactams, tetracyclines, and even colistin, has been implicated in the transmission of methicillin-resistant *Staphylococcus aureus* (MRSA) and other superbugs. Farmers and livestock and meat handlers are at risk of being colonized by the livestock-associated MRSA (LA-MRSA). Although LA-MRSA isn't as dangerous as hospital-associated MRSA (HA-MRSA), as it has animal adaptation and does not spread as easily from person to person, it is estimated that 40% of commercially sold pork meat contained methicillin-resistant *Staphylococcus aureus*. Though distinction between MRSA strains from hospitals, communities, and livestock is breaking down, LA-MRSA now poses a significant threat especially for immunocompromised individuals.

It is therefore essential to study the spread of AMR in superbugs of animal origin such as *Staphylococcus aureus*, *Salmonella*, and multidrug-resistant *Enterobacteriaceae*, especially at the human-animal-environment interface; and this can be effectively addressed only by a sustained collaborative One Health approach across human, animal, and environmental disciplines (Ewers et al. 2012).

## 2 Major AMR Drivers and Addressing Them in LMICs

Major drivers for AMR in LMICs are diverse due to the different systems of production for each animal species, wherein animal agriculture is an ancient practice and is closely linked with civilizations along with many socioeconomic factors, thus making it difficult to regulate, raise awareness, and implement control programs. This coupled with the sheer size and diversity of many LMICs calls for global solutions along with sustained local efforts.

A recent report (Ryan 2019) reviewed evidence on the economic benefits and costs of antimicrobials for the major food-producing animals across several Organisation for Economic Co-operation and Development (OECD) countries as well as Brazil and China. The findings indicate that the economic benefits are modest in modern farming systems where good production facilities, biosecurity measures, and management practices are in place. In large food animal-producing countries such as Brazil, the use of antimicrobials is an important input to enhance the competitiveness of the industry. In China, the largest producer and user of antibiotics in animal production, antibiotics are often used as a substitute for minimal sanitary animal production facilities and the lack of appropriate biosecurity on the farm. This report concludes with several key policy options and practices, in particular those that induce farmers to emphasize on the economic benefits and costs of antimicrobials and alternative interventions in production in order to stem the rise in antimicrobial resistance.

Existing factors driving AMR in LMICs include a high prevalence of infection due to poor animal disease prevention, shortage of veterinary care inclusive of weak laboratories and subsequent inadequacy in state management, shortfall in new drugs and problems with drug quality, unsafe animal feed, and inadequate hygienic practices in animal husbandry and industrial food animal production by a poorly aware farming community dispersed over large geographical areas which is out of the enforcement capacity of a weak regulator.

### 2.1 AMR in Animal Health: A Regulatory Problem

Antimicrobial use in livestock is legislated in HICs with the veterinary profession being intricately involved in enforcement. In contrast, in LMICs, the scenario for livestock production is shaped by weak regulations and regulators, irrational drug use, and poorly educated livestock farmers. The government veterinary and livestock departments in LMICs are mandated to increase production of farm animals so

as to meet the demand for high-quality animal protein, and such protein has to be produced cheaply as it should be affordable to poorer malnourished sections of the society. The governments and industry are hence antagonistic to the idea of regulating production of an already scarce resource, and rightly so, as in many LMICs, deaths due to malnutrition, especially protein deficiency and lack of access to antimicrobials, far exceed those by AMR. This has also to be seen with an understanding that animal protein may be the panacea for preventing malnutrition and stunting among children especially in the first 3 years of life in LMICs of Asia and Africa (Kaimila et al. 2019).

Even though India has one of the largest livestock and poultry populations, with broiler poultry production almost doubling every 7 years along with the concurrent use of antimicrobials, still like many other LMICs, there is no dedicated veterinary regulator which regulates the production, sale, distribution, and use of antimicrobials (Government of India 2017). As intensive animal farms are increasing due to rapid urbanization and decreasing land availability, many food animals are being reared in unhygienic environments, which make them more prone to infectious diseases, thereby emphasizing the role of antimicrobials in disease prevention and growth promotion. Significant knowledge gaps remain in areas such as the economic contributions of antimicrobials through their reduction in livestock disease burdens and their estimated impacts on hunger and poverty alleviation (Rushton et al. 2014).

Industrial food animal production (IFAP) is characterized by large-scale, highly specialized, densely stocked operations designed to maximize output at minimal cost to producers (Casey et al. 2015). Production relies heavily on inputs, including specially formulated feeds, pharmaceuticals like antimicrobials, and synthetic hormones (in cattle), the use of which has been implicated in the presence of environmental, occupational, and food-borne hazards. This model has become increasingly globalized, with multinational corporations expanding operations from HICs to Mexico and countries in Southeast Asia, Eastern Europe, and other parts of the world.

In a recent study (Lam et al. 2019) where the industrialization of food animal production in ten LMICs (Ethiopia, Myanmar, India, Vietnam, Brazil, China, Mexico, Turkey, Kenya, and Uganda) across four continents was viewed through the perspective of environmental public health, it was concluded that industrialized methods have increased livestock production to varying extents based on species and location. Expansion was promoted in some countries by explicit government policies. Animal densities, corporate structure, and pharmaceutical reliance mirrored conditions found in HICs. There were many reported weaknesses in regulation and capacity for enforcement surrounding production and animal welfare in LMICs. Global trade increasingly influences the access to inputs such as feed. LMICs are attracted to IFAP for economic development and food security, as well as the potential for increasing access to animal-source foods and the role these foods can play in alleviating malnutrition. This study concludes that IFAP methods are resource intensive and likely result in serious negative public health and environmental impacts in LMICs. The use of antimicrobials is always greater in IFAP systems where antibiotics are cheap production tools to compensate for poor hygiene

and high stocking densities in animal farms. The use of subtherapeutic doses of antibiotics for this purpose has been widely viewed to be a driver for the emergence of AMR in countries with intensive animal farming where the use of antibiotics for growth promotion (AGPs) is not prohibited or controlled legally.

Also, many LMICs do not have a system that accounts for the use or consumption of antibiotics in the animal, food, and livestock sector. Information is also lacking on the type of antibiotics, their duration of use, method of delivery, and product labeling since all these are not subject to regulatory controls. A lot of mislabeling and fraudulent claims are being made on packing of medicines. In addition, feed additives are also rampant in LMICs to entice farmers. Food production systems in LMICs of Asia, Latin America, and Africa need to innovate farming practices on an urgent basis in order to be more sustainable and productive, especially in the wake of disruptions caused by climate change like frequent cyclones, floods, and drought.

Like most of the LMICs, AMR in food animals has not been documented in India till date. Although small-scale, localized studies are available from research papers published by students of veterinary universities as part of their research dissertation, evidence from systematic studies that can be extrapolated to the state/national level is lacking. Given that there are few regulations against the use of antibiotics for therapeutic and nontherapeutic purposes in India, with no implementation protocols or surveillance programs, the emergence of AMR from antibiotic use in the animal sector is likely to be an unmeasured burden (Government of India 2017).

## 2.2 Disease Prevention for Reducing the Need of Antimicrobials in Animal Health

AMR is not only an animal health and economic concern, with implications for decreasing the efficiency of antimicrobial treatment in animals, but is also a public health concern due to the transmission of antimicrobial-resistant bacteria through the food chain. It is a well-known fact that antimicrobial usage (AMU) (e.g., in human and veterinary medicine) drives AMR (Laxminarayan et al. 2013). The risk increases when such antimicrobials are used inappropriately, in an untargeted manner (e.g., mass medication or use on non-susceptible microorganisms), at subtherapeutic doses, repeatedly, or for inappropriate periods of time. Bacteria are becoming resistant to antibiotics as a result of evolution, and the increased use of antibiotics inevitably selects for bacteria that carry antibacterial resistance genes (ARGs) that protect them against these agents.

ARGs provide bacteria with the ability to degrade and/or excrete antibiotics. They can be exchanged between different species of bacteria through horizontal gene transfer (HGT) allowing the resistance to spread through communities. Mechanisms to transfer the genes by HGT from one bacterium to another involve mobile genetic elements, and these can be transferred in three modes: (1) transformation, a process by which free DNA is incorporated into a competent cell and brings about genetic change in the recipient; (2) conjugation, a process of genetic transfer that involves cell-to-cell contact; and (3) transduction, a process by which DNA is transferred by

bacteriophage. HGT through plasmids represents one of the most difficult challenges for counteracting the dissemination of antimicrobial resistance. They contribute to the spread of relevant resistance determinants by promoting horizontal gene transfer among unrelated bacteria (Carattoli 2013). Using antibiotics in animal health to treat nonbacterial infections or to improve the growth rate of livestock will result in entry of a large amount of antibiotics to the environment, thus creating a strong selective pressure leading to many resistant bacteria. This resistance can develop in free-living bacteria that perform important functions in soil or river ecosystems (Hong et al. 2013), but then spread to bacteria that cause disease within humans and animals.

Therapeutic procedures refer to antibiotic use targeting individual diseased animals. In food animals, it is often convenient to treat entire groups by administering medication through feed or water. Mass medication procedure called metaphylaxis is aimed at the treatment of sick animals while medicating others in the group that may not be sick but exposed. For animals like poultry and duck, mass medication is the most feasible means of treatment, but this can increase the possibility of drug dispersal into the environment. These practices are currently considered as contributing to the emergence of antimicrobial resistance (Clement et al. 2019).

Better management of animal health and preventing infections in the first instance is the best way to achieve this, as reducing the number of infections reduces the number of treatments needed. The ultimate objective is to reduce the need for antimicrobials by preventing animal diseases which can be reasonably achieved by ensuring biosecurity, following good farm management practices, and implementing integrated disease control programs with an aim to minimize the occurrence of diseases and eradicate endemic diseases.

In general, some of the measures that are being adopted by animal husbandry systems in advanced countries and which should be urgently followed in LMICs for preventing diseases and reducing the need to use antimicrobials in all species are the following (European Union 2015):

1. Farm biosecurity measures and implementing hygiene such as maintaining separate clothes and boots for each unit; limiting access; making handwashing and hand disinfection facilities available close to the workplace; culling of sick members of the flock, ensuring quick removal of dead animals; following a strict schedule for cleaning and disinfecting; and performing regular disinfection across farms.
2. Improving husbandry systems by providing appropriate housing, ventilation, and environmental conditions for animals and using only safe, high-quality feed and water.
3. Implementing programs to control specific animal diseases (both viral and bacterial) by means of vaccination. Disease profiles can vary across LMICs, and each country should prioritize controls based on economic assessment of disease burden.

4. Avoiding stressful situations which can weaken immune systems of animals and make them more susceptible to infections (i.e., avoiding overcrowding), ensuring that the recommended animal population density is always adhered to.
5. Implementing other scientifically proven preventive medication systems such as probiotics, immune modulators, and herbal medication treatments to minimize disease and as safe alternatives to antimicrobials.
6. Providing economic incentives to farmers to encourage them to adopt effective biosecurity and preventive measures and to improve animal health which would have a major impact on farming systems across LMICs.

## 2.3 Responsible or Prudent Use of Antimicrobials

In LMICs, many antimicrobials are or have been used, some of which are not used in human medicine and some from classes that are used in humans. Overall, tetracyclines, sulfonamides, penicillins, and enrofloxacins are the main classes sold. However, since the use of antimicrobials varies between animal species and even between production systems, further associations between sales and resistance are hampered by the dearth of sales data by animal species. Bacitracin, tetracycline, tylosin, and anticoccidials (ionophores) are used as growth promoters in poultry production (Mehdi et al. 2018). However, some newer types of antimicrobials which are critical for human use, such as carbapenems, are not used in food animals.

Responsible use of antimicrobials will lead to more rational and targeted use, thereby maximizing the therapeutic effect and minimizing the development of AMR. The final outcome of prudent use should be an overall reduction in the use of antimicrobials, predominantly by limiting their use only to situations where they are inevitable. In such situations, antimicrobials should be used for focused therapy in accordance with the best practices as advised by the World Organisation for Animal Health (OIE), i.e., based on clinical diagnosis and, whenever possible, as per the results of microbiological susceptibility tests and by using an antimicrobial agent of as narrow spectrum as possible.

Many of the antimicrobials used in animals are also used in humans. Some of these antimicrobials are critical (WHO 2018) for preventing or treating life-threatening infections in humans, while others are highest-priority critically important antimicrobial agents (HPCIAs). Third- and-fourth generation cephalosporins and fluoroquinolones are also of critical importance to human health. Special consideration is necessary to ensure the continued efficacy of such antimicrobials and to minimize the development of resistance. These agents should not be used as preventive treatment in feed or water or in absence of clinical signs and should not be used as first line, unless justified and supported by bacteriological tests.

Problems associated with shortage of veterinary care are very profound in many LMICs; and treatment by quack practitioners and others, instead of registered veterinary doctors, increases the AMR problem multifold since the responsible and rational use of antimicrobials, proper dosages, and timely withdrawal cannot be practiced. However, there are not enough veterinarians, and this is especially acute in

LMICs where many animals will never see a veterinarian in their lifetime. This puts farmers in the challenging position where they need to make medical decisions for their livestock without adequate training. Hence, increasing access to veterinary expertise must be at the top of the global agenda, for AMR control.

## 2.4 Animal Feed as a Major Route for Abuse of Antimicrobials in LMICs

Oral antimicrobial treatment is often administered to groups of animals through medicated feed or by adding the antimicrobial to normal farm feed. Antimicrobial use for growth promotion was banned by the European Union in 2006 and heavily regulated in the United States in 2017. As many antimicrobials in LMICs are easily available directly to farmers without a valid prescription, antimicrobials often find their way to healthy food animals as a result of group medication. Group medication of antimicrobials should be administered only when microbial infections result in serious mass mortality or morbidity with strict compliance to withdrawal period so as to prevent accumulation of drug residues. Such treatment with antimicrobials via feed or water should be limited to the animals requiring treatment, and the drug delivery systems should be appropriate for the intended treatment. Where an antimicrobial is administered through medicated feed, it is important to ensure the homogeneity of distribution of the drug, so that each animal or bird obtains the required therapeutic dose for treating the disease in accordance with the veterinary prescription.

There is a major regulatory lacuna in feed safety in many LMICs as it is not considered a priority item in contrast to food safety. Many countries lack legislations for manufacture, sale, and distribution of animal feed. The feed business operators producing medicated feed must ideally be approved by the drug regulator for the manufacture of medicated feed. They must follow all legal requirements for medicated feeds and may only produce medicated feed from authorized antimicrobials. LMICs need to develop smart regulation for animal feed that involves different stakeholders, encourages improvement by self-compliance, and lowers noncompliance.

## 2.5 Raising AMR Awareness in Animal Health

It is only possible to minimize the development of AMR through the responsible use of antimicrobials if all stakeholders involved are well informed. Prudent use campaigns in the animal health sector can be targeted at specific groups, in particular farmers, veterinarians, and other professionals involved in animal production. These campaigns may include a number of approaches, for example, providing sectoral guidelines on good practice, holding seminars, and displaying posters in veterinary practices and milk collection centers.

Relevant networks and stakeholder organizations play an important role in the success of such campaigns, and they should also be supported by the competent authorities. Guidelines should also provide practical tools for implementation and should encourage the parties concerned to be proactive in taking steps to reduce the threat of AMR (Laxminarayan et al. 2013).

Campaigns may also be targeted at consumers, to encourage them to demand food that is produced in accordance with standards such as Codex standards which require minimal use of antimicrobial agents. Positive examples of best practice in animal husbandry can strengthen consumer confidence and increase public demand for food produced with minimal use of antimicrobials. Some initiatives like Know Your Farmer, Know Your Food, to drive locally produced fresh and wholesome food, can be encouraged especially as in each LMIC, the production systems are driven by socioeconomic issues influencing local patterns of consumption of animal foods.

In Asia and Africa, most farmworkers are poorly educated, and pig and broiler poultry integrators and their contract farmers have little awareness on AMR issues and the harmful effects of drug residues in foodstuffs. Animals are routinely transported live and sold through wet markets, providing opportunities for dissemination of AMR genes and organisms over large geographical areas. Further complicating the situation is that poultry litter is widely used as a fertilizer, providing opportunities for contamination of the food chain. In such a case, raising awareness to bring about changes in behavior is the only practical solution for AMR control.

Given the complexity of the situation, a deeper understanding of the economic and social drivers of behaviors and practices in LMICs that promote the emergence and spread of AMR will be required to underpin policy interventions. Awareness campaigns including those through social media therefore play an important role and need to be regularly repeated and updated.

## 2.6 Drug Quality and New Antibiotics in the Animal Health Sector

Just like in human health, addressing resistance will require interventions aimed at discovering newer molecules of antimicrobials. The debate tends to be dominated by discussions around improving drug pipelines, whereas a less spoken topic is the narrowing of the available antibiotics in animal health. For veterinary antimicrobials that have been on the market for many years, new knowledge may emerge which requires amendments to be made to the authorization terms. Lack of new drugs for veterinary use and ban of the existing drugs leads to decrease in the available antibiotics in animal health, for example, the recent ban on colistin (a common veterinary antibiotic) for use in animal health and aquaculture sectors in China, India, and many other countries. Gram-negative bacteria containing a gene known as *mcr-1* which confers resistance to this antibiotic had spread around the world at an alarming rate since its original discovery a few years earlier (Wang et al. 2018). In many LMICs, it was estimated that a quarter of hospitals possess bacteria that now harbor this gene. Colistin is now known as the "antibiotic of last resort," and in many

parts of the world, hospitals have turned to its use because pan-drug-resistant (PDR) *Klebsiella* and *Escherichia coli* were no longer responding to the last available category of human antimicrobial agent carbapenems (Forde et al. 2018), and this comparatively toxic veterinary antibiotic was the only hope.

The lack of profits from new antimicrobial molecules in human use was evident since the last 20 years, which resulted in the introduction of newer molecules in animal health to offset the cost involved in novel drug discovery. This led to the further dissemination of AMR bacteria resistant to newer antimicrobials in animal health and the environment. On expiry of global patents, many of the newer molecules are being produced as generics from contaminated and spurious raw materials and sold as poor-quality antibiotics in LMICs especially in animal health. This coupled with poor instructional strength of regulators and political interference in implementation by regulators has only deepened the crisis. Counterfeits and substandard medicines may not have sufficient active ingredients to kill the micro-organism in question. While overuse or misuse of antibiotics plays a major role in the evolution of microbes (Davies and Davies 2010), the same is true in the use of counterfeit and substandard medicine, and this is one of the contributing factors in exacerbating resistance in many LMICs. This requires urgent attention from drug regulators of LMICs for a better market surveillance of drugs, to detect and weed out substandard versions and enforce penalties on such producers.

Extreme measures like nationalizing animal antimicrobial production and procurement can also be thought about, as in most LMICs the animal healthcare for food animal production is managed by the state and integration with drug production can bring about strict controls on the usage patterns and prevent counterfeit and substandard antimicrobials effectively.

It is also important to bring new diagnostics in animal health to market that can identify disease more rapidly and accurately and integrate diagnostics with veterinary treatments to allow for early identification and treatment, as this would minimize spread and reduce the overall use of antimicrobials. This calls for urgent research and development of new, affordable, and rapid diagnostic tests to address LMIC needs in resource-limited settings.

## 2.7 Problems with Implementation of Action Plans and Weak Veterinary Laboratories

National action plans (NAPs) are the cornerstone of country-level AMR responses, and most of the LMICs have a national action plan inclusive of action for addressing AMR risk in animal health. The lack of prioritization, necessary financing, and policy framework and its poor enforcement has also contributed to reduce antibiotic use in agriculture. At present, although there is a greater understanding of the type of surveillance data which is required in view of the guidelines of the World Health Organization's (WHO) Advisory Group on Integrated Surveillance of Antimicrobial Resistance (AGISAR) (WHO-AGISAR 2017), a comprehensive surveillance is challenging given the complex AMR ecosystem, spanning human healthcare,

agriculture, and the environment, and the limited understanding of how AMR arises and is spread across these domains.

There is further limitation of laboratory support in animal health and lack of standardized protocols inclusive of internationally harmonized clear interpretative criteria, resulting in data from LMICs, which are already scarce, to be of a poor quality. There is an urgent need to upgrade animal health, veterinary microbiology laboratories doing AMR testing, and food testing laboratories doing antibiotic residue testing in animal foods, to meet the requirements of the international laboratory quality standard ISO/IEC 17025 and accredit themselves though their national accreditation bodies. This will ensure the generation of quality data which can further be used for taking regulatory action. Many LMICs are finding it difficult to find enough resources, as upgradation of laboratory network is a costly activity, but global aid and twining programs by the WHO, Food and Agriculture Organization (FAO), and OIE and other international and regional donors are helping many laboratories modernize their equipment and systems including training of technicians. The UK government's Fleming Fund is helping to tackle AMR through efforts to strengthen NAPs, particularly of many LMICs in Africa (Fleming fund 2019).

## 3 Strategies to Control AMR in Animal Health in LMICs Through One Health Action Plans

The greater need for highly interdisciplinary approaches, comprising humans, animals, and the wider environment along with socioeconomic factors, makes AMR a quintessential One Health issue. As it is already clear that LMICs face major AMR challenges from animal health and need to take urgent action and newer regulations, they cannot afford to wait for data from new surveillance to emerge as additional data will not lead to immediate changes in farming systems and antibiotic usage. Therefore, rather than waiting for data, LMIC governments need to show commitment to implement at least the proposed national action plans as most of these are derived from the WHO's Global Action Plan (GAP-AMR) which is a One Health action plan (GAP-AMR 2015).

Since the drivers of antimicrobial resistance lie in humans, animals, plants, food, and the environment, a sustained One Health response is essential to engage and unite all stakeholders around a shared vision and goals. National antimicrobial resistance action plans are at the heart of a multisectoral One Health response, but financing and capacity constraints in many LMICs need to be urgently addressed to accelerate implementation (UN-IACG 2019). Strengthening infection prevention and control in farms using available tools and ensuring access to clean water, sanitation, and hygiene in farms and community settings are central to minimizing disease transmission and the emergence and transmission of antimicrobial resistance in humans, animals, food, and the environment. Strengthening surveillance, regulatory frameworks, professional education, and oversight of antimicrobial prescription and use and increasing awareness among all stakeholders are also significant

challenges that need to be urgently addressed to ensure the responsible use of antimicrobials and to minimize resistance.

For the One Health approach to be applied, the actual AMR burden in the community needs to be estimated, but only a few published reports of community-acquired infections caused by resistant bacteria are available, but it is more likely that the numbers of cases in disease-endemic areas are already high. The example of the spread of extended-spectrum beta-lactamase (ESBL) producers in the community within the past 10 years shows us that a high rate of carbapenemase producers in *E. coli* have rapidly spread worldwide (Normann et al. 2011). However, current evidence shows that humans, rather than animals and food, are the primary source of direct ESBL resistance.

A study reported on antibiotic-resistant *K. pneumoniae* strains in broiler farms which have not been reported in clinical settings. However, plasmids with resistance genes are transferable between strains and species. Therefore, resistance-carrying plasmids detected in food-producing animals always pose a possible risk for human health (Daehre et al. 2018). The One Health European Joint Programme (One Health EJP) has recently started a MedVetKlebs project studying *Klebsiella pneumoniae* (One Health EJP 2019), from ecology to source attribution and transmission control as this organism represents a significant threat to public health when associated with multidrug resistance. *K. pneumoniae* is present in the gastrointestinal tracts of both humans and animals and is a well-known accumulator of AMR genes, yet the main reservoirs and routes of transmission between humans and animals remain undefined. Such One Health studies are also urgently needed in LMICs as this is a priority pathogen and is responsible for most of AMR deaths. Laboratory studies with international collaborations for understanding the molecular mechanisms of resistance, so as to provide insight into the factors in LMICs that promote the survival and spread of AMR genes and resistant organisms through human, animal, and environmental routes, are now the major One Health challenge. Stronger political leadership, advocacy, coordination, and accountability are needed at all levels to enable a sustained One Health response to antimicrobial resistance (UN-IACG 2019).

The National Action Plan for Brazil on Antimicrobial Resistance in Agriculture was launched in 2018 and presents objectives, strategies, and activities to be implemented till 2022. The plan targets health education, epidemiological studies, surveillance and monitoring of antimicrobial use, strengthening infection prevention and implementation of control measures, and promotion of the rational use of antimicrobials in animals. It can be expected that the data gaps on antimicrobial use will be solved by these activities. Moreover, the regulation of antimicrobial use, such as the need of veterinary prescriptions, the research of alternatives for AGPs, and education programs with the NAP Brazil, is expected to contribute to the prudent use of antimicrobials (Cardoso 2019).

In India although health is a state subject and individual states can formulate decisive actions, only two states out of the twenty-eight, viz., Kerala and Madhya Pradesh, have ramped up efforts to control AMR by developing a local strategic action plan based on the national action plan (Government of Kerala 2018). These plans guided by the WHO initially did the identification and analysis of the relevant

stakeholders followed by the mapping of available infrastructure and capabilities of these stakeholders to arrive at a One Health action plan for control of AMR. Such state-specific, locally driven One Health action plan for AMR containment based on situational analysis could serve as a model for other LMICs. The national action plans especially in large LMICs should ideally be an aggregation of subnational plans for better implementation in field level.

## 4 Conclusions

All LMICs should urgently stop wasting the antimicrobials on the WHO list of HPCIAs for human medicine as growth promoters and reduce mass medications with these agents as an essential first step before moving further toward completely phasing out the use of antimicrobials for growth promotion. Additional effort, investments, and incentives are needed to spur innovation in antimicrobial medicines, diagnostics, vaccines, waste management tools, farmer awareness, and improving quality of veterinary antibiotics and safe and effective alternatives to antimicrobials. All LMICs should begin with earnest implementation of the national antimicrobial resistance action plans so that problems in One Health response can be continually corrected and improved. LMICs should also encourage economic incentives to farmers for implementing biosecurity and vaccination.

All stakeholder groups in LMICs including governments, professionals, farmers, regulators, industry, civil society, and the private sector need to be engaged and collaborate in a massive and unprecedented effort across the human, animal, and environmental sectors, based on a shared vision and deliverable goals. The challenges of antimicrobial resistance in animal health are just as complex and multifaceted as in human medicine, but they are not unmanageable as efforts should continue with a One Health approach to push for multilateral solutions in order to curtail this global public health emergency.

## References

Carattoli A (2013) Plasmids and the spread of resistance. Int J Med Microbiol 303:298–304. https://doi.org/10.1016/j.ijmm.2013.02.001

Cardoso M (2019) Antimicrobial use, resistance and economic benefits and costs to livestock producers in Brazil. In: OECD food, agriculture and fisheries papers, no. 135. OECD Publishing, Paris. https://doi.org/10.1787/27137b1e-en

Casey JA, Kim BF, Larsen J, Price LB, Nachman KE (2015) Industrial food animal production and community health. Curr Environ Health Rep 2(3):259–271. https://doi.org/10.1007/s40572-015-0061-0

Clift C (2019) Review of progress on antimicrobial resistance: background and analysis. Chatham House, The Royal Institute of International Affairs, London

Clement M, Olabisi M, David E, Issa M (2019) Veterinary pharmaceuticals and antimicrobial resistance in developing countries. Veterinary Medicine and Pharmaceuticals. https://doi.org/10.5772/intechopen.84888

Daehre K, Projahn M, Friese A, Semmler T, Guenther S, Roesler UH (2018) ESBL-producing *Klebsiella pneumoniae* in the broiler production chain and the first description of ST3128. Front Microbiol 9:2302. https://doi.org/10.3389/fmicb.2018.02302

Davies J, Davies D (2010) Origins and evolution of antibiotic resistance. Microbiol Mol Biol Rev 74(3):417–433. https://doi.org/10.1128/MMBR.00016-10

Ewers C, Bethe A, Semmler T, Guenther S, Wieler LH (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. Clin Microbiol Infect 18:646–655. https://doi.org/10.1111/j.1469-0691.2012.03850.x

Forde BM, Zowawi HM, Harris PNA, Roberts L, Ibrahim E, Shaikh N, Deshmukh A, Sid Ahmed MA, Al Maslamani M, Cottrell K, Trembizki E, Sundac L, Yu HH, Li J, Schembri MA, Whiley DM, Paterson DL, Beatson SA (2018) Discovery of mcr-1-mediated colistin resistance in a highly virulent *Escherichia coli* lineage. mSphere 3(5):e00486–e00418. https://doi.org/10.1128/mSphere.00486-18

Government of India (2017). National Action Plan on Antimicrobial Resistance. (NAP-AMR) 2017

Government of Kerala (2018). Kerala Antimicrobial Resistance Strategic Action Plan (KARSAP) 2018

Kaimila Y, Divala O, Agapova SE, Stephenson KB, Thakwalakwa C, Trehan I, Manary MJ, Maleta KM (2019) Consumption of animal-source protein is associated with improved height-for-age Z scores in rural Malawian children aged 12–36 months. Nutrients 11:480. https://doi.org/10.3390/nu11020480

Laxminarayan R, Duse A, Wattal C, Zaidi AK, Wertheim HF, Sumpradit N, Vlieghe E, Hara GL, Gould IM, Goossens H, Greko C, So AD, Bigdeli M, Tomson G, Woodhouse W, Ombaka E, Peralta AQ, Qamar FN, Mir F, Kariuki S, Bhutta ZA, Coates A, Bergstrom R, Wright GD, Brown ED, Cars O (2013) Antibiotic resistance-the need for global solutions. Lancet Infect Dis 13(12):1057–1098. https://doi.org/10.1016/S1473-3099(13)70318-9

Lam Y, Fry JP, Nachman KE (2019) Applying an environmental public health lens to the industrialization of food animal production in ten low- and middle-income countries. Glob Health 15:40. https://doi.org/10.1186/s12992-019-0479-5

Mehdi Y, Létourneau-Montminy MP, Gaucher ML, Chorfi Y, Suresh G, Rouissi T, Brar SK, Côté C, Ramirez AA, Godbout S (2018) Use of antibiotics in broiler production: global impacts and alternatives. Anim Nutri 4(2):170–178. https://doi.org/10.1016/j.aninu.2018.03.002

Nair DVT, Venkitanarayanan K, Kollanoor Johny A (2018) Review antibiotic-resistant salmonella in the food supply and the potential role of antibiotic alternatives for control. Foods 7(10):167. https://doi.org/10.3390/foods7100167

Normann P, Naas T, Poirel L (2011) Global spread of Carbapenemase producing Enterobacteriaceae. Emerg Infect Dis 17:10. https://doi.org/10.3201/eid1710.110655

Official Journal of the European Union (2015) commission notice (2015/C 299/04); Guidelines for the prudent use of antimicrobials in veterinary medicine 2015

One Health European Joint Programme (One Health EJP) 2019 MedVetKlebs project. https://onehealthejp.eu/medvetklebs-1st-year-meeting/. Accessed 12 Dec 2019

Hong PY, Al-Jassim N, Ansari MI, Mackie RI (2013) Environmental and public health implications of water reuse: antibiotics, antibiotic resistant bacteria, and antibiotic resistance genes. Antibiotics 2:367–399. https://doi.org/10.3390/antibiotics2030367

OIE (2017) Roadmap for zoonotic tuberculosis World Health Organization (WHO), Food and Agriculture Organization of the United Nations (FAO) and World Organisation for Animal Health (OIE)

Ryan M (2019) Evaluating the economic benefits and costs of antimicrobial use in food-producing animals. In: OECD food, agriculture and fisheries papers, no. 132. OECD Publishing, Paris. https://doi.org/10.1787/f859f644-en

Rushton J, Ferreira JP, Stark KD (2014) Antimicrobial resistance: the use of antimicrobials in the livestock sector. In: OECD food, agriculture and fisheries papers, no. 68. OECD Publishing, Paris. https://doi.org/10.1787/18156797

Sivalingam P, Poté J, Prabakar K (2019) Environmental prevalence of Carbapenem resistance Enterobacteriaceae (CRE) in a tropical ecosystem in India: human health perspectives and future directives. Pathogens 8:174. https://doi.org/10.3390/pathogens8040174

The Fleming fund 2019. https://www.flemingfund.org/. Accessed 12 Dec 2019

United Nations: Inter Agency Coordination Group on AMR (2019) Report of IACG, No Time to Wait: Securing the future from drug-resistant infections 2019

Van Boeckel TP, Pires J, Silvester R, Zhao C, Song J, Criscuolo NG, Gilbert M, Bonhoeffer S, Laxminarayan R (2019) Global trends in antimicrobial resistance in animals in low- and middle-income countries. Science 365:1266. https://doi.org/10.1126/science.aaw1944

Wang R, van Dorp L, Shaw LP, Bradley P, Wang Q, Wang X, Jin L, Zhang Q, Liu Y, Rieux A, Dorai-Schneiders T, Weinert LA, Iqbal Z, Didelot X, Wang H, Balloux F (2018) The global distribution and spread of the mobilized colistin resistance gene mcr-1. Nat Commun 9:1179. https://doi.org/10.1038/s41467-018-03205-z

World Health Organization (2015). Global Action Plan on Antimicrobial Resistance. (GAP-AMR) 2015

World Health Organization (2017) Integrated Surveillance of Antimicrobial Resistance in Foodborne Bacteria: Application of a One Health Approach; Guidance from the WHO Advisory Group on Integrated Surveillance of Antimicrobial Resistance (AGISAR) 2017

World Health Organization (2018) WHO Critically Important Antimicrobials for Human Medicine 6$^{th}$ revision 2018

WHO; Ten threats to global health in 2019. https://www.who.int/emergencies/ten-threats-to-global-health-in-2019. Accessed 12 Dec 2019

# Antifungal Resistance: Current Concepts

Gagandeep Singh and Immaculata Xess

## 1 Introduction

Advances in the treatment modalities of human diseases have led to an increasing population of critical patients and patients who are immunocompromised. These populations are at an increased risk of multiple opportunistic infections, including fungal diseases. The most common fungal pathogens are *Candida*, *Aspergillus*, *Mucorales*, *Cryptococcus*, and *Pneumocystis jirovecii*. *Candida* spp. are the fourth most common cause of bloodstream infections worldwide, with mortality as high as 50% (Wisplinghoff et al. 2004). Every case of candidemia is assessed to result in an additional 3–13 days of hospital stay and up to $29,000 in healthcare costs. Antifungal resistance is often seen with *Candida* infections. A few species of *Candida* are increasingly showing resistance to the first- and second-line antifungal medications, such as fluconazole and the echinocandins. *C. glabrata* and *C. krusei* together constitute more than 70% of these resistant isolates.

*C. auris* is an emerging fungal infection which is multidrug resistant. In India, majority of *C. auris* isolates are resistant to fluconazole, up to half are resistant to voriconazole, and one-third are resistant to amphotericin B, usually reserved as a last-resort treatment. Most of the *C. auris* isolates are susceptible to echinocandins; however, echinocandin resistance is being increasingly reported. Infections due to *C. auris* are an alarming public health issue as they are difficult to identify with standard laboratory methods and they spread easily in healthcare settings.

An increase in the incidence of mold infections has been witnessed in recent times, especially *Aspergillus* spp. where the mortality rate can cross 50% (Maschmeyer et al. 2007). With India being the diabetic capital of the world, there is an increase in the numbers of cases of mucormycosis in uncontrolled diabetes (Chakrabarti et al. 2009a). *Candida* and *Aspergillus* are two important genera which

G. Singh (✉) · I. Xess
Mycology Section, Department of Microbiology, All India Institute of Medical Sciences, New Delhi, India



S. Thomas (ed.), *Antimicrobial Resistance*,
https://doi.org/10.1007/978-981-15-3658-8_7

have demonstrated a shift in drug resistance pattern recently. Among the molds, these include the non-fumigatus *Aspergillus* spp. like *Aspergillus terreus*, *Fusarium* spp., and *Scedosporium* spp. (Malani and Kauffman 2007). There is also emergence of the rarer fungal species that are difficult to treat. Selective pressure due to increased use of antifungal prophylaxis in high-risk patients and irrational use of azoles in agriculture is an important contributory factor for this shift (Richardson and Lass-Florl 2008; Rogers 2006).

## 2 Currently Available Antifungals and Mechanism of Action in Brief

There are five major classes of antifungal agents that are used to treat invasive fungal infections. These are azoles, polyenes, echinocandins, allylamines, and pyrimidine analogs. Triazoles target the biosynthesis of ergosterol that catalyzes lanosterol 14α-demethylation. It is important to highlight that the azoles are widely used in the treatment of fungal infections in crops, humans, and livestock, with 5 licensed clinical azole antifungals and 31 for crop protection. Polyenes, such as amphotericin B (AmB), have the ability to bind the ergosterol in the fungal cell membrane and destabilize the membrane functions. Liposomal AmB may be used for avoiding side effects in humans. Echinocandins inhibit β-1,3-glucan synthase enzyme, thereby inhibiting cell wall synthesis. Pyrimidine analogs, such as 5-flucytosine (5-FC), are converted into fluorinated pyrimidines that destabilize nucleic acids which results in growth arrest. Allylamines, e.g., terbinafine, are used for treating superficial dermatophytic infections; they prevent ergosterol synthesis by inhibiting squalene epoxidase enzyme.

## 3 Epidemiology of Antifungal Drug Resistance

Amphotericin B deoxycholate and 5-flucytosine were the only treatment options for invasive fungal infections for many years. Azoles were introduced in the late 1980s. The past decades have seen the development of lipid formulations of amphotericin B (liposomal AmB), broader-spectrum triazoles (voriconazole, posaconazole, and isavuconazole), and the echinocandins (caspofungin, micafungin, and anidulafungin) (Groll and Tragiannidis 2009; Pasqualotto and Denning 2008).

Intrinsic resistance to antifungals has been observed in many *Candida* species. Examples of intrinsic resistance are the resistance of *C. krusei* to fluconazole and many newly described *C. auris* strains with elevated minimum inhibitory concentrations (MICs) to numerous classes of antifungal drugs, including azoles, echinocandins, and polyenes. The widespread use of azoles has been associated with the emergence of resistant (*C. krusei*) or less susceptible species (particularly *C. glabrata*) in many patient populations (e.g., transplantation), where azoles are used for long-term prophylaxis as well as treatment of infections. Another *Candida*

species with intrinsic resistance to fluconazole as well as decreased susceptibility to the echinocandins is *Candida guilliermondii*.

The overall resistance in *Candida* spp. to fluconazole and voriconazole is considered to be around 3–6%, and the level of resistance has remained constant over a decade (Chakrabarti et al. 2009b; Pfaller et al. 2009). However, a multicentric study on candidemia in India by Chakrabarti et al. had important findings. There was a vast spectrum of agents (31 *Candida* species) causing candidemia, and azole resistance and multidrug resistance were seen in 11.8 and 1.9% of isolates (Chakrabarti et al. 2014). There has also been an increase in echinocandin resistance worldwide. In a study, *C. glabrata* bloodstream infection over a decade (2001–2010) was reviewed, and treatment outcome correlated with minimum inhibitory concentration (MIC) results, and the presence of FKS gene mutations was detected. Resistance to echinocandins increased from 4.9% to 12.3% and FLC from 18% to 30% between 2001 and 2010, respectively. Among the 78 FLC-resistant isolates, 14.1% were resistant to one or more echinocandins, and a total of 7.9% of isolates harbored a FKS mutation. The predictor of a FKS mutant strain was prior echinocandin therapy (Alexander et al. 2013).

Azole resistance in *A. fumigatus* can develop either during treatment or due to excessive use in agriculture (Rivero-Menendez et al. 2016). The environmental course of resistance development has been reported since the late 2000s and has been reported worldwide. The emergence of multiple-triazole resistance in *A. fumigatus* was reported for the first time in 2007 in patients with primary invasive aspergillosis (IA) from six different hospitals in the Netherlands (Verweij et al. 2007). Subsequently, in 2008, 28.1% of patients with resistant IA were reported from Radboud University Medical Center, also from the Netherlands (Snelders et al. 2008). The predominance of TR34/L98H resistance mechanism in patient isolates from epidemiologically unrelated patients suggested the possibility of environmental acquisition (Snelders et al. 2009, 2008). In addition, several point mutations in the *cyp51A* gene have been associated with azole resistance. These have been increasingly documented in patients with long-term azole therapy and patients with allergic and chronic sinus and pulmonary aspergillosis. A study from a referral center in United Kingdom reported azole resistance in chronic pulmonary diseases due to *Aspergillus* (Howard et al. 2009). Azole resistance mechanisms show cross-resistance to all azoles. The isolates with TR34/L98H have been found to be pan-azole resistant, whereas *A. fumigatus* isolates harboring the TR46/Y121F/T289A mechanism exhibit high VRC resistance (van Ingen et al. 2014). Isavuconazole also showed reduced in vitro and in vivo efficacy in *A. fumigatus* isolates harboring these resistance mechanisms (Gregson et al. 2013; Howard et al. 2013). These results indicate that the clinical role of azoles in the emergence of azole-resistant aspergillosis remains limited.

Chowdhary et al. have worked extensively on azole-resistant *Aspergillus fumigatus* in India (Chowdhary et al. 2011, 2013a, 2014, 2015, 2017). They uncovered the underlying mechanism of azole resistance in *Aspergillus fumigatus.* The target for azoles is the fungal lanosterol 14α-demethylase which is encoded by *cyp51A* (in molds) by inhibiting the ergosterol synthesis. This leads to accumulation

of 14α-methyl sterols that causes alterations in cell membrane permeability and stability, thereby inhibiting fungal growth. Thus, nonsynonymous mutations in the cyp51A gene can induce resistance to some or all triazole drugs (Chowdhary et al. 2014). They are the first ones to isolate multi-triazole-resistant *A. fumigatus* strains carrying the TR34/L98H mutations from India (Chowdhary et al. 2011, 2013b) and to characterize the susceptibility profile of isolates with these mutations by the CLSI method (Buil et al. 2018). Specific triazole resistance mutations have been found in isolates from patients and from environmental isolates of *A. fumigatus*. These mutations comprise tandem repeat (TR) in the promoter region of the *cyp51A* gene in combination with single or multiple point mutations (TR34/L98H, TR53, TR46/Y121F/T289A) (Chowdhary and Meis 2018).

Majority of the reports of azole resistance in *A. fumigatus* originate from Europe. However, azole resistance rates are relatively lower in Asian countries, the first reports of which were published in 2005 from Taiwan and China (Chen et al. 2005; Hsueh et al. 2005). The Indian isolates of *A. fumigatus* showed low azole resistance. In a prospective study by Dabas et al. (2018) from India, the rate of azole-resistant *A. fumigatus* in immunocompromised patients with invasive aspergillosis (IA) was found to be low (0.8%). This study reported new *cyp51A* mutations, of which one was at codon Y431C and other two were at hot spots G54R and P216L. Two more studies from a single center in India reported the presence of TR34/ L98H in 2% and 1.5% of *A. fumigatus* clinical strains, respectively (Chowdhary et al. 2011, 2015).

It is a rare phenomenon to observe resistance to amphotericin B among *Candida* spp. Increasing MICs to amphotericin B among *C. krusei* and *C. glabrata* isolates have been reported. Intrinsic polyene resistance is frequently seen in *C. lusitaniae*, *A. terreus*, and *Trichosporon beigelii*. Polyene resistance is ever more observed in *A. flavus* and also in *A. fumigatus*. According to the SENTRY program, only 11.5% of *A. fumigatus* isolates inhibited at $\leq$1 mg/mL (Castanheira et al. 2013). However, there is a need for large longitudinal studies to establish this further. Rare *Aspergillus* species, such as *A. lentulus* and *A. ustus*, showed resistance to multiple antifungal agents. Occasionally encountered molds, such as *Fusarium* spp., *Scedosporium apiospermum*, *and Lomentospora prolificans*, showed resistance to amphotericin B.

Echinocandin resistance is not a major cause of concern. Internationally, no significant change has been observed in the susceptibility of *Candida* spp. to echinocandins. Resistance to flucytosine in yeasts remains low (<2%). However, when used alone, it can develop resistance very quickly. Therefore, it should always be used in combination mainly with amphotericin B. By and large, the incidence of antifungal resistance is low; however, it remains a grave threat in the management of high-risk patients.

## 4 Mechanism of Antifungal Resistance

### 4.1 Mechanisms of Azole Resistance

There are five proposed mechanisms of resistance to azoles (Fig. 1).

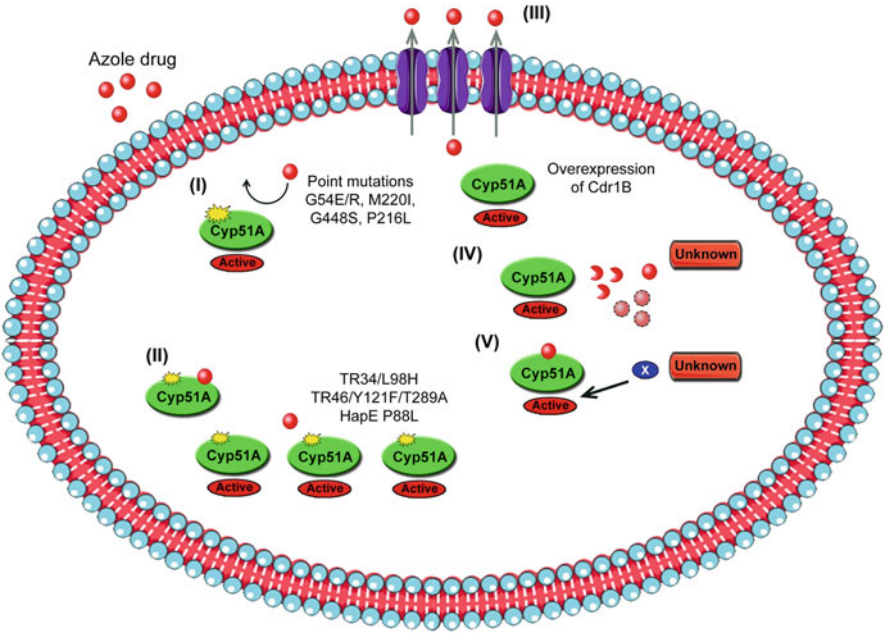


**Fig. 1** The figure depicts the five basic resistance mechanisms of a drug; and sections I, II, and III are the common mechanisms of azole resistance seen in *A. fumigatus*

### 4.1.1 Efflux Pump Activation

The activation of efflux pumps causes decrease in the drug concentrations at the site of action. The *CDR* genes of the ATP-binding cassette superfamily and the *MDR* genes of the major facilitator class encode for efflux pumps in *Candida* spp. (Sanglard et al. 1995, 1997). Upregulation of *CDR1* and *MDR1* has been demonstrated in azole-resistant *C. albicans* (White 1997). Generally, CDR gene upregulation confers pan-resistance to azoles, whereas MDR-encoded efflux pumps are narrower spectrum in action and are specific only for fluconazole.

### 4.1.2 Mutations in the HMG-CoA Reductase-Encoding Gene, *hmg1*

This is a recently reported mechanism by Rybak et al. (2019). Mutations in the HMG-CoA reductase-encoding gene, *hmg1*, were reported in majority of triazole-resistant *A. fumigatus* clinical isolates. The mutations are seen in the conserved sterol-sensing domain of *hmg1*. It is still unclear as to how the mutations in *hmg1* impart triazole, the negative regulation of *hmg1* activity, which is dependent on the sterol-sensing domain. It may be altered by residue substitutions in the sterol-sensing domain of *A. fumigatus hmg1* (Rybak et al. 2019).

### 4.1.3 Upregulation of the Target Enzymes

A few of the *Candida* isolates with reduced susceptibility to azoles have higher intracellular concentrations of *ERG11*p than do azole-susceptible strains. The

antifungal agent, therefore, can no longer effectively inhibit ergosterol synthesis. This can be brought about through gene amplification, decreased degradation of the gene product, etc. This mechanism contributes little to the overall resistance burden in *Candida* species (Lopez-Ribot et al. 1998).

### 4.1.4 Target Site Variations

Mutations in *ERG11*, the gene that encodes for the target enzyme lanosterol 14-α-demethylase, prevent the binding of azoles to the enzymatic site. Furthermore, intrinsic resistance to fluconazole in *C. krusei* isolates has been attributed to decreased affinity of *ERG11p* to the drug. Multiple amino acid substitutions in *ERG11p* have been detected. Different mutations can coexist in the same gene with additive effects (Loffler et al. 1997; Orozco et al. 1998).

### 4.1.5 Use of Bypass Pathways

The action of the azoles leads to decrease in ergosterol concentration in the fungal membrane and accumulation of the toxic products which may lead to growth arrest. Mutation of the *ERG3* gene prevents the formation of 14a-methyl-3,6-diol from 14a-methylfecosterol (Kelly et al. 1997). Replacement of ergosterol with the latter product leads to functional membranes and negates the action of azoles on the ergosterol biosynthetic pathway.

## 4.2 Mechanisms of Echinocandin Resistance

Echinocandins act by inhibiting the synthesis of β-1,3-D-glucan, which is an integral part of the fungal cell wall (Fig. 2). A defective cell wall formation leads to destruction of yeasts and aberrant hyphal forms in the molds. Echinocandins effectively inhibit *Candida* and *Aspergillus* species. *C. parapsilosis* and *C. guilliermondii* have shown higher MICs than *C. albicans* isolates. However, they are not active against *Cryptococcus*, *Mucorales*, *Fusarium*, *Scedosporium*, and *Trichosporon* species.

Secondary resistance to echinocandins in *Candida* species is associated with point mutations in the *Fks1* gene of the β-1,3-D-glucan synthase complex. Resistance mechanisms for *C. neoformans* included an echinocandin-resistant β-1,3-D-glucan synthase target, efflux pumps, etc. Echinocandin-susceptible *Candida* and *Aspergillus* isolates have the ability to grow in in vitro conditions at concentrations exceeding the MICs of caspofungin. The "eagle-like effect" or "paradoxical phenomenon" is said to be strain dependent and is possibly related to upregulation of efflux pumps.

## 4.3 Mechanisms of Polyene Resistance

The *ERG3* gene is involved in ergosterol biosynthesis, and any alterations in this gene lead to accumulation of different sterols in the fungal cell membrane. As a result, polyene-resistant isolates of *Candida*, *Cryptococcus*, etc. have low ergosterol

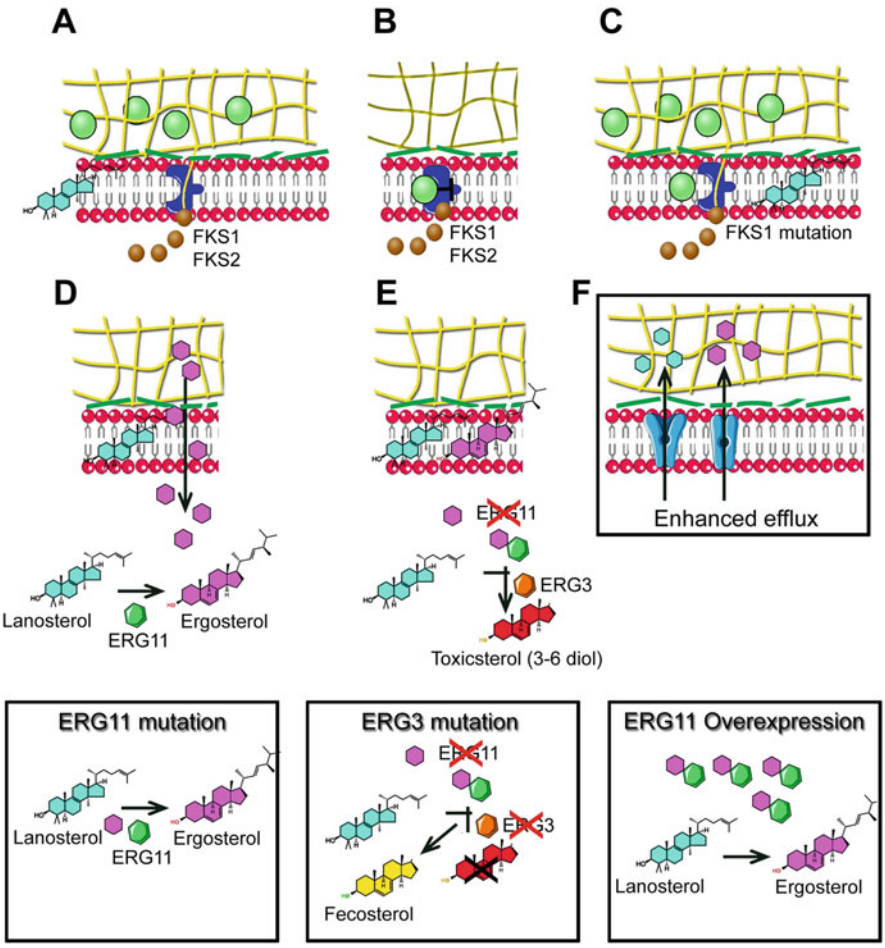


**Fig. 2** Echinocandin and azole resistance mechanisms in *Candida* spp. (**a**) Beta-1,3-glucan synthesis on the fungal membrane. (**b**) Echinocandins block cell wall synthesis by inhibiting beta-1,3-glucan synthase. (**c**) Echinocandin resistance is due to mutations of the *FKS* gene that make echinocandins less effective. (**d**) Ergosterol synthesis at the endoplasmic reticulum and uptake of azole antifungal drugs into the cytosol of the fungal cell. (**e**) Typical azole. (**f**) Various mechanisms of azole resistance are shown in boxes (Adapted from Maubon et al. 2014)

content. Other mechanisms of polyene resistance include increased catalase activity which decreases the susceptibility to oxidative damage. Many clinicians use an MIC of 1.0 mg/mL to indicate resistance to amphotericin B, as the exact resistance breakpoints have not been determined yet.

### 4.4 Mechanism of Flucytosine Resistance

Flucytosine is a pyrimidine analog that inhibits nucleic acid synthesis. A few yeasts are inherently resistant to it because of a mutation in cytosine permease that leads to impaired cellular uptake. Acquired resistance results from mutations in cytosine deaminase or uracil phosphoribosyl transferase. However, primary resistance to flucytosine in yeasts remains low (1–2%). Resistance to flucytosine develops very quickly, and therefore, it is always given in combination with other antifungals, e.g., amphotericin B, in patients with cryptococcal meningitis.

### 4.5 Mechanism of Allylamine Resistance

The most commonly used allylamine is terbinafine. It is either used as monotherapy in patients with dermatophytic infections or in combination with other drugs like voriconazole for the treatment of fusariosis or scedosporiosis. Terbinafine acts by inhibiting the enzyme squalene epoxidase, a key component in the ergosterol synthesis pathway. This leads to depletion of ergosterol and inhibition of fungal growth. Resistance to this drug has been attributed to point mutations in the target gene of squalene epoxidase. The resistance mechanism was identified as a single amino acid substitution in squalene epoxidase protein. Two common mutations seen frequently (Leu393Phe and Phe397Leu) introduce missense substitutions that result in more than a hundredfold increase in MIC than the susceptible strains.

While correlating in vitro antifungal susceptibility testing data with therapeutic outcomes (most often with a combination of *Candida* species and azoles), a pattern of "90–60" rule has been observed: fungal infections due to susceptible isolates respond to therapy approximately 90% of the time, whereas infections due to resistant isolates respond nearly 60% of the time.

## 5 Antifungal Agents and Cross-Resistance

Cross-resistance is a common occurrence among azoles as the target of action on fungi is similar. Specific mechanisms can result in cross-resistance to multiple drugs belonging to the same class. Expression of ABC transporters (e.g., CDR1) leads to cross-resistance to all azoles. Similarly, peculiar *FKS1* mutations in *C. albicans* can produce cross-resistance to all echinocandins. Cross-resistance is not seen between the echinocandin class of drugs and either the polyenes or azoles, as the sites of action are different.

## 6 Clinical Resistance to Antifungals

In vitro susceptibility testing alone may not be sufficient enough to predict clinical outcomes. The majority of patients with invasive mycoses experience treatment failure because of clinical resistance. Clinical resistance can occur due to the following reasons: (a) incorrect diagnosis of specific fungal disease, (b) failure of antifungal agents to overcome the state of immunodeficiency in such patients, (c) infections due to more virulent organisms like *Cryptococcus gattii*, (d) antifungal toxicities, (e) poor penetration of antifungal agents in some tissues, (f) subtherapeutic level of the antifungal agent, and (g) suboptimal duration of the antifungal therapy.

## 7 Therapeutic Drug Monitoring (TDM) of Antifungal Agents

Fluconazole, amphotericin B (all formulations), terbinafine, caspofungin, anidulafungin, and micafungin do not need TDM to be performed either due to reasons of concentration, efficacy, or toxicity (Table 1). The triazoles, viz. itraconazole, voriconazole, and posaconazole, should be monitored. Flucytosine does need monitoring.

### 7.1 Voriconazole

Voriconazole has nonlinear pharmacokinetics in adults and children. It takes three to five half-lives to achieve steady-state concentrations. Therefore, TDM is usually recommended after 5 days of starting treatment. A number of reasons can lead to variation in drug levels which include rapid metabolism of the drug in young children; decreased metabolism in older patients, those having hepatic impairment, and those taking proton-pump inhibitors; etc. Drug interactions especially with anti-tubercular drugs and anti-cancer agents also contribute to variation. The genetic polymorphism in *Cyp2C19A* gene may account for up to 30% of variation. Trough (pre-dose)-level target range is 1–5.5 mg/L. Voriconazole at plasma concentrations >6.0 mg/L has potential for life-threatening and serious adverse events (neurotoxic and hepatotoxic), and it is strongly recommended to lower the dosage.

**Table 1** Ideal timing of performing a TDM and target levels of various antifungal agents

| Antifungal | Ideal time for trough samples | Target trough levels (safety) |
|---|---|---|
| Itraconazole | After 7 days | 1–4 mg/L |
| Voriconazole | After 3–5 days | 1–5.5 mg/L |
| Posaconazole | After 7 days | Treatment = >1 mg/L<br>Prophylaxis = >0.7 mg/L |
| Flucytosine | Within a week | 20–40 mg/L |

### 7.2 Itraconazole

Itraconazole also has variable pharmacokinetics in adults because of nonlinear clearance, variable bioavailability of different preparations, and drug interactions. Target level by HPLC is 1–4.0 mg/L. Low levels (<0.5 mg/L) may lead to breakthrough IFIs especially in neutropenic patients. Supra-therapeutic concentrations may cause adverse events.

### 7.3 Posaconazole

Studies of posaconazole plasma levels have demonstrated large variations reflecting defects in absorption. Target plasma levels are >1.0 mg/L. Adverse effects at supra-therapeutic levels are not described with posaconazole.

### 7.4 Flucytosine

Flucytosine TDM should be performed in all patients who receive it within the first week of initiation of therapy and 3 days for neonates and patients with impaired renal function. Target levels are 20–40 mg/L (trough).

## 8 Antifungal Stewardship (AFS)

The aim of any antimicrobial stewardship program is to ensure utility of any antimicrobial agents on a long-term basis which translates to improved patient outcomes. AFS imbibes this concept; however, it has issues specific to the management of IFIs which include high mortality rates, prohibitive drug costs, and toxicities. Patients who are at a risk of developing IFIs are neutropenic patients, patients treated with chemotherapy for malignant diseases, transplant recipients, patients with chronic pulmonary disease, those on long-term steroids and other immunosuppressive drugs, etc.

AFS can deliver efficiently using a multi-pronged strategy, by prospective audit and feedback to prescribers and hospital antimicrobial teams. For the audit and feedback, the AFS team reviews patients on antifungal treatment for the suitable drug, dose, and duration. As the AFS is a unique entity, the team should have members who have sufficient experience from a range of backgrounds related to diagnosis and management of fungal infections.

Overall, there is a great need for establishing antifungal stewardships in institutions with significant antifungal use due to the growing resistance, substantial costs, and potential to optimize outcomes. This is a collaborative effort that requires the teamwork of specialists in dynamic areas among physicians, pharmacists, and microbiologists, at minimum. The success of antifungal stewardship is defined on institution-specific needs and the epidemiology of the hospital. Documented

inappropriate use of antifungals warrants opportunities for improvement and education, as part of the core elements of stewardship. Opportunities include interventions requiring infectious disease consultation for fungemia, candidemia care bundles, appropriate use of diagnostic biomarkers, therapeutic drug monitoring, and retrospective audit and feedback.

## References


Alexander BD, Johnson MD, Pfeiffer CD, Jimenez-Ortigosa C, Catania J, Booker R, Castanheira M, Messer SA, Perlin DS, Pfaller MA (2013) Increasing echinocandin resistance in *Candida glabrata*: clinical failure correlates with presence of FKS mutations and elevated minimum inhibitory concentrations. Clin Infect Dis 56(12):1724–1732. https://doi.org/10.1093/cid/cit136

Buil JB, Hagen F, Chowdhary A, Verweij PE, Meis JF (2018) Itraconazole, voriconazole, and posaconazole CLSI MIC distributions for wild-type and azole-resistant *Aspergillus fumigatus* isolates. J Fungi (Basel) 4(3):103. https://doi.org/10.3390/jof4030103

Castanheira M, Messer SA, Rhomberg PR, Pfaller MA (2013) Antifungal susceptibility patterns of a global collection of fungal isolates: results of the SENTRY Antifungal Surveillance Program (2013). Diagn Microbiol Infect Dis 85(2):200–204. https://doi.org/10.1016/j.diagmicrobio.2016.02.009

Chakrabarti A, Chatterjee SS, Das A, Panda N, Shivaprakash MR, Kaur A, Varma SC, Singhi S, Bhansali A, Sakhuja V (2009a) Invasive zygomycosis in India: experience in a tertiary care hospital. Postgrad Med J 85(1009):573–581. https://doi.org/10.1136/pgmj.2008.076463

Chakrabarti A, Chatterjee SS, Rao KL, Zameer MM, Shivaprakash MR, Singhi S, Singh R, Varma SC (2009b) Recent experience with fungaemia: change in species distribution and azole resistance. Scand J Infect Dis 41(4):275–284. https://doi.org/10.1080/00365540902777105

Chakrabarti A, Sood P, Rudramurthy SM, Chen S, Kaur H, Capoor M, Chhina D, Rao R, Eshwara VK, Xess I, Kindo AJ, Umabala P, Savio J, Patel A, Ray U, Mohan S, Iyer R, Chander J, Arora A, Sardana R, Roy I, Appalaraju B, Sharma A, Shetty A, Khanna N, Marak R, Biswas S, Das S, Harish BN, Joshi S, Mendiratta D (2014) Incidence, characteristics and outcome of ICU-acquired candidemia in India. Intensive Care Med 41(2):285–295. https://doi.org/10.1007/s00134-014-3603-2

Chen J, Li H, Li R, Bu D, Wan Z (2005) Mutations in the cyp51A gene and susceptibility to itraconazole in *Aspergillus fumigatus* serially isolated from a patient with lung aspergilloma. J Antimicrob Chemother 55(1):31–37. https://doi.org/10.1093/jac/dkh507

Chowdhary A, Kathuria S, Randhawa HS, Gaur SN, Klaassen CH, Meis JF (2011) Isolation of multiple-triazole-resistant *Aspergillus fumigatus* strains carrying the TR/L98H mutations in the cyp51A gene in India. J Antimicrob Chemother 67(2):362–366. https://doi.org/10.1093/jac/dkr443

Chowdhary A, Kathuria S, Xu J, Meis JF (2013a) Emergence of azole-resistant *Aspergillus fumigatus* strains due to agricultural azole use creates an increasing threat to human health. PLoS Pathog 9(10):e1003633. https://doi.org/10.1371/journal.ppat.1003633

Chowdhary A, Kathuria S, Xu J, Sharma C, Sundar G, Singh PK, Gaur SN, Hagen F, Klaassen CH, Meis JF (2013b) Clonal expansion and emergence of environmental multiple-triazole-resistant *Aspergillus fumigatus* strains carrying the TR(3)(4)/L98H mutations in the cyp51A gene in India. PLoS One 7(12):e52871. https://doi.org/10.1371/journal.pone.0052871

Chowdhary A, Meis JF (2018) Emergence of azole resistant *Aspergillus fumigatus* and One Health: time to implement environmental stewardship. Environ Microbiol 20(4):1299–1301. https://doi.org/10.1111/1462-2920.14055

Chowdhary A, Sharma C, Hagen F, Meis JF (2014) Exploring azole antifungal drug resistance in *Aspergillus fumigatus* with special reference to resistance mechanisms. Future Microbiol 9 (5):697–711. https://doi.org/10.2217/fmb.14.27

Chowdhary A, Sharma C, Kathuria S, Hagen F, Meis JF (2015) Prevalence and mechanism of triazole resistance in *Aspergillus fumigatus* in a referral chest hospital in Delhi, India and an update of the situation in Asia. Front Microbiol 6:428. https://doi.org/10.3389/fmicb.2015.00428

Chowdhary A, Sharma C, Meis JF (2017) Azole-resistant Aspergillosis: epidemiology, molecular mechanisms, and treatment. J Infect Dis 216:S436–S444. https://doi.org/10.1093/infdis/jix210

Dabas Y, Xess I, Bakshi S, Mahapatra M, Seth R (2018) Emergence of azole-resistant *Aspergillus fumigatus* from immunocompromised hosts in India. Antimicrob Agents Chemother 62(8): e02264–e02217. https://doi.org/10.1128/AAC.02264-17

Gregson L, Goodwin J, Johnson A, McEntee L, Moore CB, Richardson M, Hope WW, Howard SJ (2013) In vitro susceptibility of *Aspergillus fumigatus* to isavuconazole: correlation with itraconazole, voriconazole, and posaconazole. Antimicrob Agents Chemother 57 (11):5778–5780. https://doi.org/10.1128/AAC.01141-13

Groll AH, Tragiannidis A (2009) Recent advances in antifungal prevention and treatment. Semin Hematol 46(3):212–229. https://doi.org/10.1053/j.seminhematol.2009.03.003

Howard SJ, Cerar D, Anderson MJ, Albarrag A, Fisher MC, Pasqualotto AC, Laverdiere M, Arendrup MC, Perlin DS, Denning DW (2009) Frequency and evolution of Azole resistance in *Aspergillus fumigatus* associated with treatment failure. Emerg Infect Dis 15(7):1068–1076. https://doi.org/10.3201/eid1507.090043

Howard SJ, Lass-Florl C, Cuenca-Estrella M, Gomez-Lopez A, Arendrup MC (2013) Determination of isavuconazole susceptibility of Aspergillus and *Candida species* by the EUCAST method. Antimicrob Agents Chemother 57(11):5426–5431. https://doi.org/10.1128/AAC.01111-13

Hsueh PR, Lau YJ, Chuang YC, Wan JH, Huang WK, Shyr JM, Yan JJ, Yu KW, Wu JJ, Ko WC, Yang YC, Liu YC, Teng LJ, Liu CY, Luh KT (2005) Antifungal susceptibilities of clinical isolates of *Candida species, Cryptococcus neoformans*, and *Aspergillus species* from Taiwan: surveillance of multicenter antimicrobial resistance in Taiwan program data from 2003. Antimicrob Agents Chemother 49(2):512–517. https://doi.org/10.1128/AAC.49.2.512-517.2005

Kelly SL, Lamb DC, Kelly DE, Manning NJ, Loeffler J, Hebart H, Schumacher U, Einsele H (1997) Resistance to fluconazole and cross-resistance to amphotericin B in *Candida albicans* from AIDS patients caused by defective sterol delta5,6-desaturation. FEBS Lett 400(1):80–82. https://doi.org/10.1016/S0014-5793(96)01360-9

Loffler J, Kelly SL, Hebart H, Schumacher U, Lass-Florl C, Einsele H (1997) Molecular analysis of cyp51 from fluconazole-resistant *Candida albicans* strains. FEMS Microbiol Lett 151 (2):263–268. https://doi.org/10.1111/j.1574-6968.1997.tb12580.x

Lopez-Ribot JL, McAtee RK, Lee LN, Kirkpatrick WR, White TC, Sanglard D, Patterson TF (1998) Distinct patterns of gene expression associated with development of fluconazole resistance in serial *Candida albicans* isolates from human immunodeficiency virus-infected patients with oropharyngeal candidiasis. Antimicrob Agents Chemother 42(11):2932–2937

Malani AN, Kauffman CA (2007) Changing epidemiology of rare mould infections: implications for therapy. Drugs 67(13):1803–1812. https://doi.org/10.2165/00003495-200767130-00001

Maschmeyer G, Haas A, Cornely OA (2007) Invasive aspergillosis: epidemiology, diagnosis and management in immunocompromised patients. Drugs 67(11):1567–1601. https://doi.org/10.2165/00003495-200767110-00004

Maubon D, Garnaud C, Calandra T, Sanglard D, Cornet M (2014) Resistance of *Candida* spp. to antifungal drugs in the ICU: where are we now? Intensive Care Med 40(9):1241–1255. https://doi.org/10.1007/s00134-014-3404-7

Orozco AS, Higginbotham LM, Hitchcock CA, Parkinson T, Falconer D, Ibrahim AS, Ghannoum MA, Filler SG (1998) Mechanism of fluconazole resistance in *Candida krusei*. Antimicrob Agents Chemother 42(10):2645–2649. https://doi.org/10.1093/jac/dkh507

Pasqualotto AC, Denning DW (2008) New and emerging treatments for fungal infections. J Antimicrob Chemother 61:i19–i30. https://doi.org/10.1093/jac/dkm428

Pfaller MA, Diekema DJ, Gibbs DL, Newell VA, Bijie H, Dzierzanowska D, Klimko NN, Letscher-Bru V, Lisalova M, Muehlethaler K, Rennison C, Zaidi M, Global Antifungal Surveillance Group (2009) Results from the ARTEMIS DISK Global Antifungal Surveillance Study, 1997 to 2007: 10.5-year analysis of susceptibilities of non-candidal yeast species to fluconazole and voriconazole determined by CLSI standardized disk diffusion testing. J Clin Microbiol 47 (1):117–123. https://doi.org/10.1128/JCM.01747-08

Richardson M, Lass-Florl C (2008) Changing epidemiology of systemic fungal infections and the possibilities of laboratory diagnostics. Clin Microbiol Infect 14(4):5–24. https://doi.org/10.1556/AMicr.46.1999.2-3.12

Rivero-Menendez O, Alastruey-Izquierdo A, Mellado E, Cuenca-Estrella M (2016) Triazole Resistance in *Aspergillus spp*.: a worldwide problem? J Fungi (Basel) 2(3):21. https://doi.org/10.3390/jof2030021

Rogers TR (2006) Antifungal drug resistance: limited data, dramatic impact? Int J Antimicrob Agents 27:7–11. https://doi.org/10.1016/j.ijantimicag.2006.03.012

Rybak JM, Ge W, Wiederhold NP, Parker JE, Kelly SL, Rogers PD, Fortwendel JR (2019) Mutations in hmg1, challenging the paradigm of clinical triazole resistance in *Aspergillus fumigatus*. MBio 10:e00437–e00419. https://doi.org/10.1128/mBio.00437-19

Sanglard D, Ischer F, Monod M, Bille J (1997) Cloning of *Candida albicans* genes conferring resistance to azole antifungal agents: characterization of CDR2, a new multidrug ABC transporter gene. Microbiology 143:405–416. https://doi.org/10.1099/00221287-143-2-405

Sanglard D, Kuchler K, Ischer F, Pagani JL, Monod M, Bille J (1995) Mechanisms of resistance to azole antifungal agents in *Candida albicans* isolates from AIDS patients involve specific multidrug transporters. Antimicrob Agents Chemother 39(11):2378–2386. https://doi.org/10.1128/aac.39.11.2378

Snelders E, Huis In't Veld RA, Rijs AJ, Kema GH, Melchers WJ, Verweij PE (2009) Possible environmental origin of resistance of *Aspergillus fumigatus* to medical triazoles. Appl Environ Microbiol 75(12):4053–4057. https://doi.org/10.1128/AEM.00231-09

Snelders E, van der Lee HA, Kuijpers J, Rijs AJ, Varga J, Samson RA, Mellado E, Donders AR, Melchers WJ, Verweij PE (2008) Emergence of azole resistance in *Aspergillus fumigatus* and spread of a single resistance mechanism. PLoS Med 5(11):e219. https://doi.org/10.1371/journal.pmed.0050219

van Ingen J, van der Lee HA, Rijs TA, Zoll J, Leenstra T, Melchers WJ, Verweij PE (2014) Azole, polyene and echinocandin MIC distributions for wild-type, TR34/L98H and TR46/Y121F/T289A *Aspergillus fumigatus* isolates in the Netherlands. J Antimicrob Chemother 70 (1):178–181. https://doi.org/10.1093/jac/dku364

Verweij PE, Mellado E, Melchers WJ (2007) Multiple-triazole-resistant aspergillosis. N Engl J Med 356(14):1481–1483. https://doi.org/10.1056/NEJMc061720

White TC (1997) Increased mRNA levels of ERG16, CDR, and MDR1 correlate with increases in azole resistance in *Candida albicans* isolates from a patient infected with human immunodeficiency virus. Antimicrob Agents Chemother 41(7):1482–1487

Wisplinghoff H, Bischoff T, Tallent SM, Seifert H, Wenzel RP, Edmond MB (2004) Nosocomial bloodstream infections in US hospitals: analysis of 24,179 cases from a prospective nationwide surveillance study. Clin Infect Dis 39(3):309–317. https://doi.org/10.1086/421946

# "Planetary Health" Perspectives and Alternative Approaches to Tackle the AMR Challenge

Padma Venkatasubramanian, S. P. Balasubramani, and Rajan Patil

## 1 Introduction

Antimicrobials are chemical agents that kill or stop the growth of microbes including bacteria, viruses, fungi, and parasites. They can be natural or synthetic in origin. For example, penicillin is a natural antibiotic derived from the fungus *Penicillium notatum*, bleach is a synthetic disinfectant and sodium benzoate a food preservative. Antibiotics are usually specific and targeted in action, whereas disinfectants, preservatives, or antiseptics are generic antimicrobials, the latter also called biocides (Russell 2003).

Antimicrobials have indeed played a paramount role in the prevention and treatment of diseases in humans and animals, ever since the 1930s, when sulfonamide and penicillin were discovered. The 1950s and 1960s were the golden eras when novel natural and synthetic antimicrobials were discovered against deadly infectious diseases (Davies 2006). Drugs for tuberculosis (isoniazid, rifampicin, etc.), malaria (chloroquine, doxycycline, etc.), leprosy (ethambutol, pyrazinamide, etc.), cholera (erythromycin, ofloxacin, etc.), HIV (tenofovir, cotrimoxazole, etc.), and other infectious diseases saved millions of lives and have averted major epidemics. The antimicrobials had almost become a panacea and there was a growing feeling of invincibility among physicians and patients alike. They were being prescribed not only for cure of diseases but also for the prevention of infections and as growth promoters for farm animals (Ronquillo and Hernandez 2017). Little did we realize then that the indiscriminate use and the constant exposure of microbes to drugs hastens the natural process that favorably selects

P. Venkatasubramanian (✉) · R. Patil
School of Public Health, SRM Institute of Science and Technology, Chennai, India
e-mail: dean.sph.ktr@srmist.edu.in

S. P. Balasubramani
Centre for Viticulture and Small Fruit Research, Florida Agricultural and Mechanical University, Tallahassee, FL, USA



S. Thomas (ed.), *Antimicrobial Resistance*,
https://doi.org/10.1007/978-981-15-3658-8_8

resistant microbes. This led to antimicrobial resistance (AMR). Even though there are few reports indicating resistance to biocides per se, emerging evidences indicate biocides can also catalyze AMR through enhancing cross-resistance. For example, the antiseptic chlorhexidine induces AMR to colistin, an effective antibiotic against Gram-negative bacteria (FAO 2016). The "cure had become the catalyst" (Rosenblatt-Farrell 2009) and the "solution was the problem" (Landecker 2016) were some of the apt comments made by reviewers of AMR.

Anthropogenic or man-made factors including environmental pollution; bad water, sanitation, and hygiene (WASH) conditions; improper antibiotic prescription and use; etc. are known to be drivers of AMR (Rosenblatt-Farrell 2009). Common commensals like *Klebsiella pneumoniae* and *Escherichia coli* have acquired resistance to antibiotics, wreaking havoc in spotless and clean hospital intensive care units (ICUs) (WHO 2018b). There is a resurgence of Old World diseases including malaria and tuberculosis due to multiple drug resistance (MDR) and extensive drug resistance (XDR) of the pathogens (WHO 2018c). Based on the data collected from 129 countries, WHO warns us of an impending AMR apocalypse—a "post-antibiotic era"—where even common infections and minor injuries can kill (WHO 2014).

The other worry is that the last 25 years have seen a lull in the discovery of novel antimicrobials, with hardly any new class of antibiotics discovered since 1984 (Pew Charitable Trust Report 2016). Despite increasing R&D investments, no new antibiotic class has been discovered in the past 25 years. The editorial of the July 2018 issue of *Nature Biotechnology* observes the government-imposed control in antibiotic use has led to a reduction in its sales and commercial incentives, forcing multinational companies to abandon their antibiotic R&D efforts (Anonymous 2018).

Why are we in the AMR situation at all? Why is it that the experts have put their hands up in surrender and said that the only way to contain AMR is through rational use of antimicrobials (WHO-IACG-Discussion Paper 2018a)?

The AMR crisis has brought in a realization about the importance of the "ecosystem" or interconnectedness of organisms as well as the socioeconomic and political contexts within which a pathogen thrives or dies. It has forced scientists, industry, policy makers, governors, and civil society organizations to go beyond science, for public health actions to avert the AMR catastrophe (WHO-IACG-Discussion Paper 2018a). The recommendations are for multiple sectors to work in a convergent fashion, invest in R&D to discover new antimicrobials, increase awareness about AMR, and control the use of antimicrobials. However, these may not be adequate to stop the development of AMR altogether. Then, the important question remains as to how to tackle AMR.

Perhaps we may need to complement the reductionistic approaches of antimicrobial drug development with more holistic ways that are in sync with nature. The AMR crisis is a clarion call for a change from the human-centered ways that we operate at the cost of everything else on this Earth disregarding the ways of nature. Do we even understand the complex and dynamic interconnectedness that exists among all living and non-living things on this Earth? Indigenous people and their knowledge systems do seem to know, but are we willing to learn from them?

This chapter provides an opportunity to pause and reflect on the assumptions behind existing approaches, including the reductionistic processes involved in antimicrobial drug discovery. The chapter stresses on simple public health efforts that can avert infectious diseases and epidemics alongside some of the alternative ways for disease prevention. Highlighting the emerging perspectives on "Planetary Health" and "One Health," the chapter emphasizes the potential benefits of revisiting some of the principles and practices of traditional health systems that are holistic and contextual.

## 2 AMR Drivers: Knowledge and Gaps

A lot is known about factors that drive AMR but there are substantial gaps in our understanding of the complex interactions of the factors that contribute to the emergence of AMR or acceleration of the rate of its development (Holmes et al. 2016). We now know that constant antibiotic use in hospitals and animal farms, bad WASH conditions, socio-cultural behavior, poverty, and nutrition promote AMR development (WHO-IACG Discussion Paper 2018a). Evidence for these associations has mostly emerged through studies in single disciplines or a few disciplines at a time, i.e., almost in a linear fashion. However, in nature, there are multi-variate drivers, yet unknown to us, that work independently or interdependently. Systems biology approach and mathematical modeling provide scope for holistic analysis and projections but may still be inadequate to explain complex real-life situations (Raman and Chandra 2010; Fang and Casadevall 2011).

### 2.1 Mechanisms Behind AMR

Some of the known mechanisms of AMR include (1) impermeability to drugs by altering the microbial cell wall (e.g., vancomycin and Gram-negative bacteria), (2) elimination of drug through active efflux (e.g., tetracycline and *Escherichia coli*), (3) inactivation of the drug through enzymatic action (e.g., lincosamides and *Staphylococcus aureus*), (4) alteration of the drug receptor site through mutation (e.g., rifampicin and *Mycobacterium tuberculosis*), and (5) through a catabolic pathway that disintegrates the antibiotic (e.g., penicillin and *S. aureus*) (Munita and Arias 2016).

Antibiotic resistance can spread by the horizontal transmission of specific genomic resistance mechanisms and the selection/propagation of resistant strains, which is known to occur asymptomatically between humans, animals, and the environment, through what is named as the lateral antibiotic resistance genetic transfer (LARGT) pathways. Plasmids, integrons, and transposons can mediate LARGT (Chamosa et al. 2017). Microbes that were originally thought of as gut commensal, like *Klebsiella pneumoniae*, are now ubiquitous with high plasmid load and AMR gene diversity and a key AMR gene "trafficker" across ecological niches (Wyres and Holt 2018). Although inappropriate antibiotic use was considered as the main force

behind the rise of antibiotic resistance in populations, epidemiological and other studies correlate polluted environment and poor socioeconomic conditions to the spread of AMR (Hinchliffe et al. 2018; MacFadden et al. 2018).

## 2.2 Epidemiology to Understand AMR Patterns

Per WHO, information on AMR incidence, prevalence, and geographical distribution is inadequate. Most of the epidemiological figures are sourced using data from a dozen countries and extrapolated globally for nearly 200 countries (Matsunaga and Hayakawa 2018). The data on AMR comes primarily from hospitals (O'Neill 2014). Population as well as environmental prevalence studies are equally important for understanding its burden in a given setting and for informing context-specific treatment practices.

The earliest AMR was reported way back in 1940, 1 year before the first administration of penicillin for human therapeutic use; two members of the team who discovered the drug revealed that resistance to penicillin already existed (Thakur and Gray 2019). Thus, resistance to drugs was reported almost in parallel to its discovery (Fig. 1).

It is startling to realize that at least 30% of prescriptions are clinically inappropriate, inadequate, or unnecessary. A study was undertaken in a primary care setting in Scotland between 2005 and 2012 to determine the association between antimicrobial stewardship, prescriptions of certain antimicrobials (against Gram-negative bacteria) by a general practitioner, and the AMR among the patients. They found large reductions in community broad-spectrum antimicrobial use associated with stewardship interventions along with a modest reduction in resistance in coliform bacteremia and a flattening of the rising rate of AMR (Hernandez-Santiago et al. 2019).

The increase in AMR burden correlated with a 65% increase in antimicrobial consumption in humans between 2000 and 2015 in 76 countries (Klein et al. 2018). The BRIC countries and South Africa accounted for three-quarters of this growth. In a few Asian countries, resistance levels were much higher than in European countries (Matsunaga and Hayakawa 2018). However, there is also evidence that reduction in antimicrobial use does not always lead to reduced resistance, perhaps because microbes get well-adapted to carrying resistance (Livermore 2003).

Epidemiologically, the spread of AMR strains shows both local and global propensities. It usually occurs in clusters, in units where the most vulnerable people are congregated and where antimicrobial use consequently is the heaviest (Archibald et al. 1997). *Acinetobacter baumannii* emerged as an opportunistic pathogen among the wounded soldiers in the Iraq War, thus acquiring the name Iraqibacter. It is associated with nosocomial ventilator-associated pneumonias. A similar observation was made with the higher prevalence of methicillin-resistant *Staphylococcus aureus* (MRSA) in intensive care units (ICUs), where methicillin usage was much higher, than in general wards or outpatient departments within the same hospitals (Adame-Gómez et al. 2018).

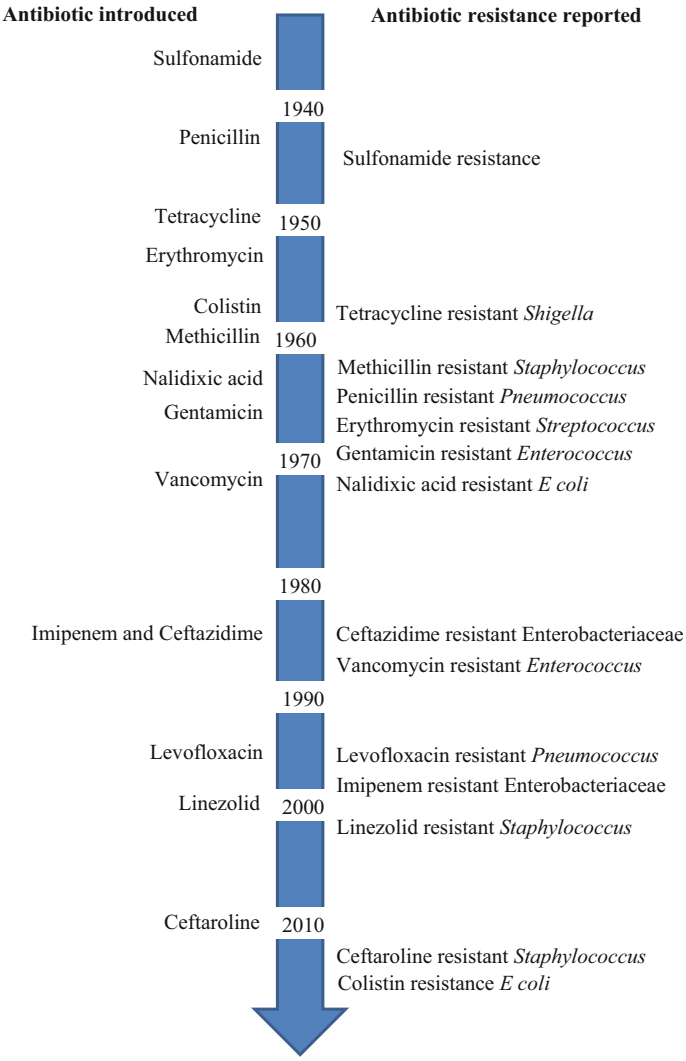


**Fig. 1** Discovery of antimicrobials and emergence of AMR

Around 200 diseases are recognized by WHO to be zoonotic, i.e., transmitted to humans from animals, and controlling them requires a consolidated approach. Recent outbreaks of swine flu, bird flu, Ebola, etc. prove humans and animals (like pigs, birds, and bats) share common pathogenicity pathways. Sub-therapeutic and therapeutic doses of antibiotics as growth promoters and medicines in farm animals cause AMR which gets transmitted to humans. There is evidence to indicate that third-generation cephalosporin-resistant *E. coli* strains can spread from livestock to humans via food consumption (Allcock et al. 2017). Analyses of genomic sequence

data suggest that a globally distributed MRSA lineage (ST5) may have originated from human-to-poultry transmission (Lowder et al. 2009). Conversely, studies have also shown that various MRSA lineages from animals also appear in humans (Baptiste et al. 2005).

The burden of MRSA is a great concern irrespective of the level of income of countries. For instance, in 15 developed European countries, 50% of the proportion of bloodstream *S. aureus* infections were caused by MRSA (O'Neill 2014). AMR spread in the community is also favored by crowding, travel, and migration (Livermore 2003). In the low- and middle-income countries (LMICs) that are undergoing rapid economic development, urbanization has resulted in overcrowding and poor water, sanitation, and hygiene (WASH) conditions that in turn increase the risk of development and spread of AMR (Allcock et al. 2017). AMR spread is also trans-geographical, to the extent that some resistant strains spread between countries and continents. Multidrug-resistant pneumococci of serotype 6B were imported from Spain to Iceland, by nasopharyngeal carriage in the children of returning holidaymakers (Klein et al. 2018).

The emergence and spread of carbapenemases such as the New Delhi metallo-beta-lactamases (NDM-1) have an enormous implication for worldwide healthcare delivery and population health because they compromise antimicrobial efficacy of almost all lactams, including the last resort carbapenems. Even though the main reservoir of NDM-1 appears to be the Indian subcontinent, it has been detected in the Balkans, the Middle East, and the UK as well (Johnson and Woodford 2013). Colistin, a polymyxin kind of antibiotic, was reserved as a drug of last resort by the WHO to combat MDR, NDM, and other AMR. However, resistance has been reported to colistin as well and the *mcr* (mobilized colistin resistance) gene detected from pigs to humans in almost every continent (Frost et al. 2019).

## 2.3 Climate Change and Environmental Drivers of AMR

It is now well established that warmer temperatures promote bacterial growth and increase AMR. Several bacteria, like *S. aureus*, thrive in temperatures between 40 and 140 °F, a range often referred to as the "danger zone." As temperatures around the world continue to rise, bacteria are expected to reproduce at a faster rate, increasing the opportunity for mutation and transmission. Studies associate the 80,000 infections and 11,000 deaths occurring every year in the USA due to MRSA with the increase in the average annual temperature by 2.6 °F above the twentieth-century average. MacFadden et al. (2018) report that a 10 °C increase in temperature across the USA was associated with a 4.2%, 2.2%, and 2.7% increase in antibiotic resistance of *E. coli*, *Klebsiella pneumoniae*, and *Staphylococcus aureus*, respectively.

AMR is not a new and recent phenomenon. Like climate change, it has been happening in nature, wherein human activity has catalyzed AMR over the last half century (Woolhouse et al. 2015). AMR is also a One World issue apart from being a One Health issue. The globalization of the food system, with increasing movement

of livestock and agricultural produce, combined with increasing human travel, facilitates the rapid spread and mixing of AMR genes that emerge (Robinson et al. 2016). Surveillance systems need to undertake environmental sampling to monitor and combat AMR. Soil and water microflora play complex and critical roles in ecosystem functions such as the recycling of carbon and nutrients. Disrupting these geo-bio-chemical vital processes by creating an imbalance may threaten "Planetary Health" (Roose-Amsaleg and Laverman 2016), potentially pushing ecosystems beyond critical environmental thresholds.

### 2.4 Gaps in Our Understanding of Nature

The fittest microbes survive and thrive in an antimicrobial environment through a natural selection process. Selection pressure of any kind, including antimicrobial usage, can drive an organism to change, adapt, survive and reproduce, and thus be naturally selected from its population (FAO 2016). In a pool of microbes, a few will inherently carry gene(s) or mechanisms that lend resistance to the drug (Fig. 2), i.e., an organism can possess resistance to an antimicrobial even in pristine environments not exposed to antibiotics (Knöppel et al. 2017; Martinez 2009). AMR genes have been detected not just in hospitals, diverse environments, and soils but also in samples from permafrost and secluded caves that have never been exposed to antibiotics (Bhullar et al. 2012). A large-scale metagenomic analysis has shown the presence of antibiotic resistance gene determinants (ARGDs) in almost all the environments sampled (Nesme et al. 2014). Considering that the natural laws are not going to change radically, AMR is here to stay. We can only hope to reduce the rate at which AMR surfaces and manage the situation (Andersson and Hughes 2011).

By design, the epistemology as well as methodology of modern science and biomedicine employed to "understand" nature is reductionist, focusing on cells, molecules, human body, etc. (Beresford 2010). Scientific reasoning breaks down the natural world into individual parts, to understand it through disciplines based on the expertise of scientists. But, nature is integrated, with sustainability as the key to the web of life (Hoffman 2016). Microbes have repeatedly demonstrated an utter disregard for man-made disciplines and sectors. Perhaps tackling AMR requires a different, more holistic approach that captures the dynamics between humans, biodiversity, and the environment. As elegantly put by Landecker (2016), "Resistance (AMR) has often flourished in blind spots generated by human categories of knowledge and action."

## 3 The Cure Lies in the Cause

Some introspection into known causes and unknown gaps can throw up sustainable solutions to the AMR problem.

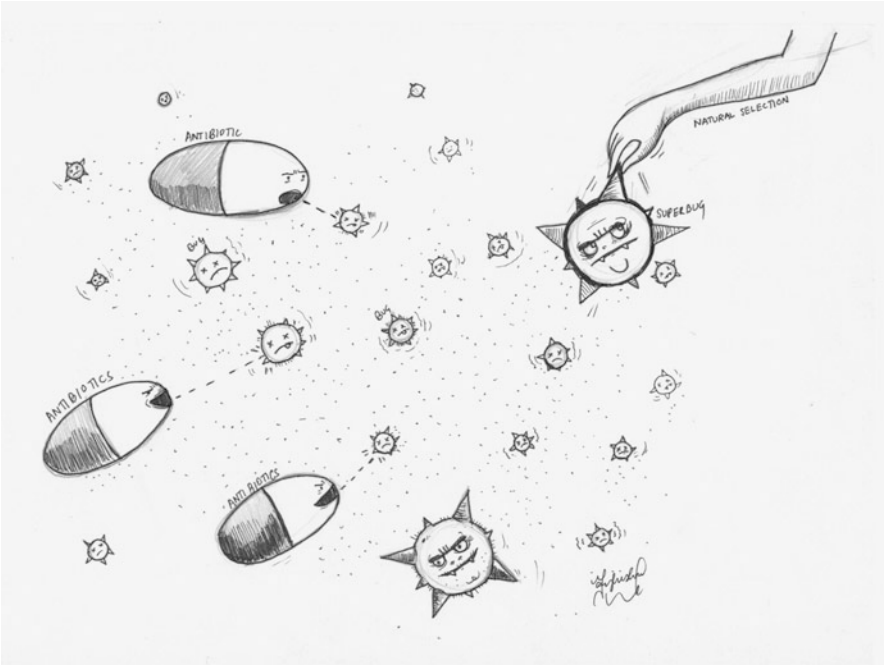


**Fig. 2** Artistic impression of natural selection: a few antimicrobial-resistant microbes get selected through a natural selection process (concept, Padma Venkatasubramanian; artist, Mr. Jaykrishnan Menon)

## 3.1 Emerging Perspectives on "Planetary Health" and "One Health"

Anthropogenic activities leading to soil, water, air, and space pollution have altered global ecosystems causing climate change, which have led to biodiversity loss as well as drastic changes to the abiotic environment.

Human gains are being pushed inequitably at the cost of the planet's health, without realizing the repercussions (Whitmee et al. 2015). The "One Health" approach emerged from evidence of the impact of overuse of antibiotics in the farm and agriculture sectors, while the "Planetary Health" perspective was based on the impact of environmental damage on human health. The crux of both perspectives is the interconnectedness and interdependency of human health on biodiversity and environmental health.

> "Planetary Health" is the health of human civilization and the state of the natural systems on which it depends (Whitmee et al. 2015)

"One Health" is an approach that calls for action by governments of countries across the world to work together in reverting AMR or at least contain the problem.

This is done so that effective antibiotics will be available for life-and-death situations rather than to further routine commercial transactions. Following this, many EU countries and the USA have banned the use of antibiotics as growth promoters for the livestock industry.

WHO defines *One Health as*

> "a concept and an approach to designing and implementing programs, policies, legislation and research in which multiple sectors communicate and work together to achieve better public health outcomes. (https://www.who.int/features/qa/one-health/en/)"

The World Organisation for Animal Health (OIE), an intergovernmental organization, was formed to collectively address the problem of human-animal-ecosystem health risks. WHO advocated a ban on the use of antimicrobials as growth promoters in animal husbandry following the steps of the EU (European Commission 2015). However, OIE has soft-pedaled the issue by avoiding the mention of growth promotion in the "OIE Strategy on AMR and the Prudent Use of Antimicrobials" (OIE 2016).

The WHO Global Plan (WHO 2015) to tackle AMR under "One Health" focuses on (1) awareness about AMR; (2) research and surveillance for knowledge and evidence base; (3) reducing incidence through better WASH, etc.; (4) optimizing AMD usage; and (5) developing economic models to sustain "One Health." This is the first formal global acknowledgment that the drivers of AMR are several including biological, social, economic, environmental, behavioral, and other factors. Yet, the implementation of the strategy is altogether a different matter.

## 3.2 Improving WASH and Infrastructure

There are historical accounts and modern evidence to show WASH (water, sanitation, and hygiene) interventions have had epidemiological impact in the decline in infectious diseases. A public health success story cited often is the fight against cholera and other communicable diseases by Londoners during the post-industrial era. Per the official UK records of 1848, this was done by cleaning the river Thames, improving the WASH conditions, creating the metropolitan sewer system, and providing better housing (https://www.thegazette.co.uk/London/issue/21764/page/3117). The main cause was mentioned as the rapid industrialization without adequate planning for development leading to crowding and grossly inadequate WASH and infrastructure amenities. A century later, the urban cities of developing countries like India, China, and Brazil grapple with a similar situation. Improvements in WASH and infrastructure interventions have been successful in preventing infections, thereby reducing AM usage and AMR (Macintyre et al. 2017). Environments (water, soil, air), humans, and animals are reservoirs that harbor pathogens and AMR genes. A roundtable discussion held in July 2017 in the UK summarizes well the possible ways improved WASH can reduce AMR. Firstly, improving WASH can prevent contact with pathogens and thereby prevent

infectious diseases. Thus, the antimicrobial use required would be less. Secondly, efforts to provide proper sanitation, e.g., ending open defecation, will break the chain of spread of resistant bacteria (Anonymous 2017). Therefore, improving WASH and infrastructure should be taken up by the developing countries, on a war footing.

## 3.3 Traditional Knowledge Systems: A Different Way of Knowing and Doing

The world-views and epistemologies of indigenous communities on health, disease, and their management are quite different from those of biomedicine. Traditional systems of medicine (TSM) such as Ayurveda or Chinese medicine are holistic and inclusive of nature and use nature for maintaining health and combating diseases (Payyappallimana and Venkatasubramanian 2016). To a person trained in biomedicine, the tenets of indigenous systems may seem to be non-scientific and vague. Contrary to this, TSM is founded on sound principles. It has very functional prescriptions that are integrated with local cultures, diet, and home remedies (Sharma et al. 2007a). One needs to make efforts to really appreciate the wealth of knowledge that exists. Ayurveda, for example, shows us ways to live in harmony with nature rather than go against ("anti") it. The ancient texts of Ayurveda not just contain details on plant drugs and formulations but also describe the philosophies, fundamental principles, clinical diagnosis of diseases, and preventive and curative practices (Sharma et al. 2007b).

The following principles/practices of TSM would be pertinent for consideration to develop alternative strategies to tackle AMR.

### 3.3.1 Botanicals and Polyherbal Preparations

Plants contain secondary metabolites such as alkaloids, flavonoids, polyphenols, triterpenoids, etc. that protect the plants from abiotic and biotic stress. Plants have been one of the main ingredients of millennia-old TSMs as well as Western medicine (Quave 2016). Traditional medical practices use whole plants as well as polyherbal formulations as crude extracts rather than isolated compounds. The complex "chemical concoctions" are less prone to microbial resistance as compared to single isolated compounds, as clearly observed in the case of artemisinin. Tea from Qinghao [*Artemisia annua* L. (Asteraceae)] has been used in traditional Chinese medicine to treat malarial fevers effectively for centuries. But within a decade of introduction of the isolated compound, artemisinin, as monotherapy for malaria, the parasite *Plasmodium falciparum* developed resistance to it (Fairhurst et al. 2012; Quave 2016). Crude plant extracts and polyherbal formulations used in TSM also perform multiple, synergistic functions from antimicrobial action to enhancing absorption, lowering toxicity, and improving immunity (Rasoanaivo et al. 2011).

Several plant compounds and extracts have been tested for their synergistic action with antibiotics to improve the outcome. Berberine from *Berberidaceae* spp. and thymol from *Thymus maroccanus* reduced the MIC of the antibiotics against *Vibrio cholerae*, *K. pneumoniae*, *S. aureus*, *and Pseudomonas aeruginosa* (Fadli et al.

2012; Cheesman et al. 2017). Similarly, *Daphne genkwa* extract was found to reduce the Fractional Inhibitory Concentration (FIC) index of oxacillin against MRSA (Kuok et al. 2017). A combination of herbal preparations with antibiotics has been shown to improve the antibiotic activity by inhibiting drug efflux and thereby act as resistance-modifying agents (Abreu et al. 2012; Shriram et al. 2018). Extracts of *Salvia officinalis*, *Cassia nigricans*, *Cichorium intybus*, and *Dorycnium pentaphyllum* reduced the FIC index of standard antibiotics like chloramphenicol, amoxicillin, gentamicin, and cephalexin against *Bacillus subtilis*, *K. pneumoniae*, *S. aureus*, and *E. coli* by at least half (Stefanovic 2017).

The Indian Systems of Medicine (ISM) use over 6000 medicinal plant species (Ved and Goraya 2007), containing >1500 medicinal plants in the Materia Medica and ~0.29 million polyherbal formulations (TKDL 2012) for treating various conditions. Even though Ayurveda is still practiced in India, it is not in mainstream medical care and its full potential is yet to be realized. Certain common medicinal plants like *Ocimum sanctum* (holy basil), *Azadirachta indica* (neem), and *Andrographis paniculata* (kalmegh) from ISM have a potential use against new infectious outbreaks like H1N1 swine flu and tackle drug-resistant pathogens including clinical isolates of *S. aureus*, *Salmonella typhimurium*, and *Candida albicans* (Ahmad and Beg 2001; Shah and Krishnamurthy 2013). Thus, botanicals and formulations used in TM can offer solutions to tackle AMR in the post-antibiotic era. However, there are issues that need to be addressed including the availability and quality control of herbs, the regulations of access and benefit sharing, and most importantly, the philosophy of the usage of herbs within the context of a traditional knowledge base in TM (Gupta and Birdi 2017; Payyappallimana and Venkatasubramanian 2016; Quave 2016).

### 3.3.2 Relation Between Man and the Universe

"Yatha pinde thatha brahmande; yatha brahmande thatha pinde"

"As the universe so the body" (Yajurveda (2nd millennium BCE))

It is important to invest in understanding the fundamental principles of TSM in order to obtain the best returns. Ayurveda, for example, looks at the macrocosm (universe) and the microcosm (human body) to be made up of the same five elements of nature, namely, earth, water, fire, air, and space (*Pancha-mahabhuthas*). The *mahabhuthas* combine to form the three humors (*tridosha*). The universal principles of Ayurveda classify everything in this universe (earth, humans, health, disease, materials, seasons, regions, etc.) through the fundamental functional unit of *tridosha.* The microcosm is inherently dependent on and affected by the macrocosm, meaning that the former cannot flourish at the cost of the latter. So, polluting the universe is like polluting our own body by vitiating the *doshas*.

There is some similarity between the Ayurvedic principles on micro- and macrocosm and the recent perspectives on "Planetary Health", since both speak about the importance of the universe/planet health for human health. However, per Ayurveda,

humans are nature and nature us, i.e., inseparable. Bearing in mind this thought process helps while designing new AMR strategies.

> "Planetary Health" is an attitude towards life and a philosophy for living. (Horton et al. 2014)

### 3.3.3 Focus on Wellness

Ayurveda places more emphasis on wellness (Svasthya) than on illness (Vyadhi). Svasthya (*Sva* + *sthya*) means to be rooted in self, meaning wellness is a state of being when one is in tune with oneself. The state of wellness is dynamic and varies between individuals (Payyappallimana and Venkatasubramanian 2016). All individuals do not fall sick or contract infections nor do they acquire infections all the time. A pathogen infects when the conditions are favorable, with compromised immunity being one of the predominant conducive factors. Therefore, building up one's immunity and resilience has been one of the important ways of TSM to fight diseases. One branch of Ayurveda called *Rasayana* spells out ways to build *vyadhikshamatva* (resistance to diseases) using herbs and lifestyle practices. For example, a newborn child is made to lick *Swarnaprashana*, a combination of gold, honey, and ghee (clarified butter), or growing children have herbal jams like Chyawanprash as tonics to build immunity, promote intellect, and develop robust functioning organs and faculties (Jyothy et al. 2014; Sharma et al. 2019). Gut health and a robust metabolism are considered pivotal for wellness. The origin of all vitiation is said to begin with a poor diet and disturbed metabolism. When the food quality is poor and/or the ingested food is not fully digested, assimilated, or eliminated, it produces *ama* (unmetabolized substances), which is the nucleus of all ailments (Balasubramani et al. 2011). Hence, before treating any disease, the gut is cleansed using herbal decoctions through a process called *panchakarma* that includes purgation and other cleansing processes following which the gut is nourished with herbal formulations to strengthen the metabolism and build resilience. Thatte et al. (2015) report regulation of pro-inflammatory cytokines, immunoglobulins, and T-cell response, post-*panchakarma* treatment. This opens the possibility for further scientific exploration. The therapeutic activities of Rasayanas are several, but the most important are as metabolic regulators, immune modulators, and anti-aging agents (Balasubramani et al. 2011; Peterson et al. 2017). Ashwagandha (*Withania somnifera*) and Triphala (a classical polyherbal preparation made with three herbs *Terminalia chebula*, *Terminalia bellerica*, and *Emblica officinalis*) are examples of botanicals/polyherbal formulations used as Rasayanas. Emerging techniques like network pharmacology have been used to demonstrate the rationale of the multifaceted benefits of Triphala (Chandran et al. 2015) and Ashwagandha (Chandran and Patwardhan 2017). Ashwagandha has also been shown to possess immune-potentiating activity supporting DPT vaccine (Gautam et al. 2004).

Recent times have seen an exponential increase in research publications on the role of the gut and the microbiome on diseases, ranging from communicable to non-communicable (Hajela et al. 2015). Fecal microbiota transplants have been

explored as a treatment to colonize the gut with beneficial microbes (Relman and Lipsitch 2018). Why not study how *panchakarma* helps gut health?

The *ways of knowing* (nature) of the millennia-old Ayurveda or Chinese medicine are different from biomedicine and were not generated using modern technologies. Thus, systems biology approach, big data analytics, and omics technologies may help bridge the gap between the seemingly esoteric principles and practices of TSM and modern biomedicine (Qiu 2007).

## 3.4 Immunity Is the Key

The human body has a well-developed and well-defined immune system whose primary job is to resist and/or eliminate infections. While the innate immune system is non-specific and provides barriers against the invading pathogens, the adaptive immune system reacts specifically by producing antibodies and cells against the pathogen. Mechanistically, innate immunity can be continuously produced as with defense through skin, intestinal tract, etc. or induced as a response to "foreign" object by secreting mediators and regulators (Porcelli 2017). Adaptive immunity can be passive (e.g., through breast milk) or active (infections and vaccination). The cells of the immune system have their own memory and can eliminate pathogens more aggressively on recurrence. Though the immune cells always keep vigilance, the immune functions can often be compromised. Thus, strengthening the immune system is a potential natural mechanism to prevent or combat infections (Hackett 2003; Baker and Isaacs 2018).

Immunostimulants are substances that activate the immune system of humans and animals and improve the body's natural resistance to infections for prevention of diseases. These substances may be from natural sources or synthetically manufactured with different chemical properties to induce the robust functioning of the immune system. Immunostimulants work by activating immune mediators like phagocytosis, properdin and complement systems, protective secretory IgA antibodies, α- and γ-interferon release, T and B lymphocytes, synthesis of specific antibodies and cytokines, and synthesis of pulmonary surfactant.

Several natural products and synthetic substances have been identified to improve resistance against infections. Pomegranate juice feeding was found to improve resistance against *Candida albicans* infection in the *Drosophila melanogaster* model (Balasubramani et al. 2014). Non-specific immunity was stimulated in fish treated with a marine microalga extract whereby they could withstand *Aeromonas hydrophila* infection (Yengkhom et al. 2019). Gjini and Brito (2016) consider integrating antimicrobial therapy and immune activation as an efficient method to fight drug resistance. A stimulated immune response can be a fitting reply to the increase in AMR and decline in antimicrobials (Handel et al. 2009).

### 3.4.1 Vaccines

Vaccines are one of the most cost-effective and best public health tools for primary prevention of infections as well as AMR. Efficacious vaccines not only protect the

**Table 1** Types of vaccines and their protection against diseases

| Vaccine type | Details | Vaccine names/diseases |
|---|---|---|
| Live, attenuated | Live but weakened/attenuated pathogens | Measles, mumps, and rubella (MMR combined vaccine), varicella (chickenpox), influenza (nasal spray), rotavirus, zoster (shingles)<br>Yellow fever |
| Inactivated/killed | Inactivated pathogens, typically using heat or chemicals such as formaldehyde | Polio (IPV)<br>Hepatitis A<br>Rabies |
| Toxoid | Inactivated toxin produced by the microbe | Diphtheria and tetanus (part of DTP combined immunization) |
| Subunit, recombinant, polysaccharide, and conjugate vaccines | Use of specific pieces of the pathogen, e.g., protein, sugar, or capsid (a casing around the pathogen)<br>This type may require booster shots to get continuous protection against diseases | Hepatitis B<br>Influenza (injection)<br>*Haemophilus influenzae* type b (Hib)<br>Pertussis (part of DTaP combined immunization), pneumococcal, meningococcal, human papillomavirus (HPV) |

immunized but can also reduce disease among unimmunized individuals in the community through indirect effects or "herd protection." The herd protection of the unvaccinated occurs when a sizeable proportion of the group is immune (John and Samuel 2000). Several population studies demonstrate the potential of vaccines in tackling AMR. For example, the introduction of a conjugate pneumococcal vaccine for infants in the USA in 2000 saw a 57% decline in disease caused by penicillin-resistant strains and a 59% decline in strains resistant to multiple antibiotics across a broad age spectrum (Gellin et al. 2001). Table 1 lists the various categories of vaccines that are widely used across the globe that offer both active and passive immunity against a range of pathogens.

AMR is maximum in areas where the pathogen numbers and proneness are high. Therefore, public health strategies such as "source drying" are used to vaccinate select high-risk groups (Andre et al. 2008). "Targeted vaccination" of food handlers decreased typhoid and hepatitis A incidence in the whole occupational groups (Fiore 2004). Another strategy is "ring vaccination" or "cocoon vaccination" used to protect those too young to be given primary vaccination by vaccinating close contacts. For example, administration of pertussis vaccine boosters of close contacts (such as parents and grandparents) led to a reduction in incidence among infants (Crowcroft et al. 2003).

The protection offered by routine vaccines is, however, not total even among the immunized. Certain segments of the population do not get protected for various reasons including age, genetic, nutritional status, and obesity (Wiedemann et al. 2016). Even with diseases that are thought to be eradicated through 100% vaccination, there can be outbreaks if there is a "chink in the armor." The outbreaks in

developed nations like the USA, the Netherlands, and Korea during the last decade have been among people who received a second or even a third dose of vaccination (https://www.nvic.org/vaccines-and-diseases/measles/measles-vaccine-effectiveness.aspx). The measles outbreak in the USA in 2019 has been attributed to unimmunized groups and travelers (https://www.cdc.gov/measles/cases-outbreaks.html). These outbreaks indicate that protection through vaccination may not be 100% because of the complex host-pathogen-environment dynamics. Considering the huge number of pathogens and associated diseases, it is practically impossible for vaccination alone to be the mode of disease prevention for an individual. It may be worthwhile exploring ways to enhance innate immunity, including through balanced nutrition and other interventions.

### 3.4.2 Microbiota and Gut Health

Billions of microorganisms reside in the human gut (called the gut microbiota); some are beneficial while others are commensals or pathogenic. An increasing body of scientific literature indicates that the gut microflora plays a major role in immunity, coordination of the nervous system, gastrointestinal functions, and homeostasis (commonly called the gut health) (Table 2). Microbiota in the large intestine prevents microbial overgrowth and infection through the formation of an ecological barrier for colonization and by inducing the host's immune system to produce immunoglobulins (IgA) and antimicrobial proteins (Shi et al. 2017). Intestinal bacteria have been shown to influence central physiological functions such as the development of lymphatic tissue, the induction of mucosal tolerance, angiogenesis, fat storage, and neuro-endocrine functions (Singer-Englar et al. 2019) (Table 2).

Some of the ways in which the gut microbiota modulates immunity are known. The GALT (gut-associated lymphoid tissue) or the mucosal immune system is a specialized secondary lymphoid organ that evolves and develops following bacterial colonization of the intestinal tract. The gut microbiome facilitates the maturation of the GALT, which "learns" to differentiate between commensal and pathogenic

**Table 2** Microbiome associated with different parts of human gut

| Organ | No. of organisms | Name of common organisms | References |
|---|---|---|---|
| Stomach | $10^2$ | *Lactobacillus*, *Candida*, *Streptococcus*, *Helicobacter pylori*, *Peptostreptococcus* | Arumugam et al. (2011) |
| Duodenum | $10^2$ | *Streptococcus*, *Lactobacillus* | Faith et al. (2013) |
| Jejunum | $10^2$ | *Streptococcus*, *Lactobacillus* | Ding and Schloss (2014) |
| Proximal ileum | $10^2$ | *Streptococcus*, *Lactobacillus* | Lozupone et al. (2012) |
| Distal ileum | $10^8$ | *Clostridium*, *Streptococcus*, *Bacteroides*, *Actinomycinae*, *Corynebacterium* | Human Microbiome Project Consortium (2012) |
| Colon | $10^{12}$ | *Bacteroides*, *Clostridium*, *Bifidobacterium*, *Enterobacteriaceae* | NIH HMP Working Group (2009) |

bacteria through toll-like receptors (TLRs) (Rooks and Garrett 2016). GALT suppresses the occurrence of an inflammatory response and promotes immunological tolerance to the microbiome's peptidoglycan. Gut microbiota induces GALT to produce immune effectors like soluble immunoglobulin (sIgA), defensins, and antimicrobial peptides (AMP) or induce local environment changes (i.e., diarrhea). The host-commensal microbiota communication triggers antimicrobial responses from the gut epithelium including the release of several antibacterial lectins, including RegIIIc, α-defensins, and angiogenins (Thaiss et al. 2016). These antibacterial effectors reduce the number of potentially pathogenic microbes and provide protection against subsequent abnormal immune responses. For instance, *Bacteroides thetaiotaomicron* triggers the production of AMPs that target other intestinal microbes. In the case of diarrhea, the host eliminates undesirable microbial communities in order to prepare niches for recolonization with more beneficial microbial populations, as a last resort to healing (Gordon et al. 2012).

A dysbiosis in the gut microbiota, due to an improper diet or antibiotics, renders the host susceptible to infections, chronic inflammation, as well as cancer. This can be resolved and gut homeostasis brought back (eubiosis) by the use of probiotics (Lazar et al. 2018).

### 3.4.3 Probiotics

Per WHO, probiotics refers to live microorganisms which, when administered in adequate amounts, confer health benefit on the host. Some of the common functions performed by probiotics include digestion of food, production of useful metabolites, destruction of pathogens, complementing the functions of the digestive enzymes, and maintenance of the pH of the digestive system.

Probiotic microbes control pathogens in several ways. They improve the intestinal barrier function and competitively exclude pathogens by reducing adherence to cells, co-aggregation, as well as production of organic acids, short-chain fatty acids, hydrogen peroxide, nitric oxide, and bacteriocins which antagonize harmful microorganisms (Wilkins and Sequoia 2017). Some of the beneficial effects of probiotics are listed in Table 3.

Further, the antimicrobial substances produced by probiotics can also disrupt biofilm. In addition to reducing the amount of antibiotic, probiotics also improve the reach of the antibiotic to its target, thereby reducing the dose required (Mantegazza et al. 2018). Thus, employing probiotics is a promising alternative to treat infections with antibiotics and tackle AMR.

## 4 Conclusions

Antimicrobial-resistant organisms occur naturally in the environment. While the susceptible organisms get killed by antimicrobials, the resistant ones are preferentially selected under certain conditions like drug overuse. AMR is also linked to several direct and indirect drivers including pollution, bad WASH and living conditions, poor immunity status, and regular use in agriculture. In this context,

**Table 3** Probiotics that can be used as an alternative to antimicrobial treatment

| S. no. | Condition | Probiotic | Effect | References |
|---|---|---|---|---|
| 1 | *Clostridium difficile* infection | *Saccharomyces boulardii*, *L. plantarum*, *L. rhamnosus* GG, *L. casei* | Reduces incidence | Goldenberg et al. (2017) |
| 2 | Antibiotic-associated diarrhea | *Bacillus* spp., *Bifidobacterium* spp., *Clostridium butyricum*, *Lactobacilli* spp., *Lactococcus* spp., *Leuconostoc cremoris*, *Saccharomyces* spp., *Streptococcus* spp. | Prevention | Hayes and Vargas (2016) |
| 3 | Travelers' diarrhea | *Saccharomyces boulardii*, *S. cerevisiae*, *L. plantarum*, *L. acidophilus*, *L. fermentum*, *L. helveticus*, *L. gasseri*, *L. bulgaricus*, *B. bifidum*, *S. thermophilus*, *E. faecium* | Prophylaxis | McFarland and Goh (2019) |
| 4 | Infectious diarrhea | *S. thermophilus*, *L. casei*, *L. reuteri*, *B. lactis* | Reduces intensity and length of disease | Van Niel et al. (2002) |
| 5 | Ventilator-associated pneumonia | Lactic acid bacteria *Pediococcus pentosaceus*, *Lactococcus raffinolactis*, *L. paracasei*, *L. plantarum*, *L. acidophilus*, *L. bulgaricus*, *L. casei*, *L. plantarum*, *B. lactis*, *Streptococcus thermophilus*, *B. breve* | Reduces risk | Watkinson et al. (2007) |
| 6 | Oral health | *Lactobacillus* and *Bifidobacterium* | Reduces pathogenic bacteria | Bonifait et al. (2009 |
| 7 | Necrotizing enterocolitis | LGG, *B. breve*, *Saccharomyces* species and mixtures of *Bacteroides bifidus*, *S. thermophilus*, *Lactobacillus acidophilus*, *B. infantis* | Beneficial for premature neonates | Thomas et al. (2017) |
| 8 | Vulvovaginitis | *Lactobacillus fermentum* LF15, *L. plantarum* LP01 | Reduces recurrence | Murina et al. (2014) |
| 9 | Recurrent urinary tract infections | *E. coli* | Protection against recurrent urinary tract infection | Darouiche et al. (2011) |

perspective on "Planetary Health" forces us to recognize the interconnectedness of humans, biodiversity, and the environment. It holds human-centered, anthropogenic actions responsible for climate change and links inequity, biodiversity loss, and

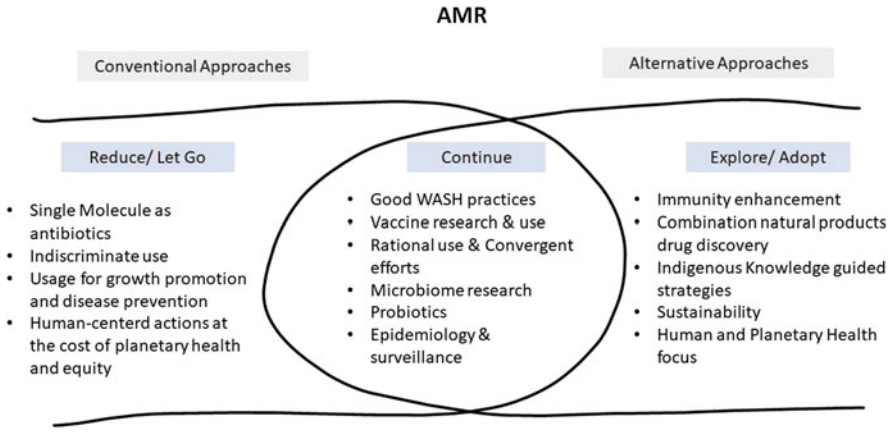


**Fig. 3** What to continue? What to reduce/drop? What to explore new?

overconsumption of the planetary resources to AMR. Resistance to antimicrobials is an *adaptive* challenge than a technical one, and strategies to tackle AMR would depend on the socio-cultural context and the environment (Hinchliffe et al. 2018). Therefore, schemes in consultation with local stakeholders would be more effective when implemented.

> The interconnected nature of people and the planet mean that solutions that benefit both the planet and human health lie within reach. (Whitmee et al. 2015)

There are certain approaches and actions that we need to let go, continue, or adopt to counter AMR (Fig. 3). The "One Health" approach needs to be promoted for a multi-sectoral and multi-disciplinary collaboration. We need to advocate for increased investments in WASH infrastructure and improved handling of waste and simultaneously educate the society for behavioral change. Strengthening the body's defense mechanisms using vaccines, gut microbiota, and probiotics can help to bypass antimicrobials and, hence, resistance. TSM like Ayurveda can provide us new perspectives and methods to boost immunity and handle infections, apart from providing new ways of thinking, of knowing nature, and of living in harmony with nature, and possible sustainable solutions against the obstinate resistant microbial population.

## References

Abreu AC, McBain AJ, Simões M (2012) Plants as sources of new antimicrobials and resistance modifying agents. Nat Prod Rep 29(9):1007–1021. https://doi.org/10.1039/c2np20035j

Adame-Gómez R, Toribio-Jimenez J, Vences-Velazquez A, Rodríguez-Bataz E, Santiago Dionisio MC, Ramirez-Peralta A (2018) Methicillin-resistant *Staphylococcus aureus* (MRSA) in artisanal cheeses in México. Int J Microbiol 2018:8760357. https://doi.org/10.1155/2018/8760357

Ahmad I, Beg AZ (2001) Antimicrobial and phytochemical studies on 45 Indian medicinal plants against multi-drug resistant human pathogens. J Ethnopharmacol 74(2):113–123

Allcock S, Young EH, Holmes M, Gurdasani D, Dougan G, Sandhu MS, Solomon L, Török ME (2017) Antimicrobial resistance in human populations: challenges and opportunities. Glob Health Epidemiol Genom 2:e4. https://doi.org/10.1017/gheg.2017.4

Andersson DI, Hughes D (2011) Persistence of antibiotic resistance in bacterial populations. FEMS Microbiol Rev 35(5):901–911. https://doi.org/10.1111/j.1574-6976.2011.00289.x

Andre FE, Booy R, Bock HL, Clemens J, Datta SK, John TJ, Lee BW, Lolekha S, Peltola H, Ruff TA, Santhosam M, Schmitt HJ (2008) Vaccination greatly reduces disease, disability, death and inequity worldwide. Bull World Health Org 86(2):140–146. https://doi.org/10.2471/blt.07.040089

Anonymous (2017) Roundtable on WASH and AMR. Building knowledge. Improving the WASH sector. SHARE report produced by UK aid, Antimicrobial Resistance Center and London School of Hygiene and Tropical Medicine. http://soapboxcollaborative.org/wp-content/uploads/2017/08/Roundtable-on-WASH and-AMR-summary-report-v4.pdf. Accessed 20 Oct 2019

Anonymous (2018) Wanted: a reward for antibiotic development. Nat Biotechnol 36(7):555. https://doi.org/10.1038/nbt.4193

Archibald L, Phillips L, Monnet D, McGowan JE Jr, Tenover F, Gaynes R (1997) Antimicrobial resistance in isolates from inpatients and outpatients in the United States: increasing importance of the intensive care unit. Clin Infect Dis 24(2):211–215

Arumugam M, Raes J, Pelletier E, Le Paslier D, Yamada T, Mende DR, Fernandes GR, Tap J, Bruls T, Batto JM, Bertalan M, Borruel N, Casellas F, Fernandez L, Gautier L, Hansen T, Hattori M, Hayashi T, Kleerebezem M, Kurokawa K, Leclerc M, Levenez F, Manichanh C, Nielsen HB, Nielsen T, Pons N, Poulain J, Qin J, Sicheritz-Ponten T, Tims S, Torrents D, Ugarte E, Zoetendal EG, Wang J, Guarner F, Pedersen O, de Vos WM, Brunak S, Doré J, MetaHIT Consortium, Antolín M, Artiguenave F, Blottiere HM, Almeida M, Brechot C, Cara C, Chervaux C, Cultrone A, Delorme C, Denariaz G, Dervyn R, Foerstner KU, Friss C, van de Guchte M, Guedon E, Haimet F, Huber W, van Hylckama-Vlieg J, Jamet A, Juste C, Kaci G, Knol J, Lakhdari O, Layec S, Le Roux K, Maguin E, Mérieux A, Melo Minardi R, M'rini C, Muller J, Oozeer R, Parkhill J, Renault P, Rescigno M, Sanchez N, Sunagawa S, Torrejon A, Turner K, Vandemeulebrouck G, Varela E, Winogradsky Y, Zeller G, Weissenbach J, Ehrlich SD, Bork P (2011) Enterotypes of the human gut microbiome. Nature 473(7346):174–180. https://doi.org/10.1038/nature09944

Baker KF, Isaacs JD (2018) Novel therapies for immune-mediated inflammatory diseases: what can we learn from their use in rheumatoid arthritis, spondyloarthritis, systemic lupus erythematosus, psoriasis, Crohn's disease and ulcerative colitis? Ann Rheum Dis 77(2):175–187. https://doi.org/10.1136/annrheumdis-2017-211555

Balasubramani SP, Venkatasubramanian P, Kukkupuni SK, Patwardhan B (2011) Plant-based Rasayana drugs from Ayurveda. Chin J Integr Med 17(2):88–94. https://doi.org/10.1007/s11655-011-0659-5

Balasubramani SP, Mohan J, Chatterjee A, Patnaik E, Kukkupuni SK, Nongthomba U, Venkatasubramanian P (2014) Pomegranate juice enhances healthy lifespan in *Drosophila melanogaster*: an exploratory study. Front Public Health 2:245. https://doi.org/10.3389/fpubh.2014.00245

Baptiste KE, Williams K, Willams NJ, Wattret A, Clegg PD, Dawson S, Corkill JE, O'Neill T, Hart CA (2005) Methicillin-resistant staphylococci in companion animals. Emerg Infect Dis 11(12):1942–1944

Beresford MJ (2010) Medical reductionism: lessons from the great philosophers. QJM 103(9):721–724. https://doi.org/10.1093/qjmed/hcq057

Bhullar K, Waglechner N, Pawlowski A, Koteva K, Banks ED, Johnston MD, Barton HA, Wright GD (2012) Antibiotic resistance is prevalent in an isolated cave microbiome. PLoS One 7(4):e34953. https://doi.org/10.1371/journal.pone.0034953

Bonifait L, Chandad F, Grenier D (2009) Probiotics for oral health: myth or reality? J Can Dent Assoc 75(8):585–590

Chamosa LS, Álvarez VE, Nardelli M, Quiroga MP, Cassini MH, Centrón D (2017) Lateral antimicrobial resistance genetic transfer is active in the open environment. Sci Rep 7(1):513. https://doi.org/10.1038/s41598-017-00600-2

Chandran U, Patwardhan B (2017) Network ethnopharmacological evaluation of the immunomodulatory activity of *Withania somnifera*. J Ethnopharmacol 197:250–256. https://doi.org/10.1016/j.jep.2016.07.080

Chandran U, Mehendale N, Tillu G, Patwardhan B (2015) Network pharmacology of ayurveda formulation triphala with special reference to anti-cancer property. Comb Chem High Throughput Screen 18(9):846–854

Cheesman MJ, Ilanko A, Blonk B, Cock IE (2017) Developing new antimicrobial therapies: are synergistic combinations of plant extracts/compounds with conventional antibiotics the solution? Pharmacogn Rev 11(22):57–72. https://doi.org/10.4103/phrev.phrev_21_17

Crowcroft NS, Booy R, Harrison T, Spicer L, Britto J, Mok Q, Heath P, Murdoch I, Zambon M, George R, Miller E (2003) Severe and unrecognised: pertussis in UK infants. Arch Dis Child 88 (9):802–806. https://doi.org/10.1136/adc.88.9.802

Darouiche RO, Green BG, Donovan WH, Chen D, Schwartz M, Merritt J, Mendez M, Hull RA (2011) Multicenter randomized controlled trial of bacterial interference for prevention of urinary tract infection in patients with neurogenic bladder. Urology 78(2):341–346. https://doi.org/10.1016/j.urology.2011.03.062

Davies J (2006) Where have all the antibiotics gone? Can J Infect Dis Med Microbiol 17 (5):287–290. https://doi.org/10.1155/2006/707296

Ding T, Schloss PD (2014) Dynamics and associations of microbial community types across the human body. Nature 509(7500):357–360. https://doi.org/10.1038/nature13178

Europen Commission (2015) Antimicrobials in agriculture and the environment: reducing unnecessary use and waste. The review on antimicrobial resistance. https://ec.europa.eu/health/amr/sites/amr/files/amr_studies_2015_am-in-agri-and env.pdf. Accessed 20 Oct 2019

Fadli M, Saad A, Sayadi S, Chevalier J, Mezrioui NE, Pagès JM, Hassani L (2012) Antibacterial activity of *Thymus maroccanus* and *Thymus broussonetii* essential oils against nosocomial infection—bacteria and their synergistic potential with antibiotics. Phytomedicine 19 (5):464–471. https://doi.org/10.1016/j.phymed.2011.12.003

Fairhurst RM, Nayyar GML, Breman JG, Hallett R, Vennerstrom JL, Duong S, Ringwald P, Wellems TE, Plowe CV, Dondorp AM (2012) Artemisinin-resistant malaria: research challenges, opportunities, and public health implications. Am J Trop Med Hyg 87 (2):231–241. https://doi.org/10.4269/ajtmh.2012.12-0025

Faith JJ, Guruge JL, Charbonneau M, Subramanian S, Seedorf H, Goodman AL, Clemente JC, Knight R, Heath AC, Leibel RL, Rosenbaum M, Gordon JI (2013) The long-term stability of the human gut microbiota. Science 341(6141):1237439. https://doi.org/10.1126/science.1237439

Fang FC, Casadevall A (2011) Reductionistic and holistic science. Infect Immun 79(4):1401–1404. https://doi.org/10.1128/IAI.01343-10

FAO (2016) Drivers, dynamics and epidemiology of antimicrobial resistance in animal production. http://www.fao.org/3/a-i6209e.pdf

Fiore AE (2004) Hepatitis A transmitted by food. Clin Infect Dis 38(5):705–715. https://doi.org/10.1086/381671

Frost I, Van Boeckel TP, Pires J, Craig J, Laxminarayan R (2019) Global geographic trends in antimicrobial resistance: the role of international travel. J Travel Med 26 pii: taz036. https://doi.org/10.1093/jtm/taz036.

Gautam M, Diwanay SS, Gairola S, Shinde YS, Jadhav SS, Patwardhan BK (2004) Immune response modulation to DPT vaccine by aqueous extract of *Withania somnifera* in experimental system. Int Immunopharmacol 4(6):841–849

Gellin B, Modlin JF, Whitney CG, Schaffner W, Butler JC (2001) Rethinking recommendations for use of pneumococcal vaccines in adults. Clin Infect Dis 33(5):662–675. https://doi.org/10.1086/322676

Gjini E, Brito PH (2016) Integrating antimicrobial therapy with host immunity to fight drug-resistant infections: classical vs. adaptive treatment. PLoS Comput Biol 12(4):e1004857. https://doi.org/10.1371/journal.pcbi.1004857

Goldenberg JZ, Yap C, Lytvyn L, Lo CK, Beardsley J, Mertz D, Johnston BC (2017) Probiotics for the prevention of *Clostridium difficile*-associated diarrhea in adults and children. Cochrane Database Syst Rev 12:CD006095. https://doi.org/10.1002/14651858.CD006095

Gordon JI, Dewey KG, Mills DA, Medzhitov RM (2012) The human gut microbiota and undernutrition. Sci Transl Med 4(137):137ps12. https://doi.org/10.1126/scitranslmed.3004347

Gupta PD, Birdi TJ (2017) Development of botanicals to combat antibiotic resistance. J Ayurveda Integr Med 8(4):266–275. https://doi.org/10.1016/j.jaim.2017.05.004

Hackett CJ (2003) Innate immune activation as a broad-spectrum biodefense strategy: prospects and research challenges. J Allergy Clin Immunol 112(4):686–694

Hajela N, Ramakrishna BS, Nair GB, Abraham P, Gopalan S, Ganguly NK (2015) Gut microbiome, gut function, and probiotics: Implications for health. Indian J Gastroenterol 34(2):93–107. https://doi.org/10.1007/s12664-015-0547-6

Handel A, Margolis E, Levin BR (2009) Exploring the role of the immune response in preventing antibiotic resistance. J Theor Biol 256(4):655–662. https://doi.org/10.1016/j.jtbi.2008.10.025

Hayes SR, Vargas AJ (2016) Probiotics for the prevention of pediatric antibiotic-associated diarrhea. Explore (NY) 12(6):463–466. https://doi.org/10.1016/j.explore.2016.08.015

Hernandez-Santiago V, Davey PG, Nathwani D, Marwick CA, Guthrie B (2019) Changes in resistance among coliform bacteraemia associated with a primary care antimicrobial stewardship intervention: a population-based interrupted time series study. PLoS Med 16(6):e1002825. https://doi.org/10.1371/journal.pmed.1002825

Hinchliffe S, Butcher A, Rahman MM (2018) The AMR problem: demanding economies, biological margins, and co-producing alternative strategies. Palgrave Commun 4:Article number: 142

Hoffman AJ (2016) The limits of intellectual reason in our understanding of the natural world. www.theconversation.com

Holmes AH, Moore LS, Sundsfjord A, Steinbakk M, Regmi S, Karkey A, Guerin PJ, Piddock LJ (2016) Understanding the mechanisms and drivers of antimicrobial resistance. Lancet 387 (10014):176–187. https://doi.org/10.1016/S0140-6736(15)00473-0

Horton R, Beaglehole R, Bonita R, Raeburn J, McKee M, Wall S (2014) From public to "Planetary Health": a manifesto. Lancet 383(9920):847. https://doi.org/10.1016/S0140-6736(14)60409-8

Human Microbiome Project Consortium (2012) Structure, function and diversity of the healthy human microbiome. Nature 486(7402):207–214. https://doi.org/10.1038/nature11234

John TJ, Samuel R (2000) Herd immunity and herd effect: new insights and definitions. Eur J Epidemiol 16(7):601–606

Johnson AP, Woodford N (2013) Global spread of antibiotic resistance: the example of New Delhi metallo-β-lactamase (NDM)-mediated carbapenem resistance. J Med Microbiol 62 (Pt 4):499–513. https://doi.org/10.1099/jmm.0.052555-0

Jyothy KB, Sheshagiri S, Patel KS, Rajagopala S (2014) A critical appraisal on Swarnaprashana in children. Ayu 35(4):361–365. https://doi.org/10.4103/0974-8520.158978

Klein EY, Van Boeckel TP, Martinez EM, Pant S, Gandra S, Levin SA, Goossens H, Laxminarayan R (2018) Global increase and geographic convergence in antibiotic consumption between 2000 and 2015. Proc Natl Acad Sci USA 115(15):E3463–E3470. https://doi.org/10.1073/pnas.1717295115

Knöppel A, Näsvall J, Andersson DI (2017) Evolution of antibiotic resistance without antibiotic exposure. Antimicrob Agents Chemother 61(11):e01495–e01417. https://doi.org/10.1128/AAC.01495-17

Kuok CF, Hoi SO, Hoi CF, Chan CH, Fong IH, Ngok CK, Meng LR, Fong P (2017) Synergistic antibacterial effects of herbal extracts and antibiotics on methicillin-resistant *Staphylococcus aureus*: a computational and experimental study. Exp Biol Med (Maywood) 242(7):731–743. https://doi.org/10.1177/1535370216689828

Landecker H (2016) Antibiotic resistance and the biology of history. Body Soc 22(4):19–52. https://doi.org/10.1177/1357034X14561341

Lazar V, Ditu LM, Pircalabioru GG, Gheorghe I, Curutiu C, Holban AM, Picu A, Petcu L, Chifiriuc MC (2018) Aspects of gut microbiota and immune system interactions in infectious diseases, immunopathology, and cancer. Front Immunol 9:1830. https://doi.org/10.3389/fimmu.2018.01830

Livermore DM (2003) Bacterial resistance: origins, epidemiology, and impact. Clin Infect Dis 36 (Suppl 1):S11–S23

Lowder BV, Guinane CM, Ben Zakour NL, Weinert LA, Conway-Morris A, Cartwright RA, Simpson AJ, Rambaut A, Nübel U, Fitzgerald JR (2009) Recent human-to-poultry host jump, adaptation, and pandemic spread of *Staphylococcus aureus*. Proc Natl Acad Sci USA 106 (46):19545–19550. https://doi.org/10.1073/pnas.0909285106

Lozupone CA, Stombaugh JI, Gordon JI, Jansson JK, Knight R (2012) Diversity, stability and resilience of the human gut microbiota. Nature 489(7415):220–230. https://doi.org/10.1038/nature11550

MacFadden DR, McGough SF, Fisman D, Santillana M, Brownstein JS (2018) Antibiotic resistance increases with local temperature. Nat Clim Chang 8(6):510–514. https://doi.org/10.1038/s41558-018-0161-6

Macintyre A, Wilson-Jones M, Velleman Y (2017) Prevention first: tackling AMR through water, sanitation and hygiene. http://resistancecontrol.info/2017/prevention-first-tackling-amr-through-water sanitation-and-hygiene/. Accessed 20 Oct 2019

Mantegazza C, Molinari P, D'Auria E, Sonnino M, Morelli L, Zuccotti GV (2018) Probiotics and antibiotic-associated diarrhea in children: a review and new evidence on *Lactobacillus rhamnosus* GG during and after antibiotic treatment. Pharmacol Res 128:63–72. https://doi.org/10.1016/j.phrs.2017.08.001

Martinez JL (2009) Environmental pollution by antibiotics and by antibiotic resistance determinants. Environ Pollut 157(11):2893–2902. https://doi.org/10.1016/j.envpol.2009.05.051

Matsunaga N, Hayakawa K (2018) Estimating the impact of antimicrobial resistance. Lancet Glob Health 6(9):e934–e935. https://doi.org/10.1016/S2214-109X(18)30325-5

McFarland LV, Goh S (2019) Are probiotics and prebiotics effective in the prevention of travellers' diarrhea: a systematic review and meta-analysis. Travel Med Infect Dis 27:11–19. https://doi.org/10.1016/j.tmaid.2018.09.007

Munita JM, Arias CA (2016) Mechanisms of antibiotic resistance. Microbiol Spectrum 4(2). https://doi.org/10.1128/microbiolspec.VMBF-0016-2015

Murina F, Graziottin A, Vicariotto F, De Seta F (2014) Can Lactobacillus fermentum LF10 and Lactobacillus acidophilus LA02 in a slow-release vaginal product be useful for prevention of recurrent vulvovaginal candidiasis? A clinical study. J Clin Gastroenterol 48(Suppl 1):S102–S105. https://doi.org/10.1097/MCG.0000000000000225

Nesme J, Cécillon S, Delmont TO, Monier JM, Vogel TM, Simonet P (2014) Large-scale metagenomic-based study of antibiotic resistance in the environment. Curr Biol 24 (10):1096–1100. https://doi.org/10.1016/j.cub.2014.03.036

NIH HMP Working Group, Peterson J, Garges S et al (2009) The NIH human microbiome project. Genome Res 19(12):2317–2323. https://doi.org/10.1101/gr.096651.109

O'Neill J (2014) Antimicrobial resistance: tackling a crisis for the health and wealth of nations. Review on antimicrobial resistance. http://amr-review.org/Publications

OIE (2016) The OIE strategy on antimicrobial resistance (AMR) and the prudent use of antimicrobials. Protecting animal health and welfare by supporting global efforts to combat antimicrobial resistance. www.oie.int/antimicrobial-resistance. Accessed 20 Oct 2019

Payyappallimana U, Venkatasubramanian P (2016) Exploring ayurvedic knowledge on food and health for providing innovative solutions to contemporary healthcare. Front Public Health 4:57. https://doi.org/10.3389/fpubh.2016.00057

Peterson CT, Denniston K, Chopra D (2017) Therapeutic uses of triphala in ayurvedic medicine. J Altern Complement Med 23(8):607–614. https://doi.org/10.1089/acm.2017.0083

Pew Charitable Trust Report (2016) A scientific roadmap for antibiotic discovery. https://www.pewtrusts.org/~/media/assets/2016/05/ascientificroadmapforantibioticdiscovery.pdf
Porcelli SA (2017) Effector mechanisms in autoimmunity and inflammation. In: Firestein GS, Budd RC, Gabriel SE, McInnes IB, O'Dell JR (eds) Kelley and Firestein's textbook of rheumatology. Elsevier, Philadelphia, pp 274–287
Qiu J (2007) Traditional medicine: a culture in the balance. Nature 448(7150):126–128
Quave CL (2016) Antibiotics from nature: traditional medicine as a source of new solutions for combating antimicrobial resistance. www.resistancecontrol.info
Raman K, Chandra N (2010) Systems biology. Resonance 15:131. https://doi.org/10.1007/s12045-010-0015-7
Rasoanaivo P, Wright CW, Willcox ML, Gilbert B (2011) Whole plant extracts versus single compounds for the treatment of malaria: synergy and positive interactions. Malar J 10 Suppl 1: S4. https://doi.org/10.1186/1475-2875-10-S1-S4
Relman DA, Lipsitch M (2018) Microbiome as a tool and a target in the effort to address antimicrobial resistance. Proc Natl Acad Sci USA 115(51):12902–12910. https://doi.org/10.1073/pnas.1717163115
Robinson TP, Bu DP, Carrique-Mas J, Fèvre EM, Gilbert M, Grace D, Hay SI, Jiwakanon J, Kakkar M, Kariuki S, Laxminarayan R, Lubroth J, Magnusson U, ThiNgoc P, Van Boeckel TP, Woolhouse ME (2016) Antibiotic resistance is the quintessential "One Health" issue. Trans R Soc Trop Med Hyg 110(7):377–380. https://doi.org/10.1093/trstmh/trw048
Ronquillo MG, Hernandez JCA (2017) Antibiotic and synthetic growth promoters in animal diets: review of impact and analytical methods. Food Control 72:255–267. https://doi.org/10.1016/j.foodcont.2016.03.001
Rooks MG, Garrett WS (2016) Gut microbiota, metabolites and host immunity. Nat Rev Immunol 16(6):341–352. https://doi.org/10.1038/nri.2016.42
Roose-Amsaleg C, Laverman AM (2016) Do antibiotics have environmental side-effects? Impact of synthetic antibiotics on biogeochemical processes. Environ Sci Pollut Res Int 23 (5):4000–4012. https://doi.org/10.1007/s11356-015-4943-3
Rosenblatt-Farrell N (2009) The landscape of antibiotic resistance. Environ Health Perspect 117(6): A244–A250. https://doi.org/10.1289/ehp.117-a244
Russell AD (2003) Biocide use and antibiotic resistance: the relevance of laboratory findings to clinical and environmental situations. Lancet Infect Dis 3(12):794–803
Shah A, Krishnamurthy R (2013) Swine flu and its herbal remedies. Int J Eng Sci 2(5):68–78
Sharma H, Chandola HM, Singh G, Basisht G (2007a) Utilization of Ayurveda in health care: an approach for prevention, health promotion, and treatment of disease. Part 2—ayurveda in primary health care. J Altern Complement Med 13(10):1135–1150. https://doi.org/10.1089/acm.2007.7017-B
Sharma H, Chandola HM, Singh G, Basisht G (2007b) Utilization of Ayurveda in health care: an approach for prevention, health promotion, and treatment of disease. Part 1—Ayurveda, the science of life. J Altern Complement Med 13(9):1011–1019
Sharma R, Martins N, Kuca K, Chaudhary A, Kabra A, Rao MM, Prajapati PK (2019) Chyawanprash: a traditional Indian bioactive health supplement. Biomolecules 9(5):E161. https://doi.org/10.3390/biom9050161
Shi N, Li N, Duan X, Niu H (2017) Interaction between the gut microbiome and mucosal immune system. Mil Med Res 4:14. https://doi.org/10.1186/s40779-017-0122-9
Shriram V, Khare T, Bhagwat R, Shukla R, Kumar V (2018) Inhibiting bacterial drug efflux pumps via phyto-therapeutics to combat threatening antimicrobial resistance. Front Microbiol 9:2990. https://doi.org/10.3389/fmicb.2018.02990
Singer-Englar T, Barlow G, Mathur R (2019) Obesity, diabetes, and the gut microbiome: an updated review. Expert Rev Gastroenterol Hepatol 13(1):3–15. https://doi.org/10.1080/17474124.2019.1543023
Stefanović OD (2017) Synergistic activity of antibiotics and bioactive plant extracts: a study against gram-positive and gram-negative bacteria, bacterial pathogenesis and antibacterial control. Sahra, IntechOpen. https://doi.org/10.5772/intechopen.72026. https://www.intechopen.com/

books/bacterial-pathogenesis-and-antibacterial-control/synergistic-activity-of-antibiotics-and-bioactive-plant-extracts-a-study-against-gram-positive-and-g

Thaiss CA, Zmora N, Levy M, Elinav E (2016) The microbiome and innate immunity. Nature 535 (7610):65–74. https://doi.org/10.1038/nature18847

Thakur S, Gray GC (2019) The mandate for a global "One Health" approach to antimicrobial resistance surveillance. Am J Trop Med Hyg 100(2):227–228. https://doi.org/10.4269/ajtmh.18-0973

Thatte U, Chiplunkar S, Bhalerao S, Kulkarni A, Ghungralkar R, Panchal F, Vetale S, Teli P, Kumbhar D, Munshi R (2015) Immunological and metabolic responses to a therapeutic course of Basti in obesity. Indian J Med Res 142(1):53–62. https://doi.org/10.4103/0971-5916.162099

Thomas JP, Raine T, Reddy S, Belteki G (2017) Probiotics for the prevention of necrotising enterocolitis in very low-birth-weight infants: a meta-analysis and systematic review. Acta Paediatr 106(11):1729–1741. https://doi.org/10.1111/apa.13902

TKDL (2012). http://www.tkdl.res.in/tkdl/langdefault/common/Home.asp?GL=Eng. Accessed 12 Aug 2019

Van Niel CW, Feudtner C, Garrison MM, Christakis DA (2002) Lactobacillus therapy for acute infectious diarrhea in children: a meta-analysis. Pediatrics 109(4):678–684

Ved DK, Goraya GS (2007) Demand and supply of medicinal plants in India. NMPB, New Delhi

Watkinson PJ, Barber VS, Dark P, Young JD (2007) The use of pre- pro- and synbiotics in adult intensive care unit patients: systematic review. Clin Nutr 26(2):182–192

Whitmee S, Haines A, Beyrer C, Boltz F, Capon AG, de Souza Dias BF, Ezeh A, Frumkin H, Gong P, Head P, Horton R, Mace GM, Marten R, Myers SS, Nishtar S, Osofsky SA, Pattanayak SK, Pongsiri MJ, Romanelli C, Soucat A, Vega J, Yach D (2015) Safeguarding human health in the Anthropocene epoch: report of The Rockefeller Foundation-Lancet Commission on "Planetary Health.". Lancet 386(10007):1973–2028. https://doi.org/10.1016/S0140-6736(15)60901-1

WHO (2014) Antimicrobial resistance: global report on surveillance. https://apps.who.int/iris/bitstream/handle/10665/112642/9789241564748_eng.pdf;jsessionid=D00C5045EE850949D5C21F07A13E2111?sequence=1. Accessed 12 Aug 2019

WHO (2015) Global action plan on antimicrobial resistance. https://www.who.int/antimicrobial-resistance/publications/global-action-plan/en/. Accessed 20 Oct 2019

WHO (2018a) Antimicrobial resistance: invest in innovation and research, and boost R&D and access IACG discussion paper. https://www.who.int/antimicrobial-resistance/interagencycoordinationgroup/IACG_AMR_Invest_innovation_research_boost_RD_and_access_110618.pdf

WHO (2018b) The detection and reporting of colistin resistance. WHO reference number/Licence: CC BY-NC-SA 3.0 IGO, Geneva

WHO (2018c) Antimicrobial resistance. https://www.who.int/en/news-room/fact-sheets/detail/antimicrobial-resistance. Accessed 12 Aug 2019

Wiedermann U, Garner-Spitzer E, Wagner A (2016) Primary vaccine failure to routine vaccines: why and what to do? Hum Vaccin Immunother 12(1):239–243. https://doi.org/10.1080/21645515.2015.1093263

Wilkins T, Sequoia J (2017) Probiotics for gastrointestinal conditions: a summary of the evidence. Am Fam Physician 96(3):170–178

Woolhouse M, Ward M, van Bunnik B, Farrar J (2015) Antimicrobial resistance in humans, livestock and the wider environment. Philos Trans R Soc Lond B Biol Sci 370 (1670):20140083. https://doi.org/10.1098/rstb.2014.0083

Wyres KL, Holt KE (2018) *Klebsiella pneumoniae* as a key trafficker of drug resistance genes from environmental to clinically important bacteria. Curr Opin Microbiol 45:131–139. https://doi.org/10.1016/j.mib.2018.04.004

Yengkhom O, Shalini KS, Subramani PA, Michael RD (2019) Stimulation of non-specific immunity, gene expression, and disease resistance in Nile Tilapia, *Oreochromis niloticus* (Linnaeus, 1758), by the methanolic extract of the marine macroalga, *Caulerpa scalpelliformis*. Veterinary World 12(2):271–276. https://doi.org/10.14202/vetworld.2019.271-276

# Use of Bacterial Cell Wall Recycle Inhibitors to Combat Antimicrobial Resistance

Ramya Raghavan and Jharna Mandal

## 1 Introduction

Way back in the 1940s, the antibiotic penicillin was discovered by Alexander Fleming where he observed that a bread mold inhibited the growth of the bacteria due to the synthesis of a specific antibacterial substance (Fleming 2001). Later the substance was purified and since then antibiotics came into clinical practice. From that historical moment, there were lots of antibiotics discovered, each having a different mechanism of action. The bugs that were killed due to antibiotics began to become smarter by manifesting resistance to the antibacterial agents (Ventola 2015; Michael et al. 2014). The various antibiotics worked either via destruction of cell wall, inhibition of protein synthesis, or inhibition of bacterial DNA synthesis. The bugs became superbugs by becoming resistant to these agents through enzymatic destruction of the antibiotic, modifications of the antimicrobial target (decreasing the affinity for the drug), a decrease in the drug uptake, activation of efflux mechanisms to extrude the harmful molecule, or global changes in important metabolic pathways via modulation of regulatory networks (Alekshun and Levy 2007; Aminov 2009; Davies and Davies 2010; Michael et al. 2014; Munita and Arias 2016). Among these various antibiotics, the most important ones are the bactericidal group of agents that target the cell wall (Fisher et al. 2005).

Bacteria adapt in a specific nature for the synthesis of their cell wall. The cell wall synthesis takes place at different layers with the help of several enzymes. Similarly the products are recycled using several enzymes for their own cell wall (Doyle et al. 1988; Lovering et al. 2012). During recycling, the peptidoglycan is split at several stages in the periplasm, inner membrane, and cytoplasm (Barreteau et al. 2008; Vollmer et al. 2008; Johnson et al. 2013). There are various enzymes involved in recycling at each of these levels (Koch 2003). These enzymes have a key role in

R. Raghavan · J. Mandal (✉)
Department of Microbiology, Jawaharlal Institute of Postgraduate Medical Education and Research (JIPMER), Puducherry, India



S. Thomas (ed.), *Antimicrobial Resistance*,
https://doi.org/10.1007/978-981-15-3658-8_9

mediating resistance to various antibiotics (Jacobs et al. 1997; Vollmer et al. 2008; Bugg et al. 2011). Now that they contribute to resistance, these have been targeted by small molecule inhibitors thereby potentiating the action of cell wall antibiotics. Since then the various recycling enzymes that contribute to resistance have been identified and targeted (Park and Uehara 2008). They are at various levels of clinical trials. The unique structure of the bacterial cell wall is complemented by the few small molecule structures to recognize both the polymer and the enzymes involved in its maintenance (Yamaguchi et al. 2012; Dik et al. 2018). As the number of life-threatening infections due to multiple organisms and the resultant usage of antibiotics are on the rise, there is an increase in resistance and hence the need for newer therapeutic strategies. For cell wall-targeting antibiotics, the emerging connection between cell wall recycling and the expression of antibacterial resistance enzymes represents a promising opportunity. Bacteria succumb to an antibiotic only after the myriad pathways for the detection and response to the presence of the antibiotic are compromised. Especially for the beta-lactam antibiotics – now and for the foreseeable, a mainstay of antibacterial chemotherapy – understanding the relationship between cell wall recycling and resistance has great potential for the preservation of their value as antibiotics (Mark et al. 2011; Yamaguchi et al. 2012).

## 2 Cell Wall Structure

The bacterial cell wall consists of a mesh-like network that protects the bacteria from osmotic stress due to a high intracellular pressure. It is made up of peptidoglycan or murein polymer comprising a peptide part and glycan part. The glycan portion is made of alternating units of *N*-acetyl glucosamine (GluNAC or NAG) and *N*-acetylmuramic acid (MurNAC or NAM) and the peptide chain attached to lactyl side chains of *N*-acetylmuramic acid. The glycan side chains are interconnected by the enzyme transglycosylase, and the peptide chains are cross-linked by a transpeptidase enzyme, also known as the penicillin-binding protein. The peptide chain is composed of –L-alanine–γ-D-glutamic acid–meso-1, 6, diaminopimelate–D-alanine–D-alanine in Gram-negative bacteria and –L-alanine–γ-D-glutamic acid–L-lysine–D-alanine–D-alanine in most of the Gram-positive bacteria (Jacobs et al. 1997).

### 2.1 Steps Involved in Cell Wall Biosynthesis in the Cytoplasm

Fructose-6-phosphate is converted to uridine diphosphate (UDP)-GluNAC with the help of the sequential enzymes GluS, GluM, and GluU. UDP-GluNAC is converted to UDP-MurNAC pentapeptide with the help of the enzyme MurA enolpyruvyl transferase, MurB reductase, and MurC-MurF ligase. On the inner cytoplasmic membrane, MraY translocase catalyzes the conversion of UDP-MurNAC pentapeptide with undecaprenyl pyrophosphate to generate lipid I that is coupled with GluNAC by the MurG transferase to yield lipid II (Mengin-Lecreulx et al. 1991;

Bouhss et al. 2004; Scheffers and Pinho 2005). Lipid II flippase translocates the lipid II into the periplasmic space with the help of several enzymes that differ in different organisms (Pomorski and Menon 2006; Ruiz 2015) (Fig. 1).

Lipid II contributes to the disaccharide chain for the growth of the glycan strand that is catalyzed by the high molecular mass penicillin-binding protein (HMM PBP) transglycosylase domain. Transpeptidase domain of HMM PBP helps in the cross-linking of the peptide side chains. Transpeptidase reaction causes displacement of terminal D-ala residue of one pentapeptide side chain by the terminal amine residue of the L-Lys (or m-DAP) residue of a neighboring strand thus providing a highly cross-linked meshwork of the bacterial cell wall. The cross-links are composed of penta-glycine bridges in Gram positives and covalent bonding in the case of Gram negatives (Johnson et al. 2013).

## 2.2 Differences in Cell Wall Structure Between Gram Positive and Gram Negative

The major difference between Gram positive and Gram negative is the absence of outer membrane in Gram positives and the peptidoglycan is of few nanometers in Gram negatives, whereas in Gram positives it is of 20–80 nm in thickness (Weidel and Pelzer 1964). In Gram-negative bacteria, there is a wide periplasmic space where the product of cell wall recycling accumulates. In Gram-positive bacteria, periplasmic space refers to the region between inner cell wall and outer surface of plasma membrane surrounded by high-density outer cell wall zone. The mono-layered peptidoglycan in Gram negatives is hydrolyzed synchronously with the new cell wall material by multi-enzyme synthesis complex (Meroueh et al. 2006).

In Gram positives which were initially thought to be non-synchronous in synthesis and turnover, recent studies suggest the presence of WalKR system, a two-component system that regulates the cell wall synthesis. This system regulates several enzymes capable of targeting the cell wall, namely, autolysins, endopeptidase, muraminidase, peptidoglycan deacylase, autolysin inhibitor/modulator, etc., thus having a balance of the cell wall lysis. The peptidoglycan background in Gram positive and Gram negative is almost similar except for the peptide composition, the length of the peptide cross-links, and the length of the peptidoglycan chains. The sugar background could be modified by *O*-acetylation, *N*-deacetylation, and the attachment of surface polymers. Secondary polymers such as teichoic acid are attached to the murein of the Gram-positive bacteria which promotes further modification to the cell wall structure (Silhavy et al. 2010).

## 2.3 Implications in the Cell Wall Turnover due to Differences in the Cell Wall of Gram-Positive and Gram-Negative Bacteria

Due to several modifications in the peptidoglycan of the Gram positives, they are resistant to hydrolases like lysozyme that affects the cell wall turnover and lysis

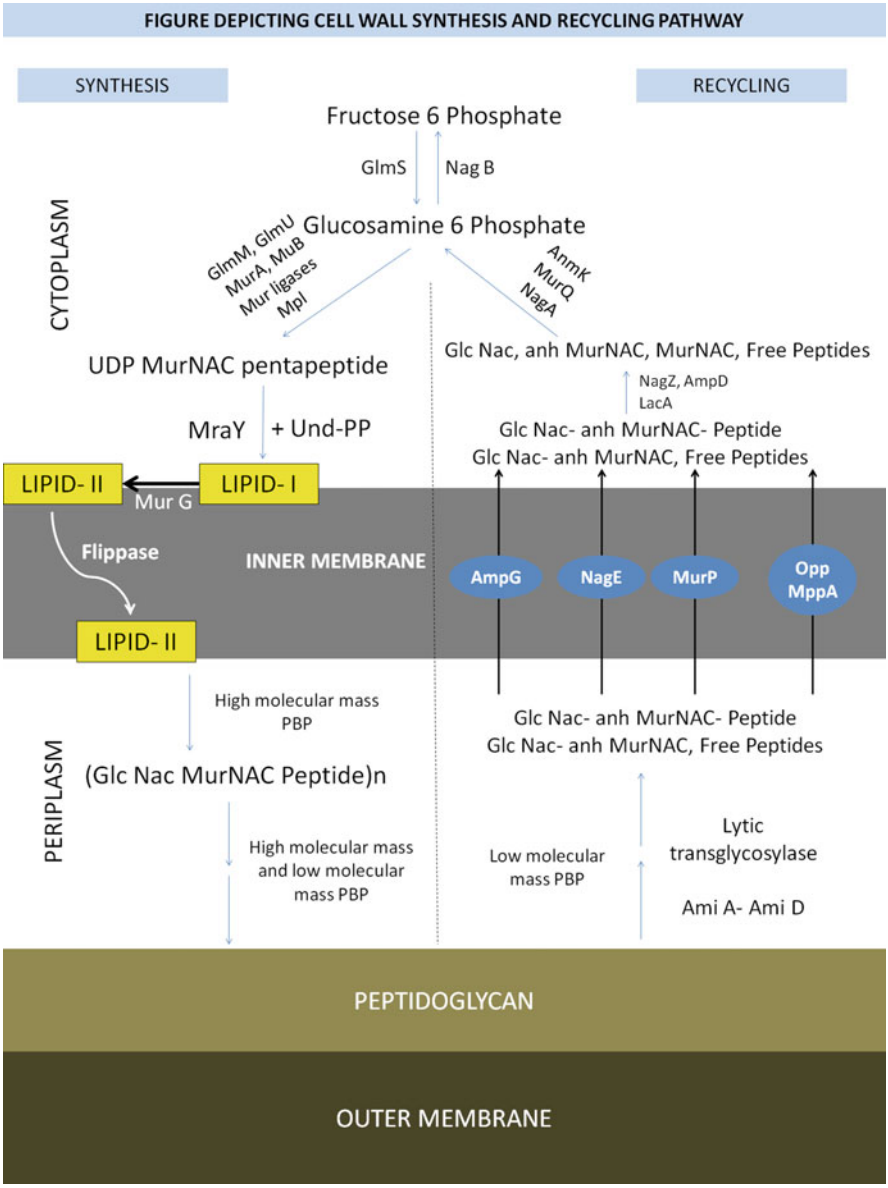


**Fig. 1** Peptidoglycan (PG) recycling and synthesis pathway in *E. coli*. The recycling and biosynthesis pathways converge in cytoplasm. GlcN-6-P is converted back to fructose-6-phosphate (Fru-6-P) through a deaminase (NagB) or undergoes a series of modifications to form the uridine diphosphate (UDP)-MurNAc pentapeptides. Lipid I and lipid II formations are aided by MraY and MurG enzymes, respectively. Periplasmic space witnesses polymerization and cross-linkage of muropeptides with the existing PG. LMM PBPs with endopeptidase (EP) activity cleave the existing PG layer

(Doyle et al. 1988). In Gram positives, along with peptidoglycan, the other cell wall structures like teichoic acid and cell wall polysaccharide are also recycled. Thus, the Gram-positive organism cell wall turnover is different when compared to that of Gram negatives.

## 3 Cell Wall Recycling in Gram-Negative Bacteria

The cell wall recycling and the enzymes involved in the process are best studied in *Escherichia coli* and *P. aeruginosa*, and these enzymes are present in three compartments. They are the periplasm, inner membrane, and cytoplasm (Fig. 1).

### 3.1 Periplasm

There are three types of enzymes in the periplasm. They are lytic transglycosylases, low molecular mass penicillin-binding protein, and amidases (Johnson et al. 2013).

#### 3.1.1 Lytic Transglycosylases (LTs)

LTs act on the peptidoglycan to release GluNAc-1,6-anhydro-MurNAc. A total of 8 LTs were identified in *E. coli* and about 11 LTs were identified in *P. aeruginosa* (Höltje et al. 1975) (Ishino et al. 1980; Dijkstra and Keck 1996; Koraimann 2003; Scheurwater et al. 2008; Lee et al. 2013, 2017). LTs are more significantly expressed during the log phase when compared to stationary phase which is in accordance to the maximum remodeling that occurs during this phase. This may also be due to structural differences in the murein sacculus during bacterial growth and to lack of cell division during the stationary phase (Vollmer et al. 1999; Blackburn and Clarke 2001, 2002; Scheurwater et al. 2008; Suvorov et al. 2008).

#### 3.1.2 Low Molecular Mass Penicillin-Binding Proteins (LMM PBPs)

These act either as endopeptidases or carboxypeptidases. Endopeptidase activity results in the hydrolysis of cross-bridge between m-DAP and D-Ala, whereas the carboxypeptidase removes the terminal peptide during cross-linking. *E. coli* has five LMM PBPs, among which there is a bifunctional enzyme, and *P. aeruginosa* has three LMM PBPs (Sauvage et al. 2008; Dhar et al. 2017).

#### 3.1.3 Amidases

The periplasmic amidases remove the amino acid chain from the muramyl moieties as well as from the recycling products like GlcNAc-anhMurNAc peptides. *E. coli* has four amidases AmiA, AmiB, AmiC, and AmiD that function as *N*-acetylmuramyl-L-alanine amidase. AmiA, AmiB, and AmiC have a high sequence homology and help in cell separation (Van Heijenoort and Van Heijenoort 1971). AmiD cleaves at both *N*-acetylmuramyl and 1,6-anhydro-*N*-acetylmuramyl peptides and plays a critical role in generating fragments for recycling. *P. aeruginosa* harbors AmiA, AmiB, AmpDh2, and AmpDh3, wherein the latter two play a major role in

recycling. Being periplasmic in nature, AmpDh2 is anchored to the inner leaflet of the outer membrane and AmpDh3 is soluble in the periplasm. These enzymes act on the peptidoglycan as well as muropeptides of various lengths that are unique to the periplasmic space. AmpDh2 hydrolyzes at the peripheries of the sacculus, which becomes soluble fractions, and AmpDh3 hydrolyzes the stem peptides on the core of the sacculus, where the change remains associated with the polymeric fraction (Yakhnina et al. 2015; Dhar et al. 2017).

## 3.2 Inner Membrane

The inner membrane enzymes take part in transporting the muropeptides from the periplasm into the cytoplasm. The most important enzyme that is identified in many of the Gram negatives is AmpG permease though it was first identified in *Enterobacter cloacae* (Korfmann and Sanders 1989). The AmpG permease in *E. coli* transports the disaccharide unit GluNac-anhMurNAc across the inner membrane to the cytoplasm (Schmidt et al. 1995). There are two AmpG homologs, AmpG and AmpP inner membrane permeases (Korfmann and Sanders 1989; Vötsch and Templin 2000), that are transcriptionally regulated by AmpR in an inducer-dependent and inducer-independent manner (Lindquist et al. 1989; Cheng and Park 2002). Alternate route of transfer of GluNAc into the cytoplasm exist for *E. coli* and *P. aeruginosa* using NagE which phosphorylates and imports GluNAc into the cytoplasm (Dhar et al. 2017).

## 3.3 Cytoplasm

In the cytoplasm, the muropeptides are processed to form lipid II which is composed of UDP-GluNAc-MurNAc pentapeptide attached to a lipid carrier which is a member of the undecaprenyl pyrophosphate group (Barreteau et al. 2008). The various enzymes in the cytoplasm are as follows:

### 3.3.1 NagZ

In the cytoplasm the β-*N*-acetylglucosaminidase processes the muropeptides. NagZ cleaves the bond between GluNac and 1,6-anhydro-MurNAc leading to independent units of GluNac and 1,6-anhydro-MurNAc peptides (Vadlamani et al. 2017). By inhibiting NagZ, the formation of the inducer molecules comprising 1,6-anhydro-MurNAc peptides would be hindered thus leading to reduced AmpC production and increased sensitivity to beta-lactams (Asgarali et al. 2009). The approach of targeting NagZ has received validation using both chemical and genetic studies and structural studies (Zamorano et al. 2010).

### 3.3.2 AmpD and LdcA

AmpD is involved in the exclusive cleavage of the peptide chain to 1,6-anhydromuramyl moieties. LD-carboxypeptidase A (LdcA) removes the

terminal D-alanine from tetrapeptide to create the tripeptide. To this tripeptide, a dipeptide D-alanine forming a pentapeptide that helps in cross-linking is added. In the final steps of recycling processes, the peptidoglycan sugars GlcNAc, MurNAc, and anhMurNAc and the peptidoglycan peptide L-Ala-ɣ-D-Glu-meso-diaminopimelic acid-D-Ala-D-Ala rejoin the peptidoglycan biosynthesis pathway in the cytoplasm.

## 4 Cell Wall Recycling in Gram-Positive Bacteria

Cell wall recycling in Gram positives was first identified in *Bacillus subtilis* of a six-gene cluster wherein the first five gene products were orthologs of the *E. coli* recycling proteins MurQ (etherase), MurR (transcriptional repressor), MurP (MurNAc phosphotransferase), AmiE (an *N*-acetylmuramyl-L-alanine-specific amidase), and NagZ (glucosaminidase). *nagZ* ortholog encoded by *B. subtilis* differs from that by *Clostridium acetobutylicum*, which also encodes orthologs of *amiE* and *murQ* as cytoplasmic enzymes, and of *murP* and *murR*. These genes strongly suggest the presence of a muropeptide recovery system that is similar, other than the cytoplasmic location for each of these identified component proteins, to the system present in *B. subtilis*. A key difference is in the mechanism of entry of muropeptide to the cytoplasm. Additional protein components that are inferred as part of the recycling system are cytoplasmic MurK kinase, catalyzing ATP-dependent 6′-phosphorylation of GlcNAc and MurNAc, and a GlmA glucosamine and glucosamine-containing muropeptide *N*-acetyl transferase. *B. subtilis* and *E. coli* encode dipeptide epimerases, catalyzing the formation of D-Ala and D-Glu dipeptides, and are presumptively involved in peptidoglycan recycling. The lytic transglycosylase-mediated liberation of anhydromuropeptides is unimportant to recycling although they have essential roles during the extensive peptidoglycan remodeling that occurs during *Bacillus* spore germination and *Staphylococcus aureus* septation. The Gram positives lack AmpG permease in contrast to Gram negatives. Also, the cytoplasmic and periplasmic amidases which are present in Gram negatives are absent in Gram positives. Lyzozymes like muraminidases are more common in Gram positives when compared to lytic transglycosylases. GluNAc'ases that cleaves peptidoglycan, amidases, and carboxypeptidases play a major role in the Gram-positive cell wall recycling. Orthologs of the recycling enzyme MurQ, which converts MurNAc-6-phosphate to GlcNAc-6-phosphate, were recognized in almost all Gram-positive bacteria. This clearly indicates that the cell wall amino sugar MurNAc can be metabolized by most Gram-positive organisms. Further catabolism involves the nag genes including *nagA*. Cell wall-derived peptides are degraded in Gram-positive bacteria since an ortholog of the muropeptide ligase is apparently absent in Gram positives, but orthologs of LdcA-like LD-carboxypeptidases and L-Ala-D/L-Glu epimerases are found (Doyle et al. 1988; Borisova et al. 2016).

Identification of a point of union between beta-lactamase repressor (BlaI) dissociation and cell wall recycling in *Bacillus licheniformis* was made recently by

Amoroso et al. The cell wall-derived dipeptide fragment, γ-D-Glu-*m*-DAP, controls the release of the BlaI (MecI) repressor from DNA. Use of a peptidoglycan fragment to control expression of resistance in this Gram-positive bacterium through repressor protein binding conceptually parallels what is seen for AmpC beta-lactamase expression system of Gram-negative bacteria (Amoroso et al. 2012).

## 5 The Process of Recycling and the Contributing Resistance

### 5.1 Beta-Lactam Resistance Linking Amp Pathway

#### 5.1.1 AmpC-AmpG-AmpR System

In this pathway, the beta-lactam antibiotics treatment breaks the balance of PG biosynthesis liberating GluNAc-anhydro-MurNAc oligopeptides in the periplasm.

GluNAc-anhydro-MurNAc oligopeptides are transported into the cytoplasm through AmpG transporter (Korfmann and Sanders 1989). GluNAc moiety is removed from the cytoplasm with the help of NagZ enzyme.

The resulting anhydro-MurNAc-tetrapeptide intermediates are the inducers of beta-lactamase expression through AmpR (Jacobs et al. 1997). AmpR is a transcriptional regulator and it acts as an activator of *ampC*. The production of *ampC* is mainly upregulated only in the presence of exogenous beta-lactam antibiotics (Wiedemann et al. 1998). The activator function of AmpR would be inhibited by a cell wall synthesis precursor UDP-MurNAc pentapeptide. This inhibition would be stopped by a point mutation in AmpR (Kong et al. 2005). Upon beta-lactam antibiotic treatment, the accumulated intracellular anhydro-MurNAc oligopeptides displace AmpR-associated UDP-MurNAc pentapeptide, triggering conformational change of AmpR and activation of *ampC* (Kong et al. 2005). During the process of peptidoglycan recycling, the AmpD would promote the dissociation of stem peptides from the anhydro-MurNAc or GluNAc-anhydro-MurNAc and hence reduce the concentrations of inducing muropeptides and overexpression of AmpC, thus helping to fine-tune its expression (Normark 1995; Kong et al. 2010; Yamaguchi et al. 2012; Lee et al. 2016).

#### 5.1.2 The BlrAB-Like Two-Component Regulatory System

In *Aeromonas* sp., the AmpC and two other chromosomally encoded beta-lactamases were regulated by BlrA regulator instead of AmpR-type regulator. Specific degraded peptidoglycan components would serve as a signal for the response regulator for production of beta-lactamase (Dhar et al. 2017).

### 5.2 Beta-Lactam Resistance Through Other Cell Wall-Related Genes

The lytic transglycosylases and low molecular mass penicillin-binding protein also moderate the beta-lactam resistance. The periplasmic LTs are the major PG

degradative enzymes that generate 1,6-anhydro-MurNAc muropeptides. The LMM PBPs comprise a group of enzymes that has endopeptidase and carboxypeptidase activity (Johnson et al. 2013).

### 5.3 Fluroquinolone Resistance

Quinolones are broad-spectrum synthetic antibiotics that inhibit DNA replication by targeting bacterial DNA gyrase or topoisomerase 2 (Drlica et al. 2008; Hernández et al. 2011). The resistance in quinolones could be due to mutations in the target genes *gyrA* and *gyrB*, overexpression of MDR efflux pumps, or plasmid-mediated resistance gene *qnr* that protects the bacterial topoisomerases from quinolone activity (Nakamura et al. 1989). In *P. aeruginosa*, MexEF-OprN is involved in the efflux of fluoroquinolones, chloramphenicol, and trimethoprim. This is under control of a positive regulator MexT and the negative regulators MexS and MvaT. *P. aeruginosa* AmpR negatively regulates the expression of the MexEF-OprN operon and MexT. Hence the *ampR* mutants are more resistant to fluoroquinolones. Thus, AmpR positively and negatively regulates resistance to beta-lactams and quinolones, respectively (Dhar et al. 2017).

### 5.4 Fosfomycin Resistance

Recent studies elucidate a connection between cell wall recycling and intrinsic fosfomycin resistance in *Pseudomonas putida.* There exists a salvage pathway that bypasses the de novo biosynthesis of UDP-MurNAc which is absent in *Enterobacteriaceae* and involves three enzymes characteristically. Similar to *E. coli*, *Pseudomonas* sp. also possesses upstream of salvage pathway enzymes, the NagZ that yields AmpD and anhMurNAc kinase (AnmK). Blocking the cell wall recycling pathway decreases the fosfomycin resistance of these strains. Thus, a combinatory therapy of fosfomycin along with peptidoglycan recycling inhibitors would be a new strategy against multidrug-resistant *P. aeruginosa* strains (Borisova et al. 2014) (Table 1).

## 6 Peptidoglycan Recycling Inhibitors

Different types of peptidoglycan recycling inhibitors are as follows (they are also depicted in Fig. 2):

### 6.1 Bulgecin

Bulgecin, a natural compound developed by Takeda Pharmaceuticals (Imada et al. 1982), when combined with other beta-lactam antibiotics, was found to inhibit the

**Table 1** The inhibitors targeting the beta-lactamase induction pathway (Stubbs et al. 2007)

| Enzymes | Function | Inhibitors |
|---|---|---|
| Lytic transglycosylases | Non-hydrolytic cleavage of peptidoglycan with concomitant formation of 1,6-anhydro-MurNAc | Bulgecin A (Skalweit 2019), NAG-thiazoline (Reid et al. 2004), hexa-*N*-acetylchitohexaose, Ivy (Clarke et al. 2010) |
| NagZ | Cleave disaccharide oligopeptides to release 1,6-anhydro-MurNAc peptide | PUGNAc, EtBuPUG (Asgarali et al. 2009; Zamorano et al. 2010) |
| AmpG | Inner membrane permease of the 1,6-GlcNAc-anhydro-MurNAc peptides | CCCP (Zhang et al. 2010) |
| AmpR | Binary regulator of AmpC | UDP-*N*-acetylmuramic acid peptides (Stubbs et al. 2007) |

lytic transglycosylases in many pathogenic *Enterobacteriaceae*, particularly *E. coli*, where beta-lactam antibiotics inhibit penicillin-binding protein 3 (PBP3) (Templin et al. 1992; Thunnissen et al. 1995; Van Asselt et al. 1999). Bulgecin A is bound in the region of the active site defined as subsite −1, −2, and − 3 and partially occludes subsite +1. An alignment of LtgA catalytic domain with Slt70 demonstrates absolute conservation of the active site of LtgA. A docked chito-oligosaccharide in the active site of LtgA mimics the glycan strand of the peptidoglycan. In the active site of LtgA, chitopentaose and bulgecin A have overlapping binding sites. This would suggest that bulgecin A could be competitive with the glycan strand. It induces cell lysis and morphology changes in the presence of beta-lactam antibiotics. The compound was not developed for several years due to unknown reasons. Tomoshige et al. developed the products of bulgecin A indicating its role in combating beta-lactam resistance. After the discovery of bulgecin compounds and LTs in *E. coli*, several LTs have been identified in many pathogenic bacteria like *P. aeruginosa*, *Acinetobacter baumannii*, *Helicobacter pylori*, *Neisseria meningitidis*, and *Campylobacter jejuni* (Skalweit 2019).

Bulgecin A-meropenem combination proves effective against various manifestations of carbapenem resistance either due to metallo-beta-lactamases or hyperproduction of AmpC. It is well established that lytic transglycosylase and PBPs form protein complexes, among which the most notable interaction is between Slt70 and PBPs 1b, 1c, 2, and 3. Combinations of enhancers with beta-lactamase inhibitor/potent anti-pseudomonal beta-lactams are the possibilities in the future antibiotic arsenal for antibiotic-resistant *P. aeruginosa* (Stubbs et al. 2007).

## 6.2 NAG-Thiazoline

NAG-thiazoline does not require the addition of beta-lactam antibiotics unlike bulgecin indicating its role in the inhibition of more than one kind of lytic transglycosylase.

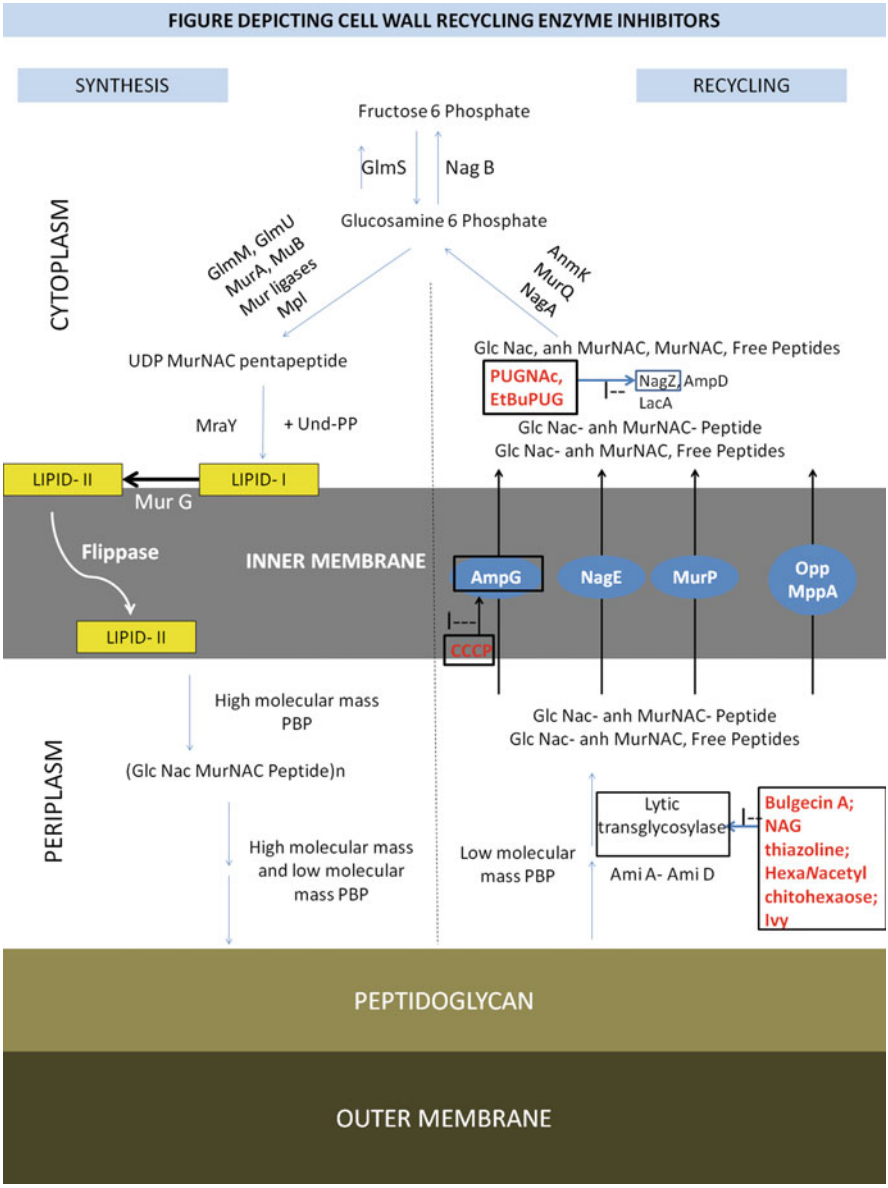


**Fig. 2** Small molecule inhibitors of cell wall recycling pathway. NagZ inhibitor—PUGNAc, EtBuPUG. AmpG inhibitor—CCCP. Lytic transglycosylase inhibitors—bulgecin A, NAG-thiazoline, Ivy, hexa-*N*-acetylchitohexaose

Cell surface hydrophobicity is greatly altered on exposure to NAG-thiazoline. The cells that were treated with NAG-thiazoline became shorter and did not adhere to cell surface due to alterations in the hydrophobicity. The cell surface

hydrophobicity was altered based on the concentrations of NAG-thiazoline (Reid et al. 2004). Cell surface hydrophobicity is associated with the adherence ability of the pathogens thus decreasing its pathogenicity. It is also capable of inhibiting the peptidoglycan biosynthetic complexes that would be responsible for cell morphology and autolysis. *Enterococcus* develops resistance using various mechanisms particularly in the variations in the stem peptides, hence resulting in decreased affinity for the antibiotic. Despite resistance shown by organisms to cell wall peptidoglycan sacculus, the peptidoglycan sacculus still contains potential targets to combat it.

## 6.3 Ivy (Inhibitor of Vertebrate Lysozyme)

The analysis of the protein encoded by *ykfE* from *E. coli* was found to be an inhibitor of C-type lysozymes and was thus renamed Ivyc for inhibitor of vertebrate lysozyme (Abergel et al. 2007). *P. aeruginosa* produces two types of Ivy proteins, namely, Ivp1 and Ivp2, though the Ivp2 function is not yet known. Both Ivyc and its homolog Ivp1 from *P. aeruginosa* contribute to lysozyme resistance, and hence they could survive in lysozyme-rich fluids, such as human saliva and breast milk, in addition to hen egg white. The proteinaceous inhibitor of vertebrate lysozymes (Ivy) is produced by Gram-negative bacteria in response to the damage of the cell wall peptidoglycan. Ivy proteins are potent inhibitors of the lytic transglycosylases, enzymes involved in the biosynthesis and maintenance of peptidoglycan. However, the essential cell wall component and target of lysozyme, PG, in Gram-negative bacteria is protected from exogenous agents by an outer membrane. Thus, the finding shows that (1) the production of Ivyc and its homologs is limited to Gram-negative bacteria rather than to Gram-positive bacteria, which possess exposed peptidoglycan, and (2) they are localized to the periplasm rather than to the external milieu. The Ivy controls the autolytic activity of Gram-negative bacteria that do not produce O-acetylated peptidoglycan. The important class of endogenous enzymes responsible for peptidoglycan lysis is the lytic transglycosylases. The LTs cleave PG with the same specificity as the lysozyme, that is, the β-1,4-glycosidic bond between MurNAc and GlcNAc residues. However, unlike lysozyme, LTs are not hydrolases as they cleave PG with the concomitant formation of an intramolecular 1,6-anhydromuramoyl reaction product (Clarke et al. 2010).

## 6.4 NagZ Inhibitor

The structure of the NagZ inhibitor complex provides the molecular basis for inhibition and enables the development of inhibitors with 100-fold selectivity for NagZ over functionally related human enzymes (Zamorano et al. 2010; Asgarali et al. 2009). By inhibiting NagZ, the formation of the inducer molecules comprising 1,6-anhydro-MurNAc peptides would be hindered thus leading to reduced AmpC production and increased sensitivity to beta-lactams. Among the several compounds

inhibiting NagZ, the most important ones are grouped as non-selective and selective. X-ray structures of NagZ bound to the non-selective *N*-acetyl-b-glucosaminidase inhibitor PUGNAc (*O*-(2-acetamido-2-deoxy-D-glucopyranosylidene) amino-*N*-phenylcarbamate) and two NagZ-selective inhibitors—EtBuPUG, a PUGNAc derivative bearing a 2-*N*-ethylbutyryl group, and MM-156, a 3-*N*-butyryl trihydroxyazepane—showed that the phenylcarbamate moiety of PUGNAc and EtBuPUG completely displaces the catalytic loop from the NagZ active site to yield a catalytically incompetent form of the enzyme. These PUGNAc-derived inhibitors reduce the minimal inhibitory concentration (MIC) values for several clinically relevant cephalosporins in both wild-type and AmpC-hyperproducing strains lacking functional AmpD. It helps in reversing the resistance to the inhibitors by exploiting the high mutational frequency of *ampC* or *ampD.* It could also be used for strains of bacteria that contain loss of function mutation in *ampD* and for reversing the resistance in many Gram-negative organisms (Vadlamani et al. 2017).

### 6.5 CCCP (Carbonyl Cyanide *m*-Chlorophenylhydrazone)

AmpG encodes a transmembrane protein that functions as a permease for 1,6-GlcNAc-anhydro-MurNAc peptides. AmpG activity is required for the peptidoglycan monomers to enter the cytoplasm and be recycled and ultimately reincorporated into the peptidoglycan. AmpG is responsible for the transport of the AmpC-inducing signal molecule, and thus the blockage of AmpG activity may provide a successful strategy for enhancing the efficacy of beta-lactams against bacteria carrying inducible AmpC. AmpG inhibition would result in the suppression of both intrinsic AmpC expression and the AmpC hyperexpression caused by AmpD mutations. CCCP is an inhibitor of proton motive force and the resistance-nodulation-division efflux pump (Pagès et al. 2005). CCCP is an AmpG permease-specific inhibitor, thus affecting cell wall recycling. It inhibits the *ampG* function rendering sensitivity to beta-lactam through suppression of *ampC.* AmpG is a transmembrane protein and is easier to be targeted thus eliminating the permeability barrier. As a part of bacterial cell wall recycling complex, *ampG* is highly conserved in most bacteria. The use of CCCP in combination with ampicillin and cefotaxime attenuated the resistance to these antibiotics close to the level of resistance exhibited by the AmpG mutant. CCCP has been tried in many *Pseudomonas* infections in cystic fibrosis patients, though the mechanism of resistance in this group of patients is not yet understood (Stubbs et al. 2007).

## 7 Summary and Conclusion

Ever since the discovery of antibiotics, bacteria have become smarter to combat their effect. Initially they developed single drug resistance followed by multidrug resistance and pan-drug resistance. Recycling pathway generates certain enzymes that provoke resistance. Peptidoglycan recycle inhibitors prove to be a promising tool for

the multidrug-resistant pathogens. Lytic transglycosylases that act on peptidoglycan are inhibited by bulgecin, NAG-thiazoline, and Ivy. Bulgecin is used in combination with beta-lactam antibiotics such as meropenem, whereas NAG-thiazoline is a stand-alone inhibitor, altering cell surface hydrophobicity and thereby inhibiting the peptidoglycan biosynthetic complex. Ivy proteins are associated with lysozyme resistance and are expressed by Gram-negative bacteria in response to damage of cell wall peptidoglycan. Sensitivity to beta-lactam antibiotics is also boosted by NagZ inhibitor which reduces AmpC production through the disruption of 1,6-anhydro-MurNAc peptide formation. The NagZ-selective inhibitors reduce minimal inhibitory concentration of cephalosporins and revert the resistance to inhibitors by targeting the mutational frequency of *ampC* or *ampD*. CCCP is specific for the inhibition of AmpG permease and disrupts proton motive force and resistance-nodulation-division efflux pumps. It is used in combinative therapies in order to weaken resistances against ampicillin and cefotaxime. The role of recycling inhibitors in eradicating antibiotic resistance would serve to be a boon in this era of pan-drug resistance. It requires continuing as a field for further research to bring more compounds for clinical efficacy and treatment.

## References

Abergel C, Monchois V, Byrne D et al (2007) Structure and evolution of the Ivy protein family, unexpected lysozyme inhibitors in Gram-negative bacteria. Proc Natl Acad Sci U S A 104:6394–6399. https://doi.org/10.1073/pnas.0611019104

Alekshun MN, Levy SB (2007) Molecular mechanisms of antibacterial multidrug resistance. Cell 128:1037–1050. https://doi.org/10.1016/j.cell.2007.03.004

Aminov RI (2009) The role of antibiotics and antibiotic resistance in nature. Environ Microbiol 11:2970–2988. https://doi.org/10.1111/j.1462-2920.2009.01972.x

Amoroso A, Boudet J, Berzigotti S et al (2012) A peptidoglycan fragment triggers β-lactam Resistance in Bacillus licheniformis. PLoS Pathog 8:e1002571. https://doi.org/10.1371/journal.ppat.1002571

Asgarali A, Stubbs KA, Oliver A et al (2009) Inactivation of the glycoside hydrolase NagZ attenuates antipseudomonal beta-lactam resistance in *Pseudomonas aeruginosa*. Antimicrob Agents Chemother 53:2274–2282. https://doi.org/10.1128/AAC.01617-08

Barreteau H, Kovac A, Boniface A et al (2008) Cytoplasmic steps of peptidoglycan biosynthesis. FEMS Microbiol Rev 32:168–207. https://doi.org/10.1111/j.1574-6976.2008.00104.x

Blackburn NT, Clarke AJ (2001) Identification of four families of peptidoglycan lytic transglycosylases. J Mol Evol 52:78–84

Blackburn NT, Clarke AJ (2002) Characterization of soluble and membrane-bound family 3 lytic transglycosylases from *Pseudomonas aeruginosa*. Biochemistry 41:1001–1013. https://doi.org/10.1021/bi011833k

Borisova M, Gaupp R, Duckworth A et al (2016) Peptidoglycan recycling in gram-positive bacteria is crucial for survival in stationary phase. mBio 7:e00923–e00916. https://doi.org/10.1128/mBio.00923-16

Borisova M, Gisin J, Mayer C (2014) Blocking peptidoglycan recycling in *Pseudomonas aeruginosa* attenuates intrinsic resistance to fosfomycin. Microb Drug Resist 20:231–237. https://doi.org/10.1089/mdr.2014.0036

Bouhss A, Crouvoisier M, Blanot D, Mengin-Lecreulx D (2004) Purification and characterization of the bacterial MraY translocase catalyzing the first membrane step of peptidoglycan biosynthesis. J Biol Chem 279:29974–29980. https://doi.org/10.1074/jbc.M314165200

Bugg TDH, Braddick D, Dowson CG, Roper DI (2011) Bacterial cell wall assembly: still an attractive antibacterial target. Trends Biotechnol 29:167–173. https://doi.org/10.1016/j.tibtech.2010.12.006

Cheng Q, Park JT (2002) Substrate specificity of the AmpG permease required for recycling of cell wall anhydro-muropeptides. J Bacteriol 184:6434–6436. https://doi.org/10.1128/JB.184.23.6434-6436.2002

Clarke CA, Scheurwater EM, Clarke AJ (2010) The vertebrate lysozyme inhibitor ivy functions to inhibit the activity of lytic transglycosylase. J Biol Chem 285:14843–14847. https://doi.org/10.1074/jbc.C110.120931

Davies J, Davies D (2010) Origins and evolution of antibiotic resistance. Microbiol Mol Biol Rev 74:417–433. https://doi.org/10.1128/MMBR.00016-10

Dhar S, Kumari H, Balasubramanian D, Mathee K (2017) Cell-wall recycling and synthesis in *Escherichia coli* and *Pseudomonas aeruginosa*—their role in the development of resistance. J Med Microbiol 67:1. https://doi.org/10.1099/jmm.0.000636

Dijkstra AJ, Keck W (1996) Identification of new members of the lytic transglycosylase family in *Haemophilus influenzae* and *Escherichia coli*. Microb Drug Resist 2:141–145. https://doi.org/10.1089/mdr.1996.2.141

Dik DA, Fisher JF, Mobashery S (2018) Cell-wall recycling of the gram-negative bacteria and the nexus to antibiotic resistance. Chem Rev 118:5952–5984. https://doi.org/10.1021/acs.chemrev.8b00277

Doyle RJ, Chaloupka J, Vinter V (1988) Turnover of cell walls in microorganisms. Microbiol Rev 52:554–567

Drlica K, Malik M, Kerns RJ, Zhao X (2008) Quinolone-mediated bacterial death. Antimicrob Agents Chemother 52:385–392. https://doi.org/10.1128/AAC.01617-06

Fisher JF, Meroueh SO, Mobashery S (2005) Bacterial resistance to beta-lactam antibiotics: compelling opportunism, compelling opportunity. Chem Rev 105:395–424. https://doi.org/10.1021/cr030102i

Fleming A (2001) On the antibacterial action of cultures of a penicillium, with special reference to their use in the isolation of *B. influenzae*. 1929. Bull World Health Organ 79:780–790

Hernández A, Sánchez MB, Martínez JL (2011) Quinolone resistance: much more than predicted. Front Microbiol 2:22. https://doi.org/10.3389/fmicb.2011.00022

Höltje JV, Mirelman D, Sharon N, Schwarz U (1975) Novel type of murein transglycosylase in *Escherichia coli*. J Bacteriol 124:1067–1076

Imada A, Kintaka K, Nakao M, Shinagawa S (1982) Bulgecin, a bacterial metabolite which in concert with beta-lactam antibiotics causes bulge formation. J Antibiot (Tokyo) 35:1400–1403

Ishino F, Mitsui K, Tamaki S, Matsuhashi M (1980) Dual enzyme activities of cell wall peptidoglycan synthesis, peptidoglycan transglycosylase and penicillin-sensitive transpeptidase, in purified preparations of *Escherichia coli* penicillin-binding protein 1A. Biochem Biophys Res Commun 97:287–293. https://doi.org/10.1016/s0006-291x(80)80166-5

Jacobs C, Frère J-M, Normark S (1997) Cytosolic intermediates for cell wall biosynthesis and degradation control inducible β-lactam resistance in gram-negative bacteria. Cell 88:823–832. https://doi.org/10.1016/S0092-8674(00)81928-5

Johnson JW, Fisher JF, Mobashery S (2013) Bacterial cell-wall recycling. Ann N Y Acad Sci 1277:54–75. https://doi.org/10.1111/j.1749-6632.2012.06813.x

Koch AL (2003) Bacterial wall as target for attack. Clin Microbiol Rev 16:673–687. https://doi.org/10.1128/CMR.16.4.673-687.2003

Kong K-F, Aguila A, Schneper L, Mathee K (2010) *Pseudomonas aeruginosa* β-lactamase induction requires two permeases, AmpG and AmpP. BMC Microbiol 10:328. https://doi.org/10.1186/1471-2180-10-328

Kong K-F, Jayawardena SR, Indulkar SD et al (2005) *Pseudomonas aeruginosa* AmpR is a global transcriptional factor that regulates expression of AmpC and PoxB β-lactamases, proteases, quorum sensing, and other virulence factors. Antimicrob Agents Chemother 49:4567–4575. https://doi.org/10.1128/AAC.49.11.4567-4575.2005

Koraimann G (2003) Lytic transglycosylases in macromolecular transport systems of Gram-negative bacteria. Cell Mol Life Sci 60:2371–2388. https://doi.org/10.1007/s00018-003-3056-1

Korfmann G, Sanders CC (1989) ampG is essential for high-level expression of AmpC beta-lactamase in *Enterobacter cloacae*. Antimicrob Agents Chemother 33:1946–1951

Lee M, Dhar S, De Benedetti S et al (2016) Corrigendum: muropeptides in *Pseudomonas aeruginosa* and their role as elicitors of β-lactam-antibiotic resistance. Angew Chem Int Ed Engl 55:12568. https://doi.org/10.1002/anie.201608482

Lee M, Hesek D, Dik DA et al (2017) From genome to proteome to elucidation of reactions for all eleven known lytic transglycosylases from *Pseudomonas aeruginosa*. Angew Chem Int Ed Engl 56:2735–2739. https://doi.org/10.1002/anie.201611279

Lee M, Hesek D, Llarrull LI et al (2013) Reactions of all *Escherichia coli* lytic transglycosylases with bacterial cell wall. J Am Chem Soc 135:3311–3314. https://doi.org/10.1021/ja309036q

Lindquist S, Lindberg F, Normark S (1989) Binding of the *Citrobacter freundii* AmpR regulator to a single DNA site provides both autoregulation and activation of the inducible ampC beta-lactamase gene. J Bacteriol 171:3746–3753

Lovering AL, Safadi SS, Strynadka NCJ (2012) Structural perspective of peptidoglycan biosynthesis and assembly. Annu Rev Biochem 81:451–478. https://doi.org/10.1146/annurev-biochem-061809-112742

Mark BL, Vocadlo DJ, Oliver A (2011) Providing β-lactams a helping hand: targeting the AmpC β-lactamase induction pathway. Future Microbiol 6:1415–1427. https://doi.org/10.2217/fmb.11.128

Mengin-Lecreulx D, Texier L, Rousseau M, van Heijenoort J (1991) The murG gene of *Escherichia coli* codes for the UDP-N-acetylglucosamine: N-acetylmuramyl-(pentapeptide) pyrophosphoryl-undecaprenol N-acetylglucosamine transferase involved in the membrane steps of peptidoglycan synthesis. J Bacteriol 173:4625–4636. https://doi.org/10.1128/jb.173.15.4625-4636.1991

Meroueh SO, Bencze KZ, Hesek D et al (2006) Three-dimensional structure of the bacterial cell wall peptidoglycan. Proc Natl Acad Sci U S A 103:4404–4409. https://doi.org/10.1073/pnas.0510182103

Michael CA, Dominey-Howes D, Labbate M (2014) The antimicrobial resistance crisis: causes, consequences, and management. Front Public Health 2. https://doi.org/10.3389/fpubh.2014.00145

Munita JM, Arias CA (2016) Mechanisms of antibiotic resistance. Microbiol Spectr 4. https://doi.org/10.1128/microbiolspec.VMBF-0016-2015

Nakamura S, Nakamura M, Kojima T, Yoshida H (1989) gyrA and gyrB mutations in quinolone-resistant strains of *Escherichia coli*. Antimicrob Agents Chemother 33:254–255. https://doi.org/10.1128/aac.33.2.254

Normark S (1995) beta-Lactamase induction in gram-negative bacteria is intimately linked to peptidoglycan recycling. Microb Drug Resist 1:111–114. https://doi.org/10.1089/mdr.1995.1.111

Pagès J-M, Masi M, Barbe J (2005) Inhibitors of efflux pumps in Gram-negative bacteria. Trends Mol Med 11:382–389. https://doi.org/10.1016/j.molmed.2005.06.006

Park JT, Uehara T (2008) How bacteria consume their own exoskeletons (turnover and recycling of cell wall peptidoglycan). Microbiol Mol Biol Rev 72:211–227. https://doi.org/10.1128/MMBR.00027-07

Pomorski T, Menon AK (2006) Lipid flippases and their biological functions. Cell Mol Life Sci 63:2908–2921. https://doi.org/10.1007/s00018-006-6167-7

Reid CW, Blackburn NT, Clarke AJ (2004) The effect of NAG–thiazoline on morphology and surface hydrophobicity of *Escherichia coli*. FEMS Microbiol Lett 234:343–348. https://doi.org/10.1016/j.femsle.2004.03.047

Ruiz N (2015) Lipid flippases for bacterial peptidoglycan biosynthesis. Lipid Insights 8:21–31. https://doi.org/10.4137/LPI.S31783

Sauvage E, Kerff F, Terrak M et al (2008) The penicillin-binding proteins: structure and role in peptidoglycan biosynthesis. FEMS Microbiol Rev 32:234–258. https://doi.org/10.1111/j.1574-6976.2008.00105.x

Scheffers D-J, Pinho MG (2005) Bacterial cell wall synthesis: new insights from localization studies. Microbiol Mol Biol Rev 69:585–607. https://doi.org/10.1128/MMBR.69.4.585-607.2005

Scheurwater E, Reid CW, Clarke AJ (2008) Lytic transglycosylases: bacterial space-making autolysins. Int J Biochem Cell Biol 40:586–591. https://doi.org/10.1016/j.biocel.2007.03.018

Schmidt H, Korfmann G, Barth H, Martin HH (1995) The signal transducer encoded by ampG is essential for induction of chromosomal AmpC beta-lactamase in *Escherichia coli* by beta-lactam antibiotics and "unspecific" inducers. Microbiol Read Engl 141(Pt 5):1085–1092. https://doi.org/10.1099/13500872-141-5-1085

Silhavy TJ, Kahne D, Walker S (2010) The bacterial cell envelope. Cold Spring Harb Perspect Biol 2(5):a000414. https://doi.org/10.1101/cshperspect.a000414

Skalweit MJ (2019) Bulgecins as β-lactam enhancers against multidrug resistant (MDR) *Pseudomonas aeruginosa*. *Pseudomonas aeruginosa*—Armory Within. https://doi.org/10.5772/intechopen.85151

Stubbs KA, Balcewich M, Mark BL, Vocadlo DJ (2007) Small molecule inhibitors of a glycoside hydrolase attenuate inducible AmpC-mediated β-lactam resistance. J Biol Chem 282:21382–21391. https://doi.org/10.1074/jbc.M700084200

Suvorov M, Lee M, Hesek D et al (2008) Lytic transglycosylase MltB of *Escherichia coli* and its role in recycling of peptidoglycan strands of bacterial cell wall. J Am Chem Soc 130:11878–11879. https://doi.org/10.1021/ja805482b

Templin MF, Edwards DH, Höltje JV (1992) A murein hydrolase is the specific target of bulgecin in *Escherichia coli*. J Biol Chem 267:20039–20043

Thunnissen AM, Rozeboom HJ, Kalk KH, Dijkstra BW (1995) Structure of the 70-kDa soluble lytic transglycosylase complexed with bulgecin A. Implications for the enzymatic mechanism. Biochemistry 34:12729–12737. https://doi.org/10.1021/bi00039a032

Vadlamani G, Stubbs KA, Désiré J et al (2017) Conformational flexibility of the glycosidase NagZ allows it to bind structurally diverse inhibitors to suppress β-lactam antibiotic resistance. Protein Sci 26:1161–1170. https://doi.org/10.1002/pro.3166

Van Asselt EJ, Thunnissen AM, Dijkstra BW (1999) High resolution crystal structures of the *Escherichia coli* lytic transglycosylase Slt70 and its complex with a peptidoglycan fragment. J Mol Biol 291:877–898. https://doi.org/10.1006/jmbi.1999.3013

Van Heijenoort Y, Van Heijenoort J (1971) Study of the N-acetylmuramyl-L-alanine amidase activity in *Escherichia coli*. FEBS Lett 15:137–141. https://doi.org/10.1016/0014-5793(71)80041-8

Ventola CL (2015) The antibiotic resistance crisis. Pharm Ther 40:277–283

Vollmer W, Joris B, Charlier P, Foster S (2008) Bacterial peptidoglycan (murein) hydrolases. FEMS Microbiol Rev 32:259–286. https://doi.org/10.1111/j.1574-6976.2007.00099.x

Vollmer W, von Rechenberg M, Höltje JV (1999) Demonstration of molecular interactions between the murein polymerase PBP1B, the lytic transglycosylase MltA, and the scaffolding protein MipA of *Escherichia coli*. J Biol Chem 274:6726–6734. https://doi.org/10.1074/jbc.274.10.6726

Vötsch W, Templin MF (2000) Characterization of a beta-N-acetylglucosaminidase of *Escherichia coli* and elucidation of its role in muropeptide recycling and beta -lactamase induction. J Biol Chem 275:39032–39038. https://doi.org/10.1074/jbc.M004797200

Weidel W, Pelzer H (1964) Bag-shaped macromolecules—a new outlook on bacterial cell walls. Adv Enzymol Relat Subj Biochem 26:193–232

Wiedemann B, Pfeifle D, Wiegand I, Janas E (1998) beta-Lactamase induction and cell wall recycling in gram-negative bacteria. Drug Resist Updat Rev Comment Antimicrob Anticancer Chemother 1:223–226

Yakhnina AA, McManus HR, Bernhardt TG (2015) The cell wall amidase AmiB is essential for *Pseudomonas aeruginosa* cell division, drug resistance and viability. Mol Microbiol 97:957–973. https://doi.org/10.1111/mmi.13077

Yamaguchi T, Blázquez B, Hesek D et al (2012) Inhibitors for bacterial cell-wall recycling. ACS Med Chem Lett 3:238–242. https://doi.org/10.1021/ml2002746

Zamorano L, Reeve TM, Deng L et al (2010) NagZ inactivation prevents and reverts beta-lactam resistance, driven by AmpD and PBP 4 mutations, in *Pseudomonas aeruginosa*. Antimicrob Agents Chemother 54:3557–3563. https://doi.org/10.1128/AAC.00385-10

Zhang Y, Bao Q, Gagnon LA et al (2010) ampG Gene of *Pseudomonas aeruginosa* and its role in β-lactamase expression. Antimicrob Agents Chemother 54:4772–4779. https://doi.org/10.1128/AAC.00009-10

# Status Quo of Omics Technologies in Analyzing the Genetic Mediators of Antimicrobial Resistance at Sub-MIC Concentrations

Merin Paul, Vidhyalakshmi Mohan, Lekshmi Narendrakumar, Iype Joseph, and Sabu Thomas

## 1 Introduction

Antibiotics to treat bacterial infections are the keystone of modern medical practice. The gross abuse of these compounds makes antimicrobial resistance (AMR) an alarming threat to global health and weakens decades of progress in the treatment of infectious diseases. According to a WHO report (2016), an estimate of 700,000 people die each year globally due to drug-resistant bacterial infections, and it will reach ten million per annum by 2050 unless a global initiative is enacted. Pioneering studies in this field are pointing out how indiscriminate use of antibiotics is contributing to resistance development and the massive abuse making it worse (Laxminarayan et al. 2013). Sir Alexander Fleming, who discovered the first antibiotic, had predicted the development of antibiotic resistance due to unintentional mistakes of the common man. Exposing the bacterial pathogens to sub-therapeutic concentrations of antibiotics increases the chances of resistance acquisition in susceptible cells (Ayukekbong et al. 2017). The bacterial cell is an excellent factory of evolutionary products and has a remarkable ability to operate in various situations. Bacteria use co-selection as a key mechanism to evolve antibiotic resistance. Co-selection mechanisms of resistance genes include co-resistance (different resistance determinants present on the same genetic element) and cross-resistance

M. Paul · L. Narendrakumar · I. Joseph · S. Thomas (✉)
Cholera and Biofilm Research Laboratory, Pathogen Biology Group, Rajiv Gandhi Centre for Biotechnology, Trivandrum, Kerala, India
e-mail: sabu@rgcb.res.in

V. Mohan
Cholera and Biofilm Research Laboratory, Pathogen Biology Group, Rajiv Gandhi Centre for Biotechnology, Trivandrum, Kerala, India

McMaster University, Hamilton, ON, Canada



S. Thomas (ed.), *Antimicrobial Resistance*,
https://doi.org/10.1007/978-981-15-3658-8_10

(the same genetic determinant responsible for resistance to antibiotics and metals) by compounds of unknown concentration in the environment. These mechanisms enhance pathogen fitness and contribute to the development of stable resistant mutants. Ever since antibiotics were introduced to treat infections, resistant bacteria have been encountered in the clinic (Zaffiri et al. 2012). Throughout the golden age of antibiotics, the only means of testing their efficiency was through experiments looking for visible inhibition of bacterial growth and the ceasing of clinical infection. While irrespective of antibiotic used, the rate of resistance development relies on the organism's genomic background and treatment strategies adopted in the clinical settings (Palmer and Kishony 2013). Current surveillance of AMR is merely focused on the reporting of phenotypic laboratory results of clinically relevant infections. This causes the generation of delayed laboratory results, incomparable data between the resistance profiles of clinical and environmental pathogens, missing out on the resistome of non-culturable pathogens, and many more.

The rapid advancement of modern DNA sequencing technologies has revolutionized the field of diagnostic biology and microbial surveillance in the past few decades. The availability of enormous raw data of genome sequences in sequence databases like NCBI and European Nucleotide Archive (ENA) has led to the development of various bioinformatics analysis tools. The technical advances of sequencing technologies help to form a new field of data analysis named "omics" that include mRNA transcript level-measuring transcriptomics; protein abundance-quantifying proteomics; cellular metabolite level-determining metabolomics; interactomics, which exposes the whole molecular interactions in cells; and fluxomics, which infers the active changes of molecules within a cell over time (Zhang et al. 2009). Omics techniques right from their infancy have been important contributors in the field of microbiology. The omics-based approach to detect AMR will be able to analyze and picturize the genomic structure of the bacterial resistome, composed of AMR genes and their precursors. Resistance development in bacteria is a complex process with multiple and interconnected drivers. With the minuscule organisms being dauntingly smart, there is an urgent need to devise curbing measures to fight the spread of AMR. If we are not putting an end to the emerging antibiotic resistance, since it is the nature's most inherent attribute where the fittest survive, nothing could be done to stop this instinct of living organisms, be it any. Hence, in this chapter, we expound these techniques which can be used to analyze and tackle the possible mechanisms of resistance acquisition at sub-lethal concentration of antibiotics in bacterial pathogens.

## 2 Factors Contributing to Antibiotic Resistance

The discovery of penicillin is considered as a milestone in the field of modern medicine to treat deadly bacterial infections. An "innovation gap" of 40 years in antibiotic development since the 1970s has gradually created a wall between the healthcare system and disease handling (Fischbach and Walsh 2009). Since 1962,

few new antibiotics have been approved to treat bacterial infections in humans such as ciprofloxacin in 1987; linezolid, a synthetic antibiotic, in 2000; daptomycin, a naturally occurring compound found in the soil saprotroph *Streptomyces roseosporus*, in 2003; and retapamulin, a semisynthetic antibiotic, in 2006 (Lock and Harry 2008). Invention and usage of new antibiotics in therapy put forward the threat of development of new resistance and also collateral resistance toward multiple antibiotics. Several factors are likely to contribute to the rapid evolution of antibiotic resistance. One is the population number of bacteria with high genomic plasticity and the presence of mutator strains within that population (Rodríguez-Rojas et al. 2013; LeClerc et al. 1996). Another reason would be the intentional or unintentional use of antibiotics which in turn creates low drug concentration in different environments (inside and outside of the human body). This concentration gradient could possibly select rare resistant mutants (Andersson and Hughes 2014). Also, the horizontal transfer of mobile genetic elements such as transposons, integrons, and plasmids between related and unrelated bacteria accelerates the spread of resistance genes (Munita and Arias 2016).

Unlike soil bacteria, human pathogens live in a different environment and it might be hard for them to face any selection pressure to evolve resistant genes because they would never have been encountered by an antibiotic-producing competitor (Fajardo and Martínez 2008). Human interference of this natural equilibrium accelerates the rate of gene exchange between microorganisms. Reports of resistance genes found in clinical pathogens are exactly the same as those found in natural sources, suggesting the prime source of resistance is environmental bacteria (Bengtsson-Palme et al. 2018; Larsson 2014). Extended use of antibiotics in veterinary medicine and agriculture practices provokes selection of resistance genes and spread of resistant bacteria in a diverse environment (Martinez 2009; Aarestrup 2005; Cabello 2006; McManus et al. 2002; Aminov 2009; Witte 1998). Mutations in genes that code for the drug targets and acquisition of foreign DNA coding for resistance through horizontal gene transfer (HGT) are the fundamental genetic mechanisms executed by bacteria to adapt the antibiotic selection (Munita and Arias 2016).

## 2.1 Gene Mutations

Genetic mutations in the genome lead to the evolution of the subpopulation of bacteria with increased survival fitness. In the presence of antibiotics, the mutant subpopulation eventually replaces the susceptible population of bacteria which cannot withstand the deleterious effect of antibiotics. Since the acquisition of resistance exerts a burden in the form of increased energy consumption in the cell, this trait is only maintained in bacteria in the presence of an antibiotic.

### 2.2 Horizontal Gene Transfer

There are classical concepts of resistance acquisition in bacteria such as transformation, transduction, and bacterial conjugation (Thomas and Nielsen 2005). Transformation is the phenomenon of incorporation of naked DNA into the host genome. Unlike transformation, transduction and conjugation are "physical processes" in which bacteriophages and bacteria themselves act as carriers of genetic material, respectively.

The shuttling of antibiotic resistance genes in the environment is associated with mobile genetic elements (MGEs) such as transposons, integrons, and plasmids. Plasmids carrying antibiotic resistance genes are involved in transferring resistance in *Enterobacteriaceae*, and some types of integrons contain a gene coding resistance to sulfonamides at the 3′ end of the integron structure (Rozwandowicz et al. 2018; Mazel 2006). A diverse array of AMR genes have been found in integrons located on plasmids, making them a common mechanism for the horizontal spread of AMR and multidrug resistance (MDR) (Moser et al. 2018). It is well known that mobile genetic elements play a major role in the transmission of AMR genes from livestock to humans (de Been et al. 2014).

## 3 Antibiotic Use and Resistance Selection: The Concept of MIC and Sub-MIC Values

Under normal conditions, the administration of the lethal dose of antibiotics may kill susceptible bacteria and spare the resistant ones. But over a time period, the MIC of the same drug against the same bacterial species may increase due to the acquisition of resistance. This is due to the presence of a subpopulation of bacteria called persister cells, which are metabolically inactive or slow-growing cells of the same population. The persister cells are a source of resistance even in the absence of antibiotic selection (Levin-Reisman et al. 2017). But due to the difficulty in culturing these cells in vitro, the exact mechanism of maintenance of the persister population is not yet known.

Recent studies shed light on the role of sub-lethal concentrations of antibiotics in the acquisition of resistance. In sub-lethal concentration of antibiotics, the tolerant cells of the bacterial population undergo spontaneous random mutations, which may be retained through the subsequent generations, rather than getting discarded. These random mutations may help to overcome the fitness cost by compensating the maintenance energy of antibiotic resistance (Andersson and Hughes 2014).

### 3.1 Minimum Inhibitory Concentration of Antibiotics

Clearing the bacterial infection without having any severe side effects on the host is the primary criterion to pass the clinical trials of a therapeutically effective drug. This makes MIC the basic concept of pharmacology. MIC is defined as the lowest

concentration of a drug which inhibits the visible growth of a target pathogen in vitro (Mouton et al. 2012). MIC was then adopted as the basis of antibiotic dosing to maintain a higher concentration in relevant body compartments which is enough to clear the infection (McKenzie 2011).

Determination of lethal concentration (MIC) of antibiotics helps to identify and differentiate susceptible wild-type and resistant mutant strains of bacteria. MIC values only provide a relative inhibitory concentration required to limit the growth of susceptible bacteria and do not give information on the sub-clones of the same bacteria, which require a higher dose of antibiotics to eliminate them. This relative difference between an "exclusively susceptible population" and a "susceptible mutant population" was explained in the light of "resistant breakpoint" in clinical microbiology. According to the British Society for Antimicrobial Chemotherapy (BSAC), a breakpoint is a chosen concentration (mg/L) of an antibiotic which defines whether a species of bacteria is susceptible or resistant to the antibiotic. The bacteria are considered as susceptible when the MIC is less than or equal to the susceptibility breakpoint, and it is considered as intermediate or resistant when the MIC is greater than the susceptibility breakpoint. There are two common breakpoint systems used in microbiology to predict resistance: (1) "clinical breakpoint" that predicts response of bacteria to antibiotics in terms of susceptible, intermediate, and resistant and (2) "epidemiological breakpoint" that clearly differentiates the minute changes in susceptibility of a wild-type population (EUCAST 2010; Winstanley and Courvalin 2011) and helps to set an early warning in therapeutic approaches.

Unlike in laboratory conditions, the evolution of bacteria toward resistance is a time-consuming process in the environment. Antibiotics like tetracyclines and fluoroquinolones with greater half-life may persist in the environment for extended time periods and exert chronic selection pressure on microbial communities (Kummerer 2009). The adsorption of antibiotics to particles will further complicate the situation due to slower release of them into the surrounding environment creating an antibiotic gradient of unknown concentration (Balbus et al. 2013; Chander et al. 2005; Córdova-Kreylos and Scow 2007). In addition, bacteria in the environment are likely to be exposed to mixtures of antibiotics rather than single compounds, which may further lower the selective concentration (Gullberg et al. 2014).

Global research on AMR always puts forward the result of the effects of antibiotics treatment on the single bacterial species rather than the microbial communities (Brosche and Backhaus 2010; Rasmussen et al. 2005). Cross-selection and co-selection of biocides and metals contribute to the selection of antibiotic-resistant genes (Pal et al. 2015). The unavoidable truth is that microbes show a sustainable degree of flexibility to high antibiotic exposure by sharing of resistance factors through horizontal gene transfer over larger time frames (Bengtsson-Palme and Larsson 2015; Flach et al. 2015; Jernberg et al. 2007; Lewis 2007; Relman 2012).

## 3.2 Sub-lethal Concentration of Antibiotics

The usage of antibiotics along with natural antibiotic biosynthesis creates an antibiotic gradient in body and environment, which results in exposure of pathogens to concentrations that are both higher and lower than the MIC. Humans, livestock, crops, and aquaculture act as antibiotic reservoirs and promote the shuttling of antibiotics and resistant bacteria between the internal and external environments (Andersson and Hughes 2014). The effects of sub-lethal concentration of antibiotics have been observed since the discovery of penicillin, but its impact on resistance development was not investigated until the early 1970s.

Antibiotic therapy in humans depends upon the specific nature of drugs such as tissue distribution and clearance of the same. Generally, the concentration of the drug in the body is high in the initial hour of treatment but can be varying over the course of time and between different body compartments. Thus, the concentration difference happening during treatment can result in low-level selection in specific tissues. Likewise, the natural production of antibiotics by microorganisms and human contamination may create a concentration gradient of mg/L to ng/L (Sandegren 2014; Baquero and Blázquez 1997). Bacteria are therefore exposed to low antibiotic concentrations in many different environments. Classical theories of resistance selection only consider the concentration of antibiotics which are related to MIC of the susceptible wild-type population (MICsusc) and MIC of the exclusively resistant population (MICres). This suggests that concentrations that are lower than the MICsusc do not inhibit the growth of susceptible bacteria and are therefore not selective. This hypothesis, which is known as the "mutant selective window" hypothesis (Drlica and Zhao 2007; Zhao and Drlica 2001), has dominated the field for a long time. Lately, several experiments have been conducted to determine the effect of sub-MIC of antibiotics in resistance selection (Baquero and Negri 1997). Several in vitro studies using *Escherichia coli*, *Salmonella* sp., *Staphylococcus aureus*, and *Pseudomonas aeruginosa* were conducted to establish the relationship of sub-MIC of antibiotics in the de novo selection of resistance and enrichment of pre-existing resistant mutations (Nair et al. 2013).

Gullberg et al. (2011) and Liu et al. (2011) published two such studies with slightly different approaches used to measure the lowest concentration of different antibiotics in selective enrichment of resistant bacteria. They used *E. coli* and *Salmonella* for their experiment. In one approach, they constructed mutants with an induced clinically relevant mutation in isogenic bacteria to produce resistant mutants with reduced susceptibility to tetracyclines, fluoroquinolones, and/or aminoglycosides. Along with the mutation, the strains also expressed cyan fluorescent protein (CFP) or yellow fluorescent protein (YFP) for differentiation of resistant and sensitive strains which can be sorted by FACS (fluorescence-activated cell sorting) analysis. Then they carried out pairwise competition experiments (wild type versus each mutant) at a range of antibiotic concentrations up to 80 generations by serial passage. The result showed that all three clinically important antibiotics increase mutant's selection at sub-MIC concentrations either via competition or de novo selection.

In the second approach, a wild-type *E. coli* and induced hypersensitive mutant by *tolC* mutation (which eliminate the activity of AcrAB-TolC multidrug efflux pump and are sensitive to several antibiotics) were engineered to express yellow and purple fluorescent proteins, respectively. The cells were allowed to grow in the presence and absence of antibiotics and their bacterial characteristics were studied in the presence of different concentrations of antibiotics and genotoxic agents. They found out that mutants of *E. coli* have greatly increased sensitivity to a wide range of antibiotics. In both experiments, they succeeded to find out the biologically relevant level of antibiotic concentration, and each can be used to quantify minimum selection concentration (MSC).

McVicker et al. in 2014 published a paper that explains the evolution of bacterial subpopulations in low antibiotic concentrations in vivo. The investigators found that exposure of isogenic resistant and susceptible strains to "sub-therapeutic" (an antibiotic dose that produced no significant effect in an in vivo model) doses of tetracycline or oxacillin results in the transformation of strain ratio in favor of the resistant population. A recent publication by Wistrand-Yuen et al. (2018) evidenced the effect of sub-MIC in resistant subpopulation selection, in which susceptible wild-type *Salmonella enterica* were exposed to constant sub-MIC of streptomycin (four-fold below the MIC, 1 mg/L) for 900 generations. They observed that a high-level resistance (up to >1024 mg/L) was developed due to novel mechanisms that are different from those observed during lethal selections evolved in several independent lineages. The cumulative effect of these mutations resulted in high-level resistance due to strong positive relation between different mechanisms: (1) alteration of the ribosomal RNA target (*gidB* mutations), (2) reduction in aminoglycoside uptake (*cyoB*, *nuoG*, and *trkH* mutations), and (3) induction of the aminoglycoside-modifying enzyme AadA (*znuA* mutations).

The experiments described above clearly show that extremely low antibiotic concentrations can select the resistance in favor of bacteria. Antibiotic concentrations in the ng per mL to μg per mL range are widespread in natural environments and are often associated with human sewage, runoff from farming activities, and effluent from industrial plants (Kummerer 2009; Thiele-Bruhn 2003; Milić et al. 2013). Accordingly, in addition to the selection of resistant strains during clinical therapy, it is likely that the selection of antibiotic resistance in ex vivo environments is an overlooked contributor to the widespread emergence of bacterial resistance on a global scale.

## 3.3 Consequences of the Sub-MIC Selection of Antibiotic Resistance

Considering a bacterial population, sub-MIC-selected resistant mutants are more problematic than that of MIC-selected cells (Andersson and Hughes 2012). Usually, a heterogeneous bacterial population contains majorly susceptible cells with a minor population of resistant and persister cells. Normally the high fitness cost halts overgrowth of resistant cells and the susceptible cells predominate. Figure 1 explains

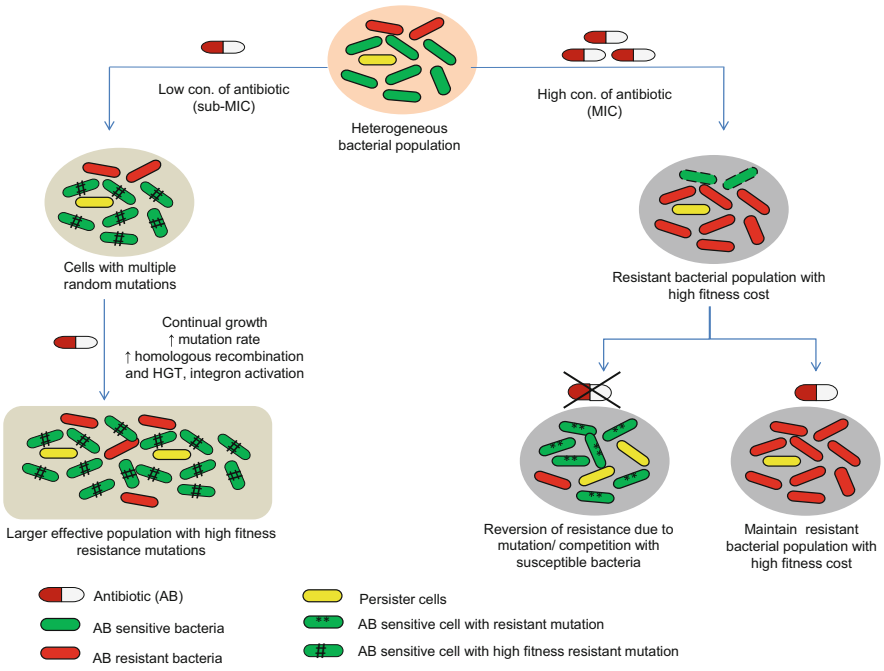


**Fig. 1** Effect of lethal and sub-lethal concentration of antibiotics in resistant mutant selection

the difference in mutation acquisition and resistance development in the presence of a lethal and sub-lethal concentration of antibiotics.

Exposure of a heterogeneous population of bacteria to higher antibiotic concentration causes immediate death of susceptible cells leaving behind the resistant cells to overgrow. Persister cells themselves do not possess any resistance mechanism, but they withstand the lethal effect of antibiotic by growth retardation. If the selection pressure (antibiotic) persists in the surrounding environment over generations, the resistant cells grow continuously, even though the maintenance cost is high. The withdrawal of antibiotic stress from medium exerts a pressure of energy burden to maintain the resistance gene/mutation. This enables the outgrowth of the susceptible bacterial population with high fitness. Thus, in conclusion, the resistance attained under high antibiotic concentration is reversible in nature.

At a low concentration of antibiotics, the bacteria gain high fitness by acquiring mutations, which is irreversible in nature (Andersson and Hughes 2010). Increased rate of stable mutations with a low fitness cost in susceptible cells occurs at a low antibiotic concentration which is successfully transferred to the next generation forming a stabilized population. These mutations contribute to differences in susceptibility (mostly of negligible clinical effect) of an individual cell, thus making the population into an effective larger population that can constantly supply multiple mutations encoding stable resistance. The non-lethal concentrations of fluoroquinolones, aminoglycosides, and beta-lactam group gain special attention

due to their increased efficiency of producing mutations at a higher rate than other antibiotic classes. These are widely used in human and livestock medicine to treat infections and as feed additives (Ysern et al. 1990; Ren et al. 1999; Miller et al. 2004; Balashov and Humayun 2002; Perez-Capilla et al. 2005; Baharoglu and Mazel 2011; Thi et al. 2011). It also effectively increases the homologous recombination, horizontal gene transfer, and activation of mobile genetic elements thus aggravating the situation (López et al. 2007; Couce and Blázquez 2009; Cantón and Morosini 2011). Since low-level antibiotics promote the expansion of resistant populations with increased fitness and high-level resistance, it mainly allows the mutants with multiple stable mutations to sustain in the population and this worsens the situation more (Mao et al. 1997; Taddei et al. 1997; Shaver et al. 2002). Therefore it is predicted that sub-MIC-selected resistant mutants will be more stable in bacterial populations than those selected by high concentrations of antibiotics.

## 4 Persister Cells and Antibiotic Resistance

Persister cells represent a specific subpopulation (typically $10^{-6}$ to $10^{-4}$ of the population) of bacterial cells that have shown transitory resistance against antibiotics. It was first reported in *Staphylococcus* sp. and later also in other genera of bacteria (Bigger 1944). Although the role of persister cells in the recurrence of bacterial infections in humans is widely accepted, the mechanism underlying their formation is still debated. The property of being in a quiescent state enables the persister cells to escape the antibiotic action.

Since the bacteria cohabited with their many counterparts in the environment, it is important to know how the cells respond against various cues. It is well established that biofilm-forming cells are more resistant than their planktonic counterparts to antibiotics. Various studies tried to explore the effect of varying concentrations of antibiotics and resistance development in the different subpopulations of bacterial cells. Miyaue and colleagues showed the *E. coli* population produces and retains an increased number of persister cells in the colony-biofilm culture than in the usual liquid culture in the presence of antibiotic-containing and nutrient-rich environment for a long period of time (Miyaue et al. 2018). It is suggestive of the "memory effect" of persister cells within the population. Bacterial memory mechanisms are related to bacterial immunity to non-self genetic materials and capable to memorize the specific DNA sequences. Miyaue proved that memory effect is also true in case of specific physiological cell state or experience and it provides a survival advantage to the bacteria in an antibiotic-containing environment.

Another study was conducted by Knudsen et al. (2016) to assess bacterial physiology and induce antibiotic tolerance in response to sub-lethal antibiotic concentrations. It was found that sub-lethal concentrations of antibiotics cause metabolic and physiological changes in cells to withstand the lethal concentrations of the same drugs. They have observed significant changes in transcriptome related to antibiotic-specific gene expression in *Listeria monocytogenes*, when it is exposed to sub-MIC of antibiotic. It also results in altered expression of several metabolic

genes which leads to a shift from aerobic to anaerobic metabolism and ethanol production. The shift in metabolism is considered as a survival strategy to avoid the generation of ROS production, a common effect of antibiotics in the bacterial cell.

Antimicrobial chemotherapy has been extensively used since the early 1900s. But the extended resistance against antibiotics makes clinicians worry about their further sustainable use. Thus it is clearly evident that exploring AMR using traditional experimental approaches is not an efficient way of understanding the underlying mechanisms of AMR development and propagation. Methods that mimic or model the conditions of complex scenarios existing in the real world are needed to understand AMR. The omics tools and the emerging systems-level computational models which can look into how AMR is transferred and acquired among the bacteria as a community are helping us to understand the system as they exist in reality. It appears that resistance arises with the interplay of the antibiotics selecting the resistant cells and the genetic elements, in turn, are specifically expressed by the antibiotic. Thus we need combinatorial approaches that can decipher the underlying mechanisms to design better treatment strategies. Hence, the rest of the chapter looks into the alternative approaches which exist and are emerging under the omics and computational bioinformatics fields in the prospect of tackling antimicrobial resistance.

## 5 Alternative Approaches to Study AMR

Sequencing techniques, though sounding inevitable today, have a long history and series of unprecedented discoveries, each of which boasts its own Nobel Prize. The development of the technique was not as fast-paced as how it got adopted and utilized in a tremendous scale after its establishment (Fig. 2). Though genomics came into light only on the commencement of the Human Genome Project and sequencing centers were established to complete the task, what fuelled the race was the sequencing of bacterial genomes. And with time, sequencing has found its way into even the average university set-up and has become a part of the PhD and project students' research now. Such transition occurred due to numerous companies trying to develop their own technologies and continuously improving them. It is reported that around 47 freely accessible bioinformatics resources have been developed till date to analyze and detect the AMR determinants alone (Hendriksen et al. 2019). Based upon this, the two decades of sequencing technology can be divided into three phases. A new phase has always arisen when the shortcomings of the previous phase are overcome. The phase 1 was when Sanger sequencing combined with cloning provided whole genome sequences. Though this technique was accurate and managed to generate complete genomes, the cost and labor involved was extremely high. Thus, independent of the cloning involved, high-throughput technologies emerged. Being based on the chemistry of DNA which was inexpensive and enabled the large-scale parallel sequencing, this marked the phase II of sequencing era but came at the cost of short reads. We currently stand at the beginning of phase III where both

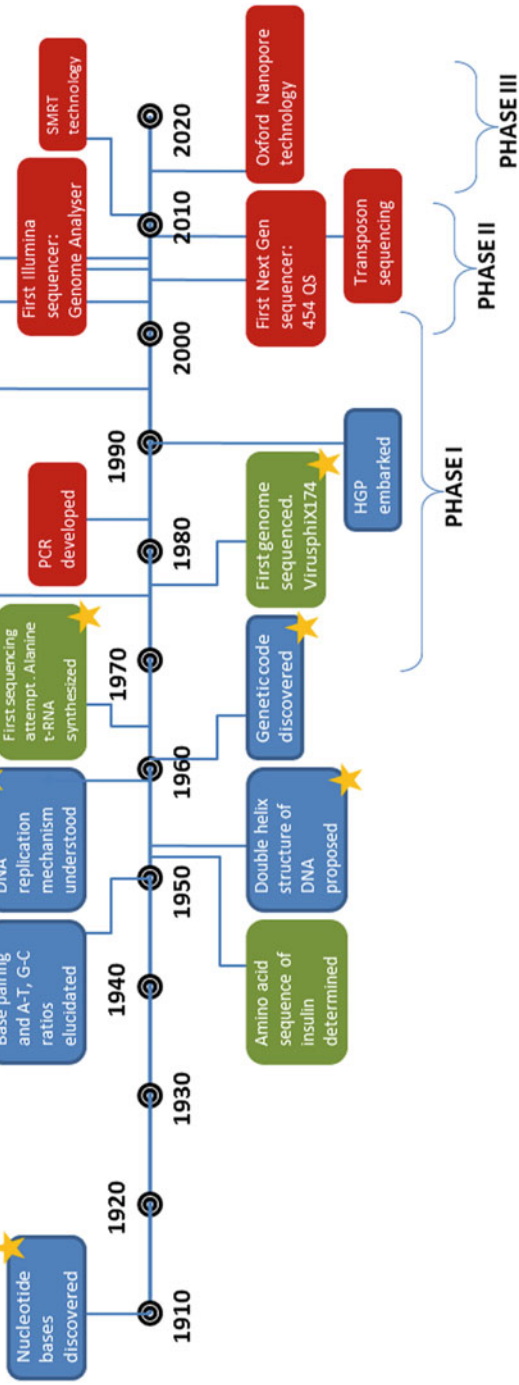


**Fig. 2** History of genomics. Series of events and developments which led to the current standing of genomics technologies. Blue—Understanding the underlying biology for the very first time. Red—Development of a technique based on a biological principle. Green—Discoveries using the techniques developed. Represents award of a Nobel Prize. Marked with left curly bracket are the three major phases of sequencing technologies

biology and chemistry are together being employed to obtain single molecules of long reads.

## 5.1 The Omics Technologies to Study the Genetic Elements

Beginning with helping in understanding organisms based on their genomes, comparative studies of different species and strains of pathogenic organisms began shedding light on the underlying mechanisms such as horizontal gene transfer, reductive evolution, and lateral gene transfer which are foundational in development and spread of antimicrobial resistance. They have also played a prime role in reverse vaccinology since the meningococcal genome was first sequenced. Genome sequences gave insights on the viable but non-culturable (VNBC) strains providing information on deficiencies of specific metabolites which in turn helped in designing axenic growth medium enabling their culturing in lab for further studies. And when both the sources and hopes were dwindling in finding targets against one of the most dreaded pathogens, *M. tuberculosis*, whole genome sequencing came to the rescue in 2005. Today, sequencing followed by protein modeling and docking techniques has risen to such a fame that it needs no introduction. Libraries of thousands of molecules can be screened simultaneously for effective binding which could quench pathogenic organisms. Gone are the days where these organisms were an enigma, and in turn today we are overwhelmed with the amount of data being generated, thanks to omics technologies. The integrated use of high-throughput omics approaches can be able to characterize the complexity of microbial cell biology. One such study was published by Ter Kuile and Westerhoff (2001) which showed that the control of glycolysis is shared in metabolic, proteomic, and genomic state of the cell.

### 5.1.1 Whole Genome Sequencing

Whole genome sequencing (WGS) involves comprehensive and exhaustive analysis of the entire genetic material of an organism including the plasmid, mitochondrial, and chloroplast DNA. It involves random shearing of the total DNA into fragments of a particular length which are then cloned into plasmid vectors. This collection of vectors with random fragments of DNA to be sequenced is called a library. This library of clones is then sequenced and the data is analyzed to figure the exact order of the fragments leading to the complete genome sequence. There are basically two approaches for the assembly of sequences, namely, de novo assembly and reference mapping. The de novo approach is used for sequencing an unknown organism which involves overlapping the sequence fragments and using those regions to trace the order of fragments in the genome. And, reference mapping is employed to identify the variations in the nucleotide sequence of a known organism such as SNPs, insertions, and deletions. These could be the entities contributing to a particular attribute with which the strain being studied varies from the reference strain, usually the wild type. The very first whole genome sequenced was of *Haemophilus influenzae* in 1995 followed by a series of model organisms until the human genome

was sequenced in 2003 (Fleischmann et al. 1995). Today it is one of the very basic data generated in a study be it in prokaryotes or eukaryotes, and the coming paragraphs describe how they are particularly useful in AMR studies.

The first *E. coli* genome was sequenced was in 1997 and it was the k-12 strain. The O157:H7 and a uropathogenic strain were then sequenced in 2001 and compared to the k-12 genome. This study identified 1.4 Mb of horizontally transferred DNA coding for 1632 proteins and 20 t-RNAs which were specific to O157:H7 strain (Hayashi 2001; Welch et al. 2002). Also, around the same time, the genomes of methicillin-resistant *Staphylococcus aureus* (MRSA) were sequenced, and it was identified that most of the resistant genes were harbored by the plasmid or mobile genetic elements. Toxin-producing pathogenecity islands and about 70 new virulence factors were identified in the study (Kuroda et al. 2001). Such studies proved the role of horizontal gene transfer in conferring AMR. With such studies arose the idea of pan-genome of a species, which is a collection of all the genes of all strains of a species sequenced till date with the subsets, a core genome and an accessory genome. The core genome is the collection of the genes which make up for the core phenotype of that particular species and is common among all strains. An accessory genome will be the set of genes which are specific to a few strains of the species conferring additional traits which give them selective advantage in a particular environment. The accessory genome is the larger and open subset of the two and keeps growing as the number of strains sequenced keeps increasing. The AMR genes which make up the resistome fall under the accessory genome. And this comparative genomics has been used since then to study new pathogens or the variants of existing one to identify the virulence factors (Xia and Wolz 2014). With rapid development of bioinformatics tools alongside sequencing techniques, the time and effort studying new strains of pathogens has been minimized to a great extent.

A similar approach can also lead us to the important mutations and SNPs involved in AMR. Genome sequencing and comparison of mutant and parent strains of *S. pneumoniae* revealed the SNPs in the quinolone resistance-determining region of *parC* and *gyrA* genes. The ParC Ser79Phe and GyrA Glu85Lys when present together lead to ciprofloxacin resistance selection (Lupien et al. 2013). Studies have also observed that the rate of mutation increases significantly under antibiotic treatment. One such study identified that prolonged exposure to sub-inhibitory concentrations of antibiotic affects the DNA repair mechanisms such as DNA mismatch and oxidative damage repair and increased expression of error-prone DNA polymerase. Thus it might be that bacteria suppress the repair mechanisms under antibiotic pressure which would accelerate their chances of acquiring advantageous mutations (Long et al. 2016). Bioinformatics pipelines are also being designed specifically for analysis of mutations involved in AMR such as RM-Seq which can be used for high-throughput screenings (Guérillot et al. 2018). WGS has also been employed to study the transformation of AMR-resistant genes by bacteriophages. For examples, genes encoding resistance to multiple antibiotics were transferred spontaneously by prophages φ11, φ12, and φ13 in *S. aureus* (Chernov et al. 2019). Also enrichment of resistance genes in phageome has been observed under antibiotic treatment in mouse model. The genes which were

identified through WGS followed by functional annotation to antibiotic resistance protein database showed that there was not only increase in resistance genes particular to the treatment antibiotic but also to other classes of antibiotics (Modi et al. 2013).

### 5.1.2 Functional Metagenomics

Another approach involves functional screening of the complete DNA pool called functional metagenomics. This method enables fishing out the unknown or new genetic elements with very little or no homology whose functions are not obvious from their sequence. This also serves as an alternative to the traditional method of gene identification through PCR amplification where identification is biased for known genes only and the new and unknown genes remain unexplored. The procedure involves cloning of total community DNA from a sample into an expression vector, creating a collection of cloned libraries. These are then transformed into an indicator strain and cultured on a selective media (here, media with antibiotic), screening for a particular phenotype (here, antibiotic resistance). Thus functional metagenomics of DNA samples both from environment and clinic has led to identification of many novel AMR genes. The functional metagenomic profiling of remote Alaskan soil sample revealed not only the presence of beta-lactamases in an area where there is no anthropogenic pressure but also that the metagenome also harbored a bifunctional beta- lactamase (combination of class C and D) with a single ORF. The class D domain conferred resistance to amoxicillin, ampicillin, and carbenicillin while the C domain conferred resistance to cephalosporins and the combined hybrid resistance to piperacillin. Thus the hybrid enzyme can hydrolyze more antibiotics than what each of the domains individually can (Boolchandani et al. 2017). In a similar study, eight among the nine resistance proteins identified from an environmental soil sample showed $<60\%$ identity to known sequences belonging to the aminoglycoside acetyltransferase enzyme family (Riesenfeld et al. 2004), and a novel tetracycline resistance gene has been identified from an oral microbiome sample (Diaz-Torres et al. 2003). But what could be the significance of studying environmental samples? One such study analyzing a series of soil samples from the USA looking for resistance genes showed that a gene named AB95_TE_1.1 (JX009365) was identical to *tetA* gene from a series of human pathogens and also an intestinal isolate. This shows the potential interconnections between the resistomes of environment, clinical pathogens, and human gut. Hence such studies open avenues to explore the dissemination of resistance genes apart from proving horizontal gene transfer (Forsberg et al. 2012).

### 5.1.3 Single-Cell Genomics

Another technique which is not fully developed or adopted is the single-cell genomics. The methods till now used a bulk population of cells in a sample, whereas in this method isolation of a single cell was performed, on which omics techniques are employed. This can give very important insights into the heterogeneous phenotypes existing in a community. High level of manipulation is possible since plasmids or viruses can be very specifically linked to a single-cell genome and mechanism of

HGT and mutations identified. Also the cellular differences existing between the sensitive, resistant, and persistent cells can be accurately pinned down. But this technique still remains in its infancy and is not widely employed yet due to the technical demand. The method involves separation of cells with flow cytometry, followed by whole genome or total RNA extraction, amplification, and sequencing. The setbacks are difficulties in establishing and performing microbial cytometry, confident isolation of single cells in microliter volumes, and eliminating the background arising from the free DNA, all of which shoot up the cost incurred too. Yet, new devices and technologies are coming up employing SCG as the underlying principle such as microfluidic chip which can make the technique feasible in the near future (Marcy et al. 2007; "Single-Cell Microbiology" 2016).

### 5.1.4 Transposon Sequencing

Though it is well known that transposons confer resistance to antibiotics, these transposons can as well be used to identify the genetic factors that make the organism resistant to certain antibiotics. This technique is called transposon sequencing (Tn-Seq) and it is based on the concept that insertion of a transposon in a gene disrupts its activity. A transposon mutant library is created through insertional mutagenesis and is then grown in a media with and without the limiting factor (here, the antibiotic). Next, the genome is extracted from mutant bacterial pool and Tn-Seq is performed, which selectively amplifies the transposon insertion location and gives the number of reads mapping at that particular insertion site. Hence comparing the number of reads of an insertion site or say a gene between the treated and untreated mutants will give their significance in the system. When the number of reads is higher in the untreated, those genes are the resistance-conferring elements. Another valuable finding would be those genes whose number of reads is higher in the presence of antibiotic. Those genes would be the elements that increase the fitness of the bacterial population in the presence of antibiotic. Thus interesting insights on AMR can be obtained at the gene level using Tn-Seq.

The major contribution of Tn-Seq to understanding AMR has been identifying the intrinsic resistant factors which are the genes in the core genome of the organism. Apart from being the housekeeping genes necessary for growth and survival of the organism, they also indirectly contribute to increased fitness of the organism in the presence of the antibiotic. One such study in *A. baumannii* has identified that genes in the core genome belonging to housekeeping processes such as phospholipid biosynthesis, phosphate regulation, and envelope homeostasis contribute to intrinsic tobramycin resistance. Another interesting observation was that there is a significant overlap between the core and the accessory genes. And four accessory genes, namely, *folA*, *folP*, *trxB*, and *gst*/ABUW_2725, situated in the resistant islands were essential for growth in the presence of antibiotic. With these being paralogs of genes in core genome, this implies that resistance island genes can function in the place of corresponding core gene under pressure (Gallagher et al. 2017). Similarly, genes encoding polytopic membrane proteins such as SAOUHSC_01025 and SAOUHSC_01050 in *S. aureus* were found to limit the action of multiple antibiotics (Rajagopal et al. 2016).

### 5.1.5 Single-Molecule Real-Time (SMRT) Sequencing

Another important and interesting aspect from the genetic point of view of AMR is the epigenetic modifications which in turn control the expression of genes. The major epigenetic modification in bacteria is methylation of adenine residues and is more prevalent than cytosine methylation. Hence single-molecule real-time sequencing came to the rescue for global analysis of methylation detecting both adenine and cytosine methylation. SMRT is a technique utilizing fluorescently labeled nucleotides and capable of both sequencing and identification of nucleotide modifications in real time. The pulse width which is the nucleotide incorporation signal and the interpulse duration which is the time between two incorporation events are unique to each nucleotide, and their corresponding methylation is observed in the template strand. Thus, as sequencing proceeds, the methylation pattern can be recorded based on the template strand (Adhikari and Curtis 2016). Though methylomes of various organisms have been analyzed by this technique, looking into AMR from an epigenetic point of view has just begun.

Only few studies have investigated the role of adenine methylation at GATC sites in *E. coli*. The adenine of GATC sites occurring about 18,000 times in the *E. coli* genome is specifically methylated by the DNA methyltransferases (DAM) on both strands of DNA. Using SMRT, the methylome of bacteria growing in the presence and absence of antibiotic stress was compared and its significance elucidated. It was found that the absence of GATC methylation in the presence of beta-lactam elicits the error-prone polymerase IV leading to increased mismatches which in turn lead to lethal DNA strand breaks by the MutH of MMR system. Thus epigenetic modifications increase the fitness of bacteria and the molecular components involved in methylation can be new targets for treatment (Cohen et al. 2016). Another study observed 175 hyper-methylated and 160 hypo-methylated genes in *M. tuberculosis* rifampicin- and iconozoid-resistant strains. Potential functional classification revealed the genes belonged to glycerolipid metabolism, nitrogen metabolism, microbial metabolism in diverse environments, ascorbate and aldarate metabolism, pentose and glucuronate interconversions, and chloroalkane and chloroalkene degradation. This study emphasizes that resistance mechanisms could be mediated by methylation (Chen et al. 2018).

## 5.2 Omics Beyond Genomics: A Systems Analysis

A biological system is an outcome of highly intricate and sophisticated layers of information processing and functioning. Different omics technologies generate data from different stages with the central dogma of genomics lying at their very foundation. But, this multilevel network can be perturbed at any layer by an incoming signal (i.e., the antibiotic). Hence a holistic approach would be necessary to evaluate the system at various levels which would give a better understanding and open new avenues for better therapeutic strategies at various levels. But the major challenge lies in the data integration from various information layers such as mRNA, proteins, and metabolites. Though there is no single sophisticated means to

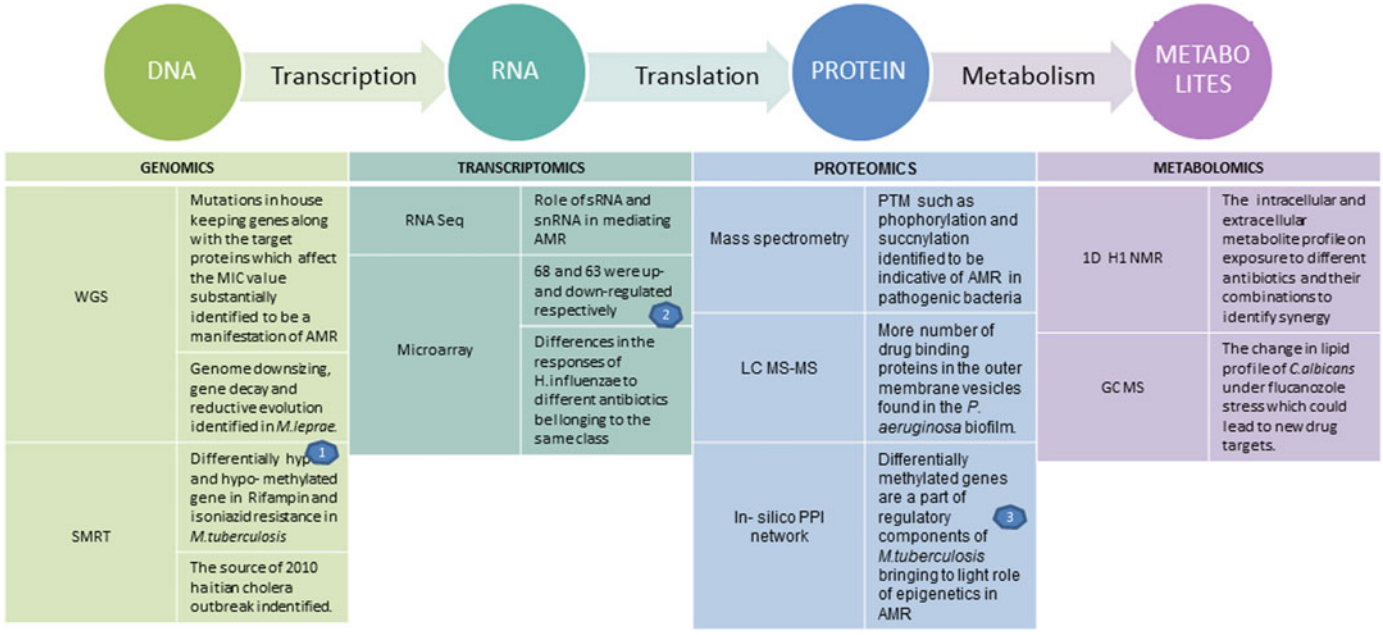


**Fig. 3** The omics techniques employed at various levels of central dogma and their outcomes in the context of antimicrobial resistance

accomplish this, utilizing the techniques in combinations of two or more and integrating the results has led to tremendous knowledge discovery in the field. Since we are concerned with the genetic elements of AMR and the relevant omics techniques applicable, elaborating the techniques beyond genomics and their findings would be beyond the scope of the chapter. Also, no particular bioinformatics tool or database has been named or emphasized here because (1) there are countless tools and scripts existing for the above approaches and new ones come up every single day and (2) there is no correct method or tool defined for any technique and it is solely based on the pipeline put together by the user based on the objective. Figure 3 shows the omics techniques utilized at various levels of central dogma and their outcomes (Cohen et al. 2015; Fondi and Liò 2015; Händel et al. 2014).

## 6 Conclusion

The biochemical and genetic mechanisms involved in antibiotic resistance are by far better known than the equally important phenomena involved in the environmental processes leading to the selection and dispersal of resistant genes and organisms. Thus, the study on antibiotic resistance evolution should simultaneously consider both the mechanisms and the environment.

The currently available data based on the studies of genetics and evolution of antibiotic resistance in bacteria is incomparable in regard of the selection that happened in natural environment. The significant part of resistance development occurs when the pathogen is exposed to non-lethal concentrations of antibiotic. It is clear that the continuous exposure of bacterial pathogens to sub-lethal concentration of antibiotics may provide increased resistance by producing spontaneous mutations. Even if these small mutations cannot create a high effect on the overall sensitivity, it will produce differences in the MIC values and gradually cause the evolution of resistance. Genome-wide analysis will provide a better idea about the contributions of mutations happening via low antibiotic concentrations in the gradual development of antibiotic resistance.

A deeper understanding of genotype-phenotype relationships will be valuable in designing improved regimens for antibiotic therapy that can exploit weaknesses in bacterial resistance phenotypes and reduce the use of inappropriate therapies that might select resistance. To address this complexity, future work needs to more systemically generate genotype-phenotype maps that take into account the variable in vivo conditions in which bacterial pathogens reside, the genetic variability of natural strains, the potential for HGT from the pan-genome, and the evolvability of foreign “resistance” genes after transfer into a novel genetic environment.

This demand of the current scenario can be overcome with the utilization of omics in a systems-level analysis as stated above. Certainly there are shortcomings to the approach, a few obvious ones being (1) integration and analysis of data from different levels of the system which have tools and formats of their own, (2) challenge of handling and analyzing big data by biologists and inadequate knowledge of

underlying biology on the perspective of computer scientists, and (3) the possible overspeculations and the need of backing up the findings of a bioinformatics approach with experimental data. Despite the drawbacks the omics technologies and bioinformatics approaches do have their advantages, namely, that (1) large-scale simulations can be performed eliminating non-probable entries thus saving a significant amount of time and resources and (2) the scope of looking into aspects of biological systems would be experimentally impossible. Therefore the combination of knowledge in both the basic biology and the bioinformatics approaches of various omics technologies would be valuable in the field of antimicrobial resistance based upon the direction the field seems to be taking toward the future.

This book chapter focuses on the genetic mechanisms through which bacteria gain resistance to antibiotics and the emerging bioinformatics and computational approaches to identify and understand them better. How the mobile genetic elements confer resistance and antibiotic usage at MIC and sub-MIC concentrations unavoidably leads to AMR through genetic alterations in the susceptible cells are looked into. The omics tools and the emerging systems-level computational models which can look into how AMR is transferred and acquired among the bacteria as a community are helping us to understand the system as they exist in reality. It appears that resistance arises with the interplay of the antibiotics selecting the resistant cells and the genetic elements in turn being specifically expressed by the antibiotic. Thus we need combinatorial approaches which can decipher the underlying mechanisms to design better treatment strategies.

## References

Aarestrup FM (2005) Veterinary drug usage and antimicrobial resistance in bacteria of animal origin. Basic Clin Pharmacol Toxicol 96(4):271–281. https://doi.org/10.1111/j.1742-7843.2005.pto960401.x

Adhikari S, Curtis PD (2016) DNA methyltransferases and epigenetic regulation in bacteria. FEMS Microbiol Rev 40(5):575–591. https://doi.org/10.1093/femsre/fuw023

Aminov RI (2009) The role of antibiotics and antibiotic resistance in nature. Environ Microbiol 11:2970–2988. https://doi.org/10.1111/j.1462-2920.2009.01972.x

Andersson DI, Hughes D (2010) Antibiotic resistance and its cost: is it possible to reverse resistance? Nat Rev Microbiol 8:260–271. https://doi.org/10.1038/nrmicro2319

Andersson DI, Hughes D (2014) Microbiological effects of sublethal levels of antibiotics. Nat Rev Microbiol 12(7):465–478. https://doi.org/10.1038/nrmicro3270

Andersson DI, Hughes D (2012) Evolution of antibiotic resistance at non-lethal drug concentrations. Drug Resist Updat 15(3):162–172. https://doi.org/10.1016/j.drup.2012.03.005

Ayukekbong JA, Ntemgwa M, Atabe AN (2017) The threat of antimicrobial resistance in developing countries: causes and control strategies. Antimicrob Resist Infect Control 6:47. https://doi.org/10.1186/s13756-017-0208-x

Baharoglu Z, Mazel D (2011) *Vibrio cholerae* triggers SOS and mutagenesis in response to a wide range of antibiotics: a route towards multiresistance. Antimicrob Agents Chemother 55:2438–2441. https://doi.org/10.1128/AAC.01549-10

Balashov S, Humayun MZ (2002) Mistranslation induced by streptomycin provokes a RecABC/RuvABC-dependent mutator phenotype in *Escherichia coli* cells. J Mol Biol 315:513–527. https://doi.org/10.1006/jmbi.2001.5273

Balbus JM, Boxall AB, Fenske RA, McKone TE, Zeise L (2013) Implications of global climate change for the assessment and management of human health risks of chemicals in the natural environment. Environ Toxicol Chem 32:62–78. https://doi.org/10.1002/etc.2046

Baquero F, Blázquez J (1997) Evolution of antibiotic resistance. Trends Ecol Evol 12(12):482–487. https://doi.org/10.1016/s0169-5347(97)01223-8

Baquero F, Negri MC (1997) Selective compartments for resistant microorganisms in antibiotic gradients. BioEssays 19(8):731–736. https://doi.org/10.1002/bies.950190814

Bengtsson-Palme J, Kristiansson E, Larsson DGJ (2018) Environmental factors influencing the development and spread of antibiotic resistance. FEMS Microbiol Rev 42(1):fux053. https://doi.org/10.1093/femsre/fux053

Bengtsson-Palme J, Larsson DGJ (2015) Antibiotic resistance genes in the environment: prioritizing risks. Nat Rev Microbiol 13:396. https://doi.org/10.1038/nrmicro3399-c1

Bigger JW (1944) Treatment of staphylococcal infections with penicillin. Lancet 244:497–500. https://doi.org/10.1016/S0140-6736(00)74210-3

Boolchandani M, Patel S, Dantas G (2017) Functional metagenomics to study antibiotic resistance. In: Sass P (ed) Antibiotics, Methods in molecular biology, vol 1520. Springer, New York, pp 307–329. https://doi.org/10.1007/978-1-4939-6634-9_19

Brosche S, Backhaus T (2010) Toxicity of five protein synthesis inhibiting antibiotics and their mixture to limnic bacterial communities. Aquat Toxicol 99(4):457–465. https://doi.org/10.1016/j.aquatox.2010.06.008

Cabello FC (2006) Heavy use of prophylactic antibiotics in aquaculture: a growing problem for human and animal health and for the environment. Environ Microbiol 8(7):1137–1144. https://doi.org/10.1111/j.1462-2920.2006.01054.x

Cantón R, Morosini MI (2011) Emergence and spread of antibiotic resistance following exposure to antibiotics. FEMS Microbiol Rev 35:977–991. https://doi.org/10.1111/j.1574-6976.2011.00295.x

Chander Y, Kumar K, Goyal SM, Gupta SC (2005) Antibacterial activity of soil-bound antibiotics. J Environ Qual 34:1952–1957. https://doi.org/10.2134/jeq2005.0017

Chen L, Li H, Chen T, Yu L, Guo H, Chen Y, Chen M, Li Z, Wang X, Zhao J, Yan H, Wang X, Zhou L, Zhou J (2018) Genome-wide DNA methylation and transcriptome changes in *Mycobacterium tuberculosis* with rifampicin and isoniazid resistance. Int J Clin Exp Pathol 11(6):3036–3045. http://www.ijcep.com/files/ijcep0075522.pdf

Chernov VM, Chernova OA, Mouzykantov AA, Lopukhov LL, Aminov RI (2019) Omics of antimicrobials and antimicrobial resistance. Expert Opin Drug Discov 14(5):455–468. https://doi.org/10.1080/17460441.2019.1588880

Cohen A, Bont L, Engelhard D, Moore E, Fernández D, Kreisberg-Greenblatt R, Oved K, Eden E, Hays JP (2015) A multifaceted "omics" approach for addressing the challenge of antimicrobial resistance. Future Microbiol 10(3):365–376. https://doi.org/10.2217/fmb.14.127

Cohen NR, Ross CA, Jain S, Shapiro RS, Gutierrez A, Belenky P, Li H, Collins JJ (2016) A role for the bacterial GATC methylome in antibiotic stress survival. Nat Genet 48(5):581–586. https://doi.org/10.1038/ng.3530

Córdova-Kreylos AL, Scow KM (2007) Effects of ciprofloxacin on salt marsh sediment microbial communities. ISME J 1(7):585–595. https://doi.org/10.1038/ismej.2007.71

Couce A, Blázquez J (2009) Side effects of antibiotics on genetic variability. FEMS Microbiol Rev 33:531–5388. https://doi.org/10.1111/j.1574-6976.2009.00165.x

De Been M, Lanza VF, de Toro M, Scharringa J, Dohmen W, Du Y et al (2014) Dissemination of cephalosporin resistance genes between *Escherichia coli* strains from farm animals and humans by specific plasmid lineages. PLoS Genet 10(12):e1004776. https://doi.org/10.1371/journal.pgen.1004776

Diaz-Torres ML, McNab R, Spratt DA, Villedieu A, Hunt N, Wilson M, Mullany P (2003) Novel tetracycline resistance determinant from the oral metagenome. Antimicrob Agents Chemother 47(4):1430–1432. https://doi.org/10.1128/AAC.47.4.1430-1432.2003

Drlica K, Zhao X (2007) Mutant selection window hypothesis updated. Clin Infect Dis 44:681–688. https://doi.org/10.1086/511642

EUCAST (2010) Breakpoint tables for interpretation of MICs and zone diameters. Version 1.1 April 2010: electronic publication. http://www.eucast.org. Accessed 2 Dec 2010, date last accessed

Fajardo A, Martínez JL (2008) Antibiotics as signals that trigger specific bacterial responses. Curr Opin Microbiol 11(2):161–167. https://doi.org/10.1016/j.mib.2008.02.006

Fischbach MA, Walsh CT (2009) Antibiotics for emerging pathogens. Science 325 (5944):1089–1093. https://doi.org/10.1126/science.1176667

Flach CF, Johnning A, Nilsson I, Smalla K, Kristiansson E, Larsson DGJ (2015) Isolation of novel IncA/C and IncN fluoroquinolone resistance plasmids from an antibiotic-polluted lake. J Antimicrob Chemother 70(10):2709–2717. https://doi.org/10.1093/jac/dkv167

Fleischmann R, Adams M, White O, Clayton R, Kirkness E, Kerlavage A (1995) Whole-genome random sequencing and assembly of *Haemophilus influenzae* Rd. Science 269(5223):496–512. https://doi.org/10.1126/science.7542800

Fondi M, Liò P (2015) Multi -omics and metabolic modelling pipelines: challenges and tools for systems microbiology. Microbiol Res 171:52–64. https://doi.org/10.1016/j.micres.2015.01.003

Forsberg KJ, Reyes A, Wang B, Selleck EM, Sommer MOA, Dantas G (2012) The shared antibiotic resistome of soil bacteria and human pathogens. Science 337(6098):1107–1111. https://doi.org/10.1126/science.1220761

Gallagher LA, Lee SA, Manoil C (2017) Importance of core genome functions for an extreme antibiotic resistance trait. MBio 8(6):e01655–e01617. https://doi.org/10.1128/mBio.01655-17

Guérillot R, Li L, Baines S, Howden B, Schultz MB, Seemann T, Monk I, Pidot SJ, Gao W, Giulieri S, Gonçalves da Silva A, D'Agata A, Tomita T, Peleg AY, Stinear TP, Howden BP (2018) Comprehensive antibiotic-linked mutation assessment by resistance mutation sequencing (RM-seq). Genome Med 10(1):63. https://doi.org/10.1186/s13073-018-0572-z

Gullberg E, Albrecht LM, Karlsson C, Sandegren L, Andersson DI (2014) Selection of a multidrug resistance plasmid by sublethal levels of antibiotics and heavy metals. MBio 5:e01918. https://doi.org/10.1128/mBio.01918-14

Gullberg E, Cao S, Berg OG, Ilbäck C, Sandegren L, Hughes D, Andersson DI (2011) Selection of resistant bacteria at very low antibiotic concentrations. PLoS Pathog 7(7):e1002158. https://doi.org/10.1371/journal.ppat.1002158

Händel N, Schuurmans JM, Feng Y, Brul S, ter Kuile BH (2014) Interaction between mutations and regulation of gene expression during development of *De Novo* antibiotic resistance. Antimicrob Agents Chemother 58(8):4371–4379. https://doi.org/10.1128/AAC.02892-14

Hayashi T (2001) Complete genome sequence of enterohemorrhagic *Escherichia coli* O157:H7 and genomic comparison with a laboratory strain K-12. DNA Res 8(1):11–22. https://doi.org/10.1093/dnares/8.1.11

Hendriksen RS, Bortolaia V, Tate H, Tyson GH, Aarestrup FM, McDermott PF (2019) Using genomics to track global antimicrobial resistance. Front Public Health 7:242. https://doi.org/10.3389/fpubh.2019.00242

Jernberg C, Löfmark S, Edlund C, Jansson JK (2007) Long-term ecological impacts of antibiotic administration on the human intestinal microbiota. ISME J 1(1):56–66. https://doi.org/10.1038/ismej.2007.3

Knudsen GM, Fromberg A, Ng Y, Gram L (2016) Sublethal concentrations of antibiotics cause shift to anaerobic metabolism in *Listeria monocytogenes* and induce phenotypes linked to antibiotic tolerance. Front Microbiol 7:1091. https://doi.org/10.3389/fmicb.2016.0109

Kummerer K (2009) Antibiotics in the aquatic environment—a review—part I. Chemosphere 75:417–434. https://doi.org/10.1016/j.chemosphere.2008.11.086

Kuroda M, Ohta T, Uchiyama I, Baba T, Yuzawa H, Kobayashi I, Cui L, Oguchi A, Aoki K, Nagai Y, Lian J, Ito T, Kanamori M, Matsumaru H, Maruyama A, Murakami H, Hosoyama A, Mizutani-Ui Y, Takahashi NK, Hiramatsu K (2001) Whole genome sequencing of methicillin-

resistant *Staphylococcus aureus*. Lancet 357(9264):1225–1240. https://doi.org/10.1016/S0140-6736(00)04403-2

Larsson DGJ (2014) Antibiotics in the environment. Ups J Med 119:108–112. https://doi.org/10.3109/03009734.2014.896438

Laxminarayan R, Duse A, Wattal C, Zaidi AKM, Wertheim HFL, Sumpradit N (2013) Antibiotic resistance—the need for global solutions. Lancet Infect Dis 13:1057–1098. https://doi.org/10.1016/S1473-3099(13)70318-9

LeClerc JE, Li BG, Payne WL, Cebula TA (1996) High mutation frequencies among *Escherichia coli* and *Salmonella* pathogens. Science 274:1208–1211. https://doi.org/10.1126/science.274.5290.1208

Levin-Reisman I, Ronin I, Gefen O, Braniss I, Shoresh N, Balaban NQ (2017) Antibiotic tolerance facilitates the evolution of resistance. Science 355(6327):826–830. https://doi.org/10.1126/science.aaj2191

Lewis K (2007) Persister cells, dormancy and infectious disease. Nat Rev Microbiol 5:48–56. https://doi.org/10.1038/nrmicro1557

Liu A, Fong A, Becket E, Yuan J, Tamae C, Medrano L et al (2011) Selective advantage of resistant strains at trace levels of antibiotics: a simple and ultrasensitive color test for detection of antibiotics and genotoxic agents. Antimicrob Agents Chemother 55(3):1204–1210. https://doi.org/10.1128/aac.01182-10

Lock RL, Harry EJ (2008) Cell-division inhibitors: new insights for future antibiotics. Nat Rev Drug Discov 7(4):324–338. https://doi.org/10.1038/nrd2510

Long H, Miller SF, Strauss C, Zhao C, Cheng L, Ye Z, Griffin K, Te R, Lee H, Chen CC, Lynch M (2016) Antibiotic treatment enhances the genome-wide mutation rate of target cells. Proc Natl Acad Sci 113(18):E2498–E2505. https://doi.org/10.1073/pnas.1601208113

López E, Elez M, Matic I, Blázquez J (2007) Antibiotic-mediated recombination: ciprofloxacin stimulates SOS-independent recombination of divergent sequences in *Escherichia coli*. Mol Microbiol 64:83–93. https://doi.org/10.1111/j.1365-2958.2007.05642.x

Lupien A, Billal DS, Fani F, Soualhine H, Zhanel GG, Leprohon P, Ouellette M (2013) Genomic characterization of ciprofloxacin resistance in a laboratory-derived mutant and a clinical isolate of *Streptococcus pneumoniae*. Antimicrob Agents Chemother 57(10):4911–4919. https://doi.org/10.1128/AAC.00418-13

Mao EF, Lane L, Lee J, Miller JH (1997) Proliferation of mutators in a cell population. J Bacteriol 179:417–422. https://doi.org/10.1128/jb.179.2.417-422.1997

Marcy Y, Ouverney C, Bik EM, Losekann T, Ivanova N, Martin HG, Szeto E, Platt D, Hugenholtz P, Relman DA, Quake SR (2007) Dissecting biological "dark matter" with single-cell genetic analysis of rare and uncultivated TM7 microbes from the human mouth. Proc Natl Acad Sci 104(29):11889–11894. https://doi.org/10.1073/pnas.0704662104

Martinez JL (2009) Environmental pollution by antibiotics and by antibiotic resistance determinants. Environ Poll 157:2893–2902. https://doi.org/10.1016/j.envpol.2009.05.051

Mazel D (2006) Integrons: agents of bacterial evolution. Nat Rev Microbiol 4(8):608–620. https://doi.org/10.1038/nrmicro1462

McKenzie C (2011) Antibiotic dosing in critical illness. J Antimicrob Chemother 66(Suppl 2):ii25–ii31. https://doi.org/10.1093/jac/dkq516

McManus PS, Stockwell VO, Sundin GW, Jones AL (2002) Antibiotic use in plant agriculture. Annu Rev Phytopathol 40:443–465. https://doi.org/10.1146/annurev.phyto.40.120301.093927

McVicker G, Prajsnar TK, Williams A, Wagner NL, Boots M et al (2014) Clonal expansion during *Staphylococcus aureus* infection dynamics reveals the effect of antibiotic intervention. PLoS Pathog 10(2):e1003959. https://doi.org/10.1371/journal.ppat.1003959

Milić N, Milanović M, Letić NG, Sekulić MT, Radonić J, Mihajlović I, Miloradov MV (2013) Occurrence of antibiotics as emerging contaminant substances in aquatic environment. Int J Environ Health Res 23(4):296–310. https://doi.org/10.1080/09603123.2012.733934

Miller C, Thomsen LE, Gaggero C, Mosseri R, Ingmer H, Cohen SN (2004) SOS response induction by b-lactams and bacterial defense against antibiotic lethality. Science 305:1629–1631. https://doi.org/10.1126/science.1101630

Miyaue S, Suzuki E, Komiyama Y, Kondo Y, Morikawa M, Maeda S (2018) Bacterial memory of persisters: bacterial persister cells can retain their phenotype for days or weeks after withdrawal from colony–biofilm culture. Front Microbiol 9:1396. https://doi.org/10.3389/fmicb.2018.01396

Modi SR, Lee HH, Spina CS, Collins JJ (2013) Antibiotic treatment expands the resistance reservoir and ecological network of the phage metagenome. Nature 499(7457):219–222. https://doi.org/10.1038/nature12212

Moser D, Lenzner B, Weigelt P, Dawson W, Kreft H, Pergl J et al (2018) Remoteness promotes biological invasions on islands worldwide. Proc Natl Acad Sci 115(37):9270–9275. https://doi.org/10.1073/pnas.1804179115

Mouton JW, Brown DFJ, Apfalter P, Cantón R, Giske CG, Ivanova M et al (2012) The role of pharmacokinetics/pharmacodynamics in setting clinical MIC breakpoints: the EUCAST approach. Clin Microbiol Infect 18(3):E37–E45. https://doi.org/10.1111/j.1469-0691.2011.03752.x

Munita JM, Arias CA (2016) Mechanisms of antibiotic resistance. Microbiol Spectrum 4(2): VMBF-0016-2015. https://doi.org/10.1128/microbiolspec.VMBF-0016-2015

Nair CG, Chao C, Ryall B, Williams HD (2013) Sub-lethal concentrations of antibiotics increase mutation frequency in the cystic fibrosis pathogen *Pseudomonas aeruginosa*. Lett Appl Microbiol 56(2):149–154. https://doi.org/10.1111/lam.12032

Pal C, Bengtsson-Palme J, Kristiansson E, Larsson DGJ (2015) Co-occurrence of resistance genes to antibiotics, biocides and metals reveals novel insights into their co-selection potential. BMC Genomics 16:964. https://doi.org/10.1186/s12864-015-2153-5

Palmer AC, Kishony R (2013) Understanding, predicting and manipulating the genotypic evolution of antibiotic resistance. Nat Rev Genet 14(4):243–248. https://doi.org/10.1038/nrg3351

Perez-Capilla T, Baquero MR, Gomez-Gomez JM, Ionel A, Martin S, Blazquez J (2005) SOS-independent induction of dinB transcription by b-lactam-mediated inhibition of cell wall synthesis in *Escherichia coli*. J Bacteriol 187:1515–1518. https://doi.org/10.1128/JB.187.4.1515-1518.2005

Rajagopal M, Martin MJ, Santiago M, Lee W, Kos VN, Meredith T, Gilmore MS, Walker S (2016) Multidrug intrinsic resistance factors in *Staphylococcus aureus* identified by profiling fitness within high-diversity transposon libraries. MBio 7(4):e00950–e00916. https://doi.org/10.1128/mBio.00950-16

Rasmussen TB, Skindersoe ME, Bjarnsholt T, Phipps RK, Christensen KB, Jensen PO 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

Relman DA (2012) The human microbiome: ecosystem resilience and health. Nutr Rev 70:S2–S9. https://doi.org/10.1111/j.1753-4887.2012.00489

Ren L, Rahman MS, Humayun MZ (1999) *Escherichia coli* cells exposed to streptomycin display a mutator phenotype. J Bacteriol 181:1043–1044

Riesenfeld CS, Goodman RM, Handelsman J (2004) Uncultured soil bacteria are a reservoir of new antibiotic resistance genes. Environ Microbiol 6(9):981–989. https://doi.org/10.1111/j.1462-2920.2004.00664.x

Rodríguez-Rojas A, Rodríguez-Beltrán J, Couce A, Blázquez J (2013) Antibiotics and antibiotic resistance: a bitter fight against evolution. Int J Med Microbiol 303(6-7):293–297. https://doi.org/10.1016/j.ijmm.2013.02.004

Rozwandowicz M, Brouwer MSM, Fischer J, Wagenaar JA, Gonzalez-Zorn B, Guerra B et al (2018) Plasmids carrying antimicrobial resistance genes in Enterobacteriaceae. J Antimicrob Chemother 73(5):1121–1137. https://doi.org/10.1093/jac/dkx488

Sandegren L (2014) Selection of antibiotic resistance at very low antibiotic concentrations. Ups J Med Sci 119(2):103–107. https://doi.org/10.3109/03009734.2014.904457

Shaver AC, Dombrowski PG, Sweeney JY, Treis T, Zappala RM, Sniegowski PD (2002) Fitness evolution and the rise of mutator alleles in experimental *Escherichia coli* populations. Genetics 162:557–566

Single-Cell Microbiology (2016) Nature. Biotechnology 34(11):1077–1077. https://doi.org/10.1038/nbt.3728

Taddei F, Godelle B, Radman M, Maynard-Smith J, Toupance B, Gouyon PH (1997) Role of mutator alleles in adaptive evolution. Nature 387:700–702. https://doi.org/10.1038/42696

Ter Kuile BH, Westerhoff HV (2001) Transcriptome meets metabolome: hierarchical and metabolic regulation of the glycolytic pathway. FEBS Lett 500(3):169–171. https://doi.org/10.1016/s0014-5793(01)02613-8

Thi TD, López E, Rodríguez-Rojas A, Rodríguez-Beltrán J, Couce A, Guelfo JR et al (2011) Effect of recA inactivation on mutagenesis of *Escherichia coli* exposed to sublethal concentrations of antimicrobials. J Antimicrob Chemother 66:531–538. https://doi.org/10.1093/jac/dkq496

Thiele-Bruhn S (2003) Pharmaceutical antibiotic compounds in soils—a review. J Plant Nutr Soil Sci 166:145–167. https://doi.org/10.1002/jpln.200390023

Thomas CM, Nielsen KM (2005) Mechanisms of, and barriers to, horizontal gene transfer between bacteria. Nat Rev Microbiol 3:711–721. https://doi.org/10.1038/nrmicro1234

Welch RA, Burland V, Plunkett G, Redford P, Roesch P, Rasko D, Buckles EL, Liou SR, Boutin A, Hackett J, Stroud D, Mayhew GF, Rose DJ, Zhou S, Schwartz DC, Perna NT, Mobley HLT, Donnenberg MS, Blattner FR (2002) Extensive mosaic structure revealed by the complete genome sequence of uropathogenic *Escherichia coli*. Proc Natl Acad Sci 99 (26):17020–17024. https://doi.org/10.1073/pnas.252529799

Winstanley T, Courvalin P (2011) Expert systems in clinical microbiology. Clin Microbiol Rev 24 (3):515–556. https://doi.org/10.1128/CMR.00061-10

Wistrand-Yuen E, Knopp M, Hjort K, Koskiniemi S, Berg OG, Andersson DI (2018) Evolution of high-level resistance during low-level antibiotic exposure. Nat Commun 9(1). https://doi.org/10.1038/s41467-018-04059-1

Witte W (1998) Medical consequences of antibiotic use in agriculture. Science 279(5353):996–997. https://doi.org/10.1126/science.279.5353.996

World Health Organization (2016) Bulletin 94:638–639. https://doi.org/10.2471/BLT.16.020916

Xia G, Wolz C (2014) Phages of *Staphylococcus aureus* and their impact on host evolution. Infect Genet Evol 21:593–601. https://doi.org/10.1016/j.meegid.2013.04.022

Ysern P, Clerch B, Castaño M, Gibert I, Barbé J, Llagostera M (1990) Induction of SOS genes in *Escherichia coli* and mutagenesis in *Salmonella typhimurium* by fluoroquinolones. Mutagenesis 5:63–66. https://doi.org/10.1093/mutage/5.1.63

Zaffiri L, Gardner J, Toledo-Pereyra LH (2012) History of antibiotics. From salvarsan to cephalosporins. J Investig Surg 25(2):67–77. https://doi.org/10.3109/08941939.2012.664099

Zhang W, Li F, Nie L (2009) Integrating multiple "omics" analysis for microbial biology: application and methodologies. Microbiology 156(2):287–301. https://doi.org/10.1099/mic.0.034793-0

Zhao X, Drlica K (2001) Restricting the selection of antibiotic-resistant mutants: a general strategy derived from fluoroquinolone studies. Clin Infect Dis 33:S147–S156. https://doi.org/10.1086/321841

# Correction to: Carbapenem Resistance in Gram-Negative Bacilli: Mechanisms and Challenges

Sarita Mohapatra and Arti Kapil

**Correction to:**
**Chapter 2 in: S. Thomas (ed.), *Antimicrobial Resistance*,**
**https://doi.org/10.1007/978-981-15-3658-8_2**

The original version of the chapter had the following error which has been corrected now.

Owing to a correction that the editor had requested, the term "bla-OXA" was replaced in the place of "bla-IMP" in Table 1(a), row no. 5, column no. 3.

The updated online version of this chapter can be found at
https://doi.org/10.1007/978-981-15-3658-8_2



S. Thomas (ed.), *Antimicrobial Resistance*,
https://doi.org/10.1007/978-981-15-3658-8_11