Muhammad Zaffar Hashmi *Editor*

Antibiotics and Antimicrobial Resistance Genes

Environmental Occurrence and Treatment Technologies

Springer

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Editor
Muhammad Zaffar Hashmi
Department of Chemistry
COMSATS University
Islamabad, Pakistan

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Preface

Antibiotics, the wonder drugs, have undoubtedly served humans in combating a variety of microbial infections. For years, antibiotics have been extensively utilized for therapeutic indications not only for humans but also across other related sectors as animal husbandry and agriculture fields. Off-set utilization and irresponsible use of antibiotics have led to the emergence of resistance. There are obviously several specialist volumes covering specific classes of antibacterial agents and describing some of their fundamental aspects. In practice, however, this type of treatise should serve as a supplement to a larger summary work that acts as a reference source. The need for a reference work reflects the universal dynamic of learning and analysis: from the general summary to the specific case, and not vice versa. The present volume therefore fills an important gap in the vast and fast-moving field of anti-infective agents. The authors of this work together combine the skills of doctors, microbiologists, and biochemists. Evidence from reported data suggests that there is immense need to educate agricultural sector, animal husbandry and healthcare to control the misuse and overuse of antibiotics. The book will be a great source of knowledge to basic microbiologists, students, clinical doctors, researchers and infectious disease specialists in the field of infectious diseases, and chemists. The book offers the most up-to-date information regarding antibiotics and AMR. It includes the involvement of contributors across the world, presenting questions of interest to readers of both developed and developing countries.

Antimicrobials are considered as the most effective drug for the treatment of various infections in humans. In the history of medicine, penicillin was marked as the beginners of the "golden era" of antibiotics. Various antibiotics from different families were produced during 1940 to 1962 for the treatment of various infections caused by a variety of bacteria. Over the past two to three decades, several new antibiotics have also been developed through structural modifications of previous antibiotics to make them remain effective. These antibiotics were extensively used in human healthcare due to their essential requirement for the treatment of bacterial infection and cancer, surgical procedures, and as prophylaxis. This has resulted in

the increase in global use of antibiotics due to which their use was at the peak between 2000 and 2010 worth $40 billion per year (O'Neill) with expected 36% increase in the future, which demonstrates their importance from societal and economic perspectives.

The book focuses on the occurrence, fate, transport, emergence, treatment and management options available for AMR. The additional focus of the book is on emerging treatment technologies for antibiotics and antimicrobial resistance genes. The book is divided into different parts such as environmental occurrence and treatment aspects covering the latest published materials. The initial chapters of the book provide information to readers regarding the entry routes of antibiotics and antimicrobial resistance, antibiotics use in hospitals and the associated waste, global and temporal trends of antibiotics and antimicrobial resistance in food animals, aquaculture, and hospital wastes and environment. Some chapters cover the spread of antimicrobial and antibiotic resistance gene. The book also addresses biomonitoring approaches, uptake mechanisms in plants and biota, and bioavailability of antibiotics and AMR. The inclusion of chapters about antibiotics and AMR toxicity, epidemiological, ecological and public health effects and surveillance and environmental risk assessment makes the book unique for the reader. These chapters provide an analysis of risk assessment of antibiotic resistance genes to help understand the environmental and socioeconomic impacts of antibiotics and AMR/ARGs. The last chapters provide management strategies, such as treatment technologies, electrochemical treatment and bioremediation technologies for environmental management. Most of the treatment technologies treat AMR by being able to degrade antimicrobial resistance genes (ARGs), lyse antimicrobial-resistant bacteria (ARBs) and/or oxidize the antibiotics whose presence in the environmental matrices contributes to the development and spread of AMR. A number of treatment technologies such as aerobic and anaerobic digestion, membrane bioreactors, composting, nanoparticles and some disinfection-based mechanisms have already been evaluated, at industrial scale, for treating the antibiotics and associated ARBs and ARGs. These chapters summarize some of the important treatment technologies, illustrating their key features and efficacy in treating AMR. Studies that provide alternative views about the contributions of various treatment technologies in selecting resistant bacteria and genes are also highlighted.

Islamabad, Pakistan Muhammad Zaffar Hashmi

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About the Book

Antibiotics are a critical component of today's healthcare. In addition to the treatment of infectious diseases in humans, they are also needed in animal healthcare. Recently, there has been a growing interest in the presence of pharmaceuticals in the environment. The release of antibiotics into the environment leads to more resistant pathogenic bacteria. The spread and accumulation of antibiotic/antimicrobial-resistant genes into multidrug-resistant pathogens is one of the most challenging problems humans already face, which has to be tackled now. Antibiotics and antimicrobial resistance (AMR) or antibiotic resistance genes (ARGs) are known as emerging environmental contaminants, and a considerable number of journal publications are available on this issue. This book summarizes and updates information about antibiotic-producing organisms, their resistance and entry routes in soil, air, water and sediment, their transport, fate, bioavailability and biomonitoring, risk assessment of antibiotics and resistance genes, and their impacts on the environment and public health. Lastly, it introduces management strategies, such as treatment technologies for managing antibiotics and AMR/ARGs-impacted environment, and bioremediation approaches.

Key Points

- Summarizes and updates information on antibiotics and AMR/ARGs production, routes of entry in the environment, and their fate and uptake in food chain. The book incorporates the results of updated models regarding the interpretation of antibiotics and AMR/ARGs in environmental and biomonitoring studies.
- Provides an analysis of risk assessment of antibiotic resistance genes to help understand the environmental and socioeconomic impacts of antibiotics and AMR/ARGs.
- Provides management strategies, such as treatment technologies and bioremediation technologies for environment management. The use of antibiotics in the human and veterinary industry resulted in the spread of antibiotics and AMR/ARGs in the environment, which has accelerated environmental pollution. Therefore, management options are required to appropriately mitigate the new environmental contaminants. Overall, the book will facilitate future examinations

of antibiotics and AMR/ARGs in the environment by providing summaries of existing data and research related to antibiotics and AMR/ARGs and offering a framework for better understanding and analysing their environmental and social impacts.

Abbreviations

AMs	Antimicrobials
AGPs	Antimicrobial growth promotants
ARGs	Antibiotic resistance genes
ARB	Antibiotic resistance bacteria
AMR	Antimicrobial resistance
AD	Anaerobic digestion
CWs	Constructed wetlands
CEC	Cation exchange capacity
EU	European Union
EMA	European Medicines Agency
ECDC	European Centre for Disease Prevention and Control
EFSA	European Food Safety Authority
ELISA	Enzyme-linked immunosorbent assay
EPA	Environmental Protection Agency
EDCs	Endocrine disruptor chemicals
GDPPC	Gross domestic product per capita
HGT	Horizontal gene transfer
JIACRA	Joint Interagency Antimicrobial Consumption and Resistance Analysis
MDR	Multidrug resistant
MGEs	Mobile genetic elements
MBRs	Membrane bioreactors
MRSA	Methicillin-resistant *Staphylococcus aureus*
PPCPs	Pharmaceutical and personal care products
RO	Reverse osmosis
VRE	Vancomycin-resistant *E. coli*
WWTPs	Wastewater treatment plants
WHO	World Health Organization

Contents

Contributors

Yusuf A. Abou Jawdeh Faculty of Agricultural and Food Sciences, American University of Beirut, Beirut, Lebanon

Muhammad Afzaal Sustainable Development Study Centre, Government College University, Lahore, Pakistan

Sarfraz Ahmed Faculty of Veterinary Sciences Narowal, Department of Basic Sciences, University of Veterinary and Animal Sciences, Lahore, Punjab, Pakistan

Toqeer Ahmed Centre for Climate Research and Development (CCRD), COMSATS University Islamabad, Islamabad, Pakistan

Essa Ali Department of Food Science and Technology, Zhejiang University of Technology, Hangzhou, People's Republic of China

Jafar Ali Key Laboratory of Environmental Nanotechnology and Health Effects, Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences, Beijing, China

Muhammad Ishtiaq Ali Environmental Microbiology Laboratory, Department of Microbiology, Quaid-i-Azam University, Islamabad, Pakistan

Muniza Almas Botany Department, GC University, Lahore, Pakistan

Saadia Andleeb Atta-ur-Rahman School of Applied Biosciences, National University of Sciences and Technology, Islamabad, Pakistan

Muzammil Anjum Department of Environmental Sciences, Pir Mehr Ali Shah Arid Agriculture University, Rawalpindi, Pakistan

Taha Arooj Botany Department, GC University, Lahore, Pakistan

Ashraf Asma Department of Zoology, Faculty of Life Sciences, Government College University Faisalabad, Faisalabad, Pakistan

Muhammad Asrar Department of Zoology, Faculty of Life Sciences, Government College University Faisalabad, Faisalabad, Pakistan

Ashfaq Ahmed Bangash Universiti Teknologi PETRONAS, Seri Iskandar, Malaysia

Isam I. Bashour Faculty of Agricultural and Food Sciences, American University of Beirut, Beirut, Lebanon

Sayyed M. A. U. S. Bukhari Department of Microbiology, Abdul Wali Khan University, Mardan, Pakistan

Nageen Bushra Department of Zoology, Faculty of Life Sciences, Government College University Faisalabad, Faisalabad, Pakistan

Susana Correia Microbiology and Antibiotic Resistance Team (MicroART), Veterinary Sciences Department, University of Trás-os-Montes and Alto Douro (UTAD), Vila Real, Portugal

Associated Laboratory for Green Chemistry (LAQV-REQUIMTE), University NOVA of Lisboa, Lisboa, Caparica, Portugal

Department of Genetics and Biotechnology, University of Trás-os-Montes and Alto Douro (UTAD), Vila Real, Portugal

Functional Genomics and Proteomics Unit, University of Tras-os-Montes and Alto Douro (UTAD), Vila Real, Portugal

Lara J. El Gemayel Faculty of Agricultural and Food Sciences, American University of Beirut, Beirut, Lebanon

Fakhar-ud-Din Department of Pharmacy, Quaid-i-Azam University, Islamabad, Pakistan

Nadim Farajalla Issam Fares Institute for Public Policy and International Affairs, Beirut, Lebanon

Mohamad T. Farran Faculty of Agricultural and Food Sciences, American University of Beirut, Beirut, Lebanon

Saima Gul Department of Chemistry, Islamia College Peshawar, Peshawar, KP, Pakistan

Rumina Hasan Department of Pathology & Laboratory Medicine, Aga Khan University, Karachi, Pakistan

Faculty of Infectious and Tropical Diseases, London School of Hygiene and Tropical Medicine, London, UK

Zahra Hasan Department of Pathology & Laboratory Medicine, Aga Khan University, Karachi, Pakistan

Ghulam Hussain Department of Physiology, Faculty of Life Sciences, Government College University Faisalabad, Faisalabad, Pakistan

Sajjad Hussain Faculty of Materials & Chemical Engineering, GIK Institute of Engineering Sciences & Technology, Topi, KP, Pakistan

Syed Makhdoom Hussain Department of Zoology, Faculty of Life Sciences, Government College University Faisalabad, Faisalabad, Pakistan

Muhammad Zeeshan Hyder Department of Biosciences, COMSATS University Islamabad, Islamabad, Pakistan

Gilberto Igrejas Associated Laboratory for Green Chemistry (LAQV-REQUIMTE), University NOVA of Lisboa, Caparica, Portugal

Department of Genetics and Biotechnology, University of Trás-os-Montes and Alto Douro (UTAD), Vila Real, Portugal

Functional Genomics and Proteomics Unit, University of Tras-os-Montes and Alto Douro (UTAD), Vila Real, Portugal

Umesh B. Jagtap Department of Botany, Government Vidarbha Institute of Science and Humanities, Amravati, Maharashtra, India

Asif Jamal Environmental Microbiology Laboratory, Department of Microbiology, Quaid-i-Azam University, Islamabad, Pakistan

Muhsin Jamal Department of Microbiology, Abdul Wali Khan University, Mardan, Pakistan

Sana Jameel Department of Biotechnology, University of Sialkot, Sialkot, Punjab, Pakistan

Muhammad Khalid Department of Chemistry, Khwaja Fareed University of Engineering & Information Technology, Rahim Yar Khan, Pakistan

Paweł Krzemiński Section of Environmental Chemistry and Technology, Norwegian Institute for Water Research (NIVA), Oslo, Norway

Irfan Liaqat Centre for Climate Research and Development (CCRD), COMSATS University Islamabad, Islamabad, Pakistan

Department of Biosciences, COMSATS University Islamabad, Islamabad, Pakistan

Tariq Mahmood Department of Environmental Sciences, Pir Mehr Ali Shah Arid Agriculture University, Rawalpindi, Pakistan

Mahnoor Majid Atta-ur-Rahman School of Applied Biosciences, National University of Sciences and Technology, Islamabad, Pakistan

Afifa Malik Sustainable Development Study Center, Government College University, Lahore, Pakistan

Zdzisław Markiewicz Department of Applied Microbiology, Faculty of Biology, Institute of Microbiology, University of Warsaw, Warsaw, Poland

Microbiology Unit, Spectrum Dermatology, Seattle, WA, USA

Safdar Ali Mirza Botany Department, Government College University, Lahore, Pakistan

Sidra Mukhtar Sustainable Development Study Center, Government College University, Lahore, Pakistan

Hussain Muhammad Munawar Department of Chemistry, Khwaja Fareed University of Engineering & Information Technology, Rahim Yar Khan, Pakistan

Rabbia Murtaza Centre for Climate Research and Development (CCRD), COMSATS University Islamabad, Islamabad Campus, Islamabad, Pakistan

Kamal Ghulam Mustafa Department of Chemistry, Khwaja Fareed University of Engineering & Information Technology, Rahim Yar Khan, Pakistan

Masooma Nazar Sustainable Development Study Center, GC University, Lahore, Pakistan

G. I. B. Obioh Environmental Biotechnology & Bioconservation Department, National Biotechnology Development Agency (NABDA), Abuja, Nigeria

José Eduardo Pereira Veterinary Sciences Department, University of Trás-os-Montes and Alto Douro, Quinta de Prados, Vila Real, Portugal

Laboratório Associado for Green Chemistry (LAQV-REQUIMTE), University NOVA of Lisboa, Lisbon, Caparica, Portugal

Patrícia Poeta Microbiology and Antibiotic Resistance Team (MicroART), Veterinary Sciences Department, University of Trás-os-Montes and Alto Douro (UTAD), Vila Real, Portugal

Associated Laboratory for Green Chemistry (LAQV-REQUIMTE), University NOVA of Lisboa, Caparica, Portugal

Magdalena Popowska Department of Applied Microbiology, Faculty of Biology, Institute of Microbiology, University of Warsaw, Warsaw, Poland

Audil Rashid Department of Environmental Sciences, Pir Mehr Ali Shah Arid Agriculture University, Rawalpindi, Pakistan

Bilal Rasool Department of Zoology, Faculty of Life Sciences, Government College University Faisalabad, Faisalabad, Pakistan

Azhar Rasool Department of Zoology, Faculty of Life Sciences, Government College University Faisalabad, Faisalabad, Pakistan

Azhar Rasul Department of Zoology, Faculty of Life Sciences, Government College University Faisalabad, Faisalabad, Pakistan

Cell and Molecular Biology Lab, Department of Zoology, Government College University Faisalabad, Faisalabad, Pakistan

Muhammad Saif Ur Rehman Department of Chemical Engineering, Khawaja Fareed University of Engineering and Information Technology, Rahim Yar Khan, Pakistan

Ammara Riaz Department of Zoology, Faculty of Life Sciences, Government College University Faisalabad, Faisalabad, Pakistan

Luqman Riaz College of Life Sciences, Henan Normal University, Xinxiang, China

Rabia Safeer Department of Environmental Sciences, Pir Mehr Ali Shah Arid Agriculture University, Rawalpindi, Pakistan

Khizar Samiullah Department of Zoology, Faculty of Life Sciences, Government College University Faisalabad, Faisalabad, Pakistan

Sumbal Sardar Atta-ur-Rahman School of Applied Biosciences, National University of Sciences and Technology, Islamabad, Pakistan

Iqra Sarfraz Department of Zoology, Faculty of Life Sciences, Government College University Faisalabad, Faisalabad, Pakistan

Zeliha Selamoglu Department of Medical Biology, Faculty of Medicine, Nigde Ömer Halisdemir University, Nigde, Turkey

Nasir Shabab Department of Zoology, Faculty of Life Sciences, Government College University Faisalabad, Faisalabad, Pakistan

Sadia Shakoor Department of Pathology & Laboratory Medicine, Aga Khan University, Karachi, Pakistan

Anila Sikandar Department of Environmental Sciences, Pir Mehr Ali Shah Arid Agriculture University, Rawalpindi, Pakistan

Vanessa Silva Microbiology and Antibiotic Resistance Team (MicroART), Veterinary Sciences Department, University of Trás-os-Montes and Alto Douro (UTAD), Vila Real, Portugal

Associated Laboratory for Green Chemistry (LAQV-REQUIMTE), University NOVA of Lisboa, Caparica, Portugal

Department of Genetics and Biotechnology, University of Trás-os-Montes and Alto Douro (UTAD), Vila Real, Portugal

Functional Genomics and Proteomics Unit, University of Tras-os-Montes and Alto Douro (UTAD), Vila Real, Portugal

Amtul Bari Tabinda Sustainable Development Study Center, Government College University, Lahore, Pakistan

Izhar Ullah Department of Pharmacy, University of The Poonch Rawalakot, Rawalakot, Azad Kashmir, Pakistan

Saad Ullah Faculty of Materials & Chemical Engineering, GIK Institute of Engineering Sciences & Technology, Topi, KP, Pakistan

Hassan Waseem Environmental Microbiology Laboratory, Department of Microbiology, Quaid-i-Azam University, Islamabad, Pakistan

Department of Biotechnology, University of Sialkot, Sialkot, Punjab, Pakistan

Department of Civil and Environmental Engineering, Michigan State University, East Lansing, MI, USA

Qingxiang Yang College of Life Sciences, Henan Normal University, Xinxiang, China

Abdullah Yasir Sustainable Development Study Center, Government College University, Lahore, Pakistan

Sandra A. Youssef Faculty of Agricultural and Food Sciences, American University of Beirut, Beirut, Lebanon

Wei Yuan School of Environmental and Municipal Engineering, North China University of Water Resources and Electric Power, Zhengzhou, China

Magdalena Zalewska Institute of Microbiology, Faculty of Biology, University of Warsaw, Warsaw, Poland

Chapter 1
Entry Routes of Antibiotics and Antimicrobial Resistance in the Environment

Paweł Krzemiński, Zdzisław Markiewicz, and Magdalena Popowska

Abstract This chapter describes the current knowledge about the entry routes of antibiotics and antimicrobial resistance in the environment. It starts with an overview of the most important entry routes being wastewater and sludge from urban wastewater treatment plants, and natural fertilizers like pig slurry and cow manure and fertilizer from poultry farming. These sites are referred to as hotspots for antibiotic resistance genes (ARGs) and antibiotic resistance bacteria (ARB), including bacteria pathogenic for humans and animals, also those mentioned on the WHO priority list of global priority pathogens and of antibiotic-resistant bacteria. All these entry routes, manure, wastewater, and aquacultures, are characterized in terms of general sources of antibiotics, ARB, and ARGs. We also analyze the European Surveillance of Veterinary Antimicrobial Consumption report on the sale of antibacterials for veterinary. Furthermore, the EU guidelines to reduce the sales of veterinary antimicrobials across Europe under the umbrella of the EU One-Health Action Plan against Antimicrobial Resistance are mentioned. In the last section we point the need to develop and standardize the guidelines and method protocols for surveillance of AMR which need to be practicable, comparable, simple, and cost-effective so that they can be applied globally.

Keywords Entry routes · Antibiotics · Antimicrobial resistance · Environment

P. Krzemiński
Section of Environmental Chemistry and Technology, Norwegian Institute for Water Research (NIVA), Oslo, Norway
e-mail: pawel.krzeminski@niva.no

Z. Markiewicz
Institute of Microbiology, Faculty of Biology, University of Warsaw, Warsaw, Poland

Spectrum Dermatology, Seattle, WA, USA
e-mail: zd.markiewicz@uw.edu.pl

M. Popowska (✉)
Institute of Microbiology, Faculty of Biology, University of Warsaw, Warsaw, Poland
e-mail: magdapop@biol.uw.edu.pl

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1.1 Introduction

Antibiotics and antimicrobial resistance (AMR) can enter the environment through very many different routes, the most important being wastewater and sludge from urban wastewater treatment plants (WWTPs), and natural fertilizers like pig slurry and cow manure and fertilizer from poultry farming. These sites are referred to as hotspots for antibiotic resistance genes (ARGs) and antibiotic resistance bacteria (ARB), including bacteria pathogenic for humans and animals. In many cases, these are multidrug-resistant (MDR) strains, where ARGs are frequently carried on mobile genetic elements, notably plasmids and transposons, that can be transferred by different mechanisms of horizontal gene transfer (HGT) not only among bacteria of the same species, but also among different species. In the following sections, we present current knowledge on antibiotics and antimicrobial resistance entry routes in the environment, and finally we present a scheme of general sources of antibiotics ARB and ARGs and degradation mechanisms of antibiotics in the environment, in an attempt to better understand the complexity of the problem of dissemination of AMR.

1.2 Characterization of Entry Routes

The entry routes of antibiotics and AMR are mainly connected with the excretion of animal urine and feces (manure) from agriculture, where a large fraction of antimicrobial agents can be released into the environment in an active form. Another source represents WWTPs, where both sludge and treated wastewater are major pathways. For example, when sewage sludge is used as fertilizer or to condition soil, or when treated wastewater is reused for irrigating arable fields, the remaining antibiotics and ARB that are reservoirs of ARGs are introduced into the environment (Krzeminski et al. 2019). Finally, the third pathway is aquaculture, where antibiotics provided with a feed are frequently overused. Perhaps of lesser importance for AMR, there are also communal rubbish dumps; however in this case a leachate from a municipal solid waste is a real threat to environmental pollution by antibiotics and other drugs that are thrown into the household rubbish bins. The presence of antibiotics in the environment creates a selective pressure, which promotes the spread of AMR among bacteria, especially with a high potential of resistance genes located on mobile genetic elements (MGEs). This, in turn, may lead to the selection of resistant strains, which are also capable of moving between different environments (including people's microbiome and the hospital environment), thereby creating the potential for the movement of ARGs and associated MGEs (further covered in Chap. 14 on the fate of ARB and their AMR genes within the environment). Considering the above, it seems extremely important to develop effective methods of wastewater, water, and soil treatment to reduce spread and proliferation of AMR in the environment.

1.2.1 Manure

Manure is characterized by a rich chemical composition and contains, among others, compounds sometimes unavailable in mineral fertilizers. The advantage of manure is also that it slowly decomposes and does not cause soil salinity. Manure provides plants with basic nutrients such as nitrogen, phosphorus, and potassium. In addition, it also contains calcium, magnesium, boron, and iron, which are sometimes unavailable in mineral fertilizers (Szogi et al. 2015; Burton and Turner 2003). Manure spread and excavated with soil (optimally at a depth of 15–20 cm) also affects the humus formation. Manure types due to their origin:

Bovine manure—used on different soils, but it is recommended for sandy substrates in which nutrients are quickly washed away. It contains fewer nutrients, but is more abundant in potassium. The bovine manure contains an average of 0.9% N; 0.5% P_2O_5; 1.2% K_2O; 1.2% CaO, and 0.2% MgO at a water content of 34%.

Horse manure—causes rapid heating of the soil which is not always a beneficial process. It can be used on heavy and light soils. Horse manure contains an average of 0.7% N; 0.3% P_2O_5; 0.8% K_2O; at a water content of 55%.

Pig manure—a good solution for sandy substrates in the cultivation of plants that produce a large amount of green matter. It is nitrogen rich. The liquid manure is a mixture of animal manure and water. It is created in the non-bedding of swine as a by-product and waste product. Among the compounds included in its composition, it is possible to distinguish compounds that are mainly a solid fraction, for example, organic compounds and phosphorus compounds, as well as components of the liquid fraction, such as nitrogen compounds and minerals in the form of sodium, potassium, and magnesium oxides. However, the content of these substances depends on the method of breeding and feeding of pigs. Pig manure contains an average of 1.1% N, 0.6% P_2O_5, 0.7% K_2O, 0.4% CaO, and 0.2% MgO at a water content of 88% (Jørgensen and Jensen 2009).

Poultry manure—usually used as an additive to other fertilizers or a component of a compost prism. It contains a very large amount of nitrogen and its use may lead to a risk of overfertilizing the plants. It is usually recommended to dilute it and/or mix with other natural fertilizers. Poultry manure is used for fertilizing soils and for the production of various types of organic substrate, e.g., for growing mushrooms. The chemical composition of this fertilizer varies depending on the species. The chicken manure contains an average of 2.8% N, 1.2% P_2O_5, 1.4% K_2O, 2.4% CaO, and 0.7% MgO at a water content of 56%. Manure of waterfowl (duck and goose) contains 0.5–1.0% N, 0.5–1.4% P_2O_5, 0.6–0.9% K_2O, 0.8–1.6% CaO, and 0.2–0.3% MgO at a water content of 30%. Nitrogen in bird manure is predominantly in the form of uric acid, which quickly decomposes into ammonia. Poultry manure is recommended in doses of 10–15 t/ha, for the same plants and at the same times as traditional manure.

Natural fertilizers like manure can be harmful to health. Therefore, in the countries of the European Union, the rules for introducing these products for sale and use have been specified. Permission for introduction of fertilizer or other means is

issued by the minister competent for agriculture. Before being placed on the market, organic fertilizers must be carefully tested in terms of both fertilizer value and safety for humans, animals, and the environment.

Organic and organic-mineral fertilizers are subject to physicochemical and biological research. Physicochemical tests determine the content of organic matter, nitrogen, phosphorus, magnesium, and heavy metals in fertilizers. In addition to organic matter and heavy metals, the scope of fertilizer testing depends on the manufacturer's declaration. Biological examinations are aimed at excluding the presence of *Salmonella* and live parasitic eggs from *Ascaris* spp., *Trichuris* spp., and *Toxocara* spp.

The biological impurities introduced together with organic fertilizers into the environment include bacteria, viruses, fungi, and invasive forms of parasites, mainly intestinal. It is also known that the survival time of pathogenic organisms in the soil is from several days to even 10 years, and on plants from several days to 1 year.

The chemical composition of natural fertilizers is variable and depends on the species, age, direction of use and way of feeding animals, as well as the storage conditions of fertilizers.

In order to use natural fertilizers rationally and in accordance with the regulations, it is necessary to determine the permissible and optimal dose of fertilizers. The permissible dose is that in which the amount of nitrogen carried in does not exceed 170 kg N/ha. The optimum dose, depending on the nutritional requirements of the plants and soil availability, may be lower than the acceptable dose. When using natural fertilizers frequently and in high doses, particular attention should be paid to the abundance of soils in phosphorus, the excessive accumulation of which may pose a threat to the aquatic environment. Manure is not active enough to fully replace fast-acting mineral fertilizers, but it can significantly reduce expenditures on the total cost of fertilizers, because it is the cheapest of all known fertilizers. Liquid manure differs from manure not only in physical values but also in chemical composition and fertilizing action. In contrast to manure, it is a liquid fertilizer, more aggressive, and thus faster affecting the soil. In addition, its use by plants is very fast, which results from the fact that most fertilizer substances are in a mineral form, e.g., nitrogen from slurry is much better used by plants than from manure. Liquid manure is a mixture of feces and urine as well as water from washing up the stands. It is created in rooms adapted to keep animals without mulch. The content of fertilizing ingredients in slurry depends on the species and age of the animals and how they are used, the type of feeding, the degree of dilution, water, etc.

It should be remembered, however, that the aforementioned fertilizers are the essence of fertilization in so-called organic farming, whose aim is to maintain or increase the fertility and biological activity of the soil and create optimal conditions for the development of plants.

In manure, antibiotics from a wide range of classes are detected in the highest levels (Hu et al. 2010; Jechalke et al. 2014; Kemper et al. 2008; Lathers 2001; Marshall and Levy 2011; Sarmah et al. 2006). The second environment with high concentration of antibiotics are soils fertilized with manure, where antibiotics are washed off with groundwater from the soil and move forward (Anjum and Krakat

2015; De La Torre et al. 2012; Williams-Nguyen et al. 2016). Also veterinary medicine were antibiotics of the same classes as in human treatment are or have been used, carries the danger of the emergence and spread of AMR (Heuer et al. 2009; Martínez 2009). This phenomenon is particularly serious in the case of the pathogens transmitted via food, e.g., *Campylobacter jejuni*, *Escherichia coli*, *Salmonella*, and *Enterococcus faecium*. The same strains may hence colonize animals and humans, yet resistance genes disseminate easily between closely related species. This creates a serious threat of extensive antibiotic and/or resistance gene dissemination (Ding and He 2010; Popowska et al. 2010, 2012; Mąka et al. 2015a, b, 2018). Since January 1, 2006, antibiotics should not be used as growth promoters (http://europa.eu). Until 2006, 90% of antibiotics used in agriculture had been destined for growth stimulation and only 10% for fighting bacterial diseases. Statistical data indicate that in the last 50 years over one million of tons of antibiotics were introduced into the environment, 50% derived from veterinary medicine and agriculture (Allen et al. 2010). Despite the existence of legal acts regulating the use of antibiotics in veterinary medicine and animal husbandry, medically important antibiotics are still routinely used for livestock (Cantas et al. 2013). Many publications report the association between antibiotics (of 24 antibiotics used in animal and/or human medicine) and bacterial AMR of *Escherichia coli*, *Enterococcus faecalis,* and *Enterococcus faecium* in liquid pig manure used as fertilizer. Reported concentrations of antibiotics in manure are from residual levels to commonly 1–10 mg/kg or mg/L, yet concentrations of more than 50 mg/kg were also reported (Hölzel et al. 2010; Massé et al. 2014). A summary of the findings is presented in Table 1.1.

The levels of antibiotics found in manure might seem generally low, but the production of manure is on a big scale: in Europe, pigs and cows are reported to jointly produce 1.27 billion tonnes of manure per year. The consolidated data from 30 EU/EEA countries shows that more than 8300 tonnes of active ingredients were sold for use in animals in 2015 alone (EMA/ESVAC 2017; ECDC/EFSA/EMA 2017). In agricultural land where farm manures (both solid manures and slurries) are applied, it is estimated that, antimicrobials are being released into the environment in the region of kilograms per hectare per year (Kemper 2008). An example of the use of fertilizers on a large scale is the United Kingdom, where about 96 million tonnes of farm manures (both solid manures and slurries) are applied to agricultural land (Defra 2010). This is a real threat of environmental contamination with antibiotics used in livestock. The final concentration of antibiotics in manure and then in the agriculture soil is the resultant of various processes, among others, antibiotic metabolism and degradation processes. Some antibiotics bind strongly to soil and sediments, which contributes to their persistence as they become inaccessible to degradation (Kumar et al. 2005; Kühne et al. 2000; Rabølle and Spliid 2000; Sengelov et al. 2003). The rate of degradation of antimicrobials in the environment is dependent on a range of conditions, for example: antibiotic concentration, chemical structure of the compound, composition and structure and sorption properties of soil, pH, temperature, availability of oxygen and microorganisms that support biodegradation (Kümmerer 2004, 2009a, b). The application of manure to land poses a threat to contamination of the aquatic environment including surrounding surface

Table 1.1 Concentration of antibiotics in manure from global sources (Massé et al. 2014)

Class of antibiotic	Antibiotic	Source	Concentration	Reference
Ionophorous	Monensin	Beef manure stockpile	120 mg/kg	Dolliver et al. (2008)
Macrolide	Tylosin	Fresh calf manure	0.11 mg/kg	Jacobsen and Halling-Sørensen (2006)
		Beef manure stockpile	8.1 mg/kg	Dolliver et al. (2008)
Sulfonamide	Sulfadiazine	Swine manure	7.1 mg/L	Chen et al. (2012)
	Sulfonamides	Swine manure	2 mg/kg DM	Jacobsen and Halling-Sørensen (2006)
Tetracycline	Chlortetracycline	Swine manure	764.4 mg/L	Pan et al. (2011)
		Swine manure	139 mg/L	Chen et al. (2012)
		Swine manure storage lagoon	1 mg/L	Campagnolo et al. (2002)
		Beef manure stockpile	6.6 mg/kg	Dolliver et al. (2008)
	Doxycycline	Swine manure	37 mg/L	Chen et al. (2012)
	Oxytetracycline	Swine manure	354 mg/L	Chen et al. (2012)
		Manure	136 mg/L	Martínez-Carballo et al. (2007)
		Cow manure	0.5–200 mg/L	Ince et al. (2013)
		Fresh calf manure	10 mg/kg	De Liguoro et al. (2003)
	Tetracycline	Swine manure	98 mg/L	Chen et al. (2012)

water and groundwater. The antibiotic concentrations reported in aquatic environments are less than 10 μg/L (Kümmerer 2009a, b), which, however, taking into account the phenomenon of promoting antibiotic resistance in sub-lethal concentrations, creates a serious risk of antibiotic resistance spread (Andersson and Hughes 2014; Cairns et al. 2017; Friman et al. 2015; Händel et al. 2013).

Manure is also the reservoir of AMR and ARGs, and thus poses a risk for animal or human health (Marshall and Levy 2011; Storteboom et al. 2007; Thanner et al. 2016). It has been shown that manure is a "hot spot" of ARB, which carrying ARGs on mobile genetic elements (MG) and via horizontal gene transfer (HGT) these ARGs can be transferred to the soil bacteria (Cytryn et al., 2017; Binh et al. 2008; Fahrenfeld et al. 2014; Wolters et al. 2014). ARB are also found in meat, generating a real risk of pathogen infections in human. Examples are *Staphylococcus aureus* (MRSA), extended-spectrum beta-lactamase (ESBL)-producing or AmpC beta-lactamase-producing *Escherichia coli*, and *Enterococcus faecalis* (Endimiani et al. 2012; EU 2015; Overesch et al. 2013; Schmidt et al. 2015; Vogt et al. 2014). These bacteria were included on the WHO priority list on February 2017, the first ever list of global priority pathogens (global PPL) and of antibiotic-resistant bacteria. Totally, 12 families of bacteria identified as posing the greatest threat to human health were mentioned (Table 1.2). These bacteria were grouped into three classes in the order of risk: I—critical, II—high, and III—medium priority. Due to the high

Table 1.2 WHO global PPL of antibiotic-resistant bacteria (WHO 2017)

Classification	Antibiotic resistant
Priority 1: Critical	
Acinetobacter baumannii	Carbapenem-resistant
Pseudomonas aeruginosa	Carbapenem-resistant
Enterobacteriaceae	Carbapenem-resistant, ESBL-producing
Priority 2: High	
Enterococcus faecium	Vancomycin-resistant
Staphylococcus aureus	Methicillin-resistant, vancomycin-intermediate and resistant
Helicobacter pylori	Clarithromycin-resistant
Campylobacter spp.	Fluoroquinolone-resistant
Salmonellae	Fluoroquinolone-resistant
Neisseria gonorrhoeae	Cephalosporin-resistant, fluoroquinolone-resistant
Priority 3: Medium	
Streptococcus pneumoniae	Penicillin-non-susceptible
Haemophilus influenzae	Ampicillin-resistant
Shigella spp.	Fluoroquinolone-resistant

level of resistance among these bacteria and the presence of MDR strains, new antimicrobial agents targeting this priority list of pathogens are needed. According to the WHO "The list was drawn up in a bid to guide and promote research and development (R&D) of new antibiotics, as part of WHO's efforts to address growing global resistance to antimicrobial medicines." It is important to realize that R&D of new antibiotics is not enough and cannot solve the problem. It is equally important to use better prevention of infections and appropriate use of existing antibiotics in humans and animals.

The European Medicines Agency (EMA) published on 15th October 2018, the eighth ESVAC (European Surveillance of Veterinary Antimicrobial Consumption) report on the sale of antibacterials for veterinary use in 2016 (EMA/ESVAC 2018). On average, sales of antibacterial agents for veterinary use in the EU decreased by 20% between 2011 and 2016. Sixteen countries experienced a reduction of 9–58%. Not all countries, however, experienced such a reduction as six countries experienced an increase of 8–68%. What can be a reason for satisfaction, the sale of critical substances, which are antibacterial substances that are important for public health, amounted to 0.21 mg/PCU, 2.70 mg/PCU, and 6.62 mg/PCU, respectively, for third and fourth generation cephalosporins, fluoroquinolones, and polymyxins. The data shows there was a drop of almost 40% in sales of polymyxins for veterinary use. This class includes colistin, which is used as a last resort treatment in patients with bacterial infections resistant to other antibiotics. Sales of third and fourth generation cephalosporins decreased by 15.4%, while sales of quinolones declined by 13.6%.

This continues the downward trend seen over the last few years and confirms that EU guidance and national campaigns promoting prudent use of antibiotics in

animals to fight antimicrobial resistance are having a positive effect. Reduction in sales is the result of combined efforts of the European Commission, EMA, EU Member States, veterinarians, farmers, and other actors in the livestock sector. EU guidance together with national campaigns for prudent use of antibiotics in animals, sales targets, and restriction of use of some antimicrobials in food-producing animals are among the actions implemented to reduce the sales of veterinary antimicrobials across Europe under the umbrella of the EU One-Health Action Plan against Antimicrobial Resistance (EU 2017; WHO—http://www.who.int/features/qa/one-health/en/). Actions to reduce the emergence and spread of AMR are also carried out.

The results of studies on AMR in soil fertilized with manure indicate that the spread of manure leads to a temporary increase in the occurrence of AMR in the manure-amended soil (Bengtsson-Palme et al. 2018; Kumar et al. 2018; Scott et al. 2018). Thus, the use of manure in agriculture contributes to the global dissemination of AMR in the environment (Heuer et al. 2011a, b; Jensen et al. 2002; Sengelov et al. 2003). Therefore, a matter of high importance is the right manure treatment strategies to reduce or eliminate the risk of the release of antibiotics and ARGs from manures to the environment. More information on AMR and ARGs in natural fertilizers is provided in Chap. 6: "Antimicrobial/antibiotic resistance gene due to manure and agricultural waste applications," and on treatment technologies for removal of antibiotics, ARB, and ARGs is posted in Chap. 19.

1.2.2 Wastewater Treatment Plants (WWTPs)

WWTPs where sewage is collected and then treated serve an essential role in the protection of human and environmental health. WWTPs therefore meet the principles outlined in the WHO concept—One-Health approach. Unfortunately, traditional WWTPs are designed to remove conventional pollutants, including organic matter, suspended solids, and nutrients like nitrogen and phosphorus. In many cases traditional WWTPs are effective in eliminating some pathogens but are not designed for the removal of antibiotics or ARGs (Agga et al. 2015; Manaia et al. 2018; Novo et al. 2013; Krzeminski et al. 2020; Pruden et al. 2013; Schwermer et al. 2018). Together with sewage to WWTPs enter antibiotic residues from different sources (hospital, pharmaceutical industry, and household) and other co-selecting factors, such as chemical pollutants (e.g., pesticides), heavy metals, and disinfectants/surfactants. Sewage microbiota is mainly composed of human commensal bacteria, which is mixed with bacteria that may be colonizing the sewage system (Cai et al. 2014; Shchegolkova et al. 2016; Wang et al. 2014). The ARB fraction in sewage may reach more than 50%, mainly in a given group: enterobacteria or enterococci (Manaia et al. 2016; Rizzo et al. 2013). Considering the very good conditions for bacterial growth in a bioreactor system such as WWTPs, the presence of these abovementioned factors causes selection pressure and the phenomenon of co-selection, which in turn promotes horizontal gene transfer (Di Cesare et al. 2016;

Schlüter et al. 2007). Besides, the mobile genetic elements (MGEs) are in fact postulated as important vectors of ARGs between bacterial strains (Dröge et al. 2000; Kim et al. 2014; Marano and Cytryn 2017; Perry and Wright 2013; Szczepanowski et al. 2009).

Analysis of the effectiveness of removing various antibiotics from wastewater in WWTPs showed that removal is achieved mainly via chemical treatment and/or bioadsorption onto particulates, and subsequent physical separation from municipal wastewater. The removal is not a result of biological degradation, which is relatively less effective in removing antibiotics from the municipal wastewater (Michael et al. 2013). For some antibiotics such as sulfonamides like sulfamethoxazole and quinolones like norfloxacin and ciprofloxacin the detected abundance in the effluent still remained high, ranging at concentrations of 119–544 ng/L, 24–175 ng/L, and 11–168 ng/L, respectively (Senta et al. 2013). Generally, at least 56 antibiotics belonging to six different classes have been widely detected at nanogram per-liter to microgram per-liter levels in sewage of East Asia, North America, Europe, and Australia (Zhang and Li 2011). Many studies have shown the occurrence of chemical contaminants including antibiotic residues and their uncontrolled emission in the environment, which contributes to the proliferation of ARB and their associated genes, especially ARGs (Berendonk et al. 2015; Berglund et al. 2015; Manaia 2017; Manaia et al. 2016; Michael et al. 2013; Rizzo et al. 2013). The results of numerous studies indicate that WWTPs are "hot spots" of ARGs and ARB (Baquero et al. 2008; Michael et al. 2013; Piotrowska et al. 2017a; Zhang et al. 2009a; b). Depending on the treatment technology used and the sources of sewage coming to a WWTP (hospital, municipal effluent), the variability of diverse ARB including multidrug-resistant (MDR) strains of clinically relevant bacteria and ARGs for important antibiotics in medicine and veterinary medicine (B-lactams, macrolides, sulfonamides, fluoroquinolones and tetracyclines) are observed (Hong et al. 2013; Michael et al. 2013; Rizzo et al. 2013). For example, β-lactamase genes (https://www.lahey.org) belonging to the AmpC, ESBL, KPC, and NDM groups have been found in bacteria in wastewater (Amador et al. 2015; Gatica et al. 2016; Khan and Parvez 2014; Picão et al. 2013; Piotrowska et al. 2017a; Varela et al. 2016; Zhang et al. 2009a, b). A study of Chen and Zhang (2013) using QPCR methods found that tetracycline resistance genes (e.g., tetA, tetB, tetC, tetD, tetE, tetG, tetK, tetL, tetM, tetO, tetP, tetS, tetX) were present in the activated sludge sampled from 15 WWTPs at different geographical locations. Others indicated the ARGs are widespread in the effluent, for example: sulfonamide resistance genes (sul1 and sul2); erythromycin resistance genes (ermB, ermF); and vancomycin resistance gene (vanA) (Bockelmann et al. 2009; Burch et al. 2013; Chen and Zhang 2013; Fahrenfeld et al. 2013; Negreanu et al. 2012; Zhang et al. 2009a, b). ARBs found at different stages of the treatment process in municipal WWTPs belong mainly to *Acinetobacter* spp., and to enterococci and *Enterobacteriaceae*, which includes those mentioned in the list of global priority pathogens list (Table 1.2). Analysis of the results published showed that many of the enterococci and *Enterobacteriaceae* were resistant to more than one antibiotic (Ferreira da Silva et al. 2006, 2007). In addition, it has been shown that for some bacteria of *Escherichia*, *Shigella* and *Klebsiella* spp. resistant to more than

two antibiotics increased from an average 11% in the raw wastewater to 21% in the treated wastewater. Similarly, the collective proportion of these *Enterobacteriaceae* which were resistant to three antibiotics increased from 5.5 to 14.1% in the treated wastewater. This observation further suggested that the conventional municipal wastewater treatment scheme does not effectively remove viable *Enterobacteriaceae* that are resistant to antibiotics. Goldstein et al. (2012) reported evidence for the presence of methicillin-resistant *Staphylococcus aureus* (MRSA) in the effluent of four U.S. WWTPs. Likewise, bacteria resistant to clinically important antibiotics, including ciprofloxacin and vancomycin, have been found in the activated sludge (Nagulapally et al. 2009). Since many similar examples can be given, the effluent of WWTPs, if not purified sufficiently, poses a threat to public health (Hong et al. 2013; Walsh et al. 2011). To analyze ARB and ARGs different approaches are used: targeted (culture-based and quantitative PCR) and non-targeted like metagenomics. However, it should be remembered that depending on the type of environment, less than 1–10% of bacteria can be culturable (Vaz-Moreira et al. 2013). That is why it seems that the combination of culture-based methods with culture-independent approaches may be the ideal way to explore the environmental resistome (Li et al. 2015; Port et al. 2014; Yang et al. 2014).

Water scarcity is a global issue, especially in certain regions like Africa, the Middle East, southern Europe, as well as the western states of America. Thus, in these regions irrigation with treated wastewater is likely an important entry route of antibiotics into soil ecosystems. Multiple studies point out the presence of ARGs and ARB in wastewater effluent (Auerbach et al. 2007; LaPara et al. 2011; Manaia et al. 2010; Munir et al. 2011; Piotrowska and Popowska 2015; Piotrowska et al. 2017a). Unfortunately, the effects of antibiotics introduced into the soil on ARGs or ARB levels in soil has been little explored (Negreanu et al. 2012; McLain and Williams 2014). Antibiotics transferred into the soil may also affect the microorganisms and other biota inhabiting the niche and also the different processes in soil (Aga et al. 2016; Majewsky et al. 2014; Williams-Nguyen et al. 2016).

1.2.3 Aquaculture

Aquacultures are an intensively developing, fastest-growing food industry in the world. Continuous intensification of fish farming, increasing the risk of disease, has resulted in the widespread application of antibiotics treatment (Bostock et al. 2010). Consequently, the occurrence of ARGs in commercially available fish meals has been reported. Han et al. (2017) found 132 AMR genes in fishmeal from Russia and from China and these resistance genes were also isolated from sediment bacteria. Hence in aquacultures, where antibiotics are used as feed additives, the number of strains non-susceptible to antibiotics is many times greater (Baquero et al. 2008). The type and amount of use of antibiotics in aquaculture depends on farming practices, different local and national regulations and government enforcement ability. However, in many countries that are major aquaculture producers, regulation and

enforcement is very weak and, it seems that inadequate. In aquaculture, antibiotics are used in range: from 1 g in Norway to 700 g in Vietnam per metric ton of production (Defoirdt et al. 2011). The low use of antibiotics in Norway is a results of a national strategy to substitute antibiotic treatment with vaccinations. The antibiotics officially approved for use in the treatment and prophylaxis of cultured aquatic animals belongs to several classes and their representatives are: oxytetracycline, florfenicol, sarafloxacin, erythromycin, sulfonamides with trimethoprim or ormethoprim (Serrano 2005; Kümmerer 2009a). The literature data indicate that up to 75 percent of antibiotics used in aquaculture may be released to the surrounding environment and the presence of antibacterial compounds strongly disturbs the microbiome composition. The application of antimicrobials affects targeted pathogens as well as a wide variety of environmental bacteria, resulting in selection of AMR strains. The presence of AMR strains increases the risk of HGT to potential human pathogens (Zhang et al. 2009a, b). Some antibiotics in aqueous medium are not biodegradable and accumulate, e.g., by the process of adsorption on solid surfaces including bottom sediments or silt (Kümmerer 2004, 2009b). As research indicates, a minimum of 75% of antibiotics added to the feed of farmed fish enters the aquatic environment and accumulate in sediments (Lalumera et al. 2004). In the review article by Caruso (2016), there are many examples of research work on AMR and HGT in aquacultures. Generally, till now ARGs have been described in bacteria responsible for fish diseases, such as *Aeromonas hydrophila, Aeromonas salmonicida, Edwardsiella tarda, Edwardsiella icttaluri, Vibrio anguillarum, Vibrio salmonicida, Pasteurella piscida, Yersinia ruckeri,* or *Piscirickettsia salmonis* (Serrano 2005; Henríquez et al. 2016). Two bacterial genera have been found in aquacultures with a high frequency: *Aeromonas* spp. and *Vibrio* spp. What is interesting, bacteria of the genus *Aeromonas* isolated from fish ponds exhibit resistance to multiple antibiotics, and the resistance genes for these therapeutics are primarily located on plasmids and integrons (Baquero et al. 2008; Piotrowska and Popowska 2014, 2015; Piotrowska et al. 2017b). What is very important, since some of the *Aeromonas* strains also cause disease in humans and become the same specific vector that connects both environments, they may transfer MGEs carrying resistance genes to pathogenic or opportunistic bacteria in the human microbiome. These data clearly indicate that aquacultures are a reservoir of antibiotic resistance genes, and therefore pose a great risk to the health and life of humans (Agersø and Petersen 2007; Cabello et al. 2013; Furushita et al. 2003; Kemper 2008; Miranda et al. 2018). The significantly greater percentage of an aquaculture's strains are non-susceptible to tetracycline, streptomycin, and erythromycin, but the resistance mechanism against those antibiotics has been explained in only 50% of the resistant isolates. Literature data indicates that the dominant mechanisms of tetracycline and erythromycin resistance are: efflux pumps, ribosomal protection, or enzymatic modification of rRNA (Miranda et al. 2003; Muziasari et al. 2014; Patterson et al. 2007; Piotrowska et al. 2017b; Tamminen et al. 2010). The latter is dominant in the streptomycin-resistant aquatic isolates (Mohapatra et al. 2008). Quinolone, florfenicol, flumequine, ampicillin, or oxolinic acid-resistant strains are found with lesser frequency in aquaculture (Miranda et al. 2018; Su et al. 2011). On the contrary, bacteria resistant to

gentamicin, kanamycin, flumequine, and enrofloxacin have been reported to account for a low percentage of the total of the isolates (Miranda and Zemelman 2002). Very often, specific ARGs are detected simultaneously during the analysis of fish farm effluents. The results of the research of coastal aquaculture located in South Korea revealed 22 ARGs encoding tetracycline resistance (tetA, tetB, tetD, tetE, tetG, tetH, tetM, tetQ, tetX, tetZ, tetBP), sulfonamide resistance (sul1, sul2), quinolone resistance (qnrD, qnrS, aac(60)-Ib-cr), b-lactams resistance (blaTEM, blaCTX, blaSHV), macrolide resistance (ermC), florfenicol resistance (floR), and multidrug resistance (oqxA) and a class 1 integrons-integrase gene (intI1) were quantified (qPCR) (Jang et al. 2018). The same ARGs have been found in the commercial fish and seafood (Ryu et al. 2012) which already creates real threats to human health. Yang et al. (2013) detected with metagenomic method 58 genes codifying for resistance against 11 antibiotics. Many of these genes are located on MGE with more than 90% similarity with transposons and plasmids described for human pathogens, which suggests the possibility of occurrence of mobility of these ARGs to human pathogenic bacteria (Yang et al. 2013; Chen et al. 2018) or the potential risk of the ARGs spreading to other environments (Muziasari et al. 2017). The AMR strain from fish farms have been detected in nearly all key countries responsible for the production of farmed fish: United States (Seyfried et al. 2010), Pakistan and Tanzania (Shah et al. 2012), Australia (Akinbowale et al. 2006), China (Su et al. 2011), and Chile (Miranda et al. 2018).

It seems obvious that the overuse of antibiotics in aquaculture, especially prophylactic use, must be stopped. Antibiotics should be used only to treat diagnosed bacterial diseases. However, the question how to do it effectively, remains unanswered. It seems that the best solution would be to change food-safety regulations that set maximum residue limits or the use of special aquaculture ecolabeling schemes. For example, in Norway, veterinarians, fish farmers, and feed producers are legally obligated to report antibiotics use and prescriptions to a government agency. Due to such strict regulations, this data is made publicly available. In Norway, such data show a 99% reduction in antibiotic use in the Norwegian salmon industry since the early 1980s (Taranger et al. 2015). Therefore, implementation of legal obligation to report antibiotics use in food animals to a government agency in all countries is urgently needed. This approach will enable meaningful comparisons between species, countries, and over time of application. It is only on the basis of such aggregate data will it be possible to demonstrate any reduction or change in antibiotics use. Another very interesting approach is introduction of the farm level certification system (https://www.asc-aqua.org/). It is known that, only 5% of farmed seafood is currently certified, but its market share is growing because of consumer awareness about this framework. It is also extremely important to raise the awareness of fish farmers and veterinarians regarding the use of medically important antimicrobial drugs in food-animal production, and the public health risks associated with antibiotic resistance. “At a joint aquaculture and agriculture industry roundtable discussion in Oxford, UK, in May 2014, participants including vets, food scientists, farmers, and representatives of the food and animal health industries agreed that the development of a ‘replace, reduce, refine’ strategy could

help drive the responsible use of antibiotics in food-producing animals" (Taranger et al. 2015). At the same time, the Aquaculture Stewardship Council (ASC) recommends development of alternative treatments and vaccines (https://www.asc-aqua.org/). There are a lot of methods capable of removing antibiotics from aquaculture systems: physical, chemical, and biological methods including adsorption, biodegradation, disinfection, membrane separation, hydrolysis, photolysis, and volatilization (Chuah et al. 2016; Feng et al. 2016). The use of the "One Health approach" seems to be extremely important in the case of aquacultures, because this framework recognizes the interconnectedness of aquaculture production and human health especially in the aspect of linkage between antibiotic use in aquaculture and AMR pathogenic bacteria in humans.

1.2.4 Airborne ARGs

In recent years, the interest in the potential spread of airborne ARGs has increased. So far, scientists have been able to demonstrate ARGs in air samples collected from places around WWTPs, for example, they detected sul2 and class 1 integrase (Li et al. 2016). Similar research was carried out in the vicinity of composting plant, cattle feed yards, metro station, and hospital, where ARGs were detected in the airborne particulate matter (PM) (Hu et al., 2018; Gao et al. 2014, 2018; McEachran et al. 2015; Zhou and Wang 2013). In addition, mexF was found in airborne samples collected from sludge and animal feces (Yang et al. 2018).

These studies indicate the main threat to human respiratory tract infections as well as the possibility of transferring ARGs to other places as a result of rainfall and thus the possibility of threatening water or soil ecosystems (Ahmed et al. 2018).

Li et al. (2018) studied 39 ARG subtypes coding resistance to seven common classes of antibiotics (aminoglycosides, β-lactams, macrolides, sulfonamides, tetracyclines, quinolones, and vancomycins) and two MGE genes (tnpA encoding transposase and intI1 encoding integrase class I), which were screened by a high-throughput real-time qPCR platform. The study samples were total PM across 19 world cities, sampled and grouped by year (2004, 2009, 2014), and by season. The obtained results allowed for the detection of 30 ARG subtypes and from all the cities the highest richness (up to 18 subtypes) of airborne ARGs was found in Beijing, compared with Bandung where only 5 subtypes of ARGs were found. The most frequent genes were resistance genes to β-lactams (blaTEM was found to be most abundant) and quinolones (qepA), followed by macrolide, tetracycline, sulfonamide, aminoglycoside, and vancomycin. The highest abundance of β-lactam resistance genes was detected in San Francisco in contrast to Johannesburg, Zurich, and Hong Kong. In each of 19 cities except Melbourne intI gene was detected, and the TnA gene was determined in only 4 cities, with the largest amount being in Beijing. It is worth noting that in general these two genes, blaTEM and qepA, as well as the class 1 integron-integrase gene, IntI1, are widespread across various external environments such as sediment, water, soil, and wastewater/sludge (Pal et al. 2016). As

is known, MGEs are associated with HGT and therefore may be responsible for the dissemination of detected ARGs, which already poses a health risk (Xie et al. 2018).

Li et al. (2018) also analyzed the bacterial communities associated with the air-borne ARGs detected for 19 cities. Fifty most abundant bacterial genera were detected, among others *Corynebacterium, Albirhodobacter*, *Burkholderia*, *Escherichia/Shigella*, *Brevundimonas*, *Streptococcus*, *Delftia*, *Serratia*, *Lactobacillus*, *Mathanosarcina*, *Bacillus*, *Raoultella*, *Acinetobacter,* or *Pseudomonas*; among them also pathogenic bacteria for humans and animals. In other work, 69 air samples were collected every 4 h continuously, both day and night, over 6 days in Beijing. Not only was the blaTEM gene detected but also the multidrug-resistant NDM-1 gene and vanB gene. In addition, increase in the abundance of ARGs in the more polluted air, as well as of MGEs tnpA and intI1, was also observed. Of the ARGs detected, the sul3 gene proved to be the most widespread among the culturable *Bacillus* isolates in the air (Zhang et al. 2019).

Although the evidence of the studies carried out so far on airborne ARGs is not sufficient to specify their public health impact and to determine the real risk to public health, the results definitely indicate the need for redefining our current air quality standards. Consequently, studies on the dissemination of ARB and ARGs require global research to demonstrate their importance for public health.

1.3 Mutual Interactions: A Conceptual Model for Understanding Entry Routes of Antibiotics, ARB, and ARGs

As comprehensively explored above, the scope and dynamics of ARB and ARGs in each of the entry routs are determined by many factors. The main ones may include: a natural, for each entry route, microbiome and resistome (biodiversity); the abundance and diversity of ARGs and ARB introduced within the different environments; the ability to mobilize genes and the ability of bacteria to survive and replicate in different ecosystems (horizontal and vertical transfer); the scope and intensity of selective pressure (i.e., presence of residual antibiotic compounds, heavy metals, biocides or detergents); and environmental conditions (e.g., temperature, moisture, pH, amount of rainfall, availability of organic matter, nutrients). In addition, there are also mutual interactions between all entry routes that can generate, through dust and air-borne aerosols, new threats of the dissemination of ARB and ARGs to humans, animals, or food. According to the One Health approach, we can additionally superimpose the transfer of AMR between people, animals, animals and humans, which complicates this scheme even further.

A conceptual model that summarizes the various factors, conditions, and interactions that impact ARB and ARGs transfer is shown in Fig. 1.1. This model is not complete and, depending on the specificity of place and climate, other factors involved in the dissemination of ARB and ARGs, such as flies, river and lake waters can probably be of importance.

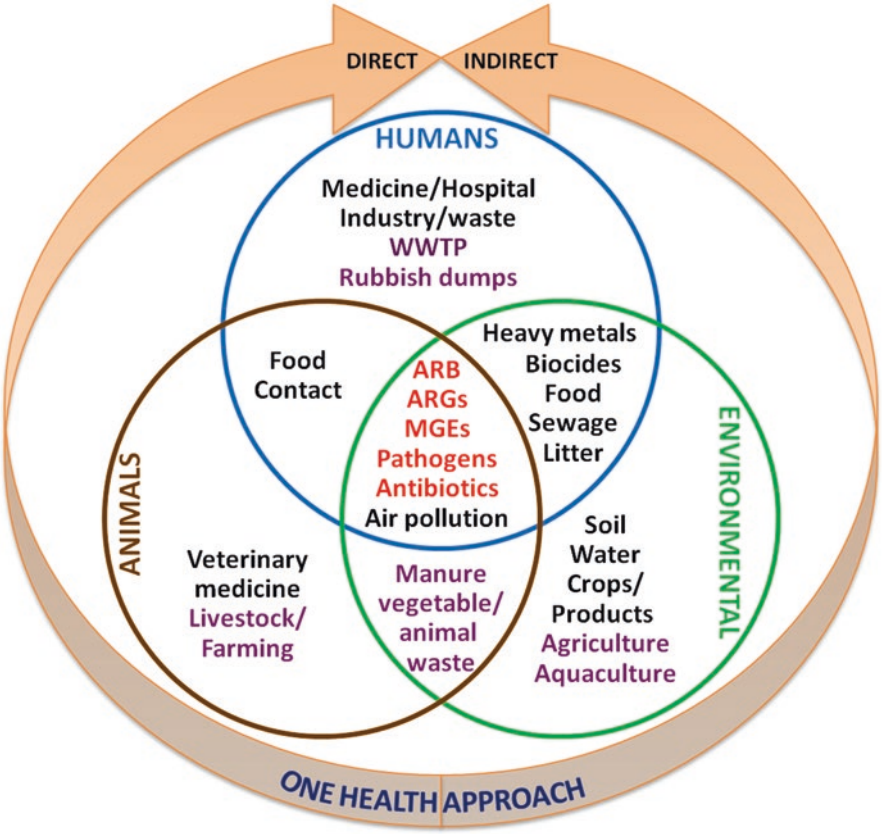

Fig. 1.1 A conceptual model, according to the One Health approach, that summarizes the interactions that impact the dissemination of antibiotics, ARB, and ARGs transfer

1.4 Summary and Perspectives

Antibiotics, ARB, and ARGs are widespread in the environment, not only in the clinical one but also in the natural one. Yet, it is difficult to clearly identify what constitutes the greatest threat to humans and animals. It is known that in the natural environment, as a result of the presence of subminimal inhibitory concentrations of antibiotics, new resistance mechanisms or resistance genes encoded on mobile genetic elements can be propagated through horizontal gene transfer. Therefore, antibiotic pressure does not eliminate as many bacteria as in a clinical environment. When high concentrations of antibiotics are used, only a single selected bacterial strain resistant to one or, more often, many antibiotics survives. In addition, in the natural environment there are co-selection mechanisms associated with resistance to heavy metals and biocides, which are also widespread in the environment. The resistance determinants for metals and biocides can be co-localized to the same

MGEs as antibiotic resistance genes, which mean that even in the absence of an antibiotic in the environment, a specific MGE is maintained and disseminated. However, WWTPs seem to be the most dangerous of the entry routes for resistant pathogenic bacteria, including MDR strains. Since the human feces are the main sources of pathogenic bacteria, the risk of human fecal contamination should not be neglected. There is definitely a greater risk of the transfer of resistance genes between pathogens in the human microbiome than the transfer of the same genes from environmental bacteria. On the other hand, there are probably more possibilities in the environment for the creation of new antibiotic resistance mechanisms and horizontal transfer events that lead to the phenomenon of co-selection.

After the antibiotics were used globally on a huge scale in the last few decades, their use as growth stimulators was prohibited in Europe. Nevertheless, their massive use in agriculture and animal husbandry in India and China makes it impossible to eliminate the problem of antibiotic resistance. Even though in many countries outside Europe there are antibiotic protection programs dedicated to the clinical environment, ARB do not recognize borders. Unfortunately, there are no regulations to monitor the problem of ARB dissemination in the environment and the main route of antibiotics, ARB, and ARGs, or use of WWTP sludge as natural fertilizers without proper treatment in agriculture. Furthermore, chemical plant protection products in agriculture as well as widespread environmental pollution with toxic compounds such as xenobiotics is also important contributing factor. All this causes the reduction of the proper microflora in a given environment, creating the niche for opportunistic and pathogenic bacteria. However, there is still insufficient knowledge about the barriers to the spread of antibiotic resistance in the environment and the importance of biodiversity in this process.

It is also necessary to develop and standardize the guidelines and method protocols for the surveillance of AMR, allowing to estimate precise values of the abundance of ARB and ARGs in WWTP discharges and to determine the fate of ARBs and ARGs. Such guidelines and protocols need to be practicable, comparable, simple, and cost-effective so that they can be applied globally.

However, to effectively limit the spread of ARB and ARGs via environmental pathways it is necessary to develop and implement new policies and regulations, which should be used globally and not locally. It should also be mentioned that the development of effective treatment strategies for removal of antibiotics, ARB, and ARGs is also extremely important.

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Chapter 2
Antibiotics Use in Hospitals and Their Presence in the Associated Waste

Luqman Riaz, Qingxiang Yang, Anila Sikandar, Rabia Safeer, Muzammil Anjum, Tariq Mahmood, Muhammad Saif Ur Rehman, Audil Rashid, and Wei Yuan

Abstract Over the last two to three decades, a large number of antibiotics from different groups have been developed to cure variety of bacterial infections in hospitals. These antibiotics, when administered orally to the patients, resulted in the detection of large amounts of antibiotics in hospital waste through excretion due to their limited metabolization in human body, coupled with the inappropriate use in the hospitals. Further, many antibiotic-resistant bacteria present in patient intestine also enters the hospital waste through excretion. The primary treatment system to treat hospital waste does not exist in developing countries. This issue is also pronounced in many developed regions and result in the release of large amount of antibiotics, antibiotic-resistant bacteria, and antibiotic resistance genes in the hospital waste. The hospital waste will create conditions that are conducive for the bacteria not only to multiply but also help to share their resistance genes with other bacteria through mobile genetic elements. Thus, the antibiotics and antibiotic-resistant bacteria that were present in the hospital waste can reach the drinking water, surface water, and marine and soil environment where they can cause variety

L. Riaz (✉) · Q. Yang
College of Life Sciences, Henan Normal University, Xinxiang, China
e-mail: yangqx@htu.edu.cn

A. Sikandar · R. Safeer · M. Anjum · T. Mahmood · A. Rashid
Department of Environmental Sciences, Pir Mehr Ali Shah Arid Agriculture University, Rawalpindi, Pakistan
e-mail: audil@uaar.edu.pk

M. S. U. Rehman
Department of Chemical Engineering, Khawaja Fareed University of Engineering and Information Technology, Rahim Yar Khan, Pakistan
e-mail: saif@kfueit.edu.pk

W. Yuan
School of Environmental and Municipal Engineering, North China University of Water Resources and Electric Power, Zhengzhou, China
e-mail: yuanwei@ncwu.edu.cn

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of problems through altering the ongoing processes in the ecosystem, toxicity to various species followed by the threat to humans through food chain.

Keywords Antibiotics · Hospitals · Associated waste

2.1 Introduction

Antimicrobials are considered as most effective drug for the treatment of various infections in humans. In the history of medicine, penicillin was marked as the beginners of the "golden era" of antibiotics. Various antibiotics from different families were produced during 1940–1962 for the treatment of various infections caused by variety of bacteria. In the past two to three decades several new antibiotics were also developed through structural modifications of previous antibiotics to make them remain effective. These antibiotics were extensively employed in human healthcare due to their essential requirement for bacterial infection treatment, surgical involvements, and prophylactic cure and in cancer. This has resulted in the increase in global use of antibiotics due to which the usage were at the peak between 2000 and 2010 worth $40 billion per year (O'Neill 2015) with expected 36% increase in the future, which demonstrate their importance from societal and economic perspective (Van Boeckel et al. 2014). Today, antibiotics have the wide range of usage in hospitals (Cabello 2006; Sarmah et al. 2006) for the cure of various diseases in hospitals but massive development and flourishing inappropriate usage (Van Boeckel et al. 2014, 2015) have resulted in many challenges. Beside of antibiotics significance in treatment of diseases in hospitals, they are of concern due to their potential genotoxic effects. Further, the detection of large number of antimicrobials in the environment poses some serious threats including alteration in aquatic ecosystem, spread of antimicrobial resistance, and threats to public health (Daughton and Ternes 1999; Boxall 2004; Runnalls et al. 2010; Brandt et al. 2015).

2.2 Antibiotics and Their Use in Hospitals: An Overview

Antibiotics are designed to have such molecular structures and functions which make them to show activity against variety of bacteria which are responsible for causing many infections related to gastrointestinal tract, respiratory tract, skin infections, and many sexually transmitted diseases. Globally about 30% increase in antibiotic consumption was observed during 2000 and 2010 reaching 50–70 billion standard units, out of which about 20% was applied in the hospitals for the treatment of infections (Van Boeckel et al. 2014). Only in the USA 23×10^6 kg of antibiotics are presently in use on annual basis, out of which half of antibiotics are used for general public in hospitals and communities (Lederberg and Harrison 1998).

The use of antibiotics mainly depend upon the economic status of the country and the population they hold. In 2000 the antibiotic consumption pattern was high in the USA, France, and Italy while it was reported to be low in many low- or medium-income countries. However a dramatic increase in antibiotic consumption was observed in many developing countries particularly in China, India, and Pakistan (Klein et al. 2018). This global increase in the use of antibiotics are putting burden of many untreated bacterial infections in humans, so it is the need of time not only to quantify the antibiotic use but also to focus on the reduction of antibiotic consumption in developed and developing nations. The antibiotic consumption pattern in hospitals of different countries over the period of time have been presented in Table 2.1.

Table 2.1 Antibiotics consumption in hospitals of various countries over the period of time

Country	Hospital	Period	Antibiotic consumption	References
Hungary	Hungarian Hospital	1996–2005	5.3 million DDDs	Benko et al. (2009)
China	Five largest children's hospitals	2002–2006	68.2, 58.4, 65.8, 65.6 and 49.9 DDD/100 bed-days	Zhang et al. (2008)
United States	42 Hospitals	2003	704 DDD/1000 patient-days/hospital[a]	Pakyz et al. (2008)
Norway	13 Hospitals	1998–1999	47.5 DDD/100 bed-days[a]	Blix and Hartug (2005)
Spain	General hospital (ICU)	1996–2000	176.16 DDD/100 s-d	Hermosilla Nájera et al. (2003)
Switzerland	Hospital Cantonal de Geneva, (HCG)	1996–2000	HCG = 400 DDD/1000 patient-days[a] SICU = 462 DDD/1000 patient-days[a] MICU = 683 DDD/1000 patient- days[a]	Loeffler et al. (2003)
Turkey	Pamukkale University Hospital	2009 and 2010	2009 = 64.5 DDD/100 bed-days[a] 2010 = 70.46 DDD/100 bed-days[a]	Akalin (2015)
Switzerland	University Hospitals	2008	114.3 DDD/100 BD	Plüss-Suard et al. (2011)
Slovenia	University and general hospitals	2007	59.4 DDDs/100 bed-days	Čižman (2011)
Denmark	University and general hospitals	2007	69.9 DDDs/100 bed-days	Čižman (2011)
Netherlands	University and general hospitals	2007	60.9 DDDs/100 bed-days	Čižman (2011)
Norway	Regional Health Enterprise (RHE)	2002–2007	72.4 DDDs/100 bed-days	Haug et al. (2011)
United States	130 US hospitals	2002–2003	792 DDD/1000 patient-days[a]	Polk et al. (2007)

[a]Mean value
DDD defined daily doses, *SICU* surgical ICU, *MICU* medical ICU

2.2.1 Classification of Antibiotics

Based on the structures and functions antibiotics have been classified into broad range of groups. Within same group the antibiotics are active against the related bacteria due to similar pharmacologic and chemical properties. Several classifications of antibiotics have been developed depending upon their action of mechanism or infection cured. The most important groups of antibiotics include aminoglycosides, β-lactams (penicillin, cephalosporins, carbapenems), cephalosporins, chloramphenicol, imidazoles, lincosamides, macrolides, quinolones, rifamycins, tetracyclines, and others (Pinheiro et al. 2019). Various groups of antibiotics and examples are presented in Fig. 2.1 and explained in following sections.

2.2.1.1 Aminoglycosides

Aminoglycosides were mostly effective against Gram-negative bacteria with limited potential to work against Gram-positive bacteria. Their history linked with the discovery of streptomycin back into 1943. Aminoglycosides kill bacteria through inhibition of protein synthesis and mostly they were applied through veins instead

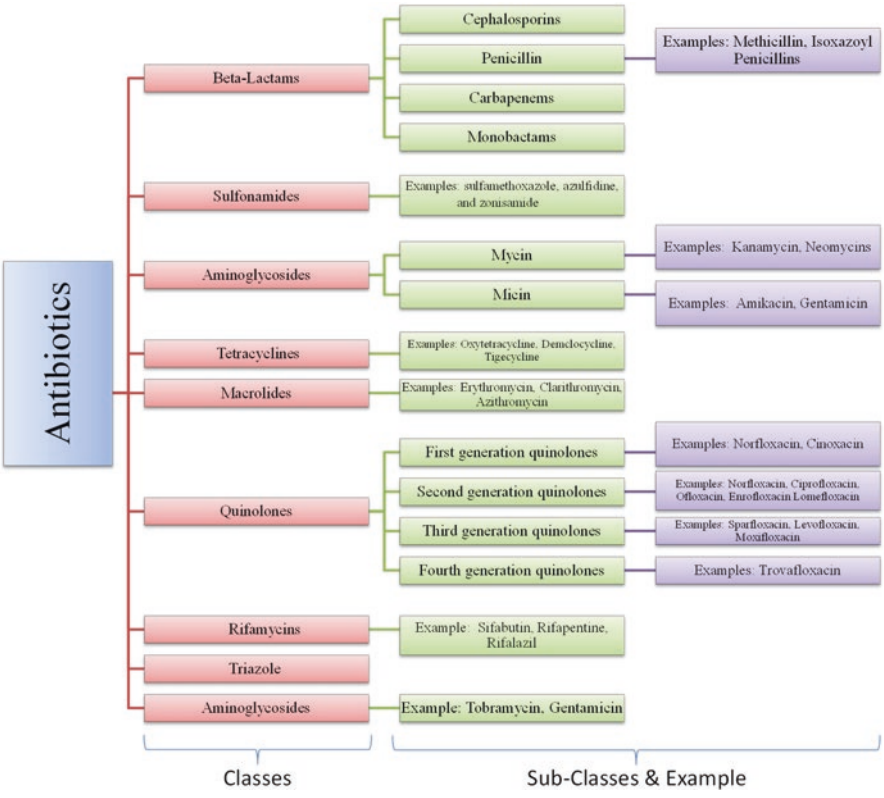

Fig. 2.1 Classification of antibiotics

of oral administration due to poor absorption during digestion. Streptomycin from this group was very effective against tuberculosis orcysticfibrosis. Moreover, these are commonly used prophylactically in premature infants (Hayward et al. 2019). However due to toxicity of this drug its use has also been limited today. Aminoglycosides are divided into two main groups:

1. Mycin (Kanamycin, Neomycins)
2. Micin (Amikacin, Gentamicin)

Aminoglycosides are derived by various species of Micromonospora and Streptomyces bacteria. Aminoglycosides produced by Streptomyces species are referred with suffix "mycin" whereas those produced from Micromonospora are referred the suffix "micin" (Farouk et al. 2015).

2.2.1.2 β-Lactams

The discovery of penicillin in 1928 led to the development of β-lactam antibiotics which consist of β-lactam ring, penicillin (amoxicillin), and cephalosporin. The β-lactams were extensively used class of antibiotics which showed activity against Gram-positive bacteria by interfering the synthesis of peptidoglycan in bacterial cell wall. In human β-lactams chemicals are most successful ever used antibiotic to cure infections due to its wide spectrum properties such as oral availability, activity, lack of toxicity, pharmacokinetics, and bactericidal action (Foster 2019). β-Lactams are further divided into two groups, which include:

(a) Cephalosporins
(b) Penicillin (methicillin, isoxazoyl penicillins, amoxicillin)
(c) Carbapenems
(d) Monobactams

Among various classes, semi-synthetic cephalosporins belong to β-lactams antibiotics which have wide applications for the treatment of human-related diseases and considered as second- or third-line therapy. According to World Health Organization (World Health Organization, 2017) cephalosporins have been declared as the most critical and highly prioritized medicine for human therapy and mainly used to combat gonorrhea infections and meningitis (BPAC 2011).

In general category, penicillin antibiotics is another β-lactam drug which have been extensively applied for curing and preventing infectious diseases. The aforementioned antibiotic is used in a drastic means for combating infections due to bacteria for many years, where its significant role in human medicine is quite popular. Penicillin are basically bicyclic organic molecules which are formed by the fusion of a β-lactam ring and a five-membered thiazolidine ring, combined at various side chains. Penicillins can be classified further into six subclasses, depending upon use, chemical structure, spectrum (extended, broad, and narrow), susceptibility to β-lactamase destruction, and source (natural, synthetic, semisynthetic). Benzyl penicillin or penicillin-G belongs to the first subclass whereas oxacillin, cloxacillin, and dicloxacillin belong to the third class (Alampanos et al. 2019).

Cephalosporin antibiotic drugs belong to the "new non-β-lactam" antibiotics which has capability to disinfect activity of *Klebsiella pneumonia*. Some novel cephalosporins including ceftolozane are applied in combination with tazobactam for the cure and treatment of *aeruginosa* infections (Peri et al. 2019).

2.2.1.3 Chloramphenicol

Chloramphenicol, also known as CAP, is a broad spectrum antibiotic group that is used for curing activity of both Gram-positive and Gram-negative bacteria (Yanovych et al. 2018). Chloramphenicol is applied to counter a broad variety of infections, which includes common cold, typhoid fever, meningitis, and bronchitis (Sharma et al. 2019). Due its side effects, this antibiotic is prohibited in many cases because of its cause of serious illnesses such as blood dyscrasias, suppression of bone marrow, gray baby syndrome, and minor effects such as headache and nausea.

2.2.1.4 Macrolides

Macrolides are a group of antibiotics medically in use for more than six decades and have the capability to inhibit a broad range of bacteria frequently used in feedlots and hospitals (Liu et al. 2014). Macrolides mainly worked against Gram-positive bacteria just like β-lactam through growth and reproduction prevention of bacteria by hindering synthesis of protein. They inhibit microbial cell growth by interfering the function of ribosome (Vázquez-Laslop and Mankin 2018). Erythromycin from this class is the most frequently used antibiotics; though bacteria has developed resistance to this group but still it is the second most prescribed group of antibiotics. Macrolide is typically a natural chemical with variety of biological activities including antiparasitic, antimalarial, and antifungal activities. A small class of natural macrolides is 12-membered macrolides, for example, pandangolide 1–4, cladospolide A and B, ozoroalide, aspergillolide, chloriolide, and balticolid (Huang et al. 2019). Macrolide chemicals have an overlapping binding site on ribosome of bacteria structurally distinct, streptogramins and lincosamides, resulting to clusters of genes encoding resistance to macrolides, called Macrolide-Lincosamide-Streptogramin resistance genes (Sutcliffe and Leclercq 2002).

2.2.1.5 Quinolones

The quinolones is an important class of synthetic antibiotics which have broad-spectrum activity against both Gram-positive and Gram-negative bacteria. Quinolones act in bactericidal manner through interference with the replication and transcription of DNA in bacteria cells. They are used in treatment of humans and food-producing animals (Zhang et al. 2017). Quinolones were mainly used to cure urinary tract infections and those which cannot be treated with other antibiotics. Besides their broad spectrum of antibacterial activity, quinolone exhibited high

pharmacological properties such as antitumor, antituberculosis, antifungal, anti-HIV, antimalarial, and antiplasmodial activities. Quinolones including fluoroquinolones have efficient in vitro antiplasmodial activity against hepatic and erythrocytic stages of CQ-sensitive and CQ-resistant *P. falciparum*. Moreover, fluoroquinolones also exhibited excellent in vivo potency, which make them efficient candidates for the chemoprophylaxis (Fan et al. 2018). Up till now four generations/classes of quinolone antibiotics have been formulated (Hernandez-Montelongo et al. 2014) which contains various types of quinolones.

1. First-generation quinolones (norfloxacin, cinoxacin)
2. Second-generation quinolones (norfloxacin, ciprofloxacin, ofloxacin, enrofloxacin, and lomefloxacin)
3. Third-generation quinolones (sparfloxacin, levofloxacin, moxifloxacin)
4. Fourth-generation quinolones (trovafloxacin)

Currently, the most common quinolones in markets are second-generation quinolone antibiotics which include norfloxacin, ciprofloxacin, ofloxacin, enrofloxacin, gemifloxacin, lomefloxacin etc. (Gidwani and Vyas 2014) that are used in various bacterial infections. Among them, ciprofloxacin has potential to cause toxicity to central nervous, liver, and kidney toxicity and hematological toxicity in the human body. In case of third- and fourth-generation quinolone, their use is very limited because of their serious side effects. Moreover, the newly developed generation of quinolones has more genotoxic potential for instance; fourth-generation quinolones are two orders of magnitude high genotoxic as compared to third-generation quinolones (Cao et al. 2019).

2.2.1.6 Rifamycin

The rifamycin antibiotics are most commonly used in hospitals and clinics for the treatment of nontuberculous mycobacterial diseases, prophylaxis, and tuberculosis (active and latent infection) infections. Rifamycin antibiotic are complex macrocyclic molecules attained from an *Actinomycete bacterium* which play a preponderant role in therapeutics as antibiotics. Rifamycin combine with the RNA polymerase β-subunit which inhibit the RNA transcription, and therefore the bacterial synthesis. The side effects of the rifamycin antibiotics are associated with the discoloration of the skin and body fluids. The main class of rifamycin includes:

1. Rifampin
2. Rifabutin
3. Rifapentine

Among semisynthetic antibiotics, two of their most applied and established rifamycin are rifampicin and rifaximi. Rifampicin is used for curing tuberculosis and inhibition of cancer cells growth in human, whereas rifaximi is applied to treat Crohn's, diarrhea, and diverticular and intestinal infections (Jimenez-Lopez et al. 2016).

2.2.1.7 Sulfonamides

Sulfonamide is broad spectrum group of antibiotics which have a range of activities against various infections caused by Gram-positive and Gram-negative bacteria. A substantial number of this group of antibiotics was discovered based on the Prontosil which was the first sulfonamide antibiotic discovered in 1932 and made commercially available. Sulfonamide antibiotics do not kill bacteria, instead they inhibit synthesis of B vitamin folate required for nucleic acid formation, and this inhibition of folic acid results in the growth and reproduction inhibition of bacteria. The main sulfonamide type RSO_2NH_2 compounds have various types of pharmacological factors which includes antiviral, anticancer, and antibacterial activities; and protease inhibition, carbonic anhydrase inhibition, cyclooxygenase 2 inhibition, and diuretic action (Demir and Köksal 2019). The sulfonamides helps to treat dysentery, meningococcal meningitis, septicemia, tonsillitis, and urinary tract infections caused by *Chlamydia trachomatis*, *E. coli*, *Enterobacter*, *Salmonella,* and *Shigella*. The most common antibiotics in sulfonamides group are sulfamethoxazole/trimethoprim, azulfidine, and zonisamide. Their use has been limited these days due to resistance developed by bacteria against these antibiotics and their hepatotoxicity effects. New efforts have been made to develop such sulfonamide group which are potent inhibitors of the Alzheimer's disease associated butyrylcholinestarase (Apaydin and Török 2019).

2.2.1.8 Tetracyclines

Tetracyclines are one of another broadly used antibiotic class especially in the USA and Europe, applied to cure bacterial infections of respiratory and urinary tract. Tetracyclines act in a similar way like sulfonamides and prevent synthesis of protein, followed by growth and reproduction inhibition. Tetracyclines appeared among the most prescribed antibiotics medicine for human application (Van Boeckel et al. 2014). Aminoglycosides play a key role for curing and treatment of Methicillin-resistant *Staphylococcus aureus* (MRSA) infections. MRSA has long been familiar as one of the main devious pathogens which is involved in nosocomial infections. It has been reported that aminoglycosides are mainly bactericidal and commonly applied synergistically in amalgamation with either glycopeptide or beta-lactam antibiotics (Goudarzi et al. 2019).

2.2.1.9 Triazole

Triazole is also known as 1,2,4-triazole and is a common pharmacophore in many drugs (Bonandi et al. 2017) which possess anti-bacterial, antifungal, antitubercular, and antitumor activities. This is the reason that triazole have wide application in clinics and hospitals to treat variety of infections and diseases (Zhang et al. 2019). Furthermore, 1, 2, 4-triazole derivatives also play an important role in the develop-

Table 2.2 Antibiotics and their activity against variety of infections

Antibiotic group	Antibiotic name	Infection treatment	Reference
β-Lactams	Cephalosporins	Gonorrhea	Barry and Klausner (2009)
	Penicillin	*V. vulnificus* infections	Wong et al. (2015)
	Carbapenems	*K. pneumoniae* infections	Tumbarello et al. (2015)
Sulfonamides	Trimethoprim	Skin infections	Michałek et al. (2015)
	Azulfidine	Ulcerative colitis	Sailaja (2014)
	Zonisamide	Epilepsy	Mukhin and Pylaeva (2015)
Aminoglycosides	Amikacin	Septicemia	Fuchs et al. (2016)
	Tobramycin	Wound-healing	Marson et al. (2018)
	Streptomycin	Buruli ulcer	Owusu et al. (2016)
Tetracyclines	Chlortetracycline ointment	Eardrum healing	Chang (2016)
	Minocycline	Acne vulgaris Acne rosacea Respiratory diseases Rheumatoid arthritis	Farahnik et al. (2015)
	Doxycycline	Acne vulgaris	Del Rosso (2015)
Macrolides	Azithromycin	Cystic fibrosis	Principi et al. (2015)
	Spiramycin	Fetal toxoplasmosis	Avci et al. (2016)
	Clarithromycin	Gastric ulcers	Dinos (2017)
Quinolones	Lomefloxacin	Chronic obstructive pulmonary disease	Zhang et al. (2015)
	Moxifloxacin	Diabetic foot infections	Devrajani et al. (2018)
	Norfloxacin	Preventing infection in patients with liver cirrhosis	Lontos et al. (2014)

ment of novel medicines by hybrid molecules. Hybrid molecules are those chemical entities which have two or more structures having different biological functions. Therefore, hybrid molecules of 1, 2, 4-triazole are marked with the dual activities, for instance the new drug may provide excellent potency against both drug-resistant bacteria and drug-sensitive (Zhang et al. 2019). The activity of various antibiotics against different infections is summarized in Table 2.2.

2.3 Misuse of Antibiotics in Hospitals

Antibiotics are critical element of today's medicine in hospitals but they are on the top of the list of misused medicines in hospitals. Mostly antibiotics are used for the nosocomial infections and the great occurrence of infectious diseases results in the extensive practice of antibiotics in hospitals that may or may not be in an appropriate way (Wolff 1993). Due to lack of proper hygiene, misuse of antibiotics and

encoded genetic elements antimicrobial resistance is becoming a great issue in the hospitals. The four major elements that are responsible for the antibiotic misuse and resistance problems in hospitals are: (1) infection, (2) patient, (3) pharmacist, and (4) physician. The role of physicians in inappropriate prescription cannot be ignored as they focus more on short-time treatment with frequent results. While the private pharmacist contribute in this problem by supplying antibiotics without verifying the physician's prescription (Weinstein 2001). The absurd taking of antibiotics affects the whole body in different ways like allergic reactions, antibiotic resistance, antibiotic-resistant gonorrhea, and toxicity (Li 2014).

In the USA about 30% of the drug budgets in hospital is allocated for antibiotics which is correlated with the inappropriate use of antibiotics usage for more than three decades even after strict control measures. Furthermore, it was reported in 2003 that out of seven billion $ spent on antibiotics usage on annual basis, about four billion $ have been spent on the treatment of infections caused by hospital-acquired infections (Guven and Uzun 2003). Wrong prescription in hospitals increases the exposure of patients and complications (Fridkin et al. 2014), so there is a need of improved prescription of antibiotics in hospitals to offer more benefit to patients than complications. Patient outcomes can be improved by using antibiotics in an appropriate way in hospitals aiming to:

1. Cure infections with an agent that requires a short time with minimum aftereffects.
2. Control the spread and development of resistant microbial agents.
3. Ensure better healthcare facilities on fair treatment charges.

To control the misuse of antibiotics many hospitals limit the recommendation of antibiotics by prescribing only one to two antibiotic drugs from each class. The effectiveness and appropriateness of antibiotics may be improved by the use of order forms and review of antibiotic treatment on next day of prescription (Davey et al. 2013). The computerized order form is the most effective intervention to ensure correct prescription of antibiotics. To prevent the misuse of antibiotics and enhancement of treatment it is the responsibility of doctors to correctly prescribe antibiotics to patients for good quality treatment at all stages.

2.4 Types of Wastes Generated in Hospitals

Hospital waste is categorized as medical and nonmedical waste. The medical waste is produced from various activities including first aid, emergency, operations, laboratories, and diagnosis, whereas the nonmedical waste includes the waste generated from kitchens, toilets, laundry, etc. There is high variability in the production of hospital waste depending upon hospital size, wards, services offered, country, season, etc. (Verlicchi et al. 2012; Al Aukidy et al. 2014). It was estimated that about 4 kg of the waste produced in hospitals contains at least 1 kg of the infectious waste (Babatola 2008), similarly 10–25% of the residual waste is proven to be toxic which intensify and promote disease transmission among humans (Tsakona et al. 2007).

In past gradual increase in the use of antibiotics was observed which has reached between 100,000 and 200,000 tons per year (Wise 2002) making antibiotics as potential contaminants due to their long-term and synergistic effects at low concentrations when they enter in the environment (Lombardo-Agüí et al. 2014; Oliveira et al. 2015). These antibiotics may associate with one another and can present genuine difficulties to human medicinal services. There are mainly two types of wastes generated in hospital, i.e., chemical waste and biological waste. This waste when enters into the environment from hospitals results in decreasing the efficiency of medicine and promoting bacterial resistance which has threatened the public and ecosystem health. The pathway of antibiotics released in hospitals have been presented in Fig. 2.2.

2.4.1 Chemical Waste

The most commonly detected chemical in the hospital waste are antibiotics due to their excessive use and consumption for the treatment of various types of infections. Sometimes antibiotics are ineffectively used and discharged as the active parent substance in the hospital waste. It was reported that antibiotics when given to

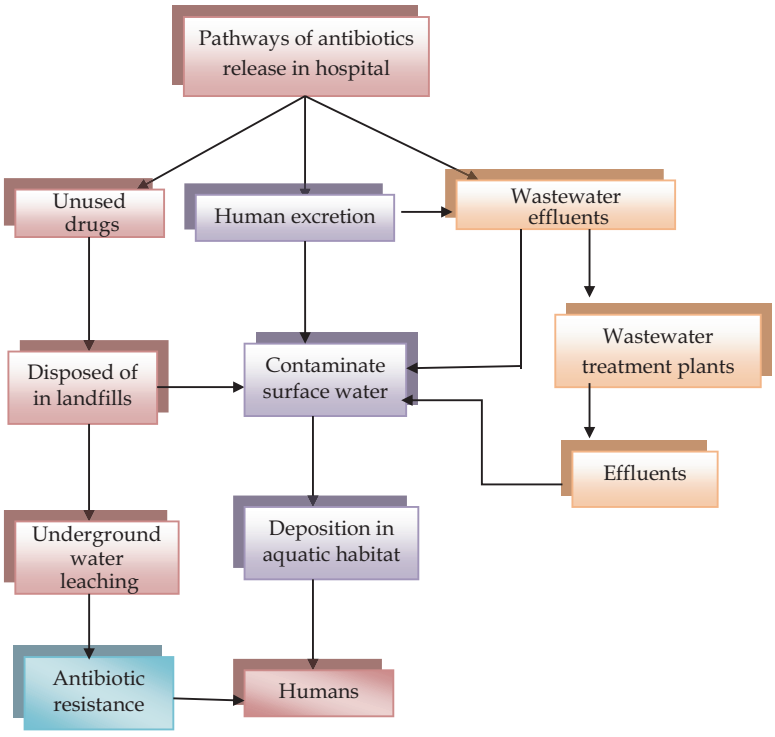

Fig. 2.2 Pathway of antibiotics from hospital waste into the environment

patients they excreted 50–80% in the urine and 4–30% in the feces as parent compound (Alcock et al. 1999; Jjemba 2006; Verlicchi et al. 2012; Al Aukidy et al. 2014). On the other hand, the metabolites produced due to consumption of antibiotics remain bioactive and sometime result in the transformation to parent compound. This was observed in case of sulfonamides when N4-acetylsulfapyridine and N4-acetylsulfamethazine has changed to the parent compound (Bonvin et al. 2012). Further these antibiotics possess high stability and low volatilization which result in the persistence of these contaminants in the hospital waste.

Hospital effluents are one of the principle sources of contamination to the natural environment. After management in the hospital and excretion from patients, these antibiotic substances are released into hospital effluents. On the other hand, sometimes unused drugs are also disposed of down drains. In hospitals various antibiotics from different groups are used with high concentrations which is an indicator that major contributor of antibiotics pollution are hospitals. Many studies reported the prevalence of antibiotics in hospital waste water (Giuliani et al. 1996; Guardabassi et al. 1998; Alder et al. 2003) due to their incomplete removal in treatment plants where the antibiotics get readily adsorbed to sludge with limited biodegradation which make them to persist in the waste water and enter into surface water through effluents (Verlicchi et al. 2010). The removal efficiency of different methods used for antibiotic residues treatment in hospital waste has been presented in Table 2.3. Fluoroquinolone group of antibiotics are considered as genotoxic and these antibiotics were widely detected in hospital effluents due to limited removal with the concentrations ranging between 3000 and 87,000 ng L^{-1} and playing major role in the growth of antibiotic-resistant bacteria (Hartmann et al. 1998). Among various classes of antibiotics, sulfonamides and fluoroquinolones persist for longer period whereas macrolides and tetracyclines persist for comparatively longer time in the absence of sunlight. On the other hand, the other two groups of antibiotics including aminoglycosides and β-lactam showed the least persistence in the environment (Huang et al. 2011). β-Lactams and fluoroquinolones were widely used in inpatients but β-lactams seldom detected in the waste water due to its rapid cleavage on hydrolysis compared to fluoroquinolones (Bréchet et al. 2014) which is persistent and resulted in the detection with high concentrations in hospital waste water (Rodriguez-Mozaz et al. 2015). The concentration of antibiotics from various groups in hospital wastewater from different countries have been presented in Table 2.4. Throughout the most recent decades, an expanding assemblage of evidence has demonstrated that antitoxins entering the environment in this manner pose potential consequences for nontarget life forms and public health (Boxall 2004; Runnalls et al. 2010; Brandt et al. 2015).

2.4.2 Biological Waste

Antibiotics use in hospitals resulted in the prevalence of their residues in hospital waste which has emerged as global concern. This happened due to the lack of primary treatment of hospital waste water or the behavior of antibiotics in treatment

Table 2.3 Removal efficiency of various methods used for the treatment of hospital waste water

Antibiotics	Treatment method	Removal efficiency	Reference
Cephalosporin	UV, Chlorination	75% and 100%	Lin et al. (2009)
Sulfadiazine	Moving Bed Biofilm Reactors	20%	Casas et al. (2015b)
Sulfamethizole	Lab-scale activated sludge, activated sludge and biofilm carriers	25%	Casas et al. (2015a)
Macrolide	Membrane Bioreactor	Between 20 and 60%	Kovalova et al. (2012)
Ciprofloxacin	Membrane Bioreactor	50%	Nielsen et al. (2013)
Amoxicillin	Bentonite adsorbent	88%	Putra et al. (2009)
Amoxicillin	NH_4Cl-induced Activated Carbon (pH 6 and 50 °C)	99%	Moussavi et al. (2013)
Sulfamethazine	Ion exchange	100%	Fernández et al. (2014)
Tetracycline	Biomass-derived Biochar adsorption	58.8 mg/g	Liu et al. (2012)
Norfloxacin	Flat sheet sponge Membrane Bioreactor and Hollow fiber sponge Membrane Bioreactor	93–99% and 62–86%	Nguyen et al. (2017)

plants. The antibiotic residues in hospital waste water continuously exert a strong selective pressure on ARBs and create favorable conditions (nutrient, pH, temperature, antibiotics) for antibiotic-resistant bacteria to promote gene transfer among other bacteria (Varela et al. 2014). This relationship between antibiotics, antibiotic-resistant bacteria, and rearrangement of bacterial communities has been presented in the past (Gros et al. 2013; Stalder et al. 2013; Bréchet et al. 2014; Varela et al. 2014; Baricz et al. 2018). Hospital waste holds variety of bacteria released from patient body and hospital equipment which includes many pathogens. The most common pathogens *Escherichia coli* and *Pseudomonas aeruginosa* responsible for causing urinary and respiratory tract infections has been reported by Tuméo et al. (2008). Further great number of vancomycin-resistant enterococci (VRE), multidrug-resistant, *Pseudomonas aeruginosa*, *Enterococcus faecalis,* and *Enterococcus faecium* were also reported in hospital effluents. It was reported that about 15% of infections in hospitals are only caused by *Pseudomonas aeruginosa* (Hocquet et al. 2016). So the hospital effluents containing these types of pathogens and antibiotic-resistant bacteria make these organisms to play a significant role in the evolution and propagation of these organisms and the related genes. These evidences raised an emerging concern not only to the human health but also to the environment (Jernberg et al. 2010).

The hospital effluents contain this type of biological waste and antimicrobial residues have induced the great level of concern today than ever in the history. This is evident from the report stated that infections may enhanced up to 120,000 during

Table 2.4 Concentration of antibiotics from various groups in hospital wastewater from different countries

Antibiotics	Concentrations in hospital waste (ng/L)	Country	References
Beta-Lactams			
Penicillin G	5200	New Mexico	Brown et al. (2006)
Amoxicillin	352,000	Germany	Kümmerer and Henninger (2003)
Ampicillin	95,300	Germany	Kümmerer and Henninger (2003)
Cephalosporin			
Cefazolin	83.4	Spain	Rodriguez-Mozaz et al. (2015)
Cefazolin	253,000	Germany	Kümmerer and Henninger (2003)
Cefotaxime	236.8	Spain	Rodriguez-Mozaz et al. (2015)
Cefuroxime	91,600	Germany	Kümmerer and Henninger (2003)
Sulfonamides			
Sulfamethoxazole	2100	New Mexico	Brown et al. (2006)
Sulfamethoxazole	4816.7	Spain	Rodriguez-Mozaz et al. (2015)
Trimethoprim	5000	New Mexico	Brown et al. (2006)
Trimethoprim	594.3	Spain	Rodriguez-Mozaz et al. (2015)
Aminoglycosides			
Gentamicin	9100	Germany	Kümmerer and Henninger (2003)
Netilmicin	7500	Germany	Kümmerer and Henninger (2003)
Tetracyclines			
Doxycycline	1100	Germany	Kümmerer and Henninger (2003)
Macrolides			
Lincomycin	2000	New Mexico	Brown et al. (2006)
Clarithromycin	941.1	Spain	Rodriguez-Mozaz et al. (2015)
Azithromycin	59.9	Spain	Rodriguez-Mozaz et al. (2015)
Roxithromycin	2189	China	Chang et al. (2010)
Quinolones			
Ciprofloxacin	15,000	Australia	Watkinson et al. (2009)

(continued)

Table 2.4 (continued)

Antibiotics	Concentrations in hospital waste (ng/L)	Country	References
Ciprofloxacin	2000	New Mexico	Brown et al. (2006)
Ciprofloxacin	13,779.7	Spain	Rodriguez-Mozaz et al. (2015)
Ciprofloxacin	26,000	Italy	Verlicchi et al. (2012)
Lomefloxacin	1162	China	Chang et al. (2010)
Norfloxacin	1620	China	Chang et al. (2010)
Ofloxacin	35,500	New Mexico	Brown et al. (2006)
Ofloxacin	14,377.8	Spain	Rodriguez-Mozaz et al. (2015)
Ofloxacin	15,000	Pakistan	Ashfaq et al. (2016)

surgery due to decrease in the efficiency of antibiotics which have caused 6300 deaths in the USA (Teillant et al. 2015). If the spread of antimicrobial resistance will continue with the evolution of pathogens, this death rate may even rise to great numbers. Recently it has been reported that a bacterial strain isolated from livestock and patient in China is multidrug resistant and can resist to colistin (Liu et al. 2016) which is considered as "last-line" medicine for such type of pathogens (Biswas et al. 2012). These evidences suggest to step up in the hospital waste management for the protection of human and ecosystem health. The problem of hospital waste management is global but it is not properly managed in the developing countries which can result in greater effects in these countries (Tudor et al. 2005).

2.5 Environmental Burden of Hospital Waste

2.5.1 Antibiotics

The antibiotics used in hospitals ends up in the environment due to limited metabolization in human body and incomplete removal in the treatment plants. Another way of these antibiotics in the environment is through the disposal of the waste through landfills (Wang et al. 2015). This heap of antibiotic agents scattered in nature could have critical ramifications for environments and human well-being, perhaps increasing sensitivities in people and propagation of antibiotic resistance. Antimicrobials are usually detected at subtherapeutic concentrations in the environment (Kümmerer 2003) but continual exposure to these drugs increased their resistance in the environment (Kümmerer 2004). Bacterial protection from antimicrobial agents has been accounted for various environment including surface water (Ash et al. 2002), sewage (Iversen et al. 2002), drinking water (Schwartz et al. 2003), soil (Burgos et al. 2005), and marine ecosystem (Kim et al. 2004). There has been a seri-

ous threat about the presence of antimicrobial in the environment that the U.K House of Lords expressed it as: “Resistance to antibiotics constitutes a major threat to public health and ought to be recognised as such more widely than it is at present” (Wise et al. 1998). The focus should be given on the appropriate use of antimicrobials, installment of primary treatment system, followed by the improvement in the treatment processes in the hospitals. Because if they will not be appropriately prescribed by the medical practitioners and eliminated in the treatment plants, they will enter in the environment and pose threat to public and ecosystem health.

2.5.2 Development of Antibiotic Resistance in Environment

Antibiotic resistance can be developed through selective pressure caused by antimicrobials or naturally by spontaneous alteration in genes due to the lack of selective pressure in the presence of antibiotics (Blair et al. 2015). Further the antimicrobial resistance can be acquired through gaining antibiotic-resistant encoded genes from other bacteria through conjugation, transformation, and transduction. Conjugation is considered as most significant process of antibiotic resistance transmission in bacteria. The circular fragment plasmids of bacterial DNA facilitate this mechanism by independently replicating the chromosome and transfer DNA fragments through the formation of pilus when bacteria are close to each other. Transformation is also another way of transmission of antibiotic resistance through “naked” DNA from cell to cell. The free DNA originated from dead bacteria enters the receiving bacteria via cytoplasm. Genetic transfer through transduction occurs by means of a vector, mostly viruses “bacteriophages” that have the ability to infect bacteria. The virus having antibiotic-resistant coded bacterial gene transfer the genetic material to receiving bacteria. When an infecting bacteriophage transfer viral DNA to receiving bacteria, it will allow replication system of bacteria to continue to replicate infecting virus till bacterial cell expires (Alanis 2005).

In hospitals various factors are responsible for the greater resistance development in bacteria including patient’s survival in severe illness with the overuse of antibiotics, lack of effective precautionary measures, and lack of restrictions on the use of antibiotics and inappropriate prescription of antibiotics to patients. So these antibiotic-resistant bacteria and pathogens originated from the hospitals when enters into the environment can disseminate their resistance to the environmental bacteria and poses some serious consequences. This leads to severe infections resulting in the reduced effectiveness of antibiotics due to resistance genes transfer between pathogens, and more expensive and complex treatments for a longer period of time (Wellington et al. 2013). Bacteria have the ability to develop multidrug resistance through complex molecular mechanism which can increase the rate of mortality and morbidity through causing new types of infections.

2.6 Conclusion

Hospitals are serving as hotspot for the different types of antibiotics, resistance genes, and human pathogens. Antibiotics were extensively employed in the hospitals to treat various infections causing the spread of antibiotics and antibiotic-resistant bacteria from human intestine to the environment which has become a global threat. The data about the antibiotics consumption is needed to control the misuse of antibiotics and to design effective strategies. Treatment technologies needed to be installed to treat hospital waste prior to their discharge into the environment. Most importantly, antibiotic steward ship is needed to minimize the misuse and waste production in the hospital and to lower the clinical cost and improvement in the hospital treatments.

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Chapter 3
Antimicrobial and Antibiotic Resistance Genes in the Environment

Muhammad Afzaal, Safdar Ali Mirza, Taha Arooj, Muniza Almas, and Sarfraz Ahmed

Abstract Antibiotic resistance and its widespread implications present predicament to healthcare. Different investigations show that environment plays a significant role in emergence of resistance in bacteria and pathogens. Though a clearer concept of the ancestral phylogenetic and environmental route resulting to clinical emergence of resistance genes is still missing as is familiarity of ecological distribution obstructions. So there is need of improved representation of how resistance genes change over the period of time, are moved, relocated and distributed in ecological setting. Here, we tried to describe the environmental and gradually improved ecological factors that play significant role to evolve resistance and its dissemination. Although movement of resistance genes is thought to be a continuous process, the immense mass of such genetic measures does not direct the organization of new resistance factors in bacterial species, except there is a selection pressure for preserving them or their fitness costs are unimportant. To allow defensive methods to work it is significant to find that what circumstances and to what degree ecological selection occurs for development of resistance. Moreover, knowledge about distribution obstructions of resistance genes is not only way to assess threats to evolving health issues, but also to stop emergence of novel resistance genes and resistant pathogens.

Keywords Antimicrobial · Antibiotic resistance genes · Environment

M. Afzaal (✉)
Sustainable Development Study Centre, GC University, Lahore, Lahore, Pakistan
e-mail: Dr.afzaal@gcu.edu.pk

S. A. Mirza · T. Arooj · M. Almas
Botany Department, GC University, Lahore, Lahore, Pakistan

S. Ahmed
Faculty of Veterinary Sciences Narowal, Department of Basic Sciences, University of Veterinary and Animal Sciences, Lahore, Punjab, Pakistan

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3.1 Introduction

In the past few years, multidrug resistance (MDR) isolates and tremendous increase in antimicrobial resistance has been recognized all over the world. The microbial isolates for which no therapeutic drugs are available or are unable to treat are now known as extreme drug resistance isolates or microbes (Paterson and Doi 2007). So, the main investigation areas that capture interest now are: how these isolates are emerging so quickly, moreover, the new emerging resistance mechanisms, the surrounding genes reorganization that helps to express, capture and transfer the resistance determinants.

According to a review on antimicrobial resistance published in 2014, the antibiotic resistance accounts for hundreds and thousands of deaths every year, and due to projected increase, WHO (World Health Organization) states this problem as the major global threat to human health (WHO 2014). To be precise, Clinical settings are the main focus of formation of resistance genes and recently the field of agriculture that aims to reduce the spread and defence of resistant isolates during antibiotic treatment. For last few years, the environmental role of resistance has been recognised as an important aspect to circulate the route of resistance but the understanding of the environmental contribution is still limited (Martinez 2008). There is limited understanding of how and under what conditions the environment helps or supports the development of resistance genes. Several investigators have focused on a so-called one-health approach which involves holistic point of view on antimicrobial and antibiotic resistance that includes human, animals and environment they are living in. The knowledge of environmental factors that facilitates the development of antibiotic resistance may allow the understanding to make models about the development of resistance and how it is circulated (Hiltunen et al. 2017). No doubt, these models will be descriptive only at first basis because most of the parameters would be unknown and will lack predictive powers, but ultimately these will help to fill the knowledge gaps for development of control strategies.

In order to define different factors, we must need to define some basic terms which have different meanings in different data available. First of all, according to the definition of resistance by Martinez et al. (2015), if the minimum inhibitory concentration of a strain is more than parental strain, then it is resistant to that antibiotic. Similarly, the definition of resistant gene becomes that the gene whose presence effects and helps the bacterium to tolerate the heavy amount of antibiotic and removal of this gene from its point will increase the susceptibility of that particular antibiotic (Martinez et al. 2007).

3.2 Antimicrobial Resistance

According to the latest statement by WHO, we are slowly entering into a post-antibiotic era, an era in which simple and earlier treatable infections by bacterial strains will kill, and moreover routine medical procedures like chemotherapy and

joint therapy replacements which totally rely on antibiotics will no longer be effective (Chan 2011). According to a report given by O'Neill in 2014 commissioned by UK government, by 2050 the antibiotic-resistant infections will be the leading cause of deaths globally.

The antibiotics are employed for treatment of bacterial infections in humans, plants and animals. These agents are widely utilized as hormones in order to enhance the meat productivity; in 2016 the use of these chemicals as growth promoters was banned in Europe (Angelakis et al. 2013; Cogliani et al. 2011). The misuse of antibiotics is considered as the greatest issue for emergence and increase in resistance but a little attention is gained by the natural environment to trigger the spread of resistance.

The resistance to these antibiotics are of two types: native or the earned one. Acquired resistance results from mutations in the DNA of pathogen or during horizontal transfer of gene during conjugation. The current subject of concern is that this acquired resistance makes the infection difficult to treat in the clinical or in other ecological settings. Antibiotics can either be original such as bread mould penicillin or they can be synthetic one or chemically modified by using the original natural antibiotic and modifying it for its better action and stability (O'Brien and Wright 2011). The two terms antibiotics and antimicrobials can be used as synonyms and are the chemicals that have microbicidal and microbistatic activity.

The antibiotic molecules are also produced by microorganisms for competition to inhibit other microbes from flourishing. So, for survival, microbes have evolved over period of time for a better machinery to defend themselves against antibiotics. Recent researches indicate that the methods of resistance against antibiotics were used even in old times, which means antibiotic resistance is an ever-evolving issue and natural process being old and is based upon shared genomics of the pathogens (Bhullar et al. 2012).

The selection of resistance already exists naturally in different environments for the pathogens without human interference. Billions of tons of the antibiotics are being used annually in parts of the world which is directly released to environment and produces considerable change in selection pressure which results in high multidrug resistance (Gaze et al. 2011). Mostly, when the antibiotics are consumed, they are directly dumped out of body without being metabolized along with the resistant bacteria. These mix with the environmental bacteria after passing through the sewage system and get mixed directly to the water and soil. Presence of other pollutants in soil and water increases the further pressure to help selection for resistance that either it should be direct or indirect. The role which environment contributes for the emerging resistance is still under observation and the answer to the role played by it depends upon the fact that for how much time the agents responsible for resistance stay in the environment in active form.

Naturally occurring bacterial strains in water as well as soil have a wide variety of these genes resistant to the antibiotics. Researches show that environment is responsible for formation and taking of resistance genes for the formation of vulnerable receptive pathogens (Wellington et al. 2013). Now researchers are concerned about the pathway that how this resistance developed at genetic level and its

dissemination in the environment (Ashbolt et al. 2013; Finley et al. 2013). The Human gets in contact with these vulnerable pathogens during food consumption and water uptake or when they interact to their environment. The next query is to what extent transmission of these vulnerable genes takes place when it interacts the environment through food chain (Leonard et al. 2015).

3.3 Origin of Resistance Genes

The factors responsible for the development of resistance in pathogens can emerge anytime. About 1030 thousand billion cells of bacteria on earth provide a huge quantity for such events to occur. Such an enormous number of pathogens provide a huge genetic unevenness and favours mutations, translocation and parallel transfer of genes (Kallmeyer et al. 2012). So, these factors are liable to come repeatedly in view. No doubt that these events are slow and are not noticed in the mainstream but are happening in the environment. There are however numerous factors which result as a hurdle to formation of new genes for multidrug resistance. This is because the formation of new genes is linked to their stability in the environment. The stability cost of these genes as new resistance genes in pathogens is really huge. This is because their occurrence and presence in the pathogen may result in disturbance of other functions taking place for pathogen survival in the cell, so these genes face strong selection pressure to hold them in new pathogen. Moreover, if the stability cost of such genes becomes negligible, it will still have low chances to develop in the pathogenic population unless a positive factor for selection works over it (Martinez 2011). Even if this positive selection pressure appears weakly, presence of it will help the new resistance genes to survive during events like genetic changes and mutations (Baquero et al. 1998).

3.4 Horizontal Spread of Antimicrobial Resistance Genes

For horizontal dissemination of antimicrobial resistance genes, following are the three eminent mechanisms known to scientists:

1. Conjugation.
2. Transduction.
3. Natural transformation.

Of these three, conjugation is most widely investigated and understood mechanism (Smillie et al. 2010; Arutyunov and Frost 2013) which involves transfer of genetic material from one bacterial cell to another via direct physical contact between the two. Natural bacterial transformation (introduction of foreign genetic material) is also a well-explored mechanism (Sparling 1966; Harford and Mergeay 1973; Prudhomme et al. 2006). Transduction is an important but also an underestimated mechanism of gene dissemination. In transduction, genetic material is transferred

from a bacterium to bacteriophage and then to another bacterium during the second infection cycle of the phage. The important role of antimicrobial resistance genes' spread was acknowledged when their natural abundance in phage particles was reported in various studies (Muniesa et al. 2004; Colomer-Lluch et al. 2011a, b, 2014a; b; Ross and Topp 2015). It's been reported through metagenomic investigation of different viromes that 50–60% of bacteriophages contain all known functional genes of bacterial origin. This finding leads to the conclusion that bacteriophages are possibly serving as diverse source of antimicrobial resistance genes and their horizontal spread (Dinsdale et al. 2008). Mazaheri et al. (2011) demonstrated the transduction-based transfer resistance conferring genes against antibiotics, from *Enterococcus gallinarum* to *E. faecalis*. Transduction mediated *in vitro* transfer of ampicillin, chloramphenicol and tetracycline among *Salmonella spp.* was reported by Schmieger and Schicklmaier (1999). Bacteriophage-based transduction calls for scientists' interest given the fact that it can transduce multiple types of genetic material including linear fragments of DNA, insertion sequences, plasmids, integrons and transposons (Schmieger and Schicklmaier 1999; Schwarz et al. 2004; Roberts 2005; Miranda et al. 2013). Contribution of transduction to spread of antimicrobial resistance genes in environment still remains poorly explored because of complicated technique-based characterization of bacteriophages, their abundance and ability to co-evolve with their host.

3.5 Assembly of Factors for Formation of Resistance

Like that of formation of new resistance gene factors, the genes can be assembled if their stability cost is not considered or if the presence of selection is reserved for those elements whose stability cost after evolving is less than their pathogen in which they are being incorporated. Now, the question arises that: How strong the gene selection pressure for the assembly of stable resistance genes is required to preserve the assembling of pre-existing genes responsible for resistance? Previously the stability cost is often linked to the factors of resistance which are moved lately to the elements of assembling them. Moreover, the stability of a gene faces pressure of maintenance due to multiple copies of a single original gene and the complexity related to is assembling during the gene expression for the formation of related proteins. So, it is obvious that for the stability of factors of resistance, the environment plays a significant and continuous role despite of the fitness cost. This leads to a better understanding that once the factors of resistance develop in the pathogenic community, it modifies itself to withstand the stability costs in the pathogen. The genes that have low stability costs then be a part of the assembly of genetic elements and a non-lethal amount of antibiotics helps for their survival in generations (Salyers and Amabile-Cuevas 1997). A very crucial point to understand here is that the genes of resistance are evolved over period of time in an antagonistic manner between different diversity of pathogens.

So, both factors responsible for formation of resistance, i.e., the native and the new factors, are selected by natural selection if they are in competition with the antibiotic forming pathogens in which they are residing and that pathogen produces more antibiotic compounds as compared to these factors. This selection helps to preserve the gene pool in ecological setting indirectly in addition to the formation of these vulnerable genes in the pathogens as they alone cannot assemble the factors of resistance. One of the features which is usually skipped while investigating is that these genes can be assembled on non-translocatable assembling elements like integrons (Gillings 2017). The stability cost of movement like these are not properly considered in most cases, but if we consider the whole phenomena of resistance development in pathogens, their method of movement helps for a better understanding in their replication as well as the functions they perform. For selection of antibiotic, the increased level of gene expression exhibited by them plays a vital role to adjust the degree of their expression. On the other hand, translocation of a gene from its original place can lead to consequences like disturbance of various other genes or the factors that can disturb the stability of pathogen, i.e., like the expression of other genes may get disturbed by movement of a certain gene. The degree of movement of gene which would not disturb the functionality of other genes to form translocatable resistance factor is particularly unspecified, but if we be able to identify it, it will help a lot in recruitment of these genes in pathogens and understanding of their incorporation in plasmids and integrons as well as in the jumping genes which are often present as tandem repeats in the plasmids. Of course, the integrons play a role in translocation of these genes in different pathogens but one cannot neglect the role of selection pressure which prevents their transfer to the genome of pathogens (Stokes and Gillings 2011).

3.6 Compatibility for Development of Resistance

In the light of different investigations, there are four basic steps that play a role in the formation of antibiotic resistance, i.e.,

1. Formation of new factors for resistance.
2. Movement and assembly of these factors.
3. Relocation of these factors from pathogens to human.
4. Dispersal of these factors.

Particularly, such events can happen in any order, e.g., they might enter into human before or after spreading or certain steps may be repeated but crucially these four are the main steps regardless of their order of occurrence. The factors that are currently part of the resistant pathogens are all formed following these four basic routes, so for development of resistance it is important to maintain these routes. If selection pressure is not considered, the resistance genes with high stability cost are liable to be vanished mainly if present on translocatable elements.

A situation with steady natural selection seems difficult with few exceptions. So, this becomes obvious that the resistance in present pathogens developed mainly due to low stability cost that resulted in formation of resistance. The translocatable genes that cannot provide a stability cost are likely to be lost and are often dropped during early stages of their appearance with translocatable genetic elements like the plasmids. So, this emphasizes over the role and importance of the surrounding environment in which these genes offer a well-established selection benefit, e.g., the atmosphere contaminated by the air pollution with antibiotic elements. These environments to which the pathogen are subjected lead to high levels of mutation causing factors that may result in formation of genes for multidrug resistance or a completely different scenario like the degree of eradication that involves pathogens with little stability cost and these pathogens are liable to survive if we remove the selection pressure, e.g., shifting to a new clean environment where the pollution factors become negligible (Gonzalez et al. 2013).

Regardless of long translocational routes for newly formed resistance genes and their spread to human micro biome, it is not surprising that the factors present in pathogens today are very hard to remove from these pathogens and appears with a little stability to their carriers. This leads to the concept that once the resistance is developed in a pathogen and it enters the microflora of human, the entire game is lost and all we can do to control is to control dispersal or vector related to it. So, it is very important to control these factors before they got incorporated in genome of pathogens and enter in human microflora. This concludes that for the control of formation of resistance genes, it is essential to identify these resistance elements in environment which are not yet part of the pathogen and by this way we can lead to a better route to deal with the new multidrug-resistant pathogens and control their development (Baquero et al. 2013).

3.7 Hazardous Effects of Environments to Human Health

A question that arises is why environmental studies are important for determining resistance in the superbugs; the answer to it lies in the fact that environment gives a better understanding to the health risks related to human health regarding the antibiotic components as well as for the animals. The understanding of it will help to form strategies to avoid spreading of resistance factors in the microflora from the ecological setting and to minimize the factors responsible for spread of these superbugs. For the development of strategies for the control of these bugs as well as for the control of genes from being incorporated in them, we need to define the environment that contributes to hazardous health risks. This is not an easy task; most of the researchers agree that the most severe situation faced nowadays is development of vulnerable pathogens due to these genes (Martinez et al. 2015). Their presence in the pathogen found in environment does not indicate high threats to health but only when are accidently encountered in human microflora.

The translocatable elements related to resistance in human microflora may spread in the environment by human waste material. So, the presence of these in ecological settings indicates that they might be transferred to this surrounding through the excretory products of human which contaminates the environment (Larsson 2010). Although we cannot skip the role of human waste pollution, basically this waste is associated with the spread of these pathogens which are previously immuned to antibiotics. So, the transfer of resistance components between human microflora is more frequent as compared to the entrance of new genes from environment. Therefore, from the point of view of clinical therapy, the role played by environment for the translocation of genes is negligible (Bengtsson-Palme and Larsson 2015). The threats can be classified into following three main routes:

1. The threats of assembling and formation of new resistance factors.
2. The threats to carry new genes previously absent in human microflora and their parallel transfer.
3. The threat of spreading the vulnerable genes of ecological pathogen to human microflora.

The natural selection pressure can affect all these routes but chiefly the first two, whereas the hurdles for spreading the third one is most significant. The selection pressure is chief component for dispersal of these vulnerable genes from ecological setting that includes the pathogens which utilizes the opportunity to develop infection in weak host. Translocation of genes occurs in all type of habitats but is chiefly seen for newly formed resistance factors due to the presence of natural selection and stability which is linked to major changes of development of these genes in human microflora. Genes with minor stability are liable to be chosen on early stages of their appearance and vanishes soon from the microflora.

Slightly high minimum selective concentration and inhibitory concentration of antibiotics in ecological setting results appearance of these resistant genes and their translocation. These environments include micro flora of animals as well as humans being cured through microbiostatic components. Information about the extreme environmental factors faced by pathogens in water treatment plants is not available but seems threatening to the pathogens as the stability pressure provided by such treatment plants results in little variety of pathogens in that environment. Moreover, low microbistatic compounds makes them ineffective against the pathogen and thus contributes to the formation of resistance. So, the untreated factory waste and water used in fields as well as in the treatment plants should be taken under consideration where the concentration of microbicidal components should be investigated (Cabello 2006). In addition, movement and translocation of new genes become high when they are exposed to microbicides and in the same manner occur at non-toxic concentrations of such components. So, once again the contaminated surrounding increases the threat but the major factor or threat is the wastewater that can hold hazardous amount of these components to bring parallel translocation of factors of resistance (Larsson 2014).

For the transfer of resistance factors to human microflora, it is important that a vector (any microbe) must accept these factors to carry it. This leads to the concept that the pathogens related to human have importance in this route of resistance

development and when associated to human microflora it might serve as a source for resistance components (Forslund et al. 2013). Translocation of resistance factors from surrounding to human microflora and role imparted by human association is not properly explored. Moreover, temporary epidermal microbes in atmosphere might serve as vector for transfer of vulnerable genes in human microflora; from these microbes these factors can be easily translocated to microflora of human even in the microbes that attack the weak hosts.

Control for most of the ecological microbes with human microbes is very little. But other vectors include domestic animals which can play role as a secondary host for transfer of these genes and serve as the organism in which these genes can flourish and translocate to human microflora (Allen et al. 2010). Chances of transfer of new resistance factors from treatment plants of water to human microflora is rare because of negligible natural selection. The understanding of role of surrounding for the spread of these genes is less explored and the major concern regarding this is the pathway for translocation of these genes. Brief pathway leads to wide threats in certain ecological setting (Andersson and Hughes 2010).

Broadly, we can conclude that the degree of threat associated to human health must be investigated regarding the vulnerable gene from surrounding to human microflora. On the other hand, involvement of surrounding with high selection for microbicides should be investigated. Thus, controlling or processing the microbicidal contaminated water and avoiding the use of microbistatic and microbicidal compounds in animals, human and hydroponics without any need lead to one of the factors to avoid development of vulnerable genes in surrounding and contribute to control the spread of it. Moreover, controlling the scattering pathways to human microflora may also serve chiefly to control the spread of these genes. To control scattering in the surrounding one should focus on the dispersal of these factors, e.g., the water treatment plants. Processed water from the plants should be treated with microbicides to control the spread of new genes and resistant pathogens. Establishing a pathway or method for treatment of polluted water will serve as a base for eradicating these resistance components from the world and the developing countries should focus over formation and designing of such pathways (Pruden et al. 2013).

3.8 Resistance Genes in Different Environments/Habitats

Antibiotic resistance, posing serious threats to human health, has always been a concern in clinical environments such as hospitals and other healthcare facilities. Accumulation of antibiotic resistance genes in natural environment is also gaining interest of researchers given the evident rise in community-acquired infections caused by resistant bacteria. Transfer of antibiotic resistance conferring plasmids from *Escherichia coli* to chicken and then from chicken to human beings have been reported by Levy et al. (1976). Sediment samples from 18 estuaries (400 km away from the coast of China) were investigated by Zhu et al. (2017) and 200 < subtypes of antibiotic-resistant genes were reported. Natural environments like air, soil, animals and glaciers are important antimicrobial genes reservoirs.

3.8.1 Ocean

Marine environment is loaded with bacteria with resistance against certain antibiotics (Baquero et al. 2008). It is believed that this resistance in bacterial communities is introduced through addition of antibiotics via wastewater treatment plants and run off from agricultural lands. Another major source of antibiotics in marine environments is release from aquaculture farms where antibiotics are used to prevent the spread of diseases commonly occurring in cages/containers with high animal densities. Apart from the introduction of antibiotics from external sources, occurrence of antibiotic resistance without any external introduction has also been proved. One such example is that of resistance genes from human pathogens which, without any known history of transfer, have been reported in marine ecosystem (Pallecchi et al. 2008; Bartoloni et al. 2009). In a metagenomic study, genes conferring fluoroquinolone resistance were found to be present in coral *Porites astreoides*-associated microbial community (Wegley et al. 2007). The presence of these genes was also explained with the presence of microbial community specialized for protection of coral but the metagenomic studies did not support this proposition. Possible presence of any anthropogenic activity-derived fluoroquinolone source was also ruled out.

3.8.2 Air

Like various other environments, air is also serving as a growing reservoir of antibiotic resistance genes. Antibiotic resistance genes and mobile genetic elements such as integrons, plasmids, prophages and transposes are gaining much needed attention. Airborne transmission of pathogens with multidrug resistance (Dijkshoorn et al. 2007) from hospital environment is contaminating the air outside the hospitals and healthcare centres too (Lis et al. 2009). Particulate matter in air play important role in transmission of these genes in urban as well as occupational (hospitals, wastewater treatment plants, cattle feed yards, etc.) environments and hence are capturing interest of researchers (Ling et al. 2013; McEachran et al. 2015; Li et al. 2016; Pal et al. 2016). Ling et al. (2013) reported the presence of *tet* genes (confer tetracycline resistance) 100–200 copies/m^3 of *tet(X)* genes near animal feed operation sites of higher animal populations and in indoor human environments of Colorado, 100–400 copies/m^3 of *tet(W)* were reported. In air samples from wastewater treatment plants of Beijing, sulfonamide resistance genes (*sul2*) were found to be more abundant than sulfonamides (Li et al. 2016). Up to 10^2 copies/m^3 and up to 10^3 copies/m^3, respectively, of airborne *sul1* and *blaSHA* were reported in California's urban parks (Echeverria-Palencia et al. 2017).

According to Hu et al. (2018), the ability of microbes to suspend in air is dependent on the adhesive surface of particulate matter available which is high on polluted days. Other factors which affect the distribution pattern of these genes are meteorological parameters, physiochemical properties and bacterial communities. These studies suggest that transmission of antimicrobial genes through air is as important as any other

way of transmission. Risk of airborne transmission varies from city to city according to their usage of antibiotics. In a comparison of airborne antibiotic resistance gene in San Diego, Beijing and New York, Beijing's air was 5–8 times richer than the other two cities (Pal et al. 2016). Similarly, airborne antibiotic resistance genes in San Diego, Bakersfield and Fresno were found to be an order of magnitude higher than Los Angeles as suggested by real-time PCR technique (Echeverria-Palencia et al. 2017). As for the third world countries, such studies regarding antibiotic resistance genes and the possible health risks that they pose are still lacking. Moreover, despite the fact that all studies regarding air pollution take account of concentration of particulate matter, abundance of antimicrobial resistance genes are entirely disregarded.

3.8.3 Soil

Soil is the richest type of microbe ecosystem and is possibly the ecosystem where the antibiotic synthesis actually evolved (D'costa et al. 2007) which makes soil the most credible source of antibiotic resistance elements. One gram of soil is said to be containing more microorganisms than there are humans of planet earth (Trevors 2010). In addition to that, <1% of these microorganisms is culturable (Singh et al. 2004; Vogel et al. 2009). A wide range of soil types, i.e., agriculture, prairie, urban and forest soil, have been and are used to formulate different medicinal and clinical applications. This speculation can be supported by the fact that about 80% of clinically used antibiotics have been procured either as natural products or as semi-synthetic derivative compounds from soil microbes (Martín and Liras 1989). The supposition that antimicrobial resistance evolved from soil microbial communities can be supported by the evidence of molecular level similarities between resistance mechanisms depicted by soil bacteria and clinical pathogens. One such case is that of soil bacterium Streptomyces, the resistance mechanism of which was found to share similarities with clinical pathogens in 1973 (Benveniste and Davies 1973). There are several pathogens which can thrive in soil and this ability gives them a chance to emerge as antibiotic-resistant strain given the formation of their potent mixture with soil bacteria. Metagenomic techniques are quite the techniques of choice for the characterization of diverse soil bacterial communities (Monier et al. 2011). These studies indicate that there are multiple resistance mechanisms used by different microbes from different types of soil. Broad activity range of these microbes enables them to resist even the newly developed antibiotics. Several soil bacteria species are naturally resistant to some antibiotics like β-lactams (Demanèche et al. 2009). Study of Alaskan soil showed the presence of genes conferring resistance against ß-lactam based antibiotics though any exposure to anthropogenically introduced antibiotics wasn't known (Allen et al. 2010), study by Torres-Cortés et al. (2011) gene encoding reductase offer resistance to trimethoprim (synthetic antibiotic interfering with tetrahydrofolic production). In soil inhabiting *E. coli,* nine different clones of genes confer resistance to five different aminoglycoside-based antibiotics and one clone confer tetracycline resistance (Riesenfeld et al. 2004). These genes shared <60% sequence similarity with already known sequences.

Table 3.1 Concentrations of different antibiotics in soil and sludge from wastewater treatment plants

Environment type	Antibiotics	Concentration (μg/g)	References
Soil	Ciprofloxacin	5.6	Hamscher et al. (2002), Thiele-Bruhn (2003), Martínez-Carballo et al. (2007), Dolliver et al. (2007), Karci and Balcioglu (2009), Hu et al. (2010), Carter et al. (2014), Van Doorslaer et al. (2014), Liu et al. (2016), Tasho and Cho (2016), Pan et al. (2017), and Łukaszewicz et al. (2018)
	Chlortetracycline	12.9	
	Difloxacin	0.0215	
	Doxycline	0.728	
	Enroflaxacin	1.3476	
	Erythromycin	0.0072	
	Linomycin	0.00117	
	Monensin	0.0000004	
	Norfloxacin	2.16	
	Ofloxacin	0.898	
	Oxytetracycline	50	
	Sulfachloropyridazine	0.0529	
	Sulfadiazine	0.0855	
	Sulfadimethoxine	0.0404	
	Sulfadoxine	0.0091	
	Sulfamethoxazole	0.0545	
	Sulfamethazine	0.2–25	
	Sulfamonomethoxine	0.00537	
	Sulfapyridine	0.00511	
	Tylosin	1.25	
	Tetracycline	2.683	
Sludge from wastewater treatment plants	Chlortetracycline	3843.79	An et al. (2015)
	Doxycycline	2104.27	
	Oxytetracycline	7369.67	
	Sulfamethoxazole	665	
	Sulfadiazine	50.32	
	Sulfamerazine	37.21	
	Sulfadimidine	27.14	
	Clarithromycin	0.3–17.6	
	norfloxacin	2.3–6800	
	Ofloxacin	3.5–5100	
	Roxithithromycin	0.1–630	
	Sulfadiazine	0.4–71.8	
	Sulfadimidine	0.2–219	
	Sulfamethoxazole	0.6–765	
	Tetracycline	2.4–313	
	Trimethoprim	0.2–870	

Table 3.2 Concentrations of different antibiotics in wastewater used for irrigation and seawater

Environment type	Antibiotics	Concentration (ng/L)	References
Wastewater used for irrigation	Ciprofloxacin	10	
		970	
		310	
	Enrofloxacin	250	Karthikeyan and Meyer (2006)
	Erythromycin	3900	
	Norfloxacin	250	
	Oxytetracycline	47,000	
	Roxithromycin	1500	
	Sulfamethoxazole	156	
		1340	Batt et al. (2006)
		310	Karthikeyan and Meyer (2006)
	Sulfamethazine	50	Karthikeyan and Meyer (2006)
		300	
	Tetracycline	560	Batt et al. (2006)
		48,000	Karthikeyan and Meyer (2006)
Seawater	Erythromycin	0.1–1900	Gaw et al. (2014)
	Clarithromycin	0.3–17.6	
	norfloxacin	2.3–6800	
	Ofloxacin	3.5–5100	
	Roxithithromycin	0.1–630	
	Sulfadiazine	0.4–71.8	
	Sulfadimidine	0.2–219	
	Sulfamethoxazole	0.6–765	
	Tetracycline	2.4–313	
	Trimethoprim	0.2–870	

3.9 Pathways of Resistance

Bacterial communities employ various ways to deal with microbicidal components to develop resistance. These methods include both pre-existing ways and the one induced using the method of genetic engineering (Cruz et al. 2002). Pre-existing methods comprise following components:

1. Utilization of previously occurring genes of pathogens.
2. Change in genes resulted from mutations that includes both pre-formed genes and newly engineered genes.

The pathway utilized by the pathogen often need the newly engineered methods involving genome of pathogens to be used to gather the genes, their addition and movement of exotic resistance factors. One of the factors that leads to formation

of resistance against microbes is formation of consortium of the pathogen in which cells stick to each other embedded in mucilage. Formation of resistance using previously formed system in the pathogen producing the antimicrobial agents naturally is well known in resistant microflora. This phenomenon is mostly observed among the edaphic samples of *Streptomyces* which shows that the common resistance pathways involves the outflow methods or targeted antimicrobial strategies (Canton and Coque 2006). Recently, the investigators come with the statement that resistance factors can even develop in ecological settings and ecological microbes without presence of ability to manufacture microbicides. Most pathways show mysterious factors for the formation of resistance in ecological bacteria which have the ability to exhibit itself in new pathogens. One of the examples is growing group of broad spectrum β- lactamases, the CTMX-M enzymes that have the most effective pathway for development of resistance including broad spectrum antibiotic class: the cephalosporins. Other examples include proteins with microbicidal effects like ciprofloxacin, levofloxacin and other proteins with topoisomerases, i.e., the Qnr-proteins. Qnr gene is associated to algal species *Shewanella* while the blaCTX-M gene is associated to bacterial strain *Kluyvera*. Both these species are not documented as antimicrobial agents but carry these resistance components, respectively (Robicsek et al. 2006). Both of these species do not show any type of natural defence or inhibition against the discussed class of antibiotics. Species with these genes are increasing and have clinical importance (Poirel et al. 2008).

3.10 Antimicrobials Co-selectors and Resistant Pathogens in Environment

Release of microbicides and various microbistatic components, for example, antiseptics and high-density metals, in surrounding habitat have ability to bring changes for the formation of multidrug-resistant bugs. These factors that contribute to formation of resistance are part of reservoir like the water body or the soil in different quantities. The solubility of these agents in these water bodies and other ecological settings is in huge amount (Kummerer 2009). Chemicals from factory waste, clinics and housing societies increase the amount of microbicidal and microbistatic chemicals including high density metals. A few medicinal companies produce different drugs and release extreme variety of antimicrobials in the surrounding which results in excessive amount of these chemicals in surrounding even more than the amount required to cure any attack (Larsson 2010). The increased resistance produced in the waste confirms that the antimicrobial resistance forms in contaminated surroundings too (Rutgersson et al. 2014). The number of contaminants on the surface of water bodies and on the surface of soil will be 1000 times less as compared to untreated wastewater.

This little number of contaminants in these water bodies is not harmful for the pathogens but is enough for the production of resistance factors (Gullberg et al. 2011). Here the query that arises is at which point antimicrobial agents lose their effect on pathogens. The quantity of antimicrobial compounds when is low in the water, development of resistance becomes dependent on translocation of resistance genes between pathogens by conjugation, which is termed as parallel gene transfer. So, the study of single strain on media plates do not give any idea about formation and development of multidrug resistance in microbes occurring in ecological setting.

The number of contaminants present in freshwater is based on the treatment plant used for processing that water and the antimicrobial chemicals used by the society that utilizes this water. Wastewater processing plants are usually designed to eradicate contaminants including dissolved and suspended solids and other elements and even the microbes but not the microbicides (Pruden et al. 2013). Waste material of animals may also have antimicrobial components used during treatment of their diseases. Once the chemicals land on soil surface, some remains active and face the stability pressure by edaphic pathogens while others requires neutralization for their activation (Subbiah et al. 2011). The knowledge about the events that occurs when antibiotics interact to microflora is important for better understanding of natural selection in the surrounding (Berendonk et al. 2015). The environment is supplemented by variety of contaminants including microbicides which provide huge pressure for natural selection as compared to a single treatment. Different metals like Ag, Cd, Cu, Mg and Zn are reported having microbicidal activities and mode of action (Seiler and Berendonk 2012). These metals are found in all environments including fields, societies, factories and all other habitats which may result in multidrug resistance development if pressure for their selection becomes negligible.

Antimicrobials and the pathogens resistant to them appear from the same origin and frequently originate mutually. Chief reservoirs for these are the discharge of factories, waste by animals, and other waste water from fields which might hold different pathogens with multidrug resistance. The release of unprocessed waste water to surrounding facilitates development of antimicrobial factors in the surrounding and this factor is hard to be removed. Even in developed countries where this water is processed properly, large variety of resistant pathogens develop in water reservoirs. The results of waste water treatment plants are contradictory; in some cases it results to decrease the quantity of pathogens in sewage and even complete removal of them is reported by some investigators while, on the other hand, it is reported to harbour huge number of resistant pathogens (Stalder et al. 2012). This huge amount depicts that may be the processing plant serves as reservoir for parallel gene transfer due to high number of pathogens and organic nutrients. That's why wastewater is given importance and is considered as main investigation tool for resistance formation in human microflora (Zhang 2016).

3.11 Mitigating Discharge of Antimicrobials in Environment

Existing rigid policies have been sluggish to correctly judge release of antibiotics and bacteria with multidrug resistance. Given the harm that antibiotic residues cause to aquatic life, three antibiotics were included in European Union Watch List of rising water pollutants in 2016. Different drug manufacturing companies have come forward and taken initiatives to diminish the high antibiotic quantities in sewage water. With the core theme of management and control of antibiotics' production, a roadmap was proposed to the United Nation in September 2016, which was signed by several prominent pharmaceutical companies.

Triclosan, antibacterial compound used in a wide range of products, along with other co-selection compounds, has been banned in different parts of the world. Wide and regular use of triclosan in personal care and cosmetic products has been addressed as an issue by the Association of Southeast Asian Nations (ASEAN 2016). Based on the studies which indicate the harmfulness (hormonal imbalance and multidrug resistance) of long-term use of triclosan and other compounds, United States Food and Drug Administration (US FDA) ordered restriction of over-the-counter drugs containing such compounds.

Vigorous regulation of co-selective compounds and antibiotics will possibly force the expansion of reasonable improvement and formulation of solutions for risk management and arouse argument of accountability for antibiotic-resistant bacteria and antibacterial residues. Debatably, the antibiotic producers, recommenders, and consumers are responsible for destructive effects which antibiotic residues offer to surroundings. Changes in the mode we undertake antibiotic resistance, chiefly in the terms of a One-Health approach, can change incentives to diminish antibiotic and to develop waste managing strategies.

Many easing strategies have already been developed that lessen or take away resistant bacteria and harmful antibiotics from waste water streams inflowing the environment. These strategies include secondary and tertiary wastewater treatment, membrane ozonation, filtration, heat treatment and UV disinfection (more effectual ways of removing feasible bacteria). The usefulness and level of effect are inconsistent and can create unintentional consequences like introduction of deadly by-products. Treatment of animal waste prior to its application to land and other simple ways to lessen water pollution can also help immensely to deal with the rising concentration of antibiotics. The major barrier in adopting these is the need of time to comprehend and minimize the risks posed by antimicrobial resistance and to build up mitigation technologies which are environmentally sustainable.

It is argued that the complication of the crisis is so immense as to be immeasurable, taking into account the probability of connections among inconceivable figures of bacteria with an apparently continual capability for gene transfer, composite mixtures of selecting chemical agents and the wide range of mechanisms of resistance-building (Smith et al. 2005).

Given the fact that wherever anthropogenic activities occur, augmented levels of antibiotics and bacterial resistance against them are witnessed. It is already recognized that underneath laboratory conditions, a few antibiotics offer selection for antibiotic resistance under their concentrations in which they are present in natural environment. Moreover, it is also known that some of the resistance genes of clinical significance that have emerged in different pathogens have actually originated in bacteria. Already available data shows that transfer of antibiotic compounds is likely to be occurred through food chains and during contact to contaminated environments. A call for evidence-based decisions emerges frequently, but with an issue as complex as antibiotic resistance, how much substantiation is enough? Acquiring enough data of the type produced by clinical trials may be impractical or such a difficult task that we risk massive delays in devising guideline for safe use of antibiotic and performance of alleviation methods.

3.12 Antibiotic and Antibiotic Extended Resistomes

Meta-genomic tools have been helping greatly in identifying resistance genes in isolates obtained from the surroundings. These genes outline parts of former machineries of bacteria which were competent in producing substances with antimicrobial activity (more commonly known now a days as natural inter-microbial signalling molecules) (Linares et al. 2006). To represent the compilation of antibiotic resistance genes identified in microorganisms (pathogenic as well as non-pathogenic bacteria), a new term was introduced: "antibiotic resistome." This term employs the idea of a sole pool of all antibiotic resistance genes known to us. It deals with the resistance genes by antibiotic producers and ancestor genes which, under suitable selective pressure, tend to develop to act as resistance elements. Largely, there are cryptic resistance genes which do not get expressed naturally in ecological isolates (Wright 2007). The idea of antibiotic resistome was first used by D'costa et al. (2006) when making a library of more than 480 strains of different species of *Streptomyces* recovered from diverse ecological sources which were then tested against 21 different antibiotics. Inside the said library, resistance genes were recognized in all strains and the resistance mechanisms were reported to be counteract against natural as well as synthetic antimicrobials. A few of the reported resistance mechanisms like inactivation mechanisms distressing telithromycin or daptomycin (two of the recently introduced antimicrobial) have not been categorized in clinical isolates yet. This meta-genomic approach has been extended and is also currently functional to resistance genes in surrounding and genetic elements taking part in transfer of resistance gene, giving way to a new term "antibiotic extended resistome" (D'Costa et al. 2007). This idea is practical for the examination of ecological variety in diverse ecosystems ranging from broad settings (entire healthcare settings) to

single patient (e.g., the intestinal section) and can be proved helpful for calculation of prospect development of antibiotic resistance. The investigation of a few of the latest antibiotic resistance induction methods in the clinical settings reinforces these approaches.

3.13 Novel Genes Formation Related to Antimicrobial Pathogens

The resistance methods in different pathogens include out flow forces of different enzymes related to class of enzymes for tetracycline, methylase and acetylases resistance which modify the aminoglycosides in pathogens for development of resistance (Canton and Coque 2006). Analogues of these classes are found in ecological bacteria and now new multidrug-resistant genes are being added to this list day by day.

3.14 Aminoglycosides Affected by Methylase Activity of rRNA

The small rRNA subunit is mainly involved in resistance for aminoglycosides (Doi and Arakawa 2007). It was first studied and identified in 2012 from *C. freundii* (Accession Number AF550415) in Poland, later on it was also discovered in Japan in the strains of *P. aeruginosa* and in late 2003 reported in *K. pneumoniae* in France. Moreover, few researches also document its presence in Enterobacters like *P. mirabilis, E. aerogenes, S. marcescens, E. coli, K. oxytoca, S. flexneri* and other *Salmonella* species. Most of the investigations in which these were reported were clinical based.

The *armA, rmtA, rmtC, rmtB* and *rmtD* genes are included in assembly of aminoglycoside methylases. Arrangement of components of these proteins suggests that these components emerged in actinomycetes first and in yeast as well as *Micromonospora* sp. (Yamane et al. 2005). These genes like other resistance genes confirm the presence of a unique initial sequence called ISCR1 similar to genes of other resistant pathogens. These are carried by plasmids to distribute among other members of microflora by process of conjugation (Doi and Arakawa 2007). This enzyme of small ribosomal subunit has also been encountered in different pathogens and is named as *blaspm1, plasmid blaampc, blaBLEE* and other enzymes responsible for modification of aminoglycosides and quinoline related resistance. Studies showed that these genes may be part of jumping genes or of the integron (Gonza'lez-zon et al. 2005).

3.15 Methyltransferase Activity Against Linezolid

Linezolid (an oxazolidinone complex) is a vigorous chemical agent against Gram-positive strains like vancomycin-resistant *Enterococcal* strains and methicillin-resistant *S. aureus*. It is an artificial compound capable to cease the reproductive activity of bacteria that ceases synthesis of protein by fastening to the ribosome large subunit (50S) at the P-site. Bacterial strains with linezolid resistance have seldom been explained. As observed and explained in experimental vancomycin-resistant *Enterococcal* and methicillin-resistant *S. aureus* strains and *in vitro* mutants of *S. pneumoniae*, resistance is conferred due to changes in domain V of 23S RNA (Wilcox 2005). Deletions in genes responsible for programming the L4 riboprotein have been studied in *S. pneumoniae* and these deletions have been found to be responsible for cross-resistance to oxazolidinones macrolides and chloramphenicol (Wolter et al. 2005). Furthermore, in *Mycobacterium tuberculosis,* efflux pumps which disturb linezolid have been recognized (Escribano et al. 2007). In recent times, a methyltransferase (encoded by the *cfr* gene) was reported to be disturbing the linezolid ribosome target and was documented in a clinical strain of *S. aureus* found in Colombia (Toh et al. 2007). This enzyme adds adenosine in 23S rRNA of 50S ribosomal subunit at position 2503. Even though linezolid is an artificial antibiotic, the *cfr* gene is thought to be of natural basis and might be accountable for resistance methods linking defence adjacent to naturally present antibiotics whose site of action overlaps that of linezolid. The Cfr methyltransferase also cause changes to lincosamides, streptogramin A, chloramphenicol, and pleuromutilins and other antibiotics. In the *S. aureus* strain mentioned above, the *cfr* gene was present in linkage to the *ermB* gene, which confers dimethylation of A2058 in 23S rRNA, hence providing resistance to erythromycin. Chromosomal location of the *ermB/cfr* operon was formerly identified (in coagulase-negative *Staphylococci* of animal origin) as transposable elements inside a conjugative plasmid (Long et al. 2006). This depicts dissemination of a resistance machinery of potential ecological basis disturbing artificial antibiotics, in this case, linezolid.

3.16 Plasmid-Mediated Quinolone Resistance Associated with Efflux Pumps

Conventionally, resistance against quinolone is basically because of targeted moderation and outflow pumps. The mechanisms of targeted moderation for formation of resistance includes mutational changes in topoisomerase that includes mutational changes in *parC* gene of Gram +ve bacteria and *gyrA* gene mutational changes in Gram -ve bacteria. While formation of resistance due to outflow pumps includes variety of families of outflow pumps. Both these procedures are

chromosome based and generally more familiar during studies of clinical settings. In recent years resistance against quinolone supported by plasmids is surprisingly originated. It is based on following three points:

1. Qnr proteins protective for topoisomerases.
2. Enzymes that modifies quinolone, i.e., AAC(6′)-Ib-cr.
3. The outflow pump, i.e., *QepA* (Perichon et al. 2007).

Possible formation and development of MFS outflow pump transport in ecological strains is exposed due to sequence studies of *qepA* gene and its position and arrangement with related genes like in actinomycetales. Examples of actinomycetales includes *N. farcinica, S. clavuligerus* and *S. globisporus*. Recent studies confirmed that this gene is related to jumping gene elements and plasmids *IncF1*, which allows its exchange between different strains of pathogens (Yamane et al. 2005). Portable formation of this struggle pathway is confirmed by these consequences (Poirel et al. 2005) but, if we consider the phenotypes of isolates, it shows that the wild strains with this gene have high minimum inhibitory concentration than those strains which are trans-conjugated. This is due to existence of other genes along with this gene which distress presence of quinolones. Even though the outflow pumps of *QepA* gene gives only a minor lift to inhibitory concentration standards, its presence may favour expansion of elevated resistance methods. In resistomes high amount of resistance against fluoroquinolones gathering methodology along with presence of this gene also favour other newly originated genes related to resistance.

3.17 Proteins Protecting Qnr Topoisomerases

Qnr gene resistance is plasmid-based resistance and was first identified in *K. pneumoniae* in 1998. Those strains were capable to produce β-lactamase (FOX-5) enzyme that effects wide diversity of cephalosporins (Martinez-Martinez et al. 1998). This novel apparatus includes the methodology to protect the topoisomerase enzymes from fluoro-quinolones. Similar to the gene *qepA*, it in spite of offering lower resistance helps for assorting strain with advanced resistance including those with changes at genetic level in topoisomerases (Rodriguez-Martinez et al. 2007). *Qnr* families include proteins, e.g., *QnrS* and *QnrA*, which allocates inconsistent resemblance to protein structure. Same comparable building structure of amino acids is recognized in strains of Mycobacterium, i.e., in *M. tuberculosis* and *M. smegmatis*. Mainly this family of gene is reported in strains of Enterobacters during clinical studies having additional pathways for resistance, e.g., the CTX-M ESBL pathway, and recently known plasmids supported outflow resistance pathways and other plasmid derived enzymes like *AmpC* enzymes and *VEB-1* enzyme related to β-lactase activity. Moreover, some other classes of enzymes that modifies aminoglycosides are also included.

Presence and origin of *Qnr* enzymes was recognized using PCR analysis of gene sequence of Gram-negative strains. The source of this gene was identified as algae species *Shewanella* found in oceans and fresh water bodies (Poirel et al. 2005). In the *Shewanella* species of algae, all the four variables of this gene *qnr* type A were recognized. The *QnrS* gene of *Vibrio splendidus* was with about 41–71% similarity in the arrangement of amino acids comparable to other species found in water including *V. parahaemolyticus, P. profundum* and *V. vulnificus* (Cattoir et al. 2007). This concludes that gene swap over happens among ecological species and species of Enterobacters and results in assumption that under the pressure of natural selection for fluoro-quinolones, these genetic factors have transmitted on moveable genes, i.e., transposons. Recent investigations show presence of the resistance factors of *qnr* related to fluoro-quinolone in species of *Aeromonas* found in river isolated from water in France.

Recently, these factors are identified in the platform known as mobile insertion cassette "mic" which are related to jumping genes of plasmids found in every environment. Both interpretations boost the prospect of distribution of these genes in ecological setting and highlights that these pathogens may serve as source of *qnr* genes in pathogens of water bodies like in case of gene responsible for resistance against tetracycline, i.e., *tet* genes. Similar inherited essentials might also have played role in recruitment of *qnr* genes, these factors includes transposons of methylase activity in ribosomes that distresses blaCTXM genes that hydrolyses wide class of cephalosporins or may also distress aminoglycosides (Cattoir et al. 2008). Actually, researchers are investigating the possible pathways for *qnr* linked classification of genes in relation to *ISCR1* gene, formerly connected to previously present genes responsible for resistance.

3.18 CTX-M ESBLs

Through current years the most productively emerged and extended resistance equipment is the CTX-M ESBLs. These *β*-lactamases were primarily elaborated in Argentina and Germany for more than two decades ago. This includes diverse varieties of this enzyme (i.e., *CTXM-8, CTXM-25, CTXM-9, CTXM-2* and *CTXM-1*), which have strong hydrolytic activity for cefotaxime and also effectively hydrolyses ceftazidime (Canton and Coque 2006). Recent investigations reveal that in *E. coli* isolated from public and veterinary strains, a spectacular boost in β-lactamases enzyme is seen. This rise comprises its connection to plasmids transferred in conjugation process in strains related to *ISs* gene including *ISEcp1* gene and *ISCR1* gene linked to phage sequences. Even though these conjugative plasmids are broadly circulated among pathogens and are smoothly translocated, it is important that the gene *CTX-M bla* and connected genes of integron class 1 are placed inside translocatable pathway like in sub-families of *Tn21*.

These genes incorporate themselves in the genes for resistance and become linked to the resistance factors working against bactericidals and play role in bacterial co-selection. Furthermore, this is not surprising to find the CTX-M genes of bacteria with relation to other genes because they are originated on same genetic pathway like the genes that code for resistance, i.e., methylase gene in 16S rRNA including *rmtB* and *armA*, fluoro-quinolone distressing genes (*qepA*), acetylases that drive out fluoro-quinolones and other *qnr* genes including its varieties A, B and S (Barlow et al. 2008). If we take it from an evolutionary point, the species of *Kluyvera* are considered as ancestors of the gene *CTX-M bla*. Similarly, the ancestor of *CTX-M1* and *2* is *K. ascorbata* and for *CTXM-8* is *K. georgiana*. The phylogenetic studies reveal that this gene have moved in species of *Kluyvera* over period of time on different chromosomes in addition to β-lactamases responsible for its translocation in species. Furthermore, during parallel gene transfer this gene has moved out of its precursor which was incorporated in chromosomes of *Kluyvera* sp., in early times. This gene become reserved in its ancestor and other strains of the same genes because they are vulnerable to wide range of cephalosporins. The importance and significance of this ancestral species and its role in determining resistance need more adequate study (Lartigue et al. 2006).

3.19 Future of Resistant "Superbugs"

Multidrug resistance might not be the only threat we will front in the future. Bacteria can also undergo mutations, recombination and horizontal gene transfer to produce genetic variety. All mentioned procedures can contribute process of selection, with the possibility for fitness-reducing mutations. Antibiotic disclosure has been revealed to boost the recombination frequencies in bacteria through the SOS response, even at sub-inhibitory levels (Beaber et al. 2004). Revelation of bacteria ecologically to altering antibiotics levels is consequently expected to produce variables with elevated rates of genetic transformation, in count to the selection for resistance. Colonies of bacteria with elevated rates of mutation are liable to swiftly attain useful mutations and also liable to rapidly produce genetic changes which are compensatory, so antibiotic contact may favour fixation of bacterial populations with raised rates of genetic changes usually. Moreover, antibiotics are repeatedly discharged into the surroundings collectively with integrons and other mobile genetic elements containing bacteria. It is not possible to calculate precisely what consequences bacterial pangenomes may have for this. Bacteria can adjust with the help of integrons and other mobile genetic elements to novel niches so mobilized genes would liable not to be constrained to confer antibiotic resistance, but might also include genes that supply a fitness benefit in terms of adjustment to varying settings. This depicts an image of a miserable upcoming where human pathogens may not only be non-sensitive to mainstream antibiotics, but also would develop into more

violent strains and increase among humans. It has already been seen a glimpse of it in Chinese hospitals, with hypervirulent *K. pneumoniae* tolerant to all available antibiotics tested (Gu et al. 2017).

3.20 Conclusion

The appearance of new resistance genes and gene factors is a continuous process. The dispersion strategies for ecological resistance are liable to the limited selection pressure and minimal dispersion routes. The variety of resistance genes in environment depicts that still there are many genes yet to be identified. The resistance genes are not likely to be eradicated even if antibiotic selection is absent. That's why these genes are already re-circulating and can emerge easily during treatment. A little is known about environmental role for formation of resistance genes but studies suggest that environment is one of the chief factors for their emergence. So, we need to focus studies on environmental factors responsible for the emergence of these genes for a better understanding of whole phenomena.

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Chapter 4
Global and Temporal Trends in the Use of Antibiotics and Spread of Antimicrobial Resistance

Iqra Sarfraz, Azhar Rasul, Ghulam Hussain, Syed Makhdoom Hussain, Khizar Samiullah, Bilal Rasool, Ammara Riaz, Muhammad Asrar, and Zeliha Selamoglu

Abstract Antibiotics, the wonder drugs, have undoubtedly served humans in combating variety of microbial infections. For years, antibiotics have been extensively utilized for therapeutic indications not only for humans but also across other related sectors as animal husbandry and agriculture fields. Irresponsible and off-set utilization of antibiotics have led to the emergence of resistance. This chapter intends to focus on the utilization of antibiotics, origin, mechanism, and consequences of antibiotic resistance. Resistance refers to the inability of drugs to work effectively. Microbes evolve resistance mechanisms via mutational adaptations, modification of genetic expression, alteration of metabolic pathways, and acquisition of resistance genes. An increasing list of microbial infections is becoming difficult to cure because of ineffectiveness of antibiotics leading to increased mortality rates. Global and temporal trends of antimicrobial usage and antibiotic resistance show that the antibiotic resistance can be highly attributed to the increased uptake of inappropriate as well as utilization of unprescribed antibiotics all over the world which can lead towards the serious consequences. Hence, the need of the hour is effectively devise ways and means to overcome this global dilemma which continues to rise day by day. Recently reported data suggests that there is immense need to educate healthcare, animal husbandry, agricultural sector to control the misuse and overuse of antibiotics.

I. Sarfraz · A. Rasul (✉) · S. M. Hussain · K. Samiullah · B. Rasool · A. Riaz · M. Asrar
Department of Zoology, Faculty of Life Sciences, Government College University Faisalabad, Faisalabad, Pakistan

G. Hussain
Department of Physiology, Faculty of Life Sciences, Government College University Faisalabad, Faisalabad, Pakistan

Z. Selamoglu
Department of Medical Biology, Faculty of Medicine, Nigde Ömer Halisdemir University, Nigde, Turkey

M. Z. Hashmi (ed.), *Antibiotics and Antimicrobial Resistance Genes*, Emerging Contaminants and Associated Treatment Technologies,
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4.1 Introduction

Antibiotics allow the body's innate defense systems to eliminate disease-causing microorganisms via their cytotoxic actions. Antibiotics mechanism of action is usually associated with the inhibition of cell membrane growth and formation of proteins, DNA, and RNAs by membrane-disrupting agents (Sass 2017). To control the microbial infections/diseases, antibiotics has served humans since times (Chandel and Budinger 2013). Modern era of antibiotics is associated with the renowned names of Fleming (Tan and Tatsumura 2015). During the nineteenth century, antibiotics were contemplated as magic bullets because of their selectivity in targeting disease-causing microbes (Aminov 2010).

Antibiotics are between the most valuable drugs that ever developed. After their exploration, it became clear that bacteria could have the ability to generate resistance against them. From some past decades, this issue was mitigated through the continuous introduction of new antibiotics. However, it has decreased down greatly in the recent years. According to World Health Organization report, the development and distribution of antibiotic-resistant bacteria in the hospitals means that everyday medical proceedings once early taken for granted could be possibly relegated to medical limbo (Levy and Marshall 2004).

Millions of antibiotics have been approved during the last 60 years since the outset of antibiotics. Utilization of these antibiotics has undoubtedly blessed human populations by saving millions of lives. But the enhanced requirement of antibiotics in various sectors leads to off-label utilization of these drugs. Regrettably, irresponsible and excessive use of these drugs led to the evolution of resistant species of microbes (Davido et al. 2016). Thus, resistance has emerged as a great matter of concern now-a-days (Table 4.1).

The fortunate discovery of Penicillin by A. Fleming, 1928, was the proof of his faith and determination (Fig. 4.1). Penicillin also target microbes via inhibition of its cell wall synthesis (Aminov 2017).

Antibiotic resistance among bacteria is an adaptive characteristic after challenge with the therapeutic drugs. This allegation is in part repetitious since no drug which lacks inhibitory action towards pathogens, regardless of whether it prohibits other organisms, will ever be noted as an anti-infective agent (Subedi et al. 2018).

Antibiotic resistance mostly occurs as a natural selection procedure where nature authorizes all the bacteria with some extent of small-level resistance. For example, an investigation proved that trimethoprim, tetracycline, sulfamethoxazole, and ampicillin have no more appearance in curing non-cholera diarrhea in Thailand. On

Table 4.1 Different types of antibiotics with their target microbes and modes of resistances

Antibiotic category	Antibiotic class	Examples	Target microbes	Mode of resistance	Reference
Protein synthesis inhibitors	Aminoglycosides	Streptomycin, Dihydrostreptomycin, Neomycin, Kanamycin, Tobramycin, Spectinomycin, Hygromycin B	*Malassezia pachydermatis, M. tuberculosis*, Aerobacter, Escherichia, Klebsiella, Salmonella, Shigella, Proteus, some strains of *Pseudomonas aeruginosa*	Phosphorylation by aminoglycoside 3′-phosphotransferase genes, β-lactams, Phosphorylation, acetylation, efflux, altered target	Etebu and Arikekpar (2016), Kapoor et al. (2017), Ramirez and Tolmasky (2017)
	Tetracyclines	Doxycycline, Chlortetracycline, Tetracycline, Metacycline, Minocycline, Oxytetracycline	*Enterobacteriaceae*, *Salmonella enterica*	Inactivation of antibiotics via enzymatic modification, Non-binding with the ribosomes	Etebu and Arikekpar (2016), Kapoor et al. (2017), Markley and Wencewicz (2018)
	Amphenicols	Chloramphenicol, Thiamphenicol, Florfenicol	Gram-positive, Gram-negative pathogens, *Bordetella bronchiseptica*	Genetic mutations, acetylation, efflux, altered target	Kadlec and Schwarz (2018), Kapoor et al. (2017)
	Macrolides	Azithromycin, Clarithromycin, Oleandomycin, Erthromycin, Roxithromycin	*Mycoplasma pneumoniae, Legionella pneumophila*, *Bordetella pertussis*, Spirochaetes, *Haemophilus Influenza*	Hydrolysis, glycosylation, phosphorylation, efflux, altered target	Etebu and Arikekpar (2016), Kapoor et al. (2017), Yang et al. (2017)

(continued)

Table 4.1 (continued)

Antibiotic category	Antibiotic class	Examples	Target microbes	Mode of resistance	Reference
Nucleic acid inhibitors	Trimethoprim	Trimethoprim	*E. coli*	Chromosomal mutations, enhanced barriers for the dissociation of substrate and its product	Abdizadeh et al. (2017)
	Sulfonamides	Sulfathiazole, Sulfamethoxazole, Sulfadiazine, Sulfamerazine	*Nocardia, E. coli, Klebsiella, Salmonella, Shigella, Enterobacter, Chlamydia trachomatis*	Folic acid sources, efflux, antibiotic degradation	Etebu and Arikekpar (2016), Kapoor et al. (2017), Vila-Costa et al. (2017)
Cell envelope inhibitors	Glycopeptide	Vancomycin, Teicoplanin	*S. aureus, E. faecium, Streptococcus bovis, E. faecalis, E. avium, E. gallinarum, lactis, Lactococcus cremoris*	Reprogramming peptidoglycan biosynthesis	Etebu and Arikekpar (2016), Kapoor et al. (2017)
	Pencillins	Amoxicillin, Ampicillin, Carbenicillin, Ticarcillin, Temocillin, Azlicillin	*Treponema pallidum, Streptococcus pneumoniae*	Point mutation hydrolysis, efflux, altered target	Etebu and Arikekpar (2016)
	β- lactams	Carbapenemes	*S. aureus, S. epidermis, E. coli, Klebsiella*	Production of β-lactamases	Etebu and Arikekpar (2016), Kapoor et al. (2017)

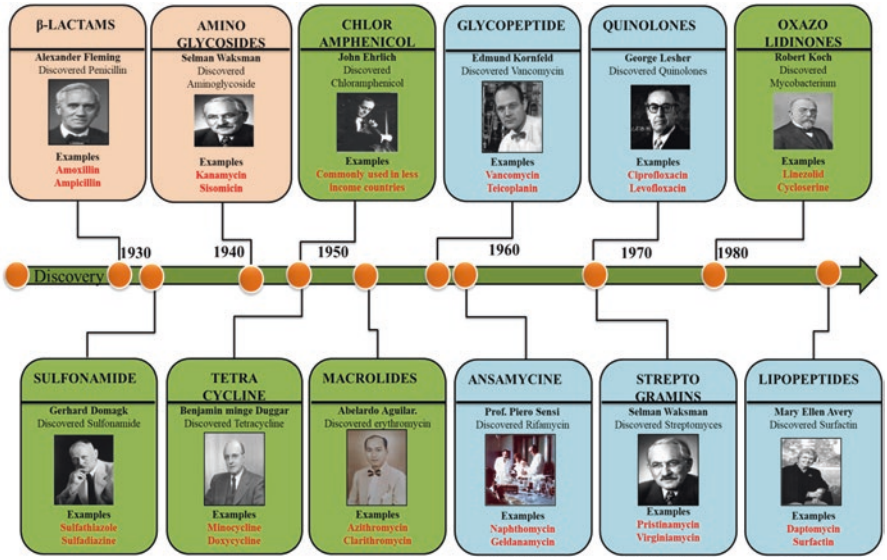

Fig. 4.1 Major classes of antibiotics and their discovery

the spot, another research was carried out in Bangladesh which revealed the efficacy of the same antibiotics in treating diarrhea disease. Indeed, resistance was recorded even before starting the use of antibiotics in fighting against the infection (Hoge et al. 1998; Rahman et al. 2017). Illegal utilization of antibiotics is liable for producing resistance in the microbes (Gootz 1990).

From the initiation of sulfonamides in 1937, the discovery of specialized systems of resistance had aggravated their curative usage. However, sulfonamide resistance was recorded in the 1930s which shows the same process that is still valid now, more than 80 years later (Chopra et al. 2002). Furthermore, methicillin was the very first of semi-synthetic penicillinase-resistance antibiotic to target *Staphylococcus aureus* and was introduced in 1961. Its resistance was documented early after its development. Similarly, fluoroquinolones were reported for treating Gram −ve bacterial ailments in 1980s but later on their resistance demonstrated that these antibiotics could also be useful to cure Gram +ve bacterial diseases (Lowy 2003). Recently, in 2002, the clinical isolates of VRSA which stands for Vancomycin-resistant *S. aureus* were found, after 44 years of discovery of Vancomycin to the market (McGuinness et al. 2017).

Antibiotic-resistant genes usually arise from the pathogenic and nonpathogenic bacteria. Bacteria obtain the genes in order to have successful survival in many adverse conditions (Martinez 2014). Mutations that cause antibiotic resistance generally occur in three different types of genes. Among them are those encoding their transporters and regulators which reduce the level of antibiotic-disinfecting factors (van Dijk et al. 2017).

Since a variety of antibiotics are produced by environmental microorganisms, it has been concluded that the origin of such bacterial-resistant genes fundamentally lie in the antibiotic producers. While recent knowledge of resistance genes in human pathogens declares that the area of microorganisms supplying these genes is much expanded. In fact, origin of just two of the resistant genes which have been obtained by human pathogens has been evidenced. These two genes include quinolone-resistant gene QnrA (in *Shewanella algae*) and CTX-M β-lactamase family (in *Kluyvera*) (Poirel et al. 2005).

4.2 Genetic Basis of Antibiotic Resistance

As antibiotics primarily aims to kill pathogenic bacteria. Nature favors the natural mechanisms that allow bacteria to be resistant towards antibiotics. Development of resistance usually occurs via genetic mutations and these genetic mutations are result of selection pressure induced by antibiotics. Bacteria have also capability to transfer genetic material with one another via formation of sex pili in a process known as conjugation, indicating that selection pressure is not the only means by which resistance in these microbes evolve (Knoppel et al. 2017; Ventola 2015).

Bacteria accumulate multiple resistance attributes with the passage of time ultimately becoming resistant to multiple classes of antibiotics. Mutations in chromosome, defects in the transportation of aminoglycosides, and enzymatic alterations are reported mechanism of resistance (Knoppel et al. 2017; Ramirez and Tolmasky 2010). Generally, resistant microbes modify the antibiotic action in one of the following ways:

- Alterations of the drug target (Drug binding site modifications) (Luthra et al. 2018)
- Decreasing the ability to uptake drug
- Activating the efflux functioning of body to remove toxic drug molecules (Kumar and Schweizer 2005)
- Reprogramming of metabolic pathways (Tenover 2006)

4.3 Biochemical Routes Associated with Antibiotic Resistance

Microbes evolve resistance by modifying various biochemical routes. Not surprisingly, bacteria develop resistance to single antibiotic class by reprogramming multiple pathways. They can develop resistance by alteration of antibiotic agents, by inhibiting the penetration/attachment of antibiotic molecule to specific target and by modification of target sites.

4.3.1 Alteration of Antibiotic Agents

The most effective mechanism adopted by bacteria to cope up with antibiotics is to manufacture enzymatic entities that add specific moieties to drug molecules; thus, inactivating the action of these drugs or these enzymes directly causes destruction of drug molecule (Wright 2005).

4.3.1.1 Chemical Modification of Antibiotic Molecules

Gram-positive and -negative bacterial species have capability to produce enzymes which can induce chemical alterations to antibiotic molecules. Most commonly catalyzed reactions by such modifying enzymes include (1) phosphorylating reactions (chloramphenicol), (2) acetylation (streptogramins), and (3) adenylating reactions (lincosamides). The common consequence of these modifications to antibiotic molecule is increased steric hindrance which leads to reduced exposure of drug molecule to its target (Wright 2005).

This type of resistance can be best explained in *Providencia stuarti* where amino and hydroxyl moieties of aminoglycosides are covalently altered ultimately conferring resistance to microbes. Another best example of enzymatic modifications of drug molecule is structural alteration of chloramphenicol by a class of acetyltransferases generally familiar as CATs (Ramirez and Tolmasky 2010; Schwarz et al. 2004)**.**

4.3.2 Inhibiting the Penetration/Attachment of Antibiotic Molecule to Specific Target

One of the best mechanisms to develop resistance is to reduce the uptake of antibiotics by cytoplasmic membranes. This phenomenon can be best described in some gram-negative bacteria. Their membrane acts as first barrier so that influx of antibiotics could be minimized. That is why the antibiotic vancomycin is not allowed to penetrate the membrane of various gram-negative bacterial species (Hancock and Brinkman 2002; Pages et al. 2008).

4.3.3 Modification of Target Sites

To minimize the effect of antibiotics, microbes have evolved modified targets either by protecting the target site or causing alteration to these targets.

4.3.3.1 Protection of Target Sites

Microbes have conferred resistance towards fluoroquinolones, tetracycline, and fusidic acid by blocking various pathways so that antibiotic could not able to reach their specific targets. Tetracycline resistance specifying determinants Tet(O) and Tet(M) was firstly reported in Streptococcus species but now occurrence of Tet(O) and Tet(M) has been documented in a range of species (Connell et al. 2003).

4.4 Global and Temporal Trends in the Use of Antibiotics

Antimicrobials, specifically antibiotics, remain the marvel medicines since the last 80 years. There is a growing concern about the dosage of antibiotics against various diseases all over the world. The reports by WHO (World Health Organization) suggests that appropriate use of medicines comprises the doses that are appropriate enough to meet the drug demands of an individual for an adequate span of time with the lowest cost and least harm to society. Deprivation of any of these factors contributes towards the irrational use of antibiotics that can lead to antibiotic resistance (Machowska and Stalsby Lundborg 2018). It was recently reported that use of antibiotics increased to 65% during the time period 2000–2015 and among that 114% increase was noticed in low- and middle-income countries (Abat et al. 2018; Klein et al. 2018).

There was a 36% increase in the use of antibiotics during the time span of 2000–2010. Out of this reported elevation of antibiotic usage, Russia, China, India, Brazil, and South Africa covers 76% of total increase. It was also observed that seasonal changes brought about the changes in the use of antibiotics. The use of carbapenems and polymixins, i.e., 45% and 13%, respectively, was highly reported (Van Boeckel et al. 2014). A significant decrease in the use of antibiotics was observed in Luxembourg, Sweden, Finland, and Norway whereas an increase in the use of antibiotics was seen in Greece and Sweden during the time period 2012–2016 (Machowska and Stalsby Lundborg 2018).

4.5 Global and Temporal Trends of Antimicrobial Resistance

Antibiotic resistance was first observed in 1980s with the introduction of lots of new antibiotics in clinical practice. This trend of discovery of new antibiotics and their efficacy continued for a short period of time and declined after the loss of interest by pharmaceutical companies (D'Costa et al. 2006).

In Europe, the data of antibiotic resistance in 30 countries on surveillance networks, e.g., EARS-NET, explains that there an increased resistance to third-generation cephalosporins in *Enterobacteriaceae* in the European countries since 10 years and the reason has not been discovered yet now (Rolain et al. 2015).

Complete data about antibiotic resistance is not available and the current scenario of antibiotics resistance is estimated to flourish in the recent times, all over the world (Rolain et al. 2016). This grim matter is particularly aggravated in the countries with high consumption of antibiotics, e.g., Greece, Italy, Romania, Cyprus, and Malta (ECDC 2017). The global use of antibiotics follows a highly fluctuating trend. Antibiotic resistance arises as a serious dilemma posing a great burden on the health and economy of patients suffering from this condition. Estimated data shows that infections with drug-resistance effects lead to 700,000 deaths per year all over the world and this figure is projected to rise up to ten million people per year by the end of 2050 costing the amount of USD 100 trillion USD globally, if no proper action plan is devised against drug resistance (O'neill 2014). In EU (European Union), 25,000 people die annually due to multidrug-resistant infections with the estimated loss of €1.5 billion per year (Machowska and Stalsby Lundborg 2018).

The rapid and unprecedented antimicrobial-resistant organisms have gained popularity in the recent times. The particular concern is accounted by the clinicians working on gram-positive type of infections while the growing incidence of gram-negative type of infections is also under consideration. Antibiotic resistance against gram-negative infections was noticed for β-lactamases in 1990s and this trend of antibiotic resistance continued to exist afterwards. Besides β-lactam, fluoroquinolones resistance is also reported for their multiple drug resistance effects in the community-onset infections round the globe (Vasoo et al. 2015).

Several pathogens like *Pseudomonas aeruginosa, Staphylococcus aureus, Mycobacterium tuberculosis,* and *Escherichia coli* are now resistant towards antibiotics such as amoxicillin, ampicillin, chloramphenicol, metronidazole, ciproxacin, gentamin, nalidixic acid, and ceftazidime. The developing countries, e.g., Nepal and Ethiopia, face the problem to single drug as well as multidrug resistance due to lack of published data, poor health policies, and lack of proper surveillance systems (Dahal and Chaudhary 2018).

Furthermore, it is worth mentioning that antimicrobial resistance also depends upon the species or strains of microbes, e.g., the members of *Bacteroides fragilis*, *Prevotella*, *Porphyromonas,* and some anaerobic gram-negative rod cells are more resistant towards penicillin and ampicillin but susceptible to other antibiotics such as clindamycin, β-lactams, and metronidazole (Eshetie et al. 2017; Schuetz 2014).

4.6 Trends in Soil, Water, and Air

Traditionally, antibiotic drug resistance is meant to be associated to clinical practices but there is a growing concern that antibiotic resistance is also linked with the environmental factors such as agricultural practices and wastewater management (Tripathi and Cytryn 2017). Antibiotics are meant to be the marvel drugs to fight against the diseases both in animals and in humans. But their left overs enter the ecosystem via soil, water, and air to induce the resistance in non-targeted microbes

and create the antibiotic-resistant genes that may pose a serious threat to human health.

Bibliometric assays performed in various parts of China, USA, America, and Europe suggest that Tetracyclines are the most abundantly found antibiotics in wastewater of these regions (Zheng et al. 2018). Broad spectrum β-lactamase-producing Enterobacteria are found in the feces of patients suffering from such infections (Woerther et al. 2013). The recent witnesses in various areas of China have shown that antibiotics and the resistance against various diseases is a matter of great concern (Qiao et al. 2018).

While talking about the antibiotic resistance in food chain, we notice that multi-drug resistance has also evolved in humans and animals acquiring high dosage of expensive drugs and prolonged hospitalization. Foodborne bacteria with drug resistance may include *Salmonella typhi*, *Campylobacter*, nontyphoidal salmonellae, or *Shigella* spp. It may be developed by the use of antimicrobials or in the fresh food that may be contaminated by irrigation water containing drug-resistant microbes (Doyle 2015).

4.7 Consequences of Antibiotic Resistance

As compared to other anti-infective agents, antibiotics are distinct as they display their effect on both the individual and the whole community via selection for resistance to their action. Antibiotics can be related to a four-edged weapon against bacteria. The very first two edges of this weapon were recognized instantly after their development (Blaser 2016).

The organisms which develop resistance against antibiotics are well known as superbugs. These superbugs are greatly responsible for causing life-threatening diseases. Consequences of such infections are provoked tremendously in various volatile conditions including natural disaster, violence, and famine. Now, multitudinal drug-resistant bacteria are the leading source of death all around the world (Lipp et al. 2002; Mendelson and Matsoso 2015).

4.8 Steps to Overcome the Antibiotic Resistance

To overcome the antibiotic resistance is the main objective of the scientists working on infectious diseases. Understanding the cell signaling networks and retaining the cellular homeostasis enable us to design effective measures against the cellular invaders:

1. Aminoacyl-tRNA synthetases (ARSs) are the enzymes responsible for the synthesis of proteins. They also act as the cell signaling mediators which play the vital role in keeping the cellular homeostasis. They are present inside the

cytoplasm of cell in the ready-to-response condition and respond to the danger signals and maintain the bodily immunity against the infectious conditions encountered by the body; that is why they may be the potential therapeutic targets to overcome antimicrobial resistance (Lee et al. 2018).
2. Increasing the bioavailability levels may reduce the dosages and can overcome the drug resistance issues (Jeong et al. 2017).
3. Drug resistance testing or antimicrobial susceptibility testing (AST) may be an effective method for the surveillance of drug resistance for microbes. This may fill up the gaps between the use of antibiotics and their efficacy, thus leading to the effective drug management with low resistance against several microbes (Schon et al. 2017).
4. Individual patient-friendly tailoring of efficacious drug treatment strategies, i.e., to design the drug dosage according to the individual person's pathological conditions (Thung et al. 2016).
5. Adsorptive materials can be utilized to remove, remediate, and recondition the wastewater and provide a safe way out for the extraction of harmful antibiotics from the fresh as well as waste water (Ahmed et al. 2015).

4.9 Concluding Remarks

Antibiotic resistance has emerged as major concern all around the world. Despite the actions taken by WHO to minimize the use of antibiotics, the utilization of antibiotics for animals, humans, and agriculture is increasing. To overcome this increasing trend of antibiotic resistance, the public healthcare and agriculture sector should establish proper surveillance strategies and reporting systems at both national and international levels. Moreover, it is recommended that the policies of antibiotic utilization should be more conventional and adhered to control the misuse of antibiotics.

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Chapter 5
Trends in Antimicrobial Use in Food Animals, Aquaculture, and Hospital Waste

Saadia Andleeb, Muhsin Jamal, Sayed M. A. U. S. Bukhari, Sumbal Sardar, and Mahnoor Majid

Abstract Antimicrobials are referred to as those drugs that can do killing or inhibition of microbes' growth. These include antibiotics, antivirals, antifungals, and antiprotozoal substances. Such pharmaceuticals have been successfully employed in food animals, aquaculture, and hospital waste. Antimicrobials are employed for the treatment and control of communicable diseases within animals and humans, for increase production in livestock and agriculture worldwide. Inadequate and overutilization of these agents have an adverse effect on the production of livestock, agriculture, and directly on human health. Microbes have developed survival strategies against these antimicrobial agents which is called antimicrobial resistance. The continuous and heavy use of antimicrobial in the aquaculture, dairy, poultry, and seafood could lead to the development of antimicrobial resistant mechanisms in microbes. Which then ultimately have various hazardous effects on different ecosystems, food web, health of aquatic organisms, animals, and humans. The developing world aquaculture appears to be one of the important contributions to food security which is the fastest growing agricultural industry in various countries. Similarly, the waste of the untreated healthcare units contain traces of antimicrobial compound and eventually leads to aquatic ecosystem and enters into food web increasing the chances of antimicrobial resistant mechanisms.

We can conclude that the increasing trends and the extensive antibiotics usage within animals, aquaculture, and hospital wastes have been entailed in the expansion of severe health issues within aquaculture, other animals, and humans. Thus, efforts on global basis are required to encourage further sensible usage of both therapeutic and prophylactic antimicrobials within animal, agriculture, and aquaculture. In order for controlling the rising bacterial species rates acquire resistance, actions should be carried out and proper rules and regulations should be exercised to edge the antibiotics usage within human, animals, and aquaculture which is not only important for antimicrobial resistance perception but then public health safety will also be guaranteed.

S. Andleeb (✉) · S. Sardar · M. Majid
Atta-ur-Rahman School of Applied Biosciences, National University of Sciences and Technology, Islamabad, Pakistan

M. Jamal · Sayed M. A. U. S. Bukhari
Department of Microbiology, Abdul Wali Khan University, Mardan, Pakistan

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5.1 Introduction

Antimicrobials (AMs) are referred to as those drugs that can either do inhibition of growth of microbes or otherwise kill them. Antifungals, antivirals, and antiprotozoal agents are also included in this category (Fair and Tor 2014). Such drugs have successfully been employed in aquaculture, hospital waste, and in food animals (Aly and Albutti 2014; Benbrook 2002; McEwen and Fedorka-Cray 2002; Tiwari and Kadu 2013) Antimicrobial are used in animals specifically to treat various kinds of microbial infectious diseases as well as to promote growth. Data from multiple sources contribute such applications of antimicrobials in animals which consist of specific doses, the resulting contraindications, and effects of withdrawal (Barragry and Powers 1994; Council 1980; McEwen and Fedorka-Cray 2002). Most of the therapeutic interventions available today are focused to treat animals with diseases. Animals can be treated individually for food animal production or they can also be treated in all groups by providing medicated feed or water. Animals like poultry and fish are mostly treated through the mass medication which is more feasible as compared to other treatment methods (Radostits 2000). Metaphylaxis is a type of mass medication which aims to recover the ailing animals while treating the other animals in the group to avert the indisposition. For those high-risk infectious diseases that arise typically after weaning or transport, prophylactic antimicrobial treatments are mostly utilized. Nonetheless, the term "therapeutics" is not consistent. For instance, the "American Veterinary Medical Association" describes "therapeutics" just like a process that includes control, treatment, and prevention of bacterial illness (Association 2006). In some parts of the world, such as in North America, some specific antimicrobial drugs might be accepted for both the control and prevention. Moreover, few growth-promoting agents might avert diseases even at their subtherapeutic quantities (Council 1999). This is a pertinent phase because the incorporation of antimicrobials even for restricted time periods can at all times be rationalized by basis of prevention of illness. Agents that promote growth frequently are delivered within comparatively lowest amounts, from 2.5 to 125 mg/kg (ppm) which is dependent upon the species and drug being treated (De Schrijver et al. 1990; Jukes 1986; K, 5–9 July 1998; Visek 1978) In the USA, "subtherapeutic" refers to the use of antimicrobials in feeds whose concentration is set at 200 g/ton for 12 weeks (Swartz et al. 1989). However, the term "non-therapeutic" which looks more accurate can include both the growth-promoting and prophylactic applications. Practically, non-therapeutic treatments are usually given to the animals in their earlier ages and are typically discontinued as they mature (Mellon et al. 2001). Antibiotic resistance is a prominent phenomenon in food animals which ascends because of the abusive antibiotic use. The association of bacteria showing drug

resistance in people and antibiotics use in animals will be under consideration (Carnevale 2005; Chiller et al. 2004; Cox and Ricci 2008; Jones and Ricke 2003; Leech and Miller 1974; Marshall and Levy 2011; Phillips et al. 2004). Antimicrobial Growth Promotants (AGPs) were supported for the first time in the middle of 1950s. At that time, small doses of antibiotics that were subtherapeutic in nature were found to enhance the fodder-to-mass ratio of the swine, cattle, and poultry. Among such drugs, procaine, penicillin, and tetracycline were the prominent ones whose therapeutic doses varied from 1/10 to 1/100 (Stokstad and Jukes 1950). For several ages, such exercise good impacts were advocated, whereas the adverse sides remained hidden. However, the microbiologists and internal medicine specialists questioned the possible side effects that occur due to the antibiotic resistance (Gorbach 2001; Howells and Joynson 1975; Lee and Lin 2004; Smith and Crabb 1957). Few of the media reports, latest releases from the press, and some present investigations have raised credible serious concerns about the safe employment of antibiotics in the aquaculture (Alderman and Hastings 1998; Goldburg et al. 2001). Industry is being emerged by the aquaculture within numerous areas of the world, and its products comprise significant supply of food with enhanced lucrative value. The production of aquaculture multiplied across the world during the years of 1994–2004, where 80–90% of the whole production is contributed by the Asian countries. The production of food fish rose to 79 million tons in the year 2010, out of which 48.19 million tons were produced in China solely. A sum of 16.59 million tons was generated by India, Thailand, Vietnam, Bangladesh, and Indonesia combined (Food and Nations 2013). Aquaculture is considered to be one of the last resorts to expand the donations made specifically for the security of food in the developing countries. In some countries it has become the leading agricultural industry with freshwater aquaculture governing the entire production of aquaculture. One of those countries where this universal phenomenon is turning presently is Africa. The aquaculture provides a good quality food at a very low price to millions of people, thereby making revenue for fishing households and farming, and also have an important role in various national and local economies (Kapetsky 1995; Kitessa et al. 2014). The antimicrobial drugs employment within aquaculture outcomes in a wide range of environment-related applications which impact a large diversity of microorganism (Rasul and Majumdar 2017). In aquaculture, antibiotics are implemented as a prophylactic or therapeutic measure or as feed additives. Human and animal wastes are the prime sources through which these drugs gain access in the pond environment. There has been abrupt rise in food-related diseases caused by antibiotic-resistant bacteria (Bondad-Reantaso et al. 2005). Only a few studies have stated a linear association among the antibiotics use in farm animals and the expansion of antibiotic resistance in animal and humans pathogens (Teuber 1999; Van Den Bogaard et al. 2002). The medications which are employed within aquaculture need legal approval by the "Center for Veterinary Medicine" governed by FDA. On accepted drug practices standard information is offered within the FDA's "Green Book" (FDA 1998). The industry of aquaculture maintains a huge variety of species and provides certain methods for the growth of aquatic animals. These methods range from simple traditional ones (where fish are grown in small

ponds) to rigorous industrial scale production systems. Similar strategies (e.g., antimicrobials usage and vaccination) are used to control the infections in the aquaculture that are also used in other disciplines of animal production. The consistent antimicrobial drugs usage within aquaculture has ended in the rising of pools of bacterial species that show resistance to antimicrobials within aquatic animals also in the overall aquatic atmosphere (Akinbowale et al. 2006; Aoki 1992; Michel et al. 2003; Miranda and Zemelman 2002; Schmidt et al. 2000).

"Selective pressure" is a phenomenon that is generated because of severe use of antimicrobial substances in aquaculture which ends in reservoir of microorganisms showing antibiotic resistance and the transfer of genes encoding resistance in the fish pathogens located in aquatic conditions (Rasul and Majumdar 2017, Sørum 2006). As a common understanding it has been accomplished that waste is renewable, and it should not be discarded in the sites that are dumping sites. There is a huge study present on the treatment of waste and techniques of recycling (Arena et al. 2003; Madu et al. 2002; Soares et al. 2013; Yay 2015). Until now, there are present various sorts of waste material that are understood very risky to be reused and recycled without pretreatment. One such type of waste is "infectious healthcare waste." According to WHO 75–90% of the waste produced across the healthcare facilities is considered as risky. It is the residual 10–25% that cannot be unnoticed (Chartier 2014). This may involve toxic or genotoxic, infectious and radioactive materials. Such waste substances pose severe occupational health hazards and environmental risks. Over the decades, hospital wastes have been meaningfully exceeded due to a trek in population, the use of disposable medical products, and number of healthcare facilities (Arab et al. 2008; Mohee 2005; Taghipour and Mosaferi 2009). Numerous advanced countries impose strict strategies concerning healthcare waste separation, transportation, storage, and disposal (Marinković et al. 2008; Tudor et al. 2005). Here, impoverished hygiene habits may end in the mingling of dangerous waste with the normal waste which might aggravate waste management problem by exceeding the price of disposal and waste treatment (Patwary et al. 2011a). Besides, inadequate healthcare practices, deprived nutrition, and shortage of immunization may rise the susceptivity of the civic to infections coming from unprocessed medical waste (Patwary et al. 2011b). The goal of the chapter is to examine the use of antimicrobial in reach "food animals," aquaculture, and presence in hospitals wastes. After manufacturing these antimicrobials are either used by humans, animal, or crops. How the antimicrobials reach to the environment is shown in Fig. 5.1. This chapter deals with (1) antimicrobials usage in production of food animals, (2) use of antimicrobials compounds in aquaculture industry for production, and (3) the use of antibiotics in healthcare unit which leads to hospital waste and their consequences, and recommendations to prevent their harmful effects and avoid misuse of antibiotics.

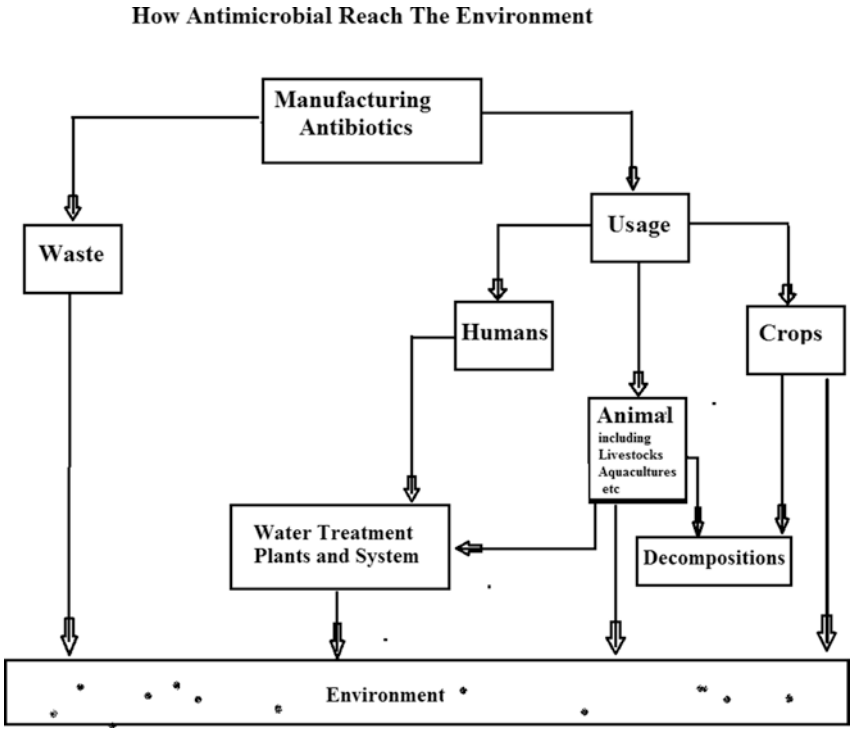

Fig. 5.1 Spread of antibiotics in the environment through various channels (O'Neill 2014)

5.2 Use of Antimicrobials in Production of Animal Food

Antimicrobials are used for the stoppage and ministration of infectious diseases in humans and animals worldwide (O'Neill 2014, 2017). Additionally, antimicrobials are employed within livestock farming promoters of growth in some countries (Page and Gautier 2012). It has been definitely recognized from observation-related study that there is relationship among antimicrobial usage (AMU) and antimicrobial resistance (AMR) within production of animal (Burow et al. 2014; Simoneit et al. 2015). Overutilization of AMR has an adverse effect on the production of livestock, either by lowering productivity of forms, or through increasing the treatment of disease costs (Tang et al. 2017). The WHO has proposed a global rise in production of meat from 218 zillion tons in 1999 to 376 zillion tons within 2030, in developing countries with comparatively bigger spikes (Vasileska and Rechkoska 2012). Several animal foods have been discussed in subsections below.

5.2.1 Beef

Beef calves are usually weaned at 7 months after which they are transported to stock or backgrounder farms. Afterwards, they are further shipped to feedlots where such calves are looked after in large groups and given high energy portions. The sizes of feedlot for beef cattle have been rising timely. In a study almost 35% of the animals were nourished on farmsteads that accounted for 32,000 head or extra, during the year 2000 (Dargatz 2000). The high rate of mortality among calves is mostly due to the pneumonia and diarrhea for which the young animals are cured on individual basis or in groups as well (Losinger and Heinrichs 1997). Bacterial strains (e.g., *Escherichia coli, Pasteurella, Salmonella* spp., and *Haemophilus* spp.) might also be included in the ailments along with the potent viral infections such as pneumonia and diarrhea. An important determinant of antimicrobial application in calves is the shipping fever complex that is a serious health concern with regard to the feedlot (Feedlot 2011). Comparatively, antimicrobials are utilized less in cow calf production (Kelch and New Jr 1993). Monensin or lasalocid have been reported as the most frequently used agents for growth increment by the survey of treatment practices for antimicrobials, carried out by US Department of Agriculture in 1999. However some of the producers also utilized neomycin and virginiamycin drug therapy (Nierenberg and Mastny 2005). In a study, different drugs were incorporated on feedlots to check the effects. A major portion, i.e., about 51.9% was administered with chlortetracycline followed by a combination of oxytetracycline on 19.3%, chlortetracycline-sulfamethazine on 16.8%, and tylosin (antimicrobial, which is included in class macrolide) on 20.3%. Furthermore, tetracycline on average were injected for 4–12 days and for 138–145 days tylosin was given. In a way to treat the animals separately, 50% of feedlots were provided with tilmicosin, tetracyclines, florfenicol, or these antimicrobials were given in variant combinations. Feedlots also employed macrolides (17.4%), cephalosporin (38.1%), fluoroquinolones (32.1%), and penicillin (31.1%) individually. For the management of control treatment or metaphylaxis, nearly about 41% of the feedlots were given drugs like tilmicosin, florfenicol, and oxytetracylcines (Church 2018; Phillips 2018).

5.2.2 Veal

Mostly in the veal industry, those dairy bulls that are culled are provided an iron restricted diet in order to grow pale muscles in these animals right after birth until they gain 400–500 pounds of weight. Though there is a variety of antimicrobials that are available to cure the enteric and respiratory diseases, very limited information is accessible about the use and levels of these drugs. For the prophylaxis, the milk replacers for these calves can consist of antimicrobials (Council 1999).

5.2.3 Dairy

Typically, most of the calves are taken out from the dams within a day after their births in the dairy farms. Afterwards, they are housed separately to prevent the infection and be fed with milk or milk replacer that might contain tetracycline. This process is continued for 6–8 weeks after which the cows are weaned and put into different groups. Drugs like sulfonamides, tetracyclines, and penicillins may be incorporated by mouth or through injection (e.g., ceftiofur) to prevent viral infections such as diarrhea and pneumonia which are serious concerns for dairy farm management (Friendship 2000). Although the dairy cows which are lactating receive some antimicrobials (penicillins, erythromycin, cephalosporins, and oxtetracyclines), they are directed via the intramammary antibiotic brew which is one of the most common way to cure the mastitis, a serious inflammation that arises due to different microorganisms (Ott 1996). These drugs are mostly administered to entire herds to keep the cows safe from mastitis during their non-lactating periods (Erskine 2000).

5.2.4 Poultry

From 1945 to 1999, production of broiler chicken was spiked from 5–40 billion pounds per year approximately. Coccidiostat are typically present in broiler rations, some of which are broad ranged antimicrobials such as ionophores and sulfonamides. For the increase in growth, drugs like bambermycin, chlortetracycline, bacitracin, and penicillin, virginiamycin, and arsenical substances are incorporated in the feed of turkeys, egg layers, and broilers. Bacitracin is a drug that is usually used for promoting the growth as well as to manage the necrotic enteritis that is an intestinal disease triggered by "*Clostridium perfringens.*" Another drug virginiamycin is also utilized for the same reason. Because of the rise of antibiotic resistance, the ancient drugs such as tetracyclines are now deemed unsuccessful. Due to this, advance and modern antibiotics, for example, fluoroquinolones are employed for curing *E. coli* diseases which are abundant in poultry (Tanner 2000). To control the mortality of the poultry, fluoroquinolones are the only permissible ones to be used for the treatment of some infections, particularly the *E. coli*. Gentamicin might be used for dipping the hatching eggs in order to reduce mycoplasma or bacterial contamination. An in vivo injection named sarafloxacin was withdrawn by its sponsor as well. Due to the perils of infections affecting the yolk sac and site abscesses caused by injections, day-old chicks can be injected with gentamicin (Usda 2007).

5.2.5 Swine

Pigs are such animals that are mostly raised in confinement right after their birth till their slaughter or in separated management systems such as nursery, grower, and finishing (Swine 2006). The antimicrobials are dominantly incorporated in the feed in low doses so as to promote the growth and prophylaxis of ailment (Agriculture 1997). Such drugs are then flushed during the last stages of production that ultimately get rid of the residues. Treatments related to therapy are also made the part of the practice; however the owners also cure the individual pigs. Most of the swine get antimicrobials in their feed after the weaning procedure is over (Dewey et al. 1997, 1999). Certain drugs like sulfonamides, ceftiofur, tiamulin, and tetracyclines are given to the animals when they are considered to be the most susceptible to infections. They also help to avert pneumonia which is predominant in swine. Bacterial diarrhea is mostly treated by doses of gentamicin, apramycin, and neomycin wherein microorganisms, for example, *E. coli* and *C. perfringens,* are also prevented. Swine-dysentery (*Serpulina hyodysenteriae*) and inflammation of the ileum (*Lawsonia intracellularis*) are some of the very serious ailments that can be treated with drugs suchlike lincomycin, tiamulin, or macrolides (Dunlop et al. 1998b). Principally, tetracyclines, tylosin, and sulfas such as sulfamethazine are the most frequently utilized antimicrobials in swine.

5.3 Antimicrobials Approachability and Approval in Animals

FDA, which is one of the most reputed national regulatory authority, usually evaluates the integration of antimicrobials in animals based on the safety levels for human consumption, the safety for animals, the efficacy, and the resulting effects on the subsequent production (Miller and Flynn 2000). The authority grounded the expected outcomes of the residues that are found in edible products on humans and the microbial effects of those drugs that are specifically employed for the subtherapeutic administration (Miller 1999). A main framework was proposed by FDA in the year 1998 that was designed specifically for assessing the antimicrobials employed in food animals. Moreover, it also helped to reduce the adverse side effects on humans such as the emergence of resistance (Medicine 1998). The thresholds for a stable human health were established by the framework particularly for the antimicrobial resistance (Medicine 2000). Furthermore, it will support the agency to meet with the drug, cosmetic act, and food that elaborates a “reasonable certainty of no harm” standard to guidelines regarding safety of human (Bager et al. 2001). A few national veterinary organizations, e.g., “American Veterinary Medical Association” and “American Association of Swine Veterinarians,” developed some structured and prudent principles. The American Association of Avian Pathologists has proposed certain protocols for the use of drugs in curing the diseases of the poultry that are very much alike the antimicrobials which are utilized in medicine for humans. It will be too early to assess the impact of such proposed programs, but if they are implemented properly, many benefits can be achieved for both the human and animals.

5.4 Antimicrobials Use Within Animals, Emergence and Spread of Antimicrobial Resistant

Certain modern reviews surveyed resistance of antibiotics evolving in a large quantity of animal species (WHO 1997). The resistance against the antimicrobials in the "zoonotic entero-pathogens" like *Yersinia, Campylobacter, Salmonella,* and certain *Escherichia coli* strains like "O157:H7" and normal flora (for example, utmost general *Escherichia coli* and *Enterococci*) is predominantly regarding for humans because of their probable transmission to humans via the food chain. Furthermore, genes that encodes resistance within commensal microorganisms are also able to be transferred to zoonotic enteropathogens (Salyers 1995). Solid evidence recommends that antimicrobial use in animals calls for developing resistance in the symbiotic (Bager et al. 1997; Dawsom et al. 1984; Dunlop et al. 1998a; Levy et al. 1976a, b; Linton et al. 1975) and in zoonotic conditions (Endtz et al. 1991; Jacobs-Reitsma et al. 1994; Low et al. 1997). Resistance might spread either directly or by indirect way through water, food, and application of waste to farming lands. It could be prolonged via transposable elements horizontal transmission like plasmids through "bacterial mating" (conjugation process). We detailed at this time the indication for animal to human bacteria transmission showing resistance on farmsteads employing antibiotics for treating and/or untherapeutic usage (McEwen and Fedorka-Cray 2002). Detail description of the antibiotic usage for different purposes and targets is given in Table 5.1.

5.5 Acquisition of Resistance by Direct Contact with Animals

There are many conducts through which animals get antibiotics resistance. One such a method is the direct dealings of the slaughterhouse and farm laborer's, veterinarian's and those that are nearby interaction with field laborers. By having nearby contact with animals which are infected and colonized, these labors are directly under the risk of being infected or colonized with bacteria showing resistance. Though primarily this spread does not cause a population-level health risk, work-related farm workers and families of such workers offer a network for the entrance of genes showing resistance into the populace and surroundings of hospital, wherever more feast into microbes is probable (Mølbak et al. 1999; Voss et al. 2005). Levy et al. stated direct feast of bacteria from animals–humans firstly. Within the chicken-caretakers gut flora he found the same strains of *E. coli* showing resistance to tetracycline, as in the chickens getting feed that is Tetracycline-laced (Levy et al. 1976a, b). A revelatory study in 2007 delivered an evidence that in poultry workers the hazard for carrying *E. coli that was showing resistance to* gentamicin was 32 times greater as related to other community members, partial of all poultry employees were occupied with strains of *E. coli* showing gentamicin resistance while only 3% of the un-poultry employees colonization was done. Similarly, the populace that was occupationally exposed was expressively at larger hazard for carrying bacteria that show multidrug resistance (Price et al. 2007). In one more study, "MRSA" was

Table 5.1 Use of antimicrobial agents in different animal-based food for product for enhancement

Antibiotics	Purpose	Antimicrobial class	Spectrum of activity	Utilization in medicine for human	References
Carbomycin B	For the treatment of poultry and prevention of respiratory disease	Macrolides	Gram-positive organisms	No	Bywater (2005)
Chloramphenicol	In the aquaculture industry for major routes of feed	Amphenicols	Broad	Yes	
Colistin	For the feed of cattle, swine and broiler	Cyclopolypeptides	Gram-negative organisms	Yes	
Efrotomycin	In production of AGP for swine	Elfamycins	Gram-negative organisms	No	
Enrofloxacin B	Employment in aquaculture for both bath and oral as well as in treatment of swine and bovine respiratory diseases	Fluoroquinolones	Broad	No	
Maduramycin	In poultry industry (coccidiostat)	Ionophores	Coccidia, Gram-positive organisms	No	Jones and Ricke (2003)
Monensin	For use as AGP in bovine; for the management of coccidiosis in poultry and cattle	Ionophores	Coccidia, Gram-positive organisms	No	
Neomycin B	Use as AGP in pig and poultry; management and control of enteritis in swine and pneumonia; prevention of infections from *E. coli* in cattle and poultry and effective management of respiratory diseases in poultry and aquaculture	Aminoglycosides	Gram-positive and -negative organisms	Yes	

Tiamulin	As AGP in swine AGP, swine treatment, enteritis, dysentery, and pneumonia	Pleuromutilins	Gram-positive organisms, mycoplasmas, spirochetes	No	McEwen and Fedorka-Cray (2002)
Tylosin B	As AGP in swine and mastitis treatment	Macrolides	Gram-positive organisms	No	
Virginiamycin	Employment of AGP in broilers	Streptogramins	Gram-positive organisms	Yes	
Ardacin	As AGP in bovine	Glycopeptides	Gram-positive organisms	No	Aarestrup et al. (2001)
Amoxicillin, B ampicillin B	Aquaculture, oral treatment of swine colibacillosis, treatment of bovine bacterial enteritis and subclinical mastitis	Aminopenicillins	Moderate	Yes	
Avilamycin	AGP for broilers	Orthosomysins	Gram-positive organisms	No	
Avoparcin B	AGP	Glycopeptides	Gram-positive organisms	No	
Oleandomycin B	As AGP in poultry and pig farms	Macrolides	Gram-positive organisms	Yes	Shotwell (2009)
Ormetoprim	AGP in poultry and prevention of fowl cholera and associated infections	Diaminopyrimidines	Broad	No	
Oxolinic acid B	In aquaculture in the form of oral feed	Quinolones	Broad	No	
Pristinamycin	AGP	Streptogramins	Gram-positive organisms	Yes	
Streptomycin B	In aquaculture in the form of a bath	Aminoglycosides	Broad	Yes	National Research Council (1999)
Sulfonamides	Use of sulfamerazine and sulfadimethoxine orally in aquaculture, use as AGP in pigs and in chickens	Sulfonamides	Broad	Yes	
Tetracyclines (oxy- and chlor-) B	Employment of AGP in cattle, poultry, swine therapeutics and management of several livestock infections and diseases. Use in control of aquatic diseases	Tetracylines	Broad	Yes	

(continued)

Table 5.1 (continued)

Antibiotics	Purpose	Antimicrobial class	Spectrum of activity	Utilization in medicine for human	References
Bacitracin/zinc bacitracin	Incorporation of AGP in livestock for the control and treatment of bacterial infections	Polypeptides	Gram-positive organisms	Yes (zinc bacitracin)	Butaye et al. (2003)
Bambermycin	For use as AGP in livestock	Phosphoglycolipids	Gram-positive organisms	No	
Carbadox	Control of dysentery in pigs	Quinoxalines	Gram-positive and -negative organisms	No	
Narasin	Poultry feed coccidiostat, prevention of necrotic enteritis in chickens,AGP for cattle	Ionophores	Coccidia, Gram-positive organisms	No	Katsunuma et al. (2007)
Nourseothricin	Use as AGP in pigs	Streptothricins	Gram-negative organisms	No	
Novobiocin	Use for staph infections treatment,use for the treatment of fowlcholera, and bovine mastitis	Aminocoumarins	Gram + organisms	Yes	
Olaquindox	Swine AGP, control of swine dysentery/enteritis	Quinoxalines	Gram-positive and -negative organisms	No	
Erythromycin*b*	For use in aquaculture through oral, bath and injections and AGP for poultry, cattle, and swine; therapy for poultry respiratory disease and bovine mastitis	Macrolides	Gram-positive organisms	Yes	Dibner and Richards (2005)
Florfenicol	For treatment of respiratory infections in cattle and pigs	Amphenicols	Broad	No	
Flumequin B	Aquaculture (oral)	Fluoroquinolones	Broad	No	
Furazolidone	Aquaculture (oral/bath)	Nitrofurans	Broad	Yes	

Procaine penicillin	AGP in poultry and swine	Beta-lactams	Gram-positive organisms	Yes	Witte (2000)
Roxarsone	AGP for poultry and swine, poultry coccidiostat, treatment of swine dysentery	Arsenicals	Coccidia	No	
Salinomycin	As AGP in pigs, management of dysentery in pigs and porcine intestinal adenomatosis, treatment of *Clostridium perfringens* in growers	Ionophores	Gram-positive organisms	No	
Spiramycin B	Swine AGP, treatment of bovine Mastitis	Macrolides	Gram-positive organisms	Yes	
Gentamicin B	For treatment of therapy of colibacillosis in swine and prevention of early Mortality in poultry	Aminoglycosides	Gram-positive and -negative organisms	Yes	Heuer et al. (2009)
Lasalocid	As AGP for livestock and rabbits and prevention of coccidiosis in poultry and sheep	Aminoglycosides	Gram-positive organisms	No	
Lincomycin	Employment as AGP in poultry and swine; therapy for swine dysentery, pneumonia, chicken necrotic enteritis, and respiratory infections	Lincosamides	Gram-positive organisms	Rare	

separated from numerous samples of pigs, chickens, cattle, and individuals from Korea. Its results displayed six out of the fifteen animal isolates comprising "*mecA*" (in *S. aureus*, it is a gene accountable for showing resistance to methicillin) that were like human isolates (Lee 2003).

5.6 Transmission of Antibiotic Resistance Through the Food Chain

Exposure to bacteria showing resistance to antimicrobials ascends through different routes. Animal products utilization or simply having contact with them are some of the main ways through which people get the antibiotic resistance. There are unquestionable evidences that foods contain large amounts of resistant microorganisms and their genes encoding resistance that have been gotten from several diverse sources of animals and within entire phases of processing. Well-documented suggestions have been found that display the increase of bacteria showing resistance among farm animals and fish products and consumer meat (Cabello 2006; Perreten 2005; Witte 2000). For example, Alexander et al. observed that the *E. coli* showing resistance to drugs was located in beef corpses after evisceration and after 20 h within the cooler and in milled beef kept for 1–8 days (Alexander et al. 2010). Others separated *Campylobacter* spp. (Ciprofloxacin resistant) from 10% to 14% of customer chicken foodstuffs (Gupta et al. 2004; Smith et al. 1999). It has been reported that 12% samples of veal, pork, mutton, lamb, turkey, beef, fowl, and game obtained within the customer market show MRSA within the Netherland and also in dairy products of cattle in Italy (Normanno et al. 2007). Consistently, widespread resistance of antibiotics has been described for bacteria among human microbes, from "market shrimp" and "farmed fish" (Duran and Marshall 2005; Heuer et al. 2009; Sørum 1999). It has also been revealed that in humans the food bacteria contain only a few genes responsible for showing resistance to antibiotics. So, providing evidence on their transmission either by consumption of food or handling, in 2001, Sorensen et al. established the hazard of usage of meat products which colonization is done by resistant bacteria, viewing that "*Enterococcus faecium"* (showing resistance to glycopeptide) separated from animal consumed by pork or chicken remained in stool of humans for about 14 days after ingesting (Sørensen et al. 2001). Donabedian et al. founded overlay designs of "pulsed-field gel electrophoresis" (PFGE) of isolates showing resistance to gentamicin from pork meat and humans also within those segregates from grocery chicken and humans. It was noticed by them that at what time an antibiotic resistance showing gene was existing within food animals, it has also been existing in selling food products from the similar species (Donabedian et al. 2003). A detail evidenced description of resistant gene transfer to food web and humans from different sources is described in Table 5.2.

Table 5.2 Key evidence for transfer of antibiotic resistance from animals to humans (Adapted from Marshall and Levy 2011)

Type transmission	Identified species	Animal host(s)	Recipient host(s)	Conferred resistance	Evidence	References
Colonization in the gut of human via direct or indirect animal touch	*E. coli*	Chickens in USA	Poultry workers	Gentamicin	The sudden spike in the gentamicin resistance phenotypically observed in poultry workers who were administering gentamicin prophylactically	Price et al. (2007)
	MRSA ST398	Dutch veal calves	Veal farmers	MDR	Strong association noted between the nasal carriage of *mecA* gene in human with greater intensity of animal contact and the number of MRSA-positive animals; use of antibiotics in animals for therapy is found to be linked with animal carriage	Graveland et al. (2010)
	E. coli	U.S. chickens	Animal caretakers, farm family	Tetracycline	Emergence of resistant *E. coli* strains with transferable plasmids in the guts of the caretakers that spread to the other members of the farm after the administration of tetracycline on a farm	Levy et al. (1976a, b)
	E. coli	Chinese swine and chickens	Farm workers	Apramycin (not used in human medicine)	Identification of *aac(3)-IV* apramycin resistance gene in humans which shared 99.3% homology with animal strains	Zhang et al. (2009)
	S. aureus, *Streptococcus* spp., *E. coli* and other Enterobacteria	French swine	Swine farmers	Erythromycin, penicillins, nalidixic acid, chloramphenicol, tetracycline, streptomycin, cotrimoxazole	Detection of phenotypic antibiotic resistance which was found to be significantly higher in the commensal floras of farmers working with pigs as compared to nonfarmers	Aubry-Damon et al. (2004)

(continued)

Table 5.2 (continued)

Type transmission	Identified species	Animal host(s)	Recipient host(s)	Conferred resistance	Evidence	References
Human infection via direct or indirect animal contact	*E. coli*	Spanish chickens (slaughtered)	Bacteremic hospital patients	Ciprofloxacin	Detection of resistant *E. coli* in birds through various molecular and epidemiological typing modalities demonstrated avian source of resistant *E. coli*	Johnson et al. (2007)
	E. coli, *Salmonella enterica* (serovar Typhimurium)	Belgian cattle (ill)	Hospital inpatients	Apramycin, gentamicin	Transfer of apramycin resistant *aac(3)-IV* gene through plasmid in animals	Chaslus-Dancla et al. (1991)
	Salmonella Newport	Beef cattle (ground beef) receiving chlortetracycline AGP	*Salmonella*-infected patients with diarrhea	Ampicillin, carbenicillin, tetracycline	Direct genetic transfer of resistance plasmid from hamburger meat to infected patients	Holmberg et al. (1984)
	Enterococcus faecium	Danish swine and chickens	Hospital patients with diarrhea	Vancomycin	Transmission of *vanA* gene cluster (Tn*1546*) reported in human and animals along with clonal spread of *E. faecium*	Hammerum et al. (2000)
	E. coli	German swine (ill)	Swine farmers, family members, community members, UTI patients	Streptothricin	Detection of resistance plasmids which are capable of transfer found in the guts of human and urinary tract infections on administration of nourseothricin as AGP in swine	Hummel et al. (1986)

5.7 Antimicrobials Usage in Aquaculture

In the evolving world, aquaculture seems to be one of the significant contributions in security of food. Currently it signifies the rapidly growing agricultural industry in several countries, with "freshwater aquafarms" governing entire production of aquaculture. This worldwide picture is reflected in Africa, where aquaculture offers good-quality food at low charges to millions of individuals, produce income for fishing and domestic farming, and have a significant role in numerous national and local economies (Kapetsky 1995; Kitessa et al. 2014).

5.7.1 Use of Antibiotics

In aquaculture industry, antibiotics are employed as therapeutic measures, prophylactic, or as feed flavors. They are often carried from animal and human wastes to the pool environment or combined farms of fish. There has been an abrupt spear in the amount of food-related diseases caused by antibiotic-resistant bacteria (ARB) (Subasinghe and Bernoth 2000). A direct connection between antibiotic usage within animals feed and development of antibiotics resistance within human and animal microbes have also been described by current studies (Teuber 1999; Van Den Bogaard et al. 2002). Bacterial illnesses although are controlled in different circumstances by maintenance of animals of definite health status, eradication, immunization, and good sanitation, for treating and in some situations avoiding some bacterial diseases, antimicrobial chemotherapy remains vitally significant (Smith et al. 2003). Appropriate employment of antimicrobials will rehabilitate certain ill animals and guarantee rapid retrieval of others. This approach will also recover the well-being of cured animals and reduce the infection and transmission to other animals or in the situation of zoonotic disease to humans. The trial is how to cleverly use antimicrobials so as to reduce resistance transmission (Kemper 2008). As related to human medicine the prophylactic usage of antimicrobial agents is more common in veterinary practice and displays husbandry system where animals are confined in close immediacy within the similar water or air patch. In these situations, group medicine characteristically includes affected animals therapeutic treatments and of non-affected contacts, the prophylactic medications. The antimicrobials are given at therapeutic doses, which obviously distinguishes this approach from the one that is used to improve production. Antibiotics incorporation in feed or using them in the form of feed additives has been mentioned as the main reason for the development of antibiotic resistance bacteria within the surrounding. Bacteria that shows resistance to antibiotics flourish in the animals gut and in the terrestrial environment when antibiotic-loaded compost and urine are administered to the land. Antibiotics are designated either to do killing of such resistant strains or to limit them (Levy 2001). Antibiotics usage at little, normally subtherapeutic concentrations, can advance performance in food-producing animals by increasing feed alteration

efficiency. Though this is perhaps not the whole cause, the upgrading echoes a decrease in "subclinical disease" (McKellar 1998). It is a general understanding that reduced concentrations of antibiotics should be used for providing protection against illnesses in fishes (Aly and Albutti 2014) as widespread antibiotics usage might end within the resistance arrival. The antibiotics use in aquaculture is no more considered as a main treatment option. Even though incorporation of antimicrobial agents within food animal's, comprising fish, control is done via good management, mainly within the USA and Europe, an extensive variety of veterinary and medical compounds that do inhibition are being employed within aquaculture (Austin 2017). There is a difference between the administration of antimicrobials in aquaculture and in terrestrial animals. Addition of drugs to the water along with feed or with no feed create a difference which results the disruption of environmental microbiota (Plumb and Hanson 2011).

Integrating those antibiotics in fish on routine basis which also use for medication of human is a hazardous act. Aquaculturists are guided to look for further preventive methods other than the antibiotic's but when antibiotics have to be administered in certain circumstances, then they have to manage merely appropriate drugs for fish (Rodgers and Furones 2009). A table of approved antibiotics used in aquaculture is given (Table 5.3). An undergone review that showed the list of chemicals and antibiotics has been proposed by the American FDA for application in aquaculture and now is categorized as novel animal medications of little controlling importance. It includes chemicals such as hydrogen, carbon dioxide gas, sodium chloride, acetic acid, or garlic even (employed as an agent that control sea lice and helminth infiltrations of marine Salmonids), onion (for treatment of exterior Crustacean parasites), and the ice employed during fish transport for reducing its rate of metabolism. (Bentzon-Tilia et al. 2016).

Besides the development of aquaculture, those diseases that are caused by numerous agents (etiological) shadowed by the death of cultivated stock have turned out to be restraining aspects in production. Therefore, usage of certain corrective trials, comprising usage of antimicrobials and medicines for monitoring the diseases have been adapted by the farmers and the hatchery workers.

The rate of utilizing antibiotics and further substances is higher within breeding places and scientific farmsteads as compared to outdated farms. Antibiotics are the most widely used drugs, among those employed in agriculture, that are used for animal health and management (Levy 1992). The use of antimicrobials in aquaculture basically started with the work of Gutsell (1946) who recognized the prospective use of antibiotics (sulfanamides for combating furunculosis). According to Kümmerer (2009a) naturally occurring chemicals like antibiotics could be classified into diverse groups such as tetracyclines, quinolones, β-lactams, sulfonamides, and macrolids. More number of antibiotics like chloramphenicol, oxolinic acid, flumequin, ciproflaxin, and others were introduced gradually with time (Austin et al. 2012). Later, these antibiotics were further categorized as antibacterials, antivirals, antifungal, antiprotozoan, antimetazoan preparations, probiotics, immunostimulants, vaccines, bactecins, hormones, growthstimulants, anesthetics, and bioremediators for both bioaugmentors and biostimulators.

Table 5.3 List of different drugs approved for aquaculture by the food and drug administration (FDA) (Adopted from Benbrook 2002)

Drug	Trade name	Species	Indications	Dosage	Withdrawal (day)	Route
Formalin (antiseptic)	Paraside-F	Salmon/Trout eggs	Control of fungi of the family Saprolegniaceae on the eggs of salmon, trout and pike	1000–2000 μL/L		Water, ambient
		Catfish, largemouth bass and bluegill	For protozoa control	15–250 μL/L (dependent on temperature, species and type of pond)		Water, ambient
		Salmonids, reared	Control of protozoa	15–250 μL/L (dependent on temperature, species and type of pond)		Water, ambient
	Parasite-S®	Eggs of salmon/ Trout	External protozoa's control	1000–2000 μL/L		Water, ambient
		Other finned fish	Control of external protozoans	15–250 μL/L (dependent on temperature, species and type of pond)		Water, ambient
		Shrimp	For controlling the fungi family Saprolegniaceae present on the eggs of all fish species	25–100 μL/L		Water, ambient

(continued)

Table 5.3 (continued)

Drug	Trade name	Species	Indications	Dosage	Withdrawal (day)	Route
Oxytetracycline HCL (antibiotic)	Terramycin® 10	Salmonids	For control of ulcer disease caused by Hemophilus piscium, furunculosis caused by Aeromonas salmonicida, bacterial hemorrhagic septicemia caused by A. liquefaciens, and pseudomonas disease	2.5–3.75 g/100 pound fish/day for 10 days	7–21	Used in feed
		Catfish	For control of bacterial hemorrhagic septicemia caused by A. liquefaciens and pseudomonas disease.	2.5–3.75 g/100 pound fish/day for 10 days	21	Used in feed
		Lobster	Control of gaffkemia caused by Aeorccus viridian	1 g/pound of feed: Sole ration for 5 days	30	Used in feed
Tricaine Methanesulfonate (MS-222) (anesethic)	Finquel	Fish	Anesthetic	15–330 mg/L	21	Water, ambient
	Tricaine-S	Fish	Anesthetic	15–330 mg/L	21	Water, ambient

Sulfadimethozine Ormetoprim Combination (antibiotic)	Romet®-30	Salmonids	Control of furunculosis in salmonids caused by Aeromonas salmonicida	50 mg/kg/day for 5 days	42	Used in feed
		Catfish	Control of enteric septicemia of catfish caused by Edwardsiella ictaluri strains	50 mg/kg/day for 5 days	3	Used in feed
	Formalin-F	Salmon/Trout eggs	Control of protozoa	1000–2000 µL/L		Water, ambient
		Catfish, largemouth bass and bluegill	Control of protozoa	15–250 µL/L (dependent on temperature, species and type of pond)		Water, ambient
		Salmonids, reared	Control of protozoa	15–250 µL/L (dependent on temperature, species and type of pond)		Water, ambient
Sulfamerazine (antibiotic)—not currently marketed	Sufamerazine in fish grade	Trout	Control of furunculosis in salmonids casued by Aeromonas salmonicida	10 g/100 pounds of fish/day	21	

It is significant to use drugs approved by the "FDA's Center for Veterinary Medicine" in aquaculture. FDA's "Green Book," containing standard information on approved drug uses (FDA 1998), also assume of entrances into the "Code of Federal Regulations," volume 5 and title 21. Such data states "Drug constituents," species, producer, route of delivery, dosage form, times of withdrawal, acceptances, and usages through species, IATP comprising dose rates and restrictions. The utmost communal path to deliver such a legal antibiotics to fishery happens by mingling with specifically formulated feed. Howbeit, fishes will transfer them mostly in unemployed form back to the environment in their feces due to the reason they do not efficiently process antibiotics. It has been assessed that 75% of antibiotics given to fish are formerly placed by process of excretion into the water (Goldburg et al. 1997). Currently in the USA there are 5 medications lawful for usage within aquaculture. Such comprise merely 3 antibiotics; (terramycin-10), (sulfamerazine), (oxytetracycline-HCl), a drug amalgamation comprising two antibiotics; ormetoprim (romet-30) and sulfadimethozine. In spite of the frequently encountered tell that there are none antibiotics administered for promoting progress within hydroponic culture; a "National -Seafood -HACCP Alliance for Training and Education Compendium" finds "growth" one of the explanations that's why manufacturers manage antibiotics (FDA 1998).

Table 5.4 enlist all the determinants reported for antibiotic resistant in aquaculture.

Table 5.4 Representative microbial antibiotic resistance determinants identified from aquaculture systems adopted from Watts et al. (2017)

Antibiotics	ARGs	Hydroponic System or Fish Species	References
Sulfonamide, sulfamethizole	sul1, sul2, sul3	Fish farms, Tianjin, China; farmed freshwater fish, Guangdong, China; gilthead seabream.	Sousa et al. (2011)
	sul1, sul2	Chilean salmon; fish farms, Tanzania and Pakistan	Shah et al. (2012)
Quinolones (oxolinic acid, ciproflaxin)	qepA, oqxAB, qnrS, aac(60)-Ib, qnrB, qnrD	Farmed freshwater fish, Guangdong, China	Tomova et al. (2015)
	qnrA, qnrB, qnrS	Aquaculture of Salmon, Chile	Buschmann et al. (2012)
B-lactam (e.g., ampicillin, amoxicillin)	blaTEM-52, blaSHV-12	Sparus aurata	Sousa et al. (2011)
	blaTEM	Fish farms, Pakistan and Tanzania	Shah et al. (2012)
Quinoxoline 1, 4-di-*N*-oxides (carbadox, olaquindox, mequindox)	oqxA	Aquaculture of Salmon, Chile	Buschmann et al. (2012)
Trimethoprim	dfrA1, dfrA5, dftA12	Chilean salmon; fish farms, Tanzania and Pakistan	Shah et al. (2012)
	dfrA12	Catfish farm, Vietnam	Nguyen et al. (2014)

(continued)

Table 5.4 (continued)

Antibiotics	ARGs	Hydroponic System or Fish Species	References
Tetracycline (tetracycline, oxytetracycline, chlortetracycline)	tetM, tetO, tetT, tetQ	Pisciculture, Tianjin, and Guangdong, China	Xiong et al. (2015)
	tetM, tetS	Japanese and Korean coastal farms, Chilean salmon	Kim et al. (2004)
	tetA, tetG	Pisciculture, Pakistan and Tanzania	Shah et al. (2012)
	tetA	Marine aquaculture, Spain and Portugal	Rodríguez-Blanco et al. (2012)
	tetA, tetB, tetK	Salmon aquaculture, Chile	Buschmann et al. (2012)
Amphenicol (chloramphenicol, florfenicol)	cmlA	Gilthead seabream	Sousa et al. (2011)
	Cat-1	Pisciculture, Tanzania and Pakistan	Shah et al. (2012)
	floR	Salmon aquaculture, Chile	Buschmann et al. (2012)
	catB	Catfish farm, Vietnam	Nguyen et al. (2014)
Macrolides (erythromycin)	mefA	Fish farms, Tanzania	Shah et al. (2012)
	ermC, ermE, ermX, ermC	Carp farms, Poland	Piotrowska et al. (2017)
Aminoglycoside (streptomycin, spectinomycin, neomycin)	aadA strA-strB	Chilean salmon, fish farms, Tanzania and Pakistan catfish farm, Vietnam; carp farms, Poland	Shah et al. (2012)
	aad1	Gilthead seabream	Sousa et al. (2011)

5.8 Strategies of Administering Antibiotics in Aquaculture

Various methods are used for administration of drugs in aquaculture as follows.

5.8.1 Medicated Feed (Oral Administration)

There are several means for drugs administration in aquaculture. One such most successful method is medicated feeds. The careful administration of drugs is necessary because treatment failure results due to few of the causative factors of disease like stress. Fish can come under great stress in cases of enhanced fish density, poor or insufficient nutrition, poor water quality, parasite infestation, and handling (Rodgers and Furones 2009).

5.8.2 Injection

Injections are mostly preferred over medicated feed when there are severe infections. This administration strategy is more suitable for those individuals that are labor intensive and time consuming such as ornamental fishes. Injection ensures immediate effect by reaching blood quickly. Injection sites include the intraperitoneal cavity and the intramuscular sites (Korostynska et al. 2016).

5.8.3 Immersion

Other than the systemic contagions, such kind of strategy of administration is recommended for external infection treatment. It has numerous drawbacks such as: it requires a separate tank, it needs more antibiotics than oral administration, and strict water to drug volume ratio adjustment is required (Korostynska et al. 2016).

5.9 Impacts of Antibiotics

Antibiotics have been specifically designed in order for killing or inhibiting the pathogenic microorganisms. They have three mechanisms of actions: by cell membranes damaging, by protein disruption or synthesis of DNA, or by doing inhibition of function of the enzyme. In human and veterinary medicine, those compounds are selected that have antibiotic activity as they are selectively toxic to cell membranes, ribosomal activity and in prokaryotic cells disrupt enzyme activity. Although antibiotics have little toxicity, there are important environment-related alarms associated with the extensive antibiotics usage. Numerous antibiotics are not broken down in the body because they are stable chemical compounds and can persist lively long after excretion. At present-day, antibiotics become a significant contributor to increasing issue of active medicinal materials mingling within the surrounding. Large amounts of antibiotics are used to avoid infection in developing world, most commonly in the fish industry (Cabello 2004).

The consistent and extensive use of antibiotics in the aquaculture environments could result in various dangers with concern to the health of aquatic organisms as well as to the humans on consumption of fishes and shrimps. In aquaculture, the antibiotics employed for either remedial or prophylactic determinations frequently mount up within aquatic animal's tissues. Toxic effects like allergies, fluctuations within the microbial fauna of intestine, and attainment of resistance to drugs arise due to existence of antimicrobial medication deposits within the tissue which are edible. Chloramphenicol remains within food used up by humans could even outcome in aplastic anemia, which progress to very brutal diseases of bone marrow. Cancer and so many other diseases are caused by nitrofuran antibiotics. One of the

reasons due to which microbial communities in the environment are affected is the abusive use of antibiotics. But the studies on the antibiotics and its effects on aquatic farms are less and the reason for this observable fact is very well explained by Kümmerer (2009b) who stated that the concentration of antibiotics were in monograms and lower micrograms per liters range and this lower concentration makes it difficult to study the side effects. Forbye, more complications arise in determining the microbial community structure when microorganisms are present in lower densities. Antibiotics play a role like an environmental barrier within the atmosphere that could possibly affect the communities of microbes. Antibiotics cause alteration in phylogenetic structure, expansion of resistance, and disturbance of environmental role within the micro-ecosystem (Ding and He 2010).

5.10 Antibiotic Resistance in Aquaculture

The humans and aquatic environment are intertwined due to the wide consumption of fish and shrimps. Thus, antibiotic resistance can pose serious threats to public health and ecosystems. The used antibiotics persist within the aquaculture surrounding for extensive period of time because they are often nonbiodegradable. Their reliable and extended stay hence encourages achievement of resistance of antibiotics in bacteria and thus promote the bacterial growth, which can flourish in the occurrence of these antibiotics and can transfer resistance to consequential generations. One of the hazards of this bacteria showing resistance is their alteration to animal and human microbes, leading to enhanced infectious illness in diverse species including humans, animals, and fish. Antibiotics have come below the attention for the previous few years because of the increased amount of diseases becoming resistant to outdated treatments. The unceasing and unselective antibiotics use in the aquaculture farms proceeded to very serious outcomes. It has paved way for the outbreak of mutagenic multidrug-resistant microbial strains which can cause communicable illnesses.

Marine ecosystems such as fishing include an extensive range of marine fish farms and other marine food-processing units. Antibiotics medicated leftover food pellets which are used by fishes as a feed and evidently antibiotics passes to the fishes. A research study has established that fishes which are wild, consume the pills containing quinolones and tetracycline residues in aquaculture zones (Tendencia and De La Peña 2001). Besides antibiotics resistance, such antibiotics accumulation occurs in the fishes and shrimps tissues. The development of resistance is a significant outcome of the use of antibiotic (Stokes et al. 2001) and this consequence may be the most significant implication of use of antibiotic in aquaculture for a number of reasons(Salyers et al. 2004). The incorporation of oxytetracycline in aquaculture has been noticed to end in a periodic shift in certain species of bacteria towards "*Enterobacteriaceae"* and is related with resistance of antibiotics (Guardabassi et al. 2000; Wollenberger et al. 2000). Samples were obtained from intestines and gills of wild and commercial fishes captured nearby fish farming were described

with high occurrences of multiple antibiotic resistance (Guardabassi et al. 2000; Rhodes et al. 2000). Furthermore, more antibiotics are reaching the natural environment in case of treatment of disease caused by resistant strains of bacteria; because for this purpose higher levels of doses are required (Bruun et al. 2003). Based on the scientific confirmation, "treatment-resistant disease" caused by the unceasing antibiotic integration in food-producing beings progress to resistance within bacteria of intestine and such resistance could formerly be passed to overall community. Antibiotics resistant *Aeromonas hydrophila, Vibrio anguillarum, Pasteurella piscida, A. salomonicida, E. icttaluri, Edward siellatarda,* and *V. salmonicida* have been established due to the extreme antibiotics use for treating bacterial diseases in fish farming aquaculture (Hernández et al. 2005). The general conclusion of many reports investigating "microbial resistance" is that, as the larger will be the frequency of resistance of antibiotics in the bacterial flora, as more antibiotic are presented into the atmosphere (Halling-Sørensen et al. 1998; Smith et al. 1994). "Resistant strains" get an advantage over the "non-resistant strains" in the occurrence of antibiotics as they dominate in the population. Bacteria showing resistance to antibiotics and the resistance encoding genes are frequently found in aquatic ecosystems (Biyela et al. 2004; Esiobu et al. 2002; Schwartz et al. 2003). Tendencia and De La Peña (2001) noticed that antibiotics resistance was related with ancient and recent usage of antimicrobial agents that was noticed to be high within shrimp pools consuming oxolinic acid within feed. Additional indication reported that there was a rise within the amount of genes that codes for tetracycline resistance (related to genes of "16S rRNA") at little exposure level of tetracyclines within aquatic environment (Knapp et al. 2008). Phylogenetic study of different categories of genes that shows resistance towards antibiotics has proposed that genetic elements for currently resistance of antibiotic has extensive past of assortment and deviation earlier the present-day era of antibiotics (Aminov and Mackie 2007). Undisputable evidences have been reported that revealed that antibiotics, when used broadly and prophylactically in animals, have adverse effect on antibiotic therapy of animals and bacterial contagion of human due to few reasons such as (a) human beings can effortlessly be diseased by "zoonotic antibiotic-resistant bacteria" and (b) microbes related with human and animal can easily share genetic elements for resistance of antibiotic as the result of horizontal gene transfer (Angulo et al. 2004; Cabello 2004; Lederberg and Harrison 1998; Mølbak 2004; Teuber 2001; Wassenaar 2005). The phytoplankton community composition, the community of zooplanktons, and even the heterogeneity of inhabitants of complex animals can also be influenced by variations in the aquatic microbiota diversity triggered by antibiotics and might change homeostasis of marine environment and generate an impact upon the more diverse life forms including marine mammals, shellfish, fish, and humans. It has now commonly being documented that the innate atmosphere ports enormous range of genes responsible for resistance to antibiotics and some bacteria of soil employing antibiotics as their only carbon source may even live in the existence of antibiotics (Dantas et al. 2008; D'costa et al. 2006; Martínez 2008; Wright 2007).

Chemical pollutants along with their toxicity can be proficiently removed from their environments by transformation methods of contaminants, biotransformation,

or bioremediation (Srinivasan et al. 2001). In aquaculture, always the antibiotics are not used in a responsible way because of which it has been described in numerous conditions that the controlled antibiotics use has not provided an only guarantee of the evading of hazards to humans. A number of national international agencies such FAO, IOE (International office of Epizootics), and WHO have previously raised the problem of careless antibiotics use within entire sectors of production with specific apprehension for the possible hazards to public health. Various regulatory authorities around the world have made strengthen or changed national regulations on antibiotics usage either in overall or within the aqua farming sector.

5.11 Antimicrobial Use in Hospital Waste

Among all the antimicrobial usage in the hospital waste antibiotics are of great concern. Safe disposal of clinical waste has become a major challenging issue in the twenty-first century owing to the increase in the numbers and types of infectious diseases being treated in hospitals and healthcare facilities. About 10% of hospital generated waste is infectious, which can be hazardous to the public (Chartier 2014). For the disinfection procedures and treatments of patients, large sum of disinfectants and antibiotics are employed in hospitals. For the antibiotics consumed by the medical patients complete metabolism is not done and via urine and ejecta its excretion occur. After usage, the unused amounts of such chemical substances enter the wastewater, doing bacteria exposure where wide biocide variety that can act like "selective pressure" are existing, and can result in the emergence of resistance (Nunez and Moretton 2007). A prominent drug named as ciprofloxacin was discovered whose dose range was between 0.7 and 124.5 μg/L in hospital discharge (Hartmann et al. 1999). In the large German hospital the concentrations of ampicillin in effluent was fluctuating from 20 to 80 μg/L (Kümmerer 2001). The concentrated doses of antibiotics founded in the effluents of hospitals were enumerated and computed were of the same degree as that of the minimum inhibitory concentrations for the liable pathogenic microbes (Kümmerer and Henninger 2003). Hospital effluents are often diluted by the municipal sewage treatment plants that moderately decrease the concentration of antibiotics as the waste water in the city is a reservoir for various antibiotic substances as well as the antiseptics coming from the houses, veterinary sources and to some extent from the livestock. Various sources such as sewage of municipal, effluents coming from the Sewage Treatment Plants (STPs), and water from surfaces and grounds contain antibiotics in the μg/L range (Golet et al. 2001; Kolpin et al. 2002; Sacher et al. 2001; Zuccato et al. 2000). Among these antibiotics, ciprofloxacin, roxithromycin, sulfonamides, and dehydrated form of erythromycin have been founded in abundance. Such drugs can easily penetrate into the soil from manure if they are utilized in animal breeding and farming. For instance, a study reported the presence of tetracyclines in concentrations of 0.2 μg per kg within loam (Hamscher et al. 2002) while some other types have also been discovered in the fish farms sediments. Resistance against the antibiotics is con-

stantly spiking because of their consistent and extensive usage in hospitals and clinics for treating various patients. Along with the antibiotics, some other antimicrobial agents, their solid, fecal, and liquid wastes have been documented as important sources contributing to the release and subsequent spread of antibiotics, bacteria resistant to antimicrobial, and AMR genes in the environment (Amaya et al. 2012; Korzeniewska et al. 2013; Leclercq et al. 2013; Mach and Grimes 1982; Varela et al. 2013; Zhang et al. 2013). Data relating to utilization of antimicrobial agents by the human in the society and medical centers has been collected by the "European Surveillance of Antimicrobial Consumption Network" (ESAC-Net). The resulting data suggests that the penicillin was the most commonly employed group of antibacterial substances, across the Europe in both the community sectors and hospital. Others include quinolones/fluoroquinolones and beta-lactam compounds (e.g., cephalosporins) for the hospital segment, and the tetracyclines and macrolides for public sector (Morris et al. 2008). Certain preventive measures have been formulated in order to manage the blooming antibiotic resistance. Utmost of solid materials and fabrics within the markets nowadays have antimicrobial properties, contain substances that do inhibition of growth or killing of large quantity of microbes over lengthy time periods but merely under certain circumstances. Because of this, utmost products consisting of antimicrobial agents accomplish at a degree which might be valuable for aesthetic determinations, comprising control of odor, and safety of material yet not essentially for infectious restraint (Page et al. 2009). There are some antimicrobial fabrics that kill considerable amount of microorganisms rapidly (i.e., within less than 10 min). This antimicrobial action type is coarsely correspondent to that which will be carried about using a low-level disinfectant. These types of antimicrobials that are added to fabrics can be advertised as agents that can preserve the material and control odor and still qualify for a "treated article exemption". It can be measured a claim which is health-related needing full FIFRA act (Insecticide, Fungicide and Rodenticide Act) evaluation and registration, if claims are made by them regarding dropping colonization of microbes (Table 5.5).

Table 5.5 Antimicrobial use in hospital wastes water

S/No.	Country	Matrix	Antibiotics	Levels (μg L^{-1})	References
1	Canada	Wastewater	TC	0.151–0.977	Miao et al. (2004)
			DC	0.038–0.046	
2	Western NY	Wastewater	TC	0.14–0.56	Batt et al. (2006)
3	Sweden	Wastewater	Doxycycline	0.064–2.480	Lindberg et al. (2004)
4	USA	Surface water	CTC	0.42–0.69	Kolpin et al. (2002)
			TC	0.11	
			OTC	0.34	
		Wastewater	TCs	0.05–1.14	Yang and Carlson (2004)
		Wastewater	TC	0.62	Batt and Aga (2005)

(continued)

Table 5.5 (continued)

S/No.	Country	Matrix	Antibiotics	Levels (μg L^{-1})	References
5	Wisconsin	Wastewater	TC	0.05–1.2	Karthikeyan and Meyer (2006)
6	Luxembourg	Wastewater	TC	1.0–85.0	Pailler et al. (2009)
			OTC	1.0–7.0	
		Rivers	TC	1.0–8.0	
			OTC	1.0–7.0	
7	Colorado	Wastewater			Yang et al. (2005)
		Influent	TC, CTC, DC, DMC	0.05–0.27	
		Effluent	CTC	0.06	
			DC	0.07	

CTC chlorotetracycline, *DMC* demeclocycline

5.12 Antibiotic Resistance in Hospital Waste

Untreated hospital waste water (HWW) poses the hazards of transmitting the bacteria showing antibiotic resistance in the environment. In some countries like Bangladesh, liquid discharge is directly released into the "municipal sewage system" and contaminates the surrounding rivers and water bodies (Adnan et al. 2013). HWW can be hazardous to public health; subsequently it might comprise several types of contaminants like chemical, radioactive, and pharmacological wastes as well as disease-causing microorganisms (Author 2003). The appearance of resistance towards antibiotics and the cause of transmission of resistance genes in environmental samples is a consequence of uncontrolled and extreme antibiotics use (Iversen et al. 2002). Generally, untreated hospital liquid waste is either released indirectly or directly into the surface water which becomes the main cause for additional waste associated diseases due to which deaths of many people occur in developing countries such as Bangladesh (Akter 2000). Water-borne illnesses such as typhoid fever, cholera, gastroenteritis, and dysentery are caused by hospital sewages that left untreated along with the intense types of infectious agents and microbes which show resistance to antibiotics (Kruse 1999).

The evolution and expansion of bacteria which show resistance to antibiotic results due to extensive utilization of antimicrobial substances within clinical locations to treat infectious diseases as well as their incorporation in aquaculture and veterinary medication is of great apprehension to community health. This specific outcome arises when the antibiotics puts a high selective pressure on the microbes, resulting in the proliferation and subsequent spreading of resistant bacteria. The genes encoding resistance can be transmitted among cells which are plasmids located or transposable elements through conjugation processes or transductive (Islam 2011; Mazel and Davies 1999; Wlse et al. 1998; Berger-Bachi 2002). Further spread of multidrug-resistant (MDR) bacteria also involves elements of DNA which arbitrate resistance showing genes incorporation (e.g., integrons) (Moura et al.

2012). Hospital wastewater is thought of a rich reservoir for the antibiotic resistance and other genetic influences that promote the probable extension of AMR to the environment. This occurs because the hospitals obtain huge amount of antimicrobial substances and pathogens of humans (Berendonk et al. 2015; Hocquet et al. 2016). In Singapore a study was conducted to find out AMR in hospital wastewater by measuring the existence of antibiotic resistance factors including resistant bacteria, antibiotic residues, and genetic determinant's (such as, ARGs, integron) in hospital effluents. Local levels of azithromycin, clarithromycin, ciprofloxacin, trimethoprim, and sulfamethoxazole were much lesser (~10 fold) than those stated in another study (Rodriguez-Mozaz et al. 2015). The antibiotics bulks employed within hospitals get free into wastes and hence on bacteria a selection pressure is created. Consequently, bacteria showing resistance and antibiotic residues exist in wastewater of hospital at concentrations which are capable to stop the growth of susceptible bacteria (Beyene and Redaie 2011). Wastewater of hospital therefore might enhance the number of microorganisms showing resistance in the recipient sewer's via selection pressure and channels of introduction (Stalder et al. 2014). The exposure of the environment with the resistant bacteria can create serious public health concerns due to the presence of transmissible genes. The resistant microbes can also act as a vector or reservoirs of the resistance genes that pose health impact as well (Keen and Patrick 2013; Nunez and Moretton 2007). *P. aeruginosa, Campylobacter, Salmonella, Clostridium, Shigella, Yersinia, Vibrio, Leptospira, S. aureus,* and total coliform groups comprising *Klebsiella, Serratia, E. coli,* and *Enterobacter* are the utmost shared bacterial microbes existing within wastewater of hospital (Anitha and Jayraaj 2012; Pandey et al. 2011).

E. coli (fecal coliform) is one of the most common members of hospital waste that contributes to contamination. It has been approved as a fecal pollution indicator (Horan 1989). This fecal coliform serves as an important zoonotic pathogen. The fecal contamination in water is indicated by the presences of *E. coli* (Von Baum and Marre 2005). *E. coli* is also known as a shared intestinal tract native of animals and humans, and the utmost communal causative agent of community acquired and nosocomial contagions (Perez et al. 2000). When exposed to antibiotics, *E. coli* could transfer genes showing resistance to antibiotics to varied microbes (Smith et al. 2007). In addition, the inconsistency in the chlorination method is an insufficient disinfection method and it might enhance the growth of antibiotic-resistant bacteria (Khleifat et al. 2006). The microflora in hospital sewage interacts with the incoming nondigestible drugs that are coming from patients and residual chemicals. Such thriving bacteria which resides within wastewater of hospital might be exposed to an extensive variety of biocides that can act as a selective pressure for resistance development. Because of inadequate antibiotic usage, wastewater of hospital comprises high numbers of microbes showing resistance as compared to wastewater (domestic) (Berge et al. 2006). One of the possible side effects of the antimicrobial agents is their destructive effect on the nonpathogenic bacteria besides the infection-

causing bacteria. As a result, the good bacteria gets replaced with the bad ones, thus making more possibilities for resistant bacterial growth (Nunez and Moretton 2007).

The impact on public health due to release and spread of resistant bacteria in recipient atmosphere could be elucidated in numerous ways. Firstly the genes showing resistance can be transferred by conjugation or transduction, if the resistant bacteria are containing transmissible gene so that the contagion triggered by such bacteria become problematic for treatment and also reduced the pool of antibiotic for treating the bacterial contagions. Secondly, the organism carrying these genes might act like vector or resistance showing genes reservoir. Thirdly, the number of nosocomial infections will be increased. Fourthly, the cost of treatment and hospitalization will be increased if infection occurs (Nunez and Moretton 2007).

Aquatic environment (drinking and recreational water) is susceptible to antimicrobial resistance because it not merely acts like a clinical resistance showing genes reservoirs, but also like an evolution and expansion channel of genes showing resistance and vectors of them (Amenu 2014). Public health impact is an utmost serious concern which necessitates urgent reply from entire shareholders. A research carried out within Belgium compared the heterotrophic bacterial isolates antimicrobial tolerance from sewage of hospital and freshwater fish farm water that show resistance to oxytetracycline. The results concluded that the heterotrophs showing resistance to the oxytetracycline from the freshwater fish farm location (22%) displayed less frequency of ampicillin tolerance as compared to oxytetracycline-resistant hospital heterotrophs (84%) (Amenu 2014) (Table 5.6).

5.13 Conclusions

The overuse and misuse of antibiotics over the last number of decades have generated a serious problem with no immediate solution in sight. Human infection with antibiotic-resistant bacterial species has increased patient morbidity and mortality rates globally. The presence of these drug compounds in waterways and foods has resulted in both water and food contamination at levels currently unknown. It is of the upmost importance to determine the effect of such contamination on human morbidity and public health safety.

Aquaculture similarly is related with new ways for individuals to get exposed to bacteria showing resistance, or resistance conferring genes to a given drug with origins within aquaculture. Due to such cause, aquaculture might be or could come for giving contribution to pool of bacteria showing resistance to antibiotic, thus activating contagions within humans dis-proportional to aquaculture's share of antibiotic usage. Given the universal nature of the crisis of resistance of antibiotic, the waste water from the hospital is related with a rise in the frequency of resistance of antibiotic.

Table 5.6 Multidrug resistance patterns of bacterial isolates from wastewater released from hospital (Adapted from Asfaw et al. 2017)

Bacteria isolates	*E. coli*	*CoNS*	*Citrobacter* spp.	*S. aureus*	*P. aeroginosa*	*Entrobacter* spp.	*Klebsiella* spp.	*Salmonella* spp.	Other isolates
Number of isolates	6	5	3	8	8	2	10	6	2
R0	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
R1	1 (16.7)	0 (0)	0 (0)	1 (12.5)	5 (62.5)	0 (0)	1 (10)	0 (0)	0 (0)
R2	0 (0)	0 (0)	1 (33.3)	2 (25)	1 (12.5)	1 (50)	2 (20)	1 (16.7)	0 (0)
R3	1 (16.7)	1 (20)	0 (0)	2 (25)	2 (25)	0 (0)	2 (20)	2 (33.3)	0 (0)
R4	2 (33.3)	2 (40)	0 (0)	1 (12.5)	NT	0 (0)	1 (10)	1 (16.7)	1 (50)
R5	0 (0)	1 (20)	0 (0)	0 (0)	NT	1 (50)	1 (10)	0 (0)	1 (50)
≥R6	2 (33.3)	1 (20)	2 (66.7)	2 (25)	NT	0 (0)	3 (30)	2 (33.3)	0 (0)

R0 not resistant for any antibiotics tested, *R1* resistant to one antibiotic, *R2* resistant to two antibiotics, *R3* resistant to three antibiotics, *R4* resistant to four antibiotics, *R5* resistant to five antibiotics, ≥*R6* resistant to six or more antibiotics, *NT* not tested, Other isolates: *Seratia* spp. (1) and *Proteus* spp. (1)

5.14 Recommendations

It is commonly approved that the dissemination and selection of bacteria showing resistance within nature would be evaded in way for ensuring operative treatment against communicable illness within humans and for maintaining an environmental balance that supports the prevalence of a bacteria flora showing susceptibility within nature.

Undiscriminating antibiotics usage within human medicine, in hospitals, animal-husbandry, and agriculture should be avoided that might outcome in the proliferation of bacteria showing resistance to antibiotics.

Keeping in considerations the speedy growth and significance of the industry of aquaculture within several areas of the ecosphere and the extensive, severe, and frequently unfettered antimicrobial substances usage within this zone of production of animal, there is need of efforts for preventing the expansion and feast of resistance of antimicrobial within aquaculture. Such efforts must be attentive on perfection of management practices, regulatory control of antimicrobial substances usage, execution of judicious use rules and monitoring of antimicrobial substances usage and resistance of antimicrobial.

Measures must also be taken to slow the rate of emergence in currently treatable bacterial species. The antibiotics usage for the conditions treatment, which are not of bacterial origin or in cases wherever there is slight or no indication of efficiency, has significantly proliferated antimicrobial resistance.

In order for controlling the rising rate of species of bacteria achieving resistance, there is need of measure for limiting the antibiotics usage as both veterinary and human therapeutics.

Not only this is significant from an "AMR" viewpoint but also for ensuring communal health safety where there's rising indication relating antibiotics to human illness particularly within child's.

It is shown by numerous studies that the communicable pathogenic species of microbes zoonotic transmission is a stern reason of human illnesses.

Therefore, the guidelines should be designed and adjusted according to the country situation and available resources, and studies related to the economical impact of implementing these guidelines should be performed in order to evaluate the effectiveness of the policy in a country. Finally, a stronger international collaboration worldwide is essential to ensure a long-term prevention of healthcare-associated bacterial transmission because these bacteria do not respect national borders.

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Chapter 6
Antimicrobial/Antibiotic Resistance Genes Due to Manure and Agricultural Waste Applications

Magdalena Zalewska and Magdalena Popowska

Abstract Since the discovery of antibiotics, they have been considered as very promising and effective drugs for every kind of bacterial infection for humans, as well as for food-producing animals. The unregulated use of this type of drugs in livestock has a significant impact on spreading of drug resistance. The presented chapter contains a short comparison between antibiotics used in medicine vs. antibiotics used in veterinary practice. Usage of antibiotics crucial for human medicine in veterinary practice is also extensively described. The chapter also contains a description of antibiotic residues, together with drug resistance genes prevalence in manure/agricultural waste and manure-amended soil. The adverse effect of antibiotic residues presence in the environment on plants and natural soil microbiome is discussed. Furthermore, the recent regulation in EU regarding antimicrobial usage in livestock, as well as manure use within EU Member States in agriculture, has been mentioned. Finally, some common conclusions on joint action to fight antimicrobial resistance have been proposed.

Keywords Antimicrobial/antibiotic resistance genes · Manure · Agricultural waste

6.1 Introduction

In the 1940s, antibiotics were considered a miraculous drug for every kind of bacterial infection. After a few years, researchers noticed that the unrestricted, unnecessary use of this type of drug could also be dangerous. In a very short time, microorganisms have developed strategies to defeat antibiotics. This capability can be created de novo or obtained from the surrounding environment (via horizontal gene transfer; HGT). Once the antibiotic was introduced to frequent use, resistant strains

M. Zalewska · M. Popowska (✉)
Institute of Microbiology, Faculty of Biology, University of Warsaw, Warsaw, Poland
e-mail: m.zalewska@biol.uw.edu.pl; magdapop@biol.uw.edu.pl

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capable of inactivating the drug become prevalent, e.g., shortly after the introduction of sulfonamides in 1937, the first resistant bacteria in late 1940 were reported (Davies and Davies 2010). Although antimicrobial resistance is a consequence of the evolutionary adaptation of microbes, the widespread and uncontrolled use of antibiotics has created an environmental pressure of anthropological origin for microorganisms to even faster overcome the presence of this type of drug in their living niche. Genes encoding resistance were 5–12 times more abundant in 2008, compared to DNA extracted from archive soil samples (collected 1940–2008 in Europe) (Knapp et al. 2010). It has been estimated that antimicrobial resistance (AMR) is responsible for 25,000 deaths per year in the European Union (EU), and 700,000 worldwide. Moreover, it has also been calculated that by 2050, AMR will cause more deaths than cancer (European Commission 2018).

Antibiotics are commonly used not only for human medicine but also in food-producing animals, especially in swine, poultry, beef, and dairy production. In herds, antibiotics have been used for veterinary purposes (disease treatment) as well as for prophylaxis and control of disease to sustain animal welfare or to improve production efficiency (e.g., as a growth promoters) (Meek et al. 2015). Approximately 70% of the antibiotics used for nonmedical purposes in domestic animals are classified by the World Health Organization (WHO) as critically useful for human medicine (e.g., third-, fourth-, fifth-generation cephalosporins, glycopeptides, macrolides and ketolides, polymyxins, quinolones) (International Office of Epizootics 2015; WHO Advisory Group on Integrated Surveillance of Antimicrobial Resistance and World Health Organization 2017). The total amount of antibiotics used in medicine as well as in veterinary per year in 2010 has reached 100,000–200,000 tons worldwide, in which an estimated two-thirds were consumed by livestock (Tasho and Cho 2016). It is also important that antibiotics are not completely digested after their consumption—15–90% is excreted with manure and urine (e.g., amoxicillin is excreted in 90%, while erythromycin only in 15%) (Kuppusamy et al. 2018). After antibiotic administration to an animal, the antibiotics metabolites, its parent compounds, bacterial species (including drug resistance ones), and antimicrobial/antibiotic resistance genes (ARG) are excreted with feces and urine. Millions of tons of antibiotics have been released into the environment, including wastewater effluents, land application of animal wastes, treatment of crops diseases, or aquaculture (Finley et al. 2013). The animal wastes (manure, wastewater) are often used for fertilizing fields, because of the similar effect of organic fertilizers to chemical ones. This approach also allows for minimizing the high costs of agriculture waste disposal. A single dairy cow produces approx. 54 kg of wet manure per day, pig approx. 6.4 kg/day, sheep 2.5 kg/day, and chicken 0.2 kg/day (Girotto and Cossu 2017). Moreover, manure is a great source of nutrients and organic matter. Arable soil fertilization with manure is a convenient way to recycle valuable nutrients, such as phosphorus and nitrogen, which are crucial for plant growth. The nutrient content for the wastes can be desirable properties for land application, but overapplication of wastes can overload soils with nitrogen and phosphorus, as well as heavy metals (which were previously added to animal feed as micronutrients). Overapplication of animal wastes or its application to saturated soils also may cause the contamination

of surrounding waters through runoff (Burkholder et al. 2007). Manure and slurry also contain microbiologically available carbon, mineral nitrogen, and water to facilitate nitrification and denitrification (Hepperly et al. 2009). Livestock wastes seem to be a great solution for organic plants cultivation (organic food production without chemicals), which has recently grown rapidly and become a huge branch of the food industry. However, antibiotic use in livestock carries the inherent risk of spreading the antibiotic resistance genes as contaminants in the surrounding environment (Awad et al. 2015). ARG and antibiotics remain considered a potential threat to human health due to the possibility of their transfer to the food chain via plant uptake from manure-fertilized soil (Verraes et al. 2013; Kumar et al. 2018). It is also important to remember that ARG is often found in bacteria together with other genes which promote resistance to other potentially harmful chemicals such as heavy metals or biocides (Singer et al. 2016). Further, the contamination of the environment with heavy metals promotes antibiotic resistance due to cross-resistance (Kumar et al. 2019).

However, antibiotics are necessary to treat numerous animal infections. It is crucial to keep these life-saving agents available for veterinary use, but strict regulations need to be introduced to that field. Specialists from varying fields, including government agencies, medical doctors, veterinarians, breeders, and farmers, need to work together to overcome the problem of antimicrobial resistance.

6.2 Antibiotics Used in Veterinary Practices vs. Antibiotics Used in Medicine

Strong scientific evidence that the use of antibiotics in food-producing animals causes antibiotic resistance evolution in bacteria does exist (Landers et al. 2012). The use of fluoroquinolones leads to the evolution of fluoroquinolone resistance in *Escherichia coli, Salmonella* spp., and *Campylobacter* spp. (Chang et al. 2015). Moreover, the outbreak of vancomycin-resistant enterococci (VRE) in Europe was directly related to the use of glycopeptide–avoparcin in livestock. Thus, avoparcin was banned in Europe in 1997, which resulted in decreased numbers of VRE isolates from domestic animals. Moreover, in 1999, also the nonmedical use of spiramycin, virginiamycin, and bacitracin was banned within the EU. However, decreased numbers of resistant isolates from farm animals were not related to decreased numbers of infections caused by VRE in humans. A similar occurrence was noticed in The United States after forbidding the use of fluoroquinolones in food-producing animals (Przenioslo-Siwczynska and Kwiatek 2013). Also, an increased prevalence of ciprofloxacin-resistant *Campylobacter* isolates in the USA was associated with the introduction of enrofloxacin into animal use. On the other hand, a low level of fluoroquinolone resistance among clinical isolates in Australia may be explained by the fact that this antibiotic was never used in agriculture (Silbergeld et al. 2008). It

should be pointed out that once acquired resistance mechanisms are passed on to the next generations of microorganisms (Jensen and Hayes 2014).

Even if antibiotic used in veterinary differ from those used in human medicine, despite small differences, it has significant structural/chemical similarities, e.g., resistance to apramycin, a veterinary drug, might promote resistance to gentamicin, a structurally similar antimicrobial drug only for human use (Graves et al. 2011). In general, the total consumption of antimicrobials was lower in humans than in food-producing animals. The most consumed antimicrobials in human medicine were fluoroquinolones, penicillins, and macrolides, while in food-producing animals, the most extensively applied antimicrobial classes were tetracyclines, penicillins, and sulfonamides. Moreover, the overall use of penicillins, cephalosporins (all generations), and fluoroquinolones were higher in humans than in food-producing animals. The antimicrobial consumption in human medicine and veterinary medicine differ significantly between the EU Member States. The countries with the highest sell of antimicrobial drugs for food-producing animals (including horses) are Spain and Italy, with the total amount of sold antibiotic, respectively, 2726.5 and 1223.4 tones. The countries with the lowest sell are Iceland and Luxemburg, with the total amount of sold antibiotic, respectively, 0.7 and 2.1 tones (2017).

The WHO have classified antimicrobial agents aiming the significance for human disease treatment as (1) critically important for human medicine, (2) highly important for human medicine, and (3) essential for human medicine. To classify antimicrobials, two criteria were proposed: (1) antimicrobial classes as the sole or one of the limited available therapies to treat severe bacterial infections in people and (2) antimicrobial classes used to deal with infections in people caused by either bacteria that may be transmitted to humans from nonhuman sources or bacteria that may acquire resistance genes from nonhuman sources. Antimicrobial classes which fulfill the first and second criteria are determined as critically important (CIA—Critically Important Antimicrobials) for human health. Antimicrobial classes which meet either the first or second criterion are classified as highly relevant (HIA—Highly Important Antimicrobials) for human medicine, and the rest of the antimicrobials used in people are termed important antimicrobials (IA) (WHO Advisory Group on Integrated Surveillance of Antimicrobial Resistance and World Health Organization 2017). A similar division was used by the World Organization for Animal Health (OIE). This organization also classified antimicrobials used in veterinary using two criteria: (1) responders rate in the questionnaire regarding veterinary antimicrobial agents—antimicrobials were reported by more than 50% of responders as a Veterinary Important Antimicrobial Agent, (2) this criterion was met when antimicrobials were identified as essential for the treatment of specific diseases with no available alternatives. Similar to the WHO classification: when an antibacterial agent fulfills first and second criteria, it was determined as a Veterinary Critical Antimicrobial Agent (VCIA), either first or second—a Veterinary Highly Important Antimicrobial agent (VHIA), and neither first nor second—a Veterinary Important Antimicrobial Agent (VIA) (International Office of Epizootics 2015). Antimicrobial classes divided according to both criteria: WHO and OIE are presented in Table 6.1.

Table 6.1 Antimicrobials currently used in the therapy of animals and human diseases (International Office of Epizootics 2015; WHO Advisory Group on Integrated Surveillance of Antimicrobial Resistance and World Health Organization 2017; ECDC/EFSA/EMA second joint report on the integrated analysis of the consumption of antimicrobial agents and occurrence of antimicrobial resistance in bacteria from humans and food-producing animals 2017)

Class of antimicrobials	Veterinary use	Veterinary importance	Medicine importance	Consumption; human vs. veterinary (mg/kg biomass)
Aminoglycosides	Septicemias; digestive, respiratory and urinary diseases; gentamicin for *Pseudomonas aeruginosa* infections *apramycin and fortimicin only used in animals*	VCIA	CIA	0.1–5.5
Ansamycins-rifamycins	Mastitis; *Rhodococcus equi* in foals *Used only in few countries*	VHIA	CIA	n.r.
Carbapenems and other penems	Carbapenem use is restricted in animals as the last resort treatment of multidrug-resistant infections in humans	–	CIA	Carbapenems are not allowed for veterinary use
Cephalosporins (3rd, 4th, 5th generation)	Septicemias, respiratory infections, mastitis *Use limited by drug resistance presence*	VCIA	CIA	3.8–0.2
Macrolides and ketolides	Respiratory infections in cattle; *Mycoplasma* infections in swine and poultry, a hemorrhagic digestive disease in pigs, liver abscesses in cattle	VCIA	CIA	Macrolides: 7.8–11.4
Penicillins	Septicemias, respiratory, urinary tract infections *Penethamate (hydroiodide) only used in animals*	VCIA	CIA	80.0–40.0
Phosphonic acid derivatives	Aquaculture *Used only in few countries*	VHIA	CIA	n.r.
Quinolones	Septicemias	VHIA	CIA	8.1–3.5
Fluoroquinolones	Septicemias; respiratory and enteric diseases	VCIA		

(continued)

Table 6.1 (continued)

Class of antimicrobials	Veterinary use	Veterinary importance	Medicine importance	Consumption; human vs. veterinary (mg/kg biomass)
Amphenicols	Aquaculture; respiratory infections in cattle, swine, poultry; pasteurellosis in cattle and pigs	VCIA	HIA	0.1–1.5
Cephalosporins (first and second generations) and cephamycins	Septicemias, respiratory infections, mastitis	VHIA	HIA	7.0–0.1
Lincosamides	Treatment of *Mycoplasma pneumonia*, infectious arthritis, hemorrhagic enteritis of pigs	VHIA	HIA	2.2–5.5
Steroid antibacterials	Ophthalmic diseases in cattle and horses	VIA	HIA	n.r.
Streptogramins	Necrotic enteritis prevention	VIA	HIA	n.r.
Sulfonamides	a wide range of diseases of different origin: bacterial, coccidial, protozoal	VCIA	HIA	30.0–18.0
Tetracyclines	Bacterial and chlamydial diseases	VCIA	HIA	3.6–50.6
Cyclic polypeptides	Septicemias; colibacillosis, salmonellosis, and urinary tract infections; gram-negative enteric infections	VHIA	IA	Polymyxins: 0.03–10.0
Pleuromutilins	Respiratory infections in pigs and poultry *Used only in few countries*	VHIA	IA	0–4.1
Arsenicals	Coccidiosis control	VIA	Only for animals	n.a.
Ionophores/ polyethers	Coccidiosis control in poultry	VHIA	Only for animals	n.a.
Orthosomycins	The enteric disease of poultry and rabbit	VIA	Only for animals	n.a.
Quinoxalines	Digestive diseases in pigs	VIA	Only for animals	n.a.
Aminocumarin	Aquaculture; local mastitis	VCIA	Only for animals	n.a.

(continued)

Table 6.1 (continued)

CIA critically important antimicrobials, *HIA* highly important antimicrobials, *IA* important antimicrobials, *VCIA* veterinary critically important antimicrobials, *VHIA* veterinary highly important antimicrobials, VIA veterinary important antimicrobials, *n.r.* not reported in ECDC/EFSA/EMA second joint report on the integrated analysis of the consumption of antimicrobial agents and occurrence of antimicrobial resistance in bacteria from humans and food-producing animals (Joint Interagency Antimicrobial Consumption and Resistance Analysis (JIACRA) Report European Centre for Disease Prevention and Control (ECDC), European Food Safety Authority (EFSA) and European Medicines Agency (EMA) (2017), *n.a.* not applicable due to usage only on animals

From 2005 to 2009, agencies responsible for the tracking of antibiotic use noticed an increase in the veterinary use of fluoroquinolones and fourth-generation cephalosporins (Fair and Tor 2014). A few first- and second-generation cephalosporins are used worldwide for mammary gland inflammation treatment in dairy cattle. In the USA, ceftiofur (third-generation cephalosporin) is the most widely used antibiotic for the treatment of mastitis in dairy cows (Chambers et al. 2015), respiratory disease in swine, ruminants, and horses, and food rot and metritis infections in dairy cattle, but also for early mortality infections in chicken and turkey (in several countries). Also fourth-generation cephalosporin, cefquinome, are being approved for veterinary use in several countries for the treatment of respiratory diseases in cattle and swine, foot rot in cattle, and mastitis in dairy cattle (Hornish and Kotarski 2002). Cephalosporins, like antibiotics, are recognized nowadays as critically important for human health. According to the WHO, third-generation cephalosporins serve as a last resort antibiotic for severe human infection treatment and are also used to treat infections transmitted from nonhuman sources (WHO Advisory Group on Integrated Surveillance of Antimicrobial Resistance and World Health Organization 2017). Moreover, cephalosporins (third and higher generations) are recognized as antibiotics selecting for cephalosporin-resistant *Salmonella* and *E. coli*. However, also third and higher generations of cephalosporins are one of the limited therapies against *Salmonella* spp. and *E. coli* infection in people, especially in children.

The extensive use of tetracyclines in food-producing animals increased between the 1950s and 1970s, and by 2001, eightfold more antibiotics were used in the USA for domestic animals than for humans. Today, tetracycline is the second most commonly used antibiotic in US agriculture, and in China, in 2013, the total usage of tetracyclines have reached 6950 tons (Lin et al. 2015). Tetracyclines are often used for the treatment of gastrointestinal, skin, and respiratory infections, infectious diseases of the locomotory organs, genitourinary tract infections, and sepsis (Daghrir and Drogui 2013). Moreover, resistance to this antibiotic can be acquired very quickly, because resistance genes are found on transposons or conjugative plasmids (Leclercq et al. 2016). The abovementioned situation happened in Japan—tetracycline was introduced to treat *Shigella* infections in 1950, and in 1956, the first tetracycline-resistant isolate was reported. Moreover, by 1960 approx. 10% of *Shigella* isolates were resistant to tetracyclines. Also, *E. coli* resistant to this antibiotic was isolated in addition to *Shigella.* Moreover, both types were classified as multidrug-resistant (carrying also chloramphenicol- and streptomycin-resistant

genes). Nowadays, 126 different genera (76 Gram-negative and 50 Gram-positive) have been identified as resistant to tetracycline (Roberts and Schwarz 2016).

Sulfamides are widely used in humans, food-producing animals, and aquaculture. In China, in 2013, the total usage of this antibiotic group have reached 7890 tons (Lin et al. 2015). Sulfonamide resistance genes, together with tetracycline resistance genes, are the most frequently isolated ARG in livestock facilities. Resistance genes can be found in different types of farms (swine, cattle, poultry). Livestock waste appeared to be a crucial source of sulfonamide resistance genes (Li et al. 2018).

Aminophenicols (chloramphenicol, thiamphenicol, azidphenicol) were introduced into everyday use in 1949. They were considered as a broad-spectrum antibiotic and have been extensively used in veterinary and human medicine. However, several side effects were associated with chloramphenicol application. Thus, the application of this antimicrobial in human medicine has been limited. It is used only to treat several life-threatening infections. The use of chloramphenicol within the EU is currently reserved only for pets and nonfood-producing animals. This drug was withdrawn for food-producing animals (in EU 1994), because of the possibility of antibiotic residues remaining in food, which may pose a risk for consumer health. Florfenicol is still available for veterinary medicine in food-producing animals only for the treatment of several diseases, such as bacterial respiratory tract infection in pig and cattle, or infectious pododermatitis in cattle (in the USA) (Roberts and Schwarz 2016).

Macrolides, which are classified as critically important antimicrobials for humans, are the second most commonly administered antimicrobials for animals (mostly pig and cattle) within the USA and are an often used antimicrobial within the EU (varying between countries from 4% in Sweden to 14% in Denmark). This group of antibiotics is used for the treatment of frequent infections, such as respiratory and genital infections, foot lesions, mastitis, and specifically for pigs—enteritis, arthritis, and pneumonia (Pyörälä et al. 2014). According to the WHO, macrolides are known to select macrolide-resistant *Campylobacter* spp. However, at the same time, this class of antibiotic is one of the limited therapies against *Campylobacter* infection for people, especially for children (other alternatives are quinolones available only for adults).

Ionophores (e.g., lasalocid, monensin, narasin, salinomycin) are the most common antimicrobials used in cattle feedlots in North America. Within the EU, ionophores are used only for veterinary purposes to control intestinal parasitic coccidiosis in poultry, where only a few or no alternatives are available (International Office of Epizootics 2015). In a recent WHO report this class of antibiotics was classified as an antimicrobial currently not used in humans (WHO Advisory Group on Integrated Surveillance of Antimicrobial Resistance and World Health Organization 2017).

The abovementioned situations underline the considerable threat related to the use and, the most important, misuse of antibiotics in agriculture. Many classes of antimicrobials are used not only for animals but also for humans. Often, antibacterial agents used for treating animal diseases are, at the same time, the drugs of last resort for people with severe, life-threatening bacterial infections.

6.3 Drug Resistance in Agriculture Wastes and Waste-Amended Soil

Briefly, antibiotic application in animals leads to its excretion into the environment, which is not surprising, because most of the antibiotics are designed to be fast removed from the treated organisms (Burkholder et al. 2007). In 2006 in the USA, the annual production of agricultural waste exceeds 200 million tons of dry mass (Graves et al. 2011). Antimicrobials may be excreted to urine and feces in unchanged form, as well as a parent form of the drug, such as active compound, prodrug, drug metabolites, and its processed products (Bártíková et al. 2016). Moreover, even when antibiotic metabolites are excreted in an altered, biologically inactive form, they can be modified back into the active compound, e.g., a sulfamethazine metabolite, *N*-4-acetylated sulfamethazine, is converted easily to an active form in liquid manure (Sarmah et al. 2006). Many antibiotics are environmentally persistent and can be detected downstream of wastewater treatment plants and adjacent fields receiving animal wastes. Furthermore, despite antibiotic remains, also a large number of resistant bacteria inhabiting the gastrointestinal tract of animals treated with antibiotics can be released into the environment. Commensals are crucial carriers of ARG, and they may act as a reservoir of resistance genes, which may flow across the microbial ecosystem. These bacteria, found in agricultural effluents, harbor ARG, as well as mobile genetic elements which promote it exchanging between bacteria (e.g., integrons) (Finley et al. 2013).

Agriculture has been identified recently as a crucial reservoir of drug resistance genes, drug-resistant bacteria, and antibiotic residues. Due to the modern model of intensive animal production, the risk of zoonotic disease is intensified—a large number of animals are kept in dense confinement and often are exposed to their waste. Moreover, animal feed is formulated with high fat and protein content from crops, fat, and proteins from animals, the addition of industrial waste streams, animal waste, and antimicrobials (Silbergeld et al. 2008). The modern intensive agricultural techniques involve many improvements compared to the traditional ways, such as pesticide application, crops fertilization, or crops irrigation. All of the advanced technologies generate pollutants which are released into the environment, e.g., heavy metals, pesticides, chemicals and reagents, polyaromatic hydrocarbons, atmospheric depositions, pollen grains, bio-aerosols, biodegradable residues, plastics, and polymers, which could be hazardous for the living beings (Kumar et al. 2019). As it was mentioned before, the presence of specific compounds in the environment, such as heavy metals or pesticides, actively promote drug resistance among microorganisms due to cross-resistance (heavy metal or pesticide resistance genes are located at the same MGE such as ARG) (Davin-Regli and Pagès 2012; Yazdankhah et al. 2018). Animal production often involves water impoundments, or lagoons for storage and treatment of liquid manure, wash water and feeding pen runoff. To prevent water penetration into groundwaters, lagoons are lined with tightly compacted soil (McNab et al. 2007). Contaminants from animal wastes can enter the environment in many ways, such as leakage from poorly constructed

manure and slurry storage lagoons or improper application on fields. Environmental conditions also play a significant role, e.g., major precipitant events can provide facilities animal wastes overflow from storage locations or promote runoff from waste-amended fields into the surrounding groundwater (Burkholder et al. 2007). However, storage or processing facilities were not designed to control pathogens or remove antibiotics and antimicrobial genes from wastes. Studies showed that pathogen could persist in swine lagoon liquid and sludge, in manure piles and a waste litter (Graves et al. 2011). The animal waste storage tanks could be a potential hot spot for antimicrobial resistance evolution because storage material consists of fecal bacteria (commensals, pathogens), and antimicrobial agents, which are kept together for a considerable period. *E. coli* isolated from dairy farm slurry were resistant to currently and previously used drugs, with at least two-thirds of cultured strains exhibiting multidrug resistance, also to beta-lactam antibiotics (Ibrahim et al. 2016). Moreover, Bartelt-Hunt and coworkers (2011) have found the veterinary pharmaceuticals in lagoons and shallow water near swine and beef cattle facilities (beef cattle facilities: sulfamethazine and sulfathiazole were found only in groundwater, while erythromycin and monensin were found in wastewater lagoon as well as in groundwater; swine facilities: tiamulin and sulfathiazole were found only in wastewater lagoon, while sulfamethazine, lincomycin, and ractopamine were found in wastewater lagoon as well as in groundwater).

The most general way to dispose of animal waste is a land application. However, according to law, livestock waste does not have to be treated in any particular way before application, despite many reports about different waste treatment strategies aiming drug residues, drug-resistant bacteria, and ARG reduction (Tien et al. 2017; Zhang et al. 2019) (described in Chap. 19). There are many general instructions on the natural fertilizer preparation before its application onto a field. Pathogens are more likely to persist in liquid or moist waste and in sludge or lagoon (Graves et al. 2011). Then, after the soil amendment, ARGs can further affect natural microorganisms from soil changing their natural resistome (Singer et al. 2016).

The soil is a natural habitat of many microorganisms participating in substantial biological processes such as decomposition of organic matter, nitrification, denitrification, ammonification, urea decomposition, or proteolysis, but also protection of plants against plant pathogens or modulating natural plant immunity (Berendsen et al. 2012). Antimicrobials present in the natural environment can alter the natural microbiome (e.g., application of manure obtained from sulfonamide SDZ-treated pigs has led to a decrease in ammonia-oxidizing bacteria and an increase in ammonia-oxidizing archaea) (Jechalke et al. 2014), which eventually results in deterioration of plant growth condition and reduced quality of produce (Grenni et al. 2018). The presence of antimicrobial residues in the natural environment can be harmful to microorganisms, but also it can accelerate the drug resistance rate among microorganisms (Bártíková et al. 2016). The selection for resistance in some pathogens also may results in increased virulence (Stevens et al. 2007). In addition to their antimicrobial activity, antibiotics can also directly affect plant growth (e.g., tetracyclines, sulfonamides, macrolides, β-lactam, phenicols), root growth and development (e.g., tetracyclines, lincosamides), photosynthesis (e.g., β-lactams), or

seed germination (e.g., tetracyclines, sulfonamides, macrolides) (Chung et al. 2017). Antibiotics, such as oxytetracycline, chlorotetracycline, sulfadoxine, trimethoprim, or lincomycin, can also accumulate in plant roots and leaves (carrot, cucumber, lettuce, pepper, potato, spinach, tomato, celery, coriander, corn, or rice) from where they can be directly transmitted to the food chain (Pan and Chu 2017).

However, the primary concern of extended antibiotic use in agriculture is the acceleration of resistance occurrence (single- or multidrug resistance, but also cross-resistance). ARGs can also be found on plants which were grown on manure-fertilized soil. Wang and coworkers (Wang et al. 2015) have found various ARG on harvested vegetables growing on soil with a history of more than 3 years of natural fertilization. They identified tetracycline resistance genes (five efflux pump genes such as *tetA, tetAP, tetC, tetG, tetL*; four ribosomal protection proteins genes such as *tetBP, tetM, tetO, tetW*, one enzymatic modification gene—*tetX*) and two sulfonamide resistance genes (*sulI* and *sulII*). Moreover, the researchers were not able to find beta-lactamase, quinolone, or erythromycin resistance genes.

Knowledge about the natural soli microbiome and resistome is still limited. There is an enormous number of microorganisms living in that environment. Many of them naturally produce antimicrobials, and as an essential part of this production process, they can also defend themselves against the harmful action of produced compounds. Most of the antimicrobials used in humans were initially isolated from a soil ecosystem (Walsh and Duffy 2013). The "innate" resistance genes are located on integral chromosomes. Resistance genes held on chromosomes belong to so-called intrinsic resistance, which appears to be independent of the selective pressure of antimicrobials present in the environment. The presence of these natural resistance mechanisms stays in line with the theory that without this "innate" resistance, producer organisms will self-destruct. However, there are some indications that this theory needs to be reanalyzed (Davies and Davies 2010). Moreover, this intrinsic mechanism can become an object of further modification due to upregulation of cellular components protecting against antimicrobial or potential target downregulation.

The other way to acquire resistance to antimicrobials is sharing mobile genetic elements (MGE), such as plasmids, by horizontal gene transfer (HGT). MGE can be acquired by transformation (uptake of naked DNA from the environment and its incorporation into bacterial DNA), conjugation (gene transfer via direct contact between two bacteria), and transduction (gene transfer via bacteriophages) (Holmes et al. 2016). These mobile elements can be transferred very quickly between different microorganisms, often across broad species divisions (Soucy et al. 2015). The transfer rate of plasmids carrying resistance genes between donor and acceptor depends on different factors—plasmid size, a transfer mechanism (via phages or transposons), energy availability, and growth rate, but it has been implying that it may be sharply reduced in the presence of high concentrations of antibiotics. However, transfer of conjugative transposons can be significantly increased in the presence of low antibiotic concentrations (ter Kuile et al. 2016). Further, acquired mechanisms are passed via vertical gene transfer to the next generations (Martínez

et al. 2007). An extensive review of acquired antimicrobial resistance genes has been given by van Hoek and coworkers (2011).

The environmental pressure exerted by different pharmaceuticals triggers SOS response in bacteria, which further accelerates HGT. Exposure to stress induces stress response pathways in microorganisms resulting in altered gene expression patterns and subsequent physiology. This mechanism is associated with error-prone polymerase expression. SOS response pathways result in increased survival of bacterial cells throught elevated mutation generation. This pathway forces microorganisms to overcome adverse environmental conditions due to evolution by direct selection (Foster 2005; Torres-Barceló et al. 2015; Singer et al. 2016). Mutation level is strongly associated with antibiotic concentration (Long et al. 2016). It was also proven that the SOS pathway helps to adapt to sublethal doses of ciprofloxacin and rifampicin in a mouse model of infection with *E. coli* (Cirz et al. 2005). In this situation, MGE can be acquired even faster, making the spread of resistance even more common (Singer et al. 2016). The idea of resistance gene acquisition makes the environment a crucial hot-point to take under careful consideration. Animal use (and misuse) of antibiotics has created unnatural (anthropogenic origin) pressure on microorganisms and accelerated the speed of acquisition of antimicrobial mechanisms.

Wastes obtained from typical farms with unrestricted access to antibiotics (e.g., in the USA, China) was investigated for antibiotic residue content. Researchers were able to identify the following active compounds: tetracyclines (oxytetracyclines, tetracycline, doxycycline, chlortetracycline), chloramphenicol, sulfonamides (sulfonamide, sulfadimethoxine, sulfachloropyridazine, sulfadiazine, sulfamethoxazole, sulfadimidine), furazolidone, and quinolones (ciprofloxacin, pefloxacin) in animal manure, after administration of antibiotics in concentration range of 0.3–173.2 mg/kg, in which oxytetracycline was the highest, up to 173.2 mg/kg, and concentration of ciprofloxacin was the lowest, 0.3 mg/kg (Hu et al. 2008). The persistence of antimicrobial residues differs between different manure types. This phenomenon was investigated on 20 commercially swine, cattle, and chicken farms in the Netherlands. Tetracyclines are in general persistent in most calves (DT90 327–86 days) and chicken manure (DT90 61–330 days), and less persistent in pig manure (DT90 171–62 days). Sulfonamides are non- to moderate persistent in different types of manures (DT90 131–1.3 days). Macrolides (with some exceptions) are moderately persistent to persistent independently of manure type (1053–1.4 days). Also, lincosamides are very persistent in manure (DT90 2320–315 days). Pleuromutilins are reported as very persistent to persistent (DT90 1124–49 days). Quinolones (except ciprofloxacin and norfloxacin) were persistent in cow manure (DT90 4685–58) and less persistent in other types of tested manure (DT90 1290–83 days) (Berendsen et al. 2018). After soil amendment with manure containing antibiotic residues, the occurrence of the veterinary drug was also proven by Wei and coworkers (Wei et al. 2016). Researches confirmed the presence of different classes of antimicrobials in soil amended with manure derived from large-scale livestock and poultry operations. These antimicrobial compounds belong to different classes such as tetracyclines, sulfonamides, fluoroquinolones, or florfenicol,

and their maximum concentrations may differ depending on soil sampling depth: for 0–20 cm—oxytetracycline 3511 μg/kg, chlortetracycline 4723 μg/kg, tetracycline 763 μg/kg, and sulfamethazine 100 μg/kg; for 20–40 cm—oxytetracycline 1549 μg/kg, ciprofloxacin 7220 μg/kg, enrofloxacin 3059 μg/kg, sulfamethazine 1316 μg/kg, sulfamethoxazole 1784 μg/kg, sulfadoxine 924 μg/kg, and chlorotetracycline 30.779 μg/kg; and for 40–60 cm—sulfamethazine 1688 μg/kg, sulfamethoxazole 1692 μg/kg, sulfadoxine 1163 μg/kg, oxytetracycline 3676 μg/kg, chlorotetracycline 86.567 μg/kg, ciprofloxacin 5305 μg/kg, and florfenicol 11.4 μg/kg. Moreover, there are also differences in antimicrobial dissipation between rhizosphere and bulk soil. Chen and coworkers (Chen et al. 2018) noticed that the dissipation of sulfamethazine and chlorotetracycline, but not tylosin, was significantly faster in the rhizosphere than in bulk soil. The presented results stay in line with other research also regarding manure-amended soil resistome analysis. Hou and coworkers noticed the presence of active antimicrobial compounds in manure (tetracyclines, sulfonamides, fluoroquinolones, and nitrofurans) and manure-amendment soil (tetracyclines, sulfonamides, fluoroquinolones) obtained from highly developed regions with the intensive animal farming operation. Moreover, a comparison between antibiotics in manure and manure-amended soil revealed a similar pattern in antimicrobial prevalence and concentrations (Hou et al. 2015).

Antimicrobial drug residues in livestock environment can contribute to the spread of ARGs among microorganisms. In various studies, the presence of antimicrobial resistance genes in waste-treated soil was confirmed. Thus, it has been identified as a hot spot regarding antimicrobial resistance spreading. Comprehensive studies on ARG prevalence in manure and soil after manure fertilization have been conducted in many countries, including different resistance mechanisms, different manure types, or various animal holding conditions. The research focused on the screening of different known genes responsible for tetracycline resistance, as well as searching for new genes were conducted on pig manure samples. Besides the identification of 17 previously known mechanisms, two novel mechanisms were described (Leclercq et al. 2016). Also, during intensive dairy production, many ARG were identified in all samples covering feces, soil, and wastewater. A total number of 34 resistance mechanisms (e.g., ribosomal protection protein, major facilitator superfamily efflux pump, resistant beta-subunit of RNA polymerase, phenicol/chloramphenicol efflux pump, macrolide efflux pump, porin modification, multidrug and toxic compound extrusion transporter, resistant DNA topoisomerase) refering to 15 antimicrobial classes, such as tetracycline, trimethoprim, sulfonamide, rifampin, polymyxin B, phenicol, macrolide-lincosamide-streptogramin, glycopeptide, fluoroquinolone, beta-lactam, bacitracin, aminoglycoside were identified (Noyes et al. 2016). An elevated level of ARG in soil and water has been related to extensive use of antibiotic in livestock. Research conducted on calves fed with milk replacer containing antibiotics (neomycin and tetracycline) in therapeutic and subtherapeutic doses confirmed the presence of four genes coding resistance to tetracyclines (*tetG, tetO, tetW, tetX*), two genes coding resistance to sulfonamides (*sul1*, *sul2*), and two genes for erythromycin resistance (*ermB, ermF*) in feces. Moreover, all calves harbored at least one *tet* gene (Thames et al. 2012). Research

conducted on poultry litter (the mixture of excreta, feathers, feed waste and bedding material) has revealed high concentration of genes conferring sulfonamide resistance (*sul1*; $6.9 \pm 1.9 \times 10^8$ copies/g), tetracycline resistance genes (*tetW* $2.6 \pm 12 \times 10^8$ copies/g), and streptomycin resistance genes (*strB*; 9.4 ± 10^6 copies/g). Also, the concentration of these genes was high above the baseline in soil following fertilization with this poultry litter (Cook et al. 2014).

There is an urgent need to look closer at animal wastes and its utilization during the food production process. Based on current knowledge, the use of different types of animal waste could be beneficial for many branches of agriculture, but at the same time, it may pose a significant threat. Clear rules and regulations are needed to handle with antimicrobials safely to make sure that the risk of AMR transfer to the food chain has been reduced to a minimum.

6.4 Recent Regulation in the EU Aiming Animal Waste Application in Agriculture

Many antimicrobial drugs have become no longer effective. Huge pharmaceutical companies are moderately interested in new drug development, because of several reasons: antibiotics are administrated for a limited time (are less profitable than drugs for chronic diseases), new drugs are immediately introduced to common usage, where new antibiotics are held as last resort antibiotics and are used only when other antibiotics are no longer sufficient. These strategies are significant in limitation of drug resistance; however, they also restrain drug companies from new antibiotic development (Fair and Tor 2014).

Multidrug resistance and its strong relationship with antibiotic use in agriculture were first noticed in 1969, and described in the Swann report (Soulsby 2007). Until then, authorities had started the long-lasting procedure of withdrawing antibiotics from everyday unregulated use in agriculture. Antibiotics were banned as growth promoters, first in Sweden in 1986, then in Denmark in the late 1990s. In 1997 avoparcin, then in 1999, also bacitracin, spiramycin, virginiamycin, and tyrosine phosphate were banned within the EU (Przenioslo-Siwczynska and Kwiatek 2013). Finally, in 2006, antibiotic use in livestock animals as growth promoters was banned within the EU (Smith 2015). A similar ban was introduced in the USA in 2017, after a vast objection (FDA website).

For years, different organizations have prepared various plans aiming at guidance and policy on antimicrobial use in food-producing animals. In 1999, the WHO prepared "The medical Impact of Antimicrobial Use in Food Animals" guidelines, which strongly recommended the progressive withdrawal of antibiotics used in humans from agricultural, establishment of national programs of antimicrobial agents usage records, and preparation of national regulation to control the distribution and sale of antimicrobials. The WHO also clearly highlights the inherent risk of antimicrobial use in food-producing animals for human health. In 1999, the

European Commission in "Opinion of the Steering Committee on Antimicrobial Resistance" officially banned the utilization of the antimicrobials used in human medicine as growth promoters for livestock. Moreover, this document emphasized the importance of the educational aspect for veterinarians, farmers, or food producers, as well as for consumers aiming the prudent use of antibiotics. The WHO has recommended the appropriate practice for animal husbandry, such as better animal keeping conditions (e.g., housing, feeding) or prevention (e.g., vaccination), as an essential tool, which help to reduce infection risk instead of preventive use of antibiotics. Since this breakthrough, many other guidelines were released, but all of them have emphasized the importance of higher awareness of consumers and food producers about the prudent use of antibiotics and the consequences of their misuse. These instructions also pointed out the importance of and national and international surveillance programs on resistance prevalence and antimicrobial usage in domestic or accompanying animals and humans. The development of risk assessment/management plans, as well as higher participation of scientific institutions focusing on these topics, was also clearly underlined (Landers et al. 2012).

Nowadays, antibiotics may be administered to animals only in justified cases and only on the recommendation and under the constant supervision of a veterinarian. The existing rules on the limitation of the use of antimicrobials in food-producing animals are described in detail in the document "Council conclusions on the next steps under a One Health approach to combat antimicrobial resistance." The report also presents alarming data provided by the OECD (Organization for Economic Cooperation and Development)—each year up to 700,000 deaths can be caused by drug-resistant pathogens. It was estimated that the economic losses associated with the current resistance scale in OECD countries would rise to approximately 0.03% in 2020, 0.07% in 2030, and 0.16% in 2050. Mentioned above predictions would lead to a total loss of around 2.9% (one billion USD) in 2050.

One of the most important breakthroughs was the call for national action plans to combat antimicrobial resistance for all EU Member States. These plans must be presented within the EU network, in order to make it available for the other Member States to exchange the best-developed practices aiming this problem. This plan should contain systemic solutions for accurate state institutions and stakeholders in the private sector. A crucial aspect of the work is the definition of measurable goals (e.g., number of diseases, the number of antimicrobials used), in order to quickly evaluate the effects of the adopted methods of action. Such a plan should also include a description of the mechanisms for implementing the new regulations and instructions on how to enforce them. A clear emphasis is also placed on educational aspects and targeted campaigns aimed at increasing citizen awareness. Also, this plan should contain a list of tools (e.g., contractual penalties) to ensure the application of the adopted program. Another vital aspect is cooperation with the pharmaceutical industry, in order to provide assistance in activities aimed at alternative solutions and to ensure the unlimited access of developed solutions on the pharmaceutical market. The plan should also aim the actions increasing awareness of users in terms of antimicrobial use, abuse, and misuse in medicine and veterinary.

EU regulations emphasize the importance of prevention of infections as well as the identification of new, alternative therapies against bacterial infections acquired from different sources. Also, proper working surveillance network is crucial. An EU action plan highlights the importance of national surveillance in order to obtain complete data on antimicrobial usage and AMR. Without a properly working network responsible for collecting data, the real size of the problem is, in fact, unknown. Moreover, the results of some particular action will also be difficult to determine without a complete dataset (Council conclusions on the next steps under a One Health approach to combat antimicrobial resistance 2016).

All mentioned regulation strongly stressed the importance of prevention and prophylaxis of infection, e.g., vaccination or biosafety, but also the development of rapid diagnostic tests to identify the source of infection to take quick, effective, and targeted action. Emphasis should be placed not only on the search for new compounds with bactericidal activity but also legal regulations and system solutions aimed at defining the problem and limiting its range (Council conclusions on the next steps under a "One Health" approach to combat antimicrobial resistance 2016).

It should be noticed, that fighting with drug resistance needs at first comprehensive knowledge on the scale of the problem (e.g., precise databases collecting information on antimicrobial agents distribution or AMR prevalence), then an appropriate solution can be applied. Also, measurable goals need to be determined (e.g., standardization of AMR testing with a focus on defined resistance genes, indicator microorganisms). Such an approach has been suggested by Berendonk and coworkers (2015). Also, Thanner and coworkers (2016) described the current state of knowledge about AMR spreading via the agricultural use of antimicrobials and discussed possible solutions. Researchers proposed several steps, which stay consistent with EU politics, including (1) identification of current knowledge on the topic (and knowledge gaps), (2) identification of current data limitation (e.g., monitoring and testing of antimicrobial agents use and antimicrobial resistance within agricultural systems; identification of factors that cause the selection, spread, and persistence of antimicrobial resistance genes and antimicrobial-resistance bacteria; understanding the transfer pathways), and (3) interpreting the risk and impact related to searching of potential solution of problem with drug resistance. Identification of all entry routes of antimicrobials (such as wastewater, industrial manufacturing or aquaculture, and agriculture; widely described in Chap. 1) is essential to limit antimicrobial resistance genes from spreading as well as preparation of a robust system for surveillance, research, and guidelines for practitioners. Furthermore, strong support from different institutions is crucial—simple rules to follow, incentives (investments into modernization), self-regulation by industry, or strong scientific evidence that cannot be misunderstood or ignored (Bloomer and McKee 2018).

In June 2017 EU One Health Action Plan against AMR was adopted. This idea identifies the health of humans, animals, and the whole ecosystem as a single network, in which one part depends on another and all of them are inseparably connected. One Health action plan has three major key objectives: (1) making the EU a region with the best practice aiming AMR management, (2) boosting research,

development, and innovation on AMR, and (3) shaping the global agenda aiming AMR. Moreover, each main goal has smaller steps that need to be taken into consideration. These smaller tasks involve better coordination and implementation of EU rules to tackle AMR; better prevention and control of AMR; precise determination of the role of the environment in AMR spreading; a stronger partnership and cooperation against AMR; improve knowledge on AMR detection; and develop new therapeutics and alternatives, new preventive vaccines, novel diagnostic tools, as well as new economic models and incentives. One of the most important things is to close knowledge gaps on AMR in the environment. The last stage involves shaping the global agenda, for which it is crucial to strengthen EU global presence, make a stronger bilateral partnership for stronger cooperation, cooperate with developing countries, as well as develop a global agenda.

The EU has also set up, within One Health approach, the Joint Programming Initiative on AMR (JPIAMR) which aims to better coordinate and align worldwide AMR research efforts. This organization, to date, has supported many research projects addressing research regarding therapeutics, diagnostics, surveillance, transmission, environment, and interventions, with funding approximately 67 million Euro. The JPIAMR mission for the years 2020–2025 is to "join forces across nations by leading the alignment, coordination, and support to Antimicrobial Resistance One Health collaborative research and global policy activities" (JPIAMR website). The primary objectives of this aim are: align national and international research programs, support and coordinate transformative research, support and coordinate the JPIAMR Virtual Research Institute, promote innovation and encourage to the adaptation of research results, for building a bridge across the gap between research and policy. Also, the JPIAMR has indicated manure and manure-amended soil as a crucial point in AMR spreading. Several proposals aiming AMR spreading via agriculture waste application on the field were granted, lately ARMIS (Antimicrobial Resistance Manure Intervention Strategies) or INART (Intervention of Antimicrobial Resistance Transfer Into the Food Chain) (fifth call). Also during the last JPIAMR call project with acronyms WAWES (Wildlife, Agricultural soils, Water environments, and antimicrobial resistance - what is known, needed and feasible for global Environmental Surveillance) (seventh call), which was more focused on the surveillance of antimicrobial resistance bacteria using the One Health multi-sectoral approach, were granted.

6.5 Summary

Prudent use of antibiotics in humans and animals is crucial to fighting with antimicrobial resistance among microorganisms, which have become a substantial human health problem. It is assumed that the global consumption of antimicrobials in food-producing animals and humans will rise by 67% between 2010 (63,151 tons ± 1,560) and 2030 (105,596 ± 3,605), from which approx. 2/3 will be due to the increasing number of food-producing animals. It is considered that developing

countries still using antimicrobials from nonmedical purposes, will increase their use in domestic animals by 99% in 2010–2030, contributing to an increase of 67% worldwide (Bloomer and McKee 2018). The usage of antimicrobials in veterinary medicine is very high, but also the number of food-producing animals is large. It is essential to keep a perfect balance between prudent administration of drugs and maintaining animal welfare. Moreover, animal waste needs to be treated in order to dispose of any factors leading to AMR spread (Pruden et al. 2013). With no doubt, the excessive and unnecessary use of antimicrobials in food-producing animals accelerates the resistance of different microorganisms to various agents. Many scientific data addressing this topic have been collected to date. However, still many open questions does exist, e.g., places where HGT of resistance domains is most probable, also concentrations of antimicrobials used on animals which lead to resistance and, especially, environments which supports the ARG transfer via HGT or dissemination pathways of ARG from selective environments to animal and human pathogens (European Medicines Agency 2015). First of all, it is essential to fully determine all entry routes of ARG into the environment and define further the impact on human health. The limited knowledge identifying mechanisms and pathways involved in transmission on genetic, cellular, and population levels is problematic. Surveillance systems are not sufficient on collecting the data regarding the use of antimicrobial agents for animal and plant health, specific ARG in zoonotic agents and commensal bacteria in livestock, or amount of ARG and antimicrobial agents and ARG in manure. It is also essential to identify all pathways of ARG moves, such as routes/places where human- and animal-associated bacteria can acquire ARG form environment or nonpathogenic bacteria, potential risk of antimicrobial agent use on plants (e.g., direct antimicrobial resistance genes uptake by plant), as well as rate of spontaneous removal of ARG from soil or another environment. After identification of all crucial points and routes of ARG transmission, quantitative and qualitative goals need to be assessed for evaluation of chosen parameters such as analysis of fixation, prevalence, sorption, and potency of antimicrobial agents in soil, wastes, and sediments. Measurable values are required to establish proper risk management systems, which also comes from proper surveillance systems (Thanner et al. 2016).

Current awareness of AMR transfer routes is high, but still many knowledge gaps that need to be filled does exist. All efforts are being undertaken to combat drug resistance, which is considered a life-threatening problem for animals and humans. Complex solutions are being prepared and planned, but efforts of just one sector cannot prevent or eliminate the problem only a joint effort will bring the expected results.

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Chapter 7
Bio-monitoring of Antibiotics and AMR/ARGs

Muhammad Afzaal, Sidra Mukhtar, Masooma Nazar, Afifa Malik, Amtul Bari Tabinda, Abdullah Yasir, Ashfaq Ahmed Bangash, Sarfraz Ahmed, Azhar Rasool, and Muhammad Khalid

Abstract Evidence of increasing antibiotics and their bio-monitoring in different resources has received worldwide attention. This study provides a better understanding of antibiotics, antimicrobial resistance, and antibiotic resistance genes. The antibiotics used in intensive amounts results in their constant discharge into the receiving environment and consequent extensive propagation of antibiotic resistance genes, therefore causing potential threats for public and ecosystem health.

Keywords Bio-monitoring · Antibiotics · AMR/ARGs

M. Afzaal (✉) · S. Mukhtar · M. Nazar · A. Malik · A. B. Tabinda · A. Yasir
Sustainable Development Study Centre, Government College University, Lahore, Lahore, Pakistan
e-mail: dr.afzaal@gcu.edu.pk

A. A. Bangash
Universiti Teknologi PETRONAS, Seri Iskandar, Malaysia

S. Ahmed
Department of Basic Sciences, Faculty of Veterinary Science Narowal, University of Veterinary and Animal Sciences, Lahore, Punjab, Pakistan

A. Rasool
Department of Zoology, Faculty of Life Sciences, Government College University Faisalabad, Faisalabad, Pakistan

M. Khalid
Department of Chemistry, Khwaja Fareed University of Engineering & Information Technology, Rahim Yar Khan, Pakistan

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7.1 Introduction

For the treatment of bacterial infections antibiotics have been broadly used since the initiation of penicillin in 1929 (Qiu et al. 2018). They are artificial determined mixtures as their constant contribution into the environment and perpetual existence (Pan and Chu 2018). The main antibiotics normally utilized in China are macrolides, sulfonamides, and fluoroquinolones, donating nearly 15%, 20%, and 12% of the entire utilized, individually (Du et al. 2017). The fact of particular concern is that, given the partition properties and chemical durability of antibiotics which are utilized (Zhao et al. 2018). Some of these compounds are partly biologically degraded in test settings; while maximum persevere in marine structures (Chen et al. 2017). Therefore, these mixtures easily gather in environment in different sector for example in water, residue and thus characterize a possibly crucial environmental difficulty with respects to both ecological hazard and human health risk.

Nowadays, antibiotics are used as medicine for humans and utilized in animal husbandry and aquaculture (Yang et al. 2018). However, antibiotics that are inadvertently dumped into the environment cause a prolong thread to ecosystems and human health. These antibiotics may gather in food cycle and became more dangerous antibiotic persistence genes (ARGs) moved among environmental bacteria and human pathogens (Bengtsson-Palme and Larsson 2015). In 2010, almost 57 million kg antibiotics was utilized in animal farming worldwide. Misuse of antibiotics can create undesirable results because antibiotics are a costly mean to avoid and cure disease in different agricultural fields (Dungan et al. 2018).

7.2 Antimicrobial Resistance (AMR)/Antibiotic Resistance Genes (ARGs)

Antimicrobial protection (AMR) is a wider terminology, including resistance to medicine for treatment of contaminations affected by further pathogens in the same way, like parasites (e.g., malaria), viruses (e.g., HIV), and fungi (e.g. *Candida*). Antimicrobial protection (AMR) has occurred for millions of years and is a predictable evolutionary significance of microbial struggle in the environment. The spreading of antimicrobial protection (AMR) has developed into a predominant worldwide community health concern. Antibiotics are present in domestic wastewater, created from incompletely processed medicines used by individuals and discarding of pristine antibiotics. In human and agricultural waste antibiotic resistance bacteria and other gene resistance antibiotic can be assembled and then utilized or discharge into aquatic and terrestrial milieus (Ashbolt et al. 2013).

These AMR impurities cause human health hazards as formerly treatable contaminations are currently attractive resilient to conservative antibiotics (Laxminarayan et al. 2013). The investigation showed that certain situations might be used like catalyst

for antibiotic-resistant bacteria propagation and thereby serving wastewater treatment projects (Rizzo et al. 2013). The environmental sources for antibiotic resistance genes (ARGs) are water and soil medium (Taylor et al. 2011). It has been established that few antibiotic resistance genes are components of early soil microbiome (D'Costa et al. 2011). In few areas the environmental guidelines of ARGs have been seen to be developing below man-made compressions. Here is problem that growing man-made utilization of antibiotics is donating to discerning stress and enhanced threat of horizontal gene transmission to individual pathogens (Qiu et al. 2012).

The great public health threat faced these days is antimicrobial resistance. Toxicity caused by resistant organisms when rise its limit then it form treatment failure, increase injury, death and expenses related with increase burden on healthcare benefits and reduction in production.

As stated in current analysis of antimicrobial protection, in case present trends remain, ten million deaths are forecast to happen yearly through the year 2050, with a worldwide financial price of $100 billion USD by this period (Leonard et al. 2018a, b).

ARGs are examined modern pollutants and can cause possible health threats to individuals globally (Zhu et al. 2013). In microbes the ARGs might be moved to native ecological bacteria through horizontal gene transmission (Gillings et al. 2015). In agricultural soil treatment of antibiotics was known as significant issue due to the formation and circulation of antibiotic resistance genes (Kristiansson et al. 2011). ARGs must be transferred towards underground earth strata and perhaps pollute groundwater by vesicle transportation (Pan and Chu 2018).

The occurrence of antibiotic fight genes (ARGs) rises dangers to public health that involved global anxiety. A large number of groups and governments have increased observing of antibiotic-resistant bacteria and ARGs. The World Health Organization (2014) started a worldwide investigation of antibiotic resistance in 114 countries, among their initial investigation report published in April 2014; the Public Health Agency of Canada also prepare a 4-year investigation report on antibiotic-resistant organisms in Canada (2014); the U.K. Parliament released a note titled "Antibiotic resistance in the environment" (2013) and the U.S. White House has delivered an managerial instruction on national policy (2014) for fighting antibiotic resistance (Yang et al. 2016).

Important, translational and clinical research must deliver novel solutions to report disease prevention, diagnosis and treatment, develop new products for clinical use, and give information for the ideal usage of fresh and present medicines. by, preclinical study in considering how bacteria fight activities of antibiotics either extra objective for antimicrobial remedy might be recognized and browbeaten is also essential for confirming that the medicine channel is frequently refilled with contender lead mixtures with medical possibility, as novel and current antibiotics will continue to develop archaic owing to the mixture for protection. Study into novel mixtures of complexes (multiple antibiotics used simultaneously, or antibiotics joint with further anti-infective) also grips huge potential, as do the formation of mixture that might constrain the appearance and transmission of antibiotic resistance.

Additional economic research requires descriptive drivers of AMR in low- and middle-income countries, appealing native investors to confirm extensive application. A multifaceted method including small as well as moderate capacity enterprises (SMEs) to increase the proficiency of research and lower costs should be encouraged, in agreement with existing research programs, such as the Inventive Medicines Initiative Joint Undertaking program (IMI)1 with the New Drugs for Bad Bugs (ND4BB) initiative, the Biotech companies in Europe combating Antimicrobial Resistance Alliance (BEAM)2, the ENABLE project3 with the participation of EFPIA companies, research bodies, SMEs and third parties, and the DRIVE-AB4 (Driving reinvestment in R&D) project that planned push and pull motivations to policy makers.

7.3 Occurrence and Distribution of Antibiotics

The existence and dispersal of antibiotics were studied in surface water and residue gather from the chief rivers of Shenzhen, China. Qiu et al. (2018) examined 20 antibiotics in river water and residue trials obtained from the chief rivers. Subsequently the complete proportions ranged from up to 1075.687 ng per L by the average value of 244.992 ng per L within 31 water samples and up to 2728.810 ng per g in 31 samples of residues. Between them, the amounts of sulfadiazine and sulfathiazole in residue were significantly greater than those noticed in other regions and linked with additional antibiotics. Sulfadiazine was known as causing the main hazard towards algae founded on a hazard valuation of water samples. Furthermore, metagenomics examination showed that the comparative profusions of antibiotic resistance genes and microbes exist in the residue samples, and important associations were recognized between microorganism, antibiotics, and ARGs. Particularly, sul1 gene was the major plentiful antibiotic resistance genes and Proteobacteria was the major plentiful microorganism (51.7%), followed by Bacteroidetes (18.4%). Therefore, these outcomes shows that the antibiotics presented in natural water courses may perhaps help as a main choosy force that encourages the propagation of antibiotic-resistant bacteria (ARB), thereby pointedly changing the configuration of bacterial groups. The growth of active resistance to antibiotics between microorganisms can offer increase to the propagation of fatal pathogens. So some antibiotics might longer be operative in handling different medical conditions, thereby decreasing the treatment choices for specific pathogens. The information acquired in this study should reinforce our considerate of antibiotic pollution in environment and the major impact of antibiotics on microbial community (Qiu et al. 2018).

In the Canadian Arctic, discharge of public wastewater into tundra wetlands is common routine. There is a lack of information about presence of antibiotic protective bacteria and the antibiotic resistance genes in wastewater Arctic regions. This investigation was imitated on the chance of ARGs within tundra wetland environments affected by man-made wastewater resources in Arctic regions. Two wetlands were studied in the summer season of 2016 in the Inuit populations of

Naujaat and Sanikiluaq in Canada. At the time of spring season Genetic DNA was removed from both soil and water and at the end of summer in wetlands a suite of nine medically related ARGs (sul1, sul2, mecA, vanA, qnrS, ermB, tetO, blaTEM, blaCTX-M) and an integron gene (int1) were examined via quantitative polymerase chain reaction (qPCR). Water quality and hydrological analysis were accompanied in combination with the microbiological sampling. Gene goals constantly existed in wastewater and all over both wetlands excluding for mecA and vanA and. In the spring season concentration of ARGs was greater in stream by the cause of shortened hydraulic retention times (b2 days), which linked through lessened treatment performance. Determined by wastewater concentration of AGRs was higher above baseline sites in tundra wetlands and hydrology environment had a large influence on their longitudinal division and stages.

The use of dairy wastewater to farming land is broadly adopted technique to water and nutrients recycle. In this study moderate level field plots were watered six times per month with dairy wastewater (100%), 50% diluted wastewater with canal water and diluted wastewater mixed with copper sulfate about 50 mg Cu L^{-1}, whereas canal water is given to control plots. Furthermore half plots were sown by wheat and remaining was left as barren land. Twice a month soil samples were gathered and prepared to control the existence and prosperity of antibiotic protective genes [blaCTX-M-1, sul1, erm(B), tet (B), tet(X), and tet(M)] and an integron-integrase class 1 gene (intI1) through quantitative real-time PCR. Sul1 and tet(X) were only noticed (3 out of 32 samples) in soil before the application of wastewater. However, the existence and comparative plenty of maximum genes [tet(M), erm(B), sul1 and intI1] improved intensely following wastewater irrigation and ranks were sustained throughout the whole investigation time. The single gene that is not identified in soil which is treated through wastewater was BlaCTX-M-1 that is linked to its deficiency in the dairy wastewater. In most cases it was found that in soil comparative gene levels were established statistically related between the treatments, irrespective of the percentage of wastewater and existence or absence of different plants. The main outcome of this study is that irrigation by the dairy wastewater prominently increases the basin of antibiotic resistance genes (ARGs) and intI1 in soils. Although finding of these genes hardly arose in soil watered only with canal water. Furthermore, raised intensities of copper in the wastewater and treated soil did not generate a related rise of the antibiotic resistance gene levels (Dungan et al. 2018).

In current years, presence of different antibiotics and antibiotic resistance gene in municipal wastewater has turned out to be a significant problem for the defense of public health as municipal or pool wastewater is continuously utilized for agricultural requirements, for example, irrigation and crop growing. The extensive existence of several antibiotics in wastewater and cultivable soils may possibly apply collection stress on ecological microorganisms, subsequently in the propagation of antibiotic confrontation in microorganisms. The presence and dispersal of sulfamethazine, tetracycline (TC), and the consistent antibiotic resistance genes were examined within six agricultural locations in the Pearl River Delta state in China. At the two various depths, such as one is 0–10 cm and the other one is 10–20 cm, irrigated soil and irrigation water were analyzed, respectively. In irrigation water entire

concentrations of tetracycline and sulfamethazine were calculated from 69.3 to 234 ng/L and from 4 to 58.2 ng/L accordingly, whereas in irrigation soil whole concentrations of tetracycline and sulfamethazine ranged from 5 to 21.9 μg/kg and 1.30 to 4.20 μg/kg correspondingly. After long time period irrigation with municipal and pool wastewater in the agricultural field, the remaining tetracycline and sulfamethazine and their consistent antibiotic resistance genes in soils were prominently greater in lake irrigated soils (Dongguan and Shenzhen) compared to municipal wastewater irrigated soils (Foshan, Guangzhou, Huizhou and Zhongshan).

The total concentrations of antibiotics and their antibiotic resistance genes were prominently greater in irrigation water than irrigated soils. This showed that wastewater was the main resource of antibiotics in soil milieus. The municipal and pool wastewater were significant sources of antibiotics and their respective ARGs. That have been involved actively handling formerly their release into the environment. Further elements, for example, physicochemical characteristics, application of compost, irrigation water resources and farming models also disturb the antibiotic amounts and abundance of ARGs. The remaining antibiotic concentrations linked statistically with the consequent antibiotic-resistant genes in irrigated soils and irrigation water. Both of which decreased with rising soil deepness, showing that the actual concentration of antibiotics in the surrounding environment put assortment pressure upon the microbes in the atmosphere.

7.4 Environmental Drivers of Antimicrobial Resistance

To fight the risk of human health and bio-security from antimicrobial resistance, considerate of its instruments and drivers is wanted. Appearance of antimicrobial resistance in microorganisms is a normal thing; so far antimicrobial resistance has been focused by antimicrobial contact in healthcare, agriculture, and the environment.

Forward conduction is affected by standards of contamination regulator, hygiene, contact to fresh water, contact to guaranteed excellence antimicrobials and diagnostics, transportable and immigration. Policies to decrease antimicrobial conflict by eliminating antimicrobial discriminating stress only trust upon confrontation reporting appropriateness rate, a consequence not continually obvious. Reducing struggle must be consequently be measured widely, by conflict appliance, microorganism, antimicrobial drug, host, and setting; similar to novel drug innovation, wide reaching, multidisciplinary investigation is required transversely these five stages, interweaved through the healthcare, agriculture, and environment sectors. Intellectual, combined methods, careful of probable unintentional outcomes, are required to confirm constant, global contact to actual antimicrobials (Holmes et al. 2016).

Certain for the complicated nature of the problem, the attention has prolonged from human and animal antibiotic use to the human effect on conflict in the environment. The connection among the animal and human part are sound studied and directed to strategy variations in certain areas of the world. Such controlling advan-

tages are still lost in the environmental field which is frequently not involved in the One Health method to hold the worldwide conflict problem.

The direct discharge of multidrug resilient bacteria from healthcare sceneries and animal farms into the environment and the pollution of the environment with great absorptions of antibiotics generate a hazardous conflict reservoir. Current met genomics studies emphasized the part of movable hereditary rudiments (mobilome) as environmental contaminants and their part in co-assembling of confrontation factors and parallel allocation from environmental bacteria to pathogens and vice versa. Best levels of antibiotic remains in wastewater plants, fresh waters but also retrieved water supply systems owing to tolerant pollution from antibiotic manufacturing plants apply extraordinary collection stress in nature. Addressing this difficulty needs intensive policy actions and needs to be involved in the One Health approach of current global initiatives (Theuretzbacher 2016).

7.5 Risks to Human and Animal Health

The wide-ranging usage of antibiotics foremost to the quick spread of antibiotic fighting postures extraordinary health dangers to humans, but to date there is still lack of a quantitative model to properly assess the risks (Ben et al. 2018). Environmental distribution of ARBs is progressively recognized as a possible direction for the extent of AMR (Marshall and Levy 2011). Various environmental sections might give to the distribution of resilient pathogens and commensal bacteria linked through humans and animals like. Resourceful pathogens such as *Acinetobacter* spp. and *Pseudomonas* spp. are situated inside diverse environmental sections. Therefore, sewage, wastewater treatment plants, agricultural and veterinary hospital effluents (Zhang et al. 2009), drinking water (consumed either by humans or domesticated animals in adjacent connection with humans), entertaining water, air-borne aerosols, dust, wildlife fauna and polluted food from agriculture or aquaculture are all courses allowing the possible spread of bacteria and ARGs among hosts from the surroundings (Singer et al. 2016).

7.5.1 Food from Agricultural Crops

Through farming in mud to which animal manure is applied, crops and irrigation water may be polluted with resilient bacteria. An amount of studies have explored the occurrence of AMR bacteria in or on vegetables and fruits. Meanwhile renewed produce is frequently expended raw, ingesting might consequence in the digestion of resilient bacteria that, dependent on the bacterial species, are capable to colonies in gut or pass through the intestine, thus posing a potential public health risk (FAO 2016). For example, a study from the Netherlands exposed that third group cephalosporin resilient fecal Entero-bacteria were separated from 2.7%, 1.3%, and 1.1%

of supermarket vegetables, iceberg lettuce from farms, and agricultural soil, respectively (Blaak et al. 2014).

7.5.2 Food from Aquatic Population

The existence and feast of ARB and ARGs in zones chosen for fish farming (marine and fresh water) has dramatically enlarged through current years (Topp et al. 2017). The presentation of antimicrobials to the aquatic environment might choose for ARGs not merely in fish pathogens, but also in environmental bacteria (Muziasari et al. 2016). Maximum resistance has been informed contrary to oxy-tetra-cycline, tetracycline, ampicillin and florfenicol (Caruso 2016), but some ARGs coding for resistance to quinolones and β-lactams can be found in fish pathogens, human pathogens and aquatic bacteria. Furthermore, Cabello and colleagues suggested that the use of antimicrobials in aquaculture, notably the use of colistin in Asian aquaculture, could be correlated with the emergence of the plasmids encoded mobile colistin resistance (MCR) determinants (Cabello et al. 2017).

7.5.3 Drinking Water Contamination

The occurrence and confrontation designs of several bacteria isolated from drinking water dispersal methods have been lately described. For example, in Romania, multiple AMR *E. coli* straining sequestered from drinking water were originated to port ARGs encoding resistance to aminoglycosides, beta lactams, tetracyclines and trimethoprim–sulfamethoxazole (Cernat et al. 2007). A strain of *E. coli* carrying the $bla_{CTX\text{-}M\text{-}1}$ IncI1/ST3 plasmid was isolated in France from drinking water. The plasmid was identical to those found in animals and humans (Madec et al. 2016). In another German study, the *van*A and *amp*C ARGs were detected in drinking water biofilms (Schwartz et al. 2003).

7.5.4 Recreational Places Contamination

Antibiotic-resistant bacteria (ARB) have been noticed in freshwater environments and direct absorption of water from recreational locations (e.g., seawater, lakes) is a route by which the population can be straight visible (Leonard et al. 2015). In England, Leonard and colleagues revealed that 0.12% of E. coli separated from superficial waters were resilient to third generation cephalosporins (3GCs) and could represent a human contact danger for water workers (Leonard et al. 2015). Leonard et al. also used a targeted metagenomic approach to estimate contact to *E. coli* in UK shoreline washing waters carrying all known ARGs, concluding that

all exposure events product in digestion of at least one *E. coli* associated ARG (>100 million events per year) and 2.5 million events per year are likely to occur involving ingestion of 100 *E. coli* borne ARGs (Leonard et al. 2018a). The relationship between exposure, colonization, and infection with AMR opportunistic pathogens is uncertain and there are no dose-response data available for colonization, infection, or HGT (horizontal gene transfer) of ARGs from ingested bacteria to those of the microbiome. A two-dimensional study of surfers and non-surfers in the UK found that surfers were different periods as per possible to be populated by 3GC-resistant *E. coli* and >4 times as likely to be colonized by $bla_{CTX\text{-}M}$ *E. coli* suggesting an association between coastal bathing water exposure and gut colonization by ARB (Leonard et al. 2018b).

7.6 Bio-monitoring of Different Antibiotics

Bio-observing of antibiotics can reveal a complete contact quantity of antibiotics not merely from medical exploitation and self-prescription, but also from agriculture and surrounding environment. The extent of inner contact dosage can be utilized to study the link among contact and particular effects more precisely than treatment and survey (Wang et al. 2015). Antibiotics expended in human being and farm animal medicine can group between populations, animal, agricultural food, and the atmosphere like that the antibiotic outlines between them are linked with all other. The bio-monitoring of antibiotics in human beings can also show the complete application position of antibiotics in different areas to certain degree. While the human body absorbs different antibiotics, a significant amount is evacuated in urine through free or grouped types. The antibiotics incoming from the human body can be metabolized to manifold metabolites (Kümmerer 2009). Therefore, the possible contact biomarkers of antibiotics are urinary antibiotics which offer a suitable and consistent method in human inhabitants to measure antibiotic body loads (Wang et al. 2014).

The capability of blue mussel biologically known as Mytilus edulis was utilized to perform as a possible antibiotic bio-pointer in oceanic waters which is experimentally verified through the investigation of the kinetics of two special veterinary antibiotics such as oxolinic acid (OA) and oxytetracycline (OTC). Antibiotic digestion was quick in the spongy fractions of mussels. Oxolinic acid was increased rapidly removed though oxytetracycline was unrestricted extra gently like half-life in viscera¼3.9 days. In gills and viscera, oxolinic acid and oxytetracycline were specially gathered correspondingly. Bio-accumulation issues were low, i.e., highest: 2 for oxytetracycline in viscera in accord with the low. It was expected that the complex bioaccumulation design oxytetracycline was linked to its required inorganic and organic compounds that run to its movement reserve. The antibiotics were determined in shells, i.e., oxytetracycline half-life¼8.3 days. Most farm animal and human used antibiotics such as tetracyclines and sulphonamides that have short logKow (<2) and

should imperceptibly collect in mussel. In the aquatic environment, these powers limit the use of blue mussel to bio-monitor antibiotics (Le Bris and Pouliquen 2004).

The ultra-performance liquid chromatography combined with quadrupole time-of-flight mass spectrometry was applied to discover the antibiotic body load of Chinese school children. The total urinary attentions, both free and conjugated, and 18 typical antibiotics such as 5 macrolides, 3 tetracyclines, 2 β-lactams, 4 sulfonamides and 4 quinolones were dignified among 1064 students in school employed from 3 carefully and geologically separate areas in China during 2013. In urine examples all of 18 antibiotics were noticed with the recognition incidences reaching from about 0.4 to 19.6%. Generally, in 58.3% of urine samples, the antibiotics were noticed and this discovery occurrence stretched at 74.4% in one education location. Out of them, 47.8% of the urine examples had a quantity of mass attention of whole antibiotics between minimum 0.1 and 20.0 ng/mL. Eight antibiotics had their attentions of overhead 1000 ng/mL in various urine samples. Four human antibiotics, 3 farm animal antibiotics, and 11 human and veterinary antibiotics were originated generally in 19.9%, 6.3%, and 49.4% of urine samples, correspondingly. In different study areas, the discovery occurrences and absorption stages of antibiotics in urine samples changed accordingly. In general, a total of 137 mixtures of antibiotics and 20 mixtures of antibiotic groups were originated regarding diverse contacts. In more than 20% of urine samples, two or additional antibiotics or groups were noticed alongside. On the foundation of a practice investigation, polluted food or environment may be pertinent contact foundations for tetracyclines, sulfonamides, and quinolones (Wang et al. 2015).

Bio-monitoring of antibiotics in urine can amount the entire contact of human towards several antibiotics from clinical usage and antibiotic remains in foodstuff and marine ecosystem. Numerous populace-founded trainings have slow antibiotics or their metabolites in urine. A research study showed in Korean society stated that different antibiotics such as roxithromycin, trimethoprim, ciprofloxacin, and enrofloxacin were noticed in 30–65% of urine samples. Almost 20 antibiotics in urine were slow and generally noticed in 78.4% of children and 41.6% of pregnant women in two preceding educations led in China. These lessons have established a wide contact towards antibiotics in human and higher an anxiety around its possible health problems. In present study, analyst's examination of contact to antibiotics and lead a health hazard valuation between 284 school children in Shanghai. The significant factors such as age, family income, sex, screen time, veterinary food ingestion and clinical use were utilized for antibiotic predictions in children urine. There were possible health hazards for children with the exposure to antibiotics (Wang et al. 2018).

The link among antibiotic custom throughout pregnancy and neonatal birth consequences has established substantial courtesy. According to Maternal Psychological and Environmental Assessments of Kids Cohort Study, 369 pregnant women were arbitrarily designated into this study. As the board antibiotics in meconium, 18 mutual antibiotics of 6 groups such as 1 phenicols, 3 fluoroquinolones, 6 β-lactams, 4 sulfonamides, 3 tetracyclines and 1 lincosamides were designated. The dimension was directed by ultra-performance liquid chromatography joined to quadru-pole

time-of-flight mass spectrometry platform. Out of 18 antibiotics, 12 were originated in 62.1% of the meconium along with recognition charges reaching from 0.3% to 43.9%. About three antibiotics with the uppermost discovery charges were chlortetracycline (43.9%), chloramphenicol (10.8%) and penicillin (16.5%), correspondingly. The maximum antibiotic absorption among noticed antibiotics was penicillin which is 24,243.15 μg/kg. Pregnancy exposure to sure antibiotics was linked with changed fetal development and growth, which might disturb the usual development route of infants and children in future life (Zhao et al. 2019).

7.7 Conclusion

This study endow with a better understanding of the spread and providence of antibiotics and their bio-monitoring by covering of antimicrobial and antibiotic resistance in various environments. The exploration of other ecological factors influencing the antibiotic spread can be conducted to better predict the fate of antibiotic resistance and bio-monitoring. The exhaustive utilization of antibiotics in different fields resulted in their constant discharge into the receiving environment and the consequent prevalent propagation of antimicrobial resistance (AMR) and antibiotic resistance genes (ARGs), therefore causing prospective threats for public and ecosystem health.

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Chapter 8
Mechanism of Antibiotics Uptake in Plants

Lara J. El Gemayel and Isam I. Bashour

Abstract Veterinary antibiotics are globally resorted to for therapeutic usages of infectious diseases in humans as well as in intensive farming systems. The latter are designed to perform effectively at small doses and are proven to be excreted from the body through feces; around 70–90% is not digested and excreted into the environment as their parent compound or metabolite, thus explaining the large amount of antibiotics released in the environment with manure or in wastewater. A large amount of antibiotics has been detected in different crops with concentrations varying from no detection to 487 mg/kg. Consequently, increasing attention and studies are being done on the uptake of pharmaceutical compounds by plants grown in different medium such as soil or nutrient solutions. The main route of entry of these antibiotics into the plant is the roots, and the ability of the antibiotic to be absorbed by the plants relies primarily on its physicochemical properties and its ability to pass through membranes. To measure the ability of an antibiotic to move from root to shoot, the translocation factor is resorted to. This factor is defined as the transfer of antibiotics from roots to leaves and/or shoots. It is calculated as the ratio of the concentration of antibiotics in the leaf to that in the root (leaf concentration/root concentration). Several studies demonstrate the different factors included in the uptake, accumulation, and translocation mechanisms of antibiotics by plants grown in contaminated media.

Keywords Antibiotic mechanism · Antibiotic translocation · Antibiotic uptake · Transpiration stream

L. J. El Gemayel · I. I. Bashour (✉)
Faculty of Agricultural and Food Sciences, American University of Beirut, Beirut, Lebanon
e-mail: ib02@aub.edu.lb

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8.1 Introduction

In 2010, the worldwide consumption of veterinary antibiotics by livestock was 63,000 tons minimum, and it was projected to rise in 2030 to 106,600 tons (Van Boeckel et al. 2015). Ötker and Akmehmet-Balcıoğlu (2005) inform in their article that in veterinary medicine, antibiotics have been extensively used as a growth promoter or for therapeutic reasons. Due to animal's poor gut absorption, following administration of antibiotics, around 75% are excreted through feces and 90% through urine (El Gemayel 2018; Halling-Sørensen 2001). They are excreted either as the parent compound and/or as metabolites, which are subsequently added to the environment by spreading animal manure as a mean of fertilization onto agricultural lands, direct addition by grazing livestock, and the release of wastewater (Pan and Chu 2017a). A study sponsored by the USDA in 2007 revealed that some vegetables take up antibiotics when cultivated in a soil amended with livestock manure (Tasho and Cho 2016). The vegetables were planted in a greenhouse, on soil fertilized with liquid hog manure comprising sulfamethazine (Dolliver et al. 2007). This antibiotic was found in the leaves of crops. In another study done by Youssef and Bashour (2017) on the absorption of gentamicin, oxytetracycline, and tylosin by lettuce and radish in a pot experiment demonstrated that manure addition increased the absorption of the three antibiotics by the tested crops.

Several factors affect the uptake, accumulation, translocation, and transformation of the antibiotic when taken by the plant. The primary factors are the different physicochemical properties such as ionization behavior and hydrophobicity of the antibiotic. Also, the physiological nature of the plant and its tissues, the different soil properties like pH, cation exchange capacity (CEC), and organic matter content, contact duration, as well as the concentration of the antibiotics, all impact the uptake and accumulation of antibiotic compounds by crops. Several studies have proven so far that antibiotics accumulate in different parts of the crops grown in the contaminated media (El Gemayel 2018; Wu et al. 2015) and their primary route of entry into the plants is through their roots and aerial tissues (Bartrons and Peñuelas 2017); most of the antibiotics available in the soil are polar compounds detaining ionization of functional groups. Roots absorb antibiotics either through diffusion of dissolved compounds into roots or through mass flow (Miller et al. 2016): ionizable compounds traverse roots by a combination of diffusion of the neutral fraction and electrostatics interactions of the ionic fraction whereas neutral compounds intercross the root-cell membrane with a partition coefficient identical to that from water into octanol. The main sources of pharmaceutical compounds through aerial tissues are done through the deposition of aerosols and volatized compounds, direct contact (ionic fraction uptake or diffusion) with amendment materials or irrigation as well as through the translocation from the root tissues (Trapp and Legind 2011).

Scientists have been extensively scrutinizing antibiotics role in the environment; nevertheless, the disparities in the experimental designs and analytical methods adopted makes the comparison among studies harder. Goldstein et al. (2014) inform that hydrophilic compounds in equilibrium with water will move to the xylem from

which nonionic pharmaceutical compounds are collected in leaves, mainly transported in the course of the transpiration stream and ionic pharmaceutical compounds that are repelled by the negatively charged cytosol and cell walls might be stuck in the phloem thus accumulating more there. Contrarily, hydrophobic lipid compounds will mainly be retained by roots. Bartha et al. (2014) stated that once in the plant cells, metabolic enzymes like cytochrome hydrolase and alter the pharmaceutical compounds to create several transformation products which are ultimately incorporated or mineralized into the plant tissues. Compared to the parental compounds, pharmaceutical metabolite compound concentrations and biological activities are analogous or greater (Bahlmann et al. 2014); however, less is known about their uptake mechanism by plants.

Despite the different studies performed, several limitations are still present: their consequences on the ecosystems are still vague as well as the understanding of their uptake mechanism and bioaccumulation by the plants grown in contaminated media. The different sections below will further discuss the classification and types of antibiotics, the mechanism of uptake and translocation by plants as well as their metabolism in the plants.

8.2 Classification and Types of Antibiotics

Antibiotics are antimicrobial organic substances that are produced from natural microorganisms such as fungi or bacteria or through industrial synthesis: synthetic or semisynthetic chemical compounds (Khan et al. 2008; Wang et al. 2015). They are compounds that are recognized to fight infections triggered by bacteria in both: animals and humans. The general term "antibiotic" symbolizes any organic molecule class that kills or inhibits microbes via specific interactions with microbial targets. The source of the class or compound is not taken into consideration (Michael et al. 2013). Examples of man-made antibiotics with no natural origins are trimethoprim, fluoroquinolones, and sulfonamides (Coates et al. 2011). Most naturally occurring antibiotics have been chemically improved to provide developed properties of the drug. Examples of the former are: tetracyclines, streptogramins, glycopeptides, beta-lactams, aminoglycosides, macrolides, and lincosamides.

Antibiotics can be classified as narrow or broad spectrum, bacteriostatic or bactericidal, or based on their modes of action. Bactericidal are antibiotics that kill bacteria by interfering with either the development of the bacterium's cell content or cell wall such as beta-lactams, fluoroquinolones, and aminoglycosides. Bacteriostatic, on the other hand, are the ones that keep the bacteria in its stationary phase of growth. Sulfonamides, macrolides, and tetracycline groups of antibiotics are bacteriostatic. However, few antibiotics could be both bacteriostatic and bactericidal: this depends on the dosage, the state of the invading bacteria, and the period of exposure (El Gemayel 2018; Pankey and Sabath 2004).

8.2.1 Based on Their Spectrum of Activity

Antibiotics or antibacterial agents can be classified based on their target specification. They could either be broad or narrow spectrum. The narrow spectrum antibacterials are the ones which can act upon a narrow range of microorganisms (Clatworthy et al. 2007). In other words, they either act specifically against Gram-negative or Gram-positive bacteria and the broad-spectrum ones work against a broad range of pathogenic bacteria, involving both Gram-negative and Gram-positive bacteria. For medical treatments, the narrow spectrum antibacterials are favored over the broad-spectrum antibacterials and are considered as perfect antibacterials. This is because in the body, narrow spectrum antibiotics do not destroy as many of the natural microorganisms as the broad-spectrum antibiotics.

Examples of broad-spectrum antibacterials are quinolones, aminoglycosides including gentamicin, chloramphenicol, tetracycline, and oxytetracycline. Examples of narrow spectrum antibacterials are beta-lactamase (first generation: penicillin G, penamecillin), sulfonamides, and glycopeptide (El Gemayel 2018; Ullah and Ali 2017).

8.2.2 Based on Their Mechanism of Action

The mode of action is one of the most essential factors connected to each antibacterial compound. Different antibiotics may have diverse modes of action and that is due to their structure's nature and to the extent of their affinity to some target sites inside bacterial cells. Antibiotics can also be divided based on their target sites in the bacterium (Kohanski et al. 2010). The major antibiotic functions are responsible of inhibiting bacterial growth and cell membrane function, cell wall synthesis, protein and nucleic acid synthesis, etc. Antibacterials thus can be divided into four groups: inhibitors of membrane function, inhibitors of cell wall synthesis, inhibitors of nucleic acid synthesis, and inhibitors of protein synthesis (Ullah and Ali 2017). Table 8.1 lists few of the principal antibiotics with different mechanisms of action and spectrum of activity.

Table 8.1 List of few antibiotics with distinctive modes of action and spectrum of activity

Antibiotic class	Spectrum of activity	Mechanism of action
Aminoglycosides	Broad spectrum	Protein synthesis inhibitors
Macrolides	Broad spectrum	
Tetracyclines	Broad spectrum	
Fluoroquinolones	Broad spectrum	Nucleic acid inhibitors
Beta-Lactams	Broad spectrum	Cell wall synthesis inhibition

Source: Brown et al. (2017) and El Gemayel (2018)

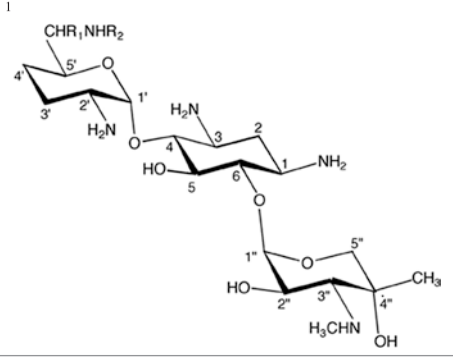
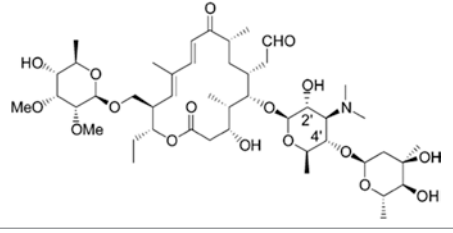
8.2.3 Based on Their Chemical Formula/Structure

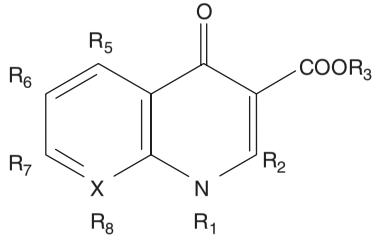
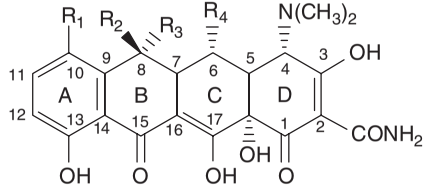
The chemical structure of antibiotics is the sole property which separates one antibiotic from another. Diverse skeleton comprising antibiotics exhibit different behaviors. For this reason, it is fundamental to categorize antibacterials based on their chemical structure. Besides, classifying antibiotics is essential as similar structural components detain analogous patterns of effectiveness, toxicity, and additional related properties (Ullah and Ali 2017). Moreover, it is the chemical structure of the antibiotics that determines all its chemical, physical, pharmacological, clinical, and lastly microbiological properties (Béahdy 1974). Table 8.2 below elaborates on the characteristics of some antibiotics that differentiate one antibiotic class from another.

8.3 Mechanism of Uptake and Translocation of Antibiotics in Plants

Nikaido and Thanassi (1993) proposed a partially energy-dependent uptake mechanism for tetracycline based on bacteria accumulating it; tetracycline is similarly structured as oxytetracycline. Based on this, through the OmpF and OmpC porins channels, tetracycline passes by the outer membrane of Gram-negative bacteria channels (Schnappinger and Hillen 1996), this way chelating an ion M^{2+} as a complex of cation–tetracycline $[M - tc]^{+}$. This cationic compound is attracted by the Donnan potential which is across the outer membrane, thus causing its accumulation in the periplasm. There, the tetracycline complex metal ion possibly dissociates liberating uncharged tetracycline which is a weakly lipophilic molecule that can pass across the lipid bilayer regions of the inner cytoplasmic membrane. Correspondingly, it is this electroneutral, lipophilic form of the antibiotic that is thought to be diffused across the cytoplasmic membrane of the Gram-positive bacteria. Chopra and Roberts (2001) inform that the uptake of tetracycline is driven by the DpH component of the proton-motive force as well as it is energy dependent. Despite the lack of information on the uptake mechanism of oxytetracycline, based on its uptake and diffusion process in the bacteria, Kong et al. (2007) postulate that oxytetracycline uptake by alfalfa roots is an active process, thus requiring energy and aquaporins in root cell membranes might not contribute to its uptake. Further research should investigate the uptake mechanism of other veterinary antibiotics into crop roots, root to shoot translocation, and accumulation in the crops as their accumulation has been hypothesized to pose hazards to humans. In addition to the energy-dependent uptake of some antibiotics such as oxytetracycline, Dodgen et al. (2015) state that transpiration is the driving mechanism for the uptake and the transport of antibiotics within the plant; antibiotics properties greatly contribute to this process. It has also been established through several studies that from the growth media, plants are able to uptake pharmaceutical compounds through their roots (Kong et al. 2007).

Table 8.2 List of antibiotics classes with their respective characteristics

Antibiotic class	Examples	Main skeleton	Characteristics
Aminoglycoside	Gentamicin, streptomycin	1 	Two aminosugars joined by glycosidic bond to an aminocyclitol
Macrolides	Tylosin	2 	Consist of a macrocyclic lactone ring, typically 14-, 15-, or 16-member to which one or more deoxy sugars could be linked

Quinolones and fluoroquinolones	Enrofloxacin, ciprofloxacin (second generation)	[3] 	Quinolones are quinine derived structural units. An added fluorine at position 6 is named fluoroquinolone
Tetracyclines	Oxytetracycline	[4] 	Four rings hydrocarbon containing compounds

Source: El Gemayel (2018)
Yoshizawa et al. (1998)[1], Phan et al. (2004)[2], Smith et al. (2001)[3], and Sarmah et al. (2006)[4]

Antibiotics have been shown to detain a wide range of physicochemical properties: from highly hydrophobic to extremely hydrophilic chemicals. Some antibiotics become either acids or basis (ionizable compounds), depending on the pH of the water or soil, may be dissociated in them. Several studies have demonstrated that the properties like dissociation and hydrophobicity of pharmaceuticals significantly affect the uptake and accumulation of an organic compound in plants (Wu et al. 2015). As mentioned earlier, one antibiotic accumulates differently than the other due to the different physicochemical properties they detain, especially their hydrophobicity and dissociation. In the parts below, the antibiotic root uptake, translocation of antibiotics within plants, and the factors affecting antibiotics uptake by plants will be discussed.

8.3.1 Root Uptake

The interpretation of organic compounds' uptake into plant roots is generally based on the hydrophobicity of the compound. Wu et al. (2013) observed in their study that a positive linear relationship is present for neutral pharmaceutical compounds between the chemical hydrophobicity and the root uptake, thus inferring that the hydrophobicity of neutral compounds play a primary role in its uptake by roots. This deduction cannot be applied to ionic compounds (Wu et al. 2013), where other mechanisms like electrical repulsion or attraction as well as ion trap can affect the collection in roots. Generally, ions cross biomembranes like the tonoplast or plasma membrane at a rate slower than that of neutral molecules (Trapp and Legind 2011); molecular dissociation consequently leads to a lower accumulation in the roots. Goldstein et al. (2014) demonstrated that ionic pharmaceuticals displayed a lower uptake than the neutral ones. Calderón-Preciado et al. (2011) identified ionic compounds in crops at a low concentration and explained that this could be due to the repulsion of the anions by the negative electrical potential of the plant cell membrane. Also, Pan and Chu (2017b) stated that in crop roots, the bioaccumulation factor (BCF) of the roots was high for sulfamethazine and erythromycin ranging from 0.07 to 0.15 and 0.06 to 0.16, respectively. In crop leaves/shoots, sulfamethazine and erythromycin accumulation was much lower with both BCF of leaf/shoot being less than 0.06; in most crops tissues, both antibiotics were not detected. In the leaves/shoots of carrots and tomatoes, sulfamethazine was determined only under the highest antibiotic treatment (wastewater with 20 μg/L antibiotic). In the case of tomatoes, erythromycin was only detected in its leaf/shoot at a BCF of 0.03–0.06 whereas no leaf/shoot accumulation was perceived in lettuce and carrot; except at a concentration of 20 μg/L antibiotic in wastewater. Under all treatments, no sulfamethazine was detected in the lettuce leaf/shoot. Herklotz et al. (2010) demonstrated that in the roots of cabbage grown hydroponically, the concentration of sulfamethoxazole was higher than that in the leaves. In an experiment run by El Gemayel (2018) where radish and lettuce were grown in nutrient solutions contaminated with tylosin, enrofloxacin, and oxytetracycline separately indicated that

enrofloxacin was absorbed by both lettuce and radish, tylosin accumulated the most in lettuce leaves at an average of 343.83 ng/g and in roots at an average of 218.94 ng/g and all parts of the radish accumulated tylosin at an average of 222.98 ng/g for roots, 384.26 ng/g for leaves, and 407.45 ng/g for bulbs. For oxytetracycline, the sequence of accumulation of oxytetracycline in radish crop goes as follows: roots > bulbs ≥ leaves. Overall, in both radish and lettuce, the sequence of distribution was: tylosin > enrofloxacin > oxytetracycline (El Gemayel 2018).

Acidic pharmaceutical compounds can form at least two species through dissociation: the anion with its undissociated acid. Consequently, Wu et al. (2015) stated that at the cell membrane, plant cells detain a negative electrical potential. Trapp (2009) informs that usually anions are poorly absorbed by plants, this causing the negatively charged anion to be repulsed. Wu et al. (2015) define ion trap as the mechanism that could lead acidic compounds to accumulate in plant cells. When the external solution has a pH lower than that of the cells, it is possible for the undissociated acid present outside the cell to diffuse rapidly into the cell. Nevertheless, once it enters the cell where the pH is higher, the weak acid might be dissociated. As it is less likely for an ion to permeate membranes compared to its neutral molecule, the acid remains trapped inside the cell; Wu et al. (2015) inform that basic pharmaceutical compounds can dissociate and form cationic or neutral compounds. Three different processes might be the reason for plant uptake: (1) the negative charge on the plasmalemma causes an electrical attraction of the cation. This process could clarify the moderate root uptake of basic polar pharmaceutical compounds (i.e., atenolol, meprobamate, and primidone); (2) accumulation in the vacuole by ion trap; and (3) separating on the root solids, considered mostly for very lipophilic compounds.

8.3.2 Translocation of Antibiotics Within Plants

Wu et al. (2015) inform that within the plants, the translocation of pharmaceutical compounds is also associated to the hydrophobicity and dissociation potential of a compound. A negative correlation was perceived between the translocation of pharmaceutical compounds from roots to leaves and log (Dow), the pH-adjusted octanol–water partition coefficient; this shows that in the roots, hydrophobic compounds tend to remain there with limited redistribution within the plant. On another note, following the transpiration stream, hydrophilic compounds were prone to move to leaves in the water stream (Wu et al. 2013). Tanoue et al. (2012) stated that intermediate polarity pharmaceutical compounds having a log (Dow) between 0.5 and 3 might be transported to plant shoots easily.

To describe the transfer ability of an antibiotic to move from root to shoot, the translocation factor is resorted to. Pan and Chu (2017c) define the translocation factor to be the transfer of antibiotics from roots to leaves and/or shoots. It is calculated as the ratio of the concentration of antibiotics in the leaf to that in the root (leaf concentration/root concentration). When the translocation factor is less than 1, then

this means that the translocation of the antibiotic from roots to leaves or fruits are restricted. When it is greater than 1, then the translocation is not restricted (Pan and Chu 2017c). When plants absorb antibiotics, they are transferred to shoots, leaves, and fruits through a passive diffusion via the phloem or xylem into the symplastic pathway (Pan and Chu 2017b). Consequently, these compounds are moved into roots through the Casparian strip and are then carried to fruits by the phloem or to leaves by the xylem (Miller et al. 2016). In plants, the xylem transports water, organic compounds, and nutrients from root to shoot through the transpiration stream it provides. The stem holds the lower concentration of antibiotics since it is only a conductive channel (Liu et al. 2013). Therefore, it is possible that an increase in the transpiration rate could speed the uptake of antibiotics from the soil into the plant (Pan and Chu 2017c). Also, Pan and Chu (2017c) indicate that the translocation of chloramphenicol, tetracyclines, and lincomycin was higher in leafy vegetables than in carrots, corn, radish, pepper, and others. This signifies that the transportation process of these antibiotics mainly occurs through the xylem. After being absorbed, most of the tetracyclines henceforth exist in the cytosol and symplast of plants (pH ~7.2) under their neutral form. Subsequently, they are moved more freely and stored at a higher concentration in leaves or fruits (Pan and Chu 2017c). Additionally, the tetracyclines that are more water-soluble are translocated more easily in plants through water mass flow (Pan and Chu 2017c). Kong et al. (2007) primarily suggested that the transport of oxytetracycline into the roots of alfalfa is energy-dependent, which necessitates selective binding sites and secondarily that the uptake of oxytetracycline is aquaporin independent as water channels are not the mean through which oxytetracycline enters into the roots.

Dodgen et al. (2015) studied the effect of transpiration on plant accumulation and translocation of basic, acidic, and neutral PPCPs (Pharmaceutical and Personal Care Products); it was demonstrated that a significant positive correlation is present between the values of the translocation factor and that of the transpired masses for neutral and basic pharmaceutical compounds whereas no relationship was detected for acidic pharmaceutical compounds. This insinuates that the translocation of neutral and basic pharmaceutical compounds from roots to leaves was greatly affected by transpiration. For acidic pharmaceutical compounds, a mechanism other than transpiration interferes: ion trap, as previously discussed, might cause their accumulation in plants. Also, they reported that the translocation factor of basic pharmaceutical compounds was much greater than that of acidic or neutral pharmaceutical compounds; thus basic compounds are more likely to translocate from roots to leaf tissues than acidic and neutral ones. Also, when comparing identical crops to each other, factors like climatic conditions, irrigation schedule, and growing period lead to accumulation difference in the crops. Wu et al. (2010) confirmed that in shoots of tomato plants grown in manure-fertilized soil, 400 ng/g of carbamazepine accumulated, whereas in the fruit, a lower concentration accumulated. These results demonstrate that carbamazepine is translocated through the plant by the transpiration stream and it accumulates at significantly greater concentrations in the leaves rather than in other parts of the crop (Sabourin et al. 2012; Wu et al. 2010).

Nevertheless, the passage of organic compounds in the phloem and xylem is still unclear as it chiefly depends on the ability of the antibiotic to cross membranes. Consequently, as it has been hypothesized that the accumulation of antibiotics into plants are hazardous to human and animal health, then further research should tackle the subject of uptake, accumulation, and translocation mechanisms of different veterinary antibiotics into plants.

8.3.3 Factors Affecting Antibiotic Uptake by Plants

Several antibiotics are adsorbed and fixed onto soil particles depending on the physicochemical properties of the antibiotic, type of soil, quality and content of soil organic matter, soil pH, soil minerals, main climatic conditions, and other environmental aspects (Tasho and Cho 2016). Veterinary antibiotics are relatively easy to adsorb to soil particles as they are organic compounds displaying a wide range of functional groups as well as they can be amphoteric, amphiphilic, or ionic. It is the interaction of veterinary antibiotics with organic matter and clay minerals that causes their binding, sorption, and fixation on the soil matrix. The different binding mechanisms involve van der wall interactions, anion exchange, cation bridging, and electrostatic attraction (Jeon et al. 2014). Due to the structure of tetracyclines, they are known to strongly adsorb to soil, organic matter, and minerals; hence their extractability is reduced, which in turn reduces their accessibility for plant uptake (El Gemayel 2018; Zhang et al. 2016). In the case of oxytetracycline, Youssef and Bashour (2017) results showed that oxytetracycline was not absorbed by lettuce grown in soil with and without manure and was only accumulated in radish roots with no significant translocation to the leaves. This could be explained by the oxytetracycline charge letting it adsorb to the soil, thus not being available for the crops to absorb it. This was confirmed in another experiment performed by El Gemayel (2018) where oxytetracycline was absorbed by lettuce roots at an average of 20.43 ng/g and radish bulb and leaves at 30.98 ng/g and 22.76 ng/g, respectively. Some other factors that limit the availability of residual pollutants for absorbance by the crops from the soil are: degradation of residues, soil bound formation of residues, and antibiotic loss via leaching in-between crop harvesting and manure fertilization (Sabourin et al. 2012).

In some studies, erythromycin and sulfamethazine were either not detected in plant tissues or detected in the roots only. This was recognized to be due to the low adsorption ability of sulfamethazine in the soil in a way where most of it was leached out; thus, less was available for plant uptake. Additionally, sulfamethazine is highly lipophilic, meaning it favors translocating in a lipophilic content, which is only found in roots (Goldstein et al. 2014). On another note, erythromycin held the heaviest molecular weight, thus making it hard for it to pass through the cell membranes. In soils and crops, erythromycin is essentially a cation, where its permeability via root membranes is greatly reduced. Tanoue et al. (2012) state that the ionization process can diminish the translocation of ionized organic compounds to

shoots. Several studies demonstrate that the accumulation of antibiotics is species specific even if the crops are grown in the same irrigated soil (Calderón-Preciado et al. 2011; Kang et al. 2013). Also, it was demonstrated that the nature of the soil played a significant role in the uptake of antibiotics by controlling its bioavailability (Pan and Chu 2017a). Consequently, based on the antibiotics' physicochemical properties and their application methods, plant species, growth, and transpiration rates, antibiotics have several translocation behaviors in several crops.

When it comes to the accumulation and translocation of antibiotics in crops, they are affected by different mechanisms and factors. Some of these factors are the quality of the water and soil properties, the nature and concentration of the antibiotic applied, as well as the crop species (Pan and Chu 2017b). Pan and Chu (2017b) proved in their study that when placed in an antibiotic-contaminated media such as contaminated wastewater or soil amended with animal manure, crops have the ability to absorb different types of antibiotics. Nevertheless, the different types of antibiotics demonstrated different translocation potential in the crops. For example, tetracycline, norfloxacin, and chloramphenicol were available in shoots/leaves at a higher concentration than the other two antibiotics, and in the roots, the translocation of sulfamethazine and erythromycin was more extensive. In a study performed by El Gemayel (2018), oxytetracycline accumulated the least in lettuce leaves at an average of 6.83 ng/g compared to enrofloxacin and tylosin which accumulated at an average of 59.39 and 343.83 ng/g, respectively. Also, the physicochemical properties of the antibiotics deemed to be positively correlated to the accumulation of the antibiotics in the crops from the contaminated media. A higher level of residual antibiotics was identified in different crop tissues at higher antibiotic levels in contaminated media (El Gemayel 2018). As the level of enrofloxacin increased in the pot from 0, 5, to 10 mg/kg, its accumulation in cucumber leaves and fruits increased significantly (El Gemayel 2018). Compared to soil amended manure, contaminated wastewater promotes antibiotic absorption in plant more due to the continuous irrigation with it, thus promoting antibiotic absorption by the crops (Pan and Chu 2017b). Also, it was proven that the antibiotic level in wastewater and animal manure disturbed the translocation ability in the crops. In tomato, the translocation factor of chloramphenicol was higher at a concentration of 20 μg/L of contaminated wastewater than in 2 μg/L. These results show that the higher the antibiotic level, the higher the translocated quantity within the crop. Closely, in El Gemayel (2018) study, it was indicated that there is a significant difference between the control and the two other enrofloxacin levels (5 and 10 mg/kg) but no significant difference between the 5 and 10 mg/kg level or between the roots, bulbs, or leaves of radish crops. This indicated that as the concentration of enrofloxacin increases in the media, its uptake and accumulation does not significantly increase and is distributed all through the plant parts root:bulbs:leaves at 1:1:1 ratios (uniform concentrations).

In crops, pharmaceutical compounds can be taken up and transported via mass flow through the transpiration stream as well as via an active uptake (Dettenmaier et al. 2008). Numerous studies have demonstrated that there is a correlation between the antibiotic accumulation in the plant and its respective logarithm octanol/water

partition coefficient (log KOW), which is the ratio of a chemical's concentration in the octanol phase to its concentration in the aqueous phase of a two-phase octanol/water system. The latter suggests that for antibiotic accumulation and translocation, an optimal hydrophobicity value is available and organic compounds that are either highly lipophilic (log KOW > 4) or highly polar (log KOW < 1) will not be significantly absorbed by crops (Dettenmaier et al. 2008; Herklotz et al. 2010).

8.4 Metabolism of Antibiotics in Plants

Wu et al. (2015) stated that following uptake, organic compounds like polycyclic aromatic hydrocarbons and pesticides were detected metabolized in plant tissues. Among the first people to study the metabolism of pharmaceuticals in plant tissues were Bartha et al. (2010) and Huber et al. (2012). The latter suggested that the detoxification mechanisms of xenobiotics in plants were very similar to the ones in the mammalian system. In brief, detoxification can be divided into three different phases: the first phase (phase I) is the activation reactions, in the second phase (phase II), the compound conjugates with glucose, amino acids, glutathione… known as small biomolecules, which increase the mobility and hydrophilicity of the parent compound. Then, these conjugated molecules can go through phase III which consists of storage in the plant vacuole, degradation/cleavage, and bound residues in the cell walls are formed or transport in the plant can occur.

Podlipná et al. (2013) scrutinized in reed, in vitro, the biotransformation of antiparasites used for killing parasitic worms, two benzimidazole anthelmintics (flubendazole and albendazole), and detected 5 flubendazole and 10 albendazole metabolites which were mainly phase II metabolites. Also, Macherius et al. (2012) studied in intact carrot plants as well as in carrot cell cultures the metabolism of triclosan, methyl triclosan, and triclocarban (antibacterials and antifungals). Even though a fast metabolism of triclosan was seen, triclocarban and methyl triclosan were unchanged in the cell cultures; all metabolites were phase II metabolites (conjugates). Also, in intact carrot crops, the total amount of triclosan conjugates was five times more than the total amount of triclosan itself.

Wu et al. (2015) claims that to date, few studies were done on the transformation of pharmaceutical compounds after their uptake by plants. In plants, disregarding the pharmaceutical compounds metabolites and conjugates might lead to a great underestimation of the pharmaceutical compounds uptake. An essential method for a better knowledge of the accumulation, translocation, and fate of pharmaceutical compounds in plants is the 14C-labeling approach. Nevertheless, the current limitation is the lack of 14C-labeled pharmaceutical compounds available, thus preventing deeper experimentations. Dodgen et al. (2013), using the 14C-labeled compounds, assessed the accumulation of naproxen and diclofenac, as well as two pharmaceutical compounds used as endocrine disrupting chemicals: nonylphenol and bisphenol A. They discovered that almost all the 14C residues in the plant tissues were non-extractable. This shows that following their uptake by the plants,

these chemicals principally existed as conjugated residues and only a few of them were extractable.

8.5 Conclusion

Antibiotics are compounds recognized to fight infections triggered by bacteria in both humans and animals. Nowadays, veterinary antibiotics are primarily used in animal husbandry as growth promoters. These antibiotics are classified based on their spectrum of activity: whether they act upon a narrow or a broad spectrum of bacteria, based on their mechanism of action, which relies on the antibiotics' structure and the extent of its affinity to its target site in the bacterium and at last based on its chemical formula or structure which is the sole classification that unquestionably differs one antibiotic from another. It has been demonstrated in previous studies that the structure/size of the antibiotic does not always play a crucial role in its uptake and accumulation by the plant; however, its charge does. Consequently, this opens the question of the uptake mechanism and translocation of antibiotics in plants.

Although a lot of information remain to be studied and discovered regarding the uptake mechanism of veterinary antibiotics by plants grown in a contaminated media, in the few researches available on the matter, the different uptake and translocation mechanisms proposed are electrical attraction or repulsion, ion trap, uptake through the transpiration stream, mass flow, passive or energy dependent. Kong et al. (2007) suggested an uptake process of oxytetracycline, claiming that it is similar to its uptake and diffusion mechanism in a bacterium. The latter concluded that oxytetracycline uptake is energy dependent; nevertheless, additional research should tackle the uptake mechanism of other veterinary antibiotics in crops as well as their accumulation and translocation.

The physicochemical properties of the antibiotics are considered among the primary characteristics defining their ability and chance to be absorbed, accumulated, and translocated by crops. These different properties range from extremely hydrophilic to highly hydrophobic compounds or ionizable compounds. Thus, hydrophobic or neutral compounds are more likely to be absorbed easily by plant roots than an ionic compound; a study demonstrated their lower accumulation than neutral ones. Nevertheless, some studies demonstrated that hydrophobic compounds are more likely to be retained in the roots and not translocated throughout the crop, whereas through the transpiration stream, hydrophilic compounds are more likely to be transported to shoots. Also, Pan and Chu (2017c) state that as the transpiration rate increases, the uptake of antibiotics from the contaminated media into the crop increases as well due to the xylem route which transports water, nutrients, and organic compounds from roots to shoots (detaining the lower concentration of antibiotics) via the transpiration stream.

Just like in mammals, the detoxification mechanism occurs in antibiotics present in plants; this detoxification process has been divided into three distinctive phases:

activation reaction, compound conjugation with biomolecule, and storage/degradation in the vacuole or cell wall. Some studies demonstrated that the total amount of some antibiotics' conjugates was found at a much higher rate in plants than the total amount of the antibiotic itself. This highlights a point where disregarding the pharmaceutical compound metabolites and conjugates might lead to an underestimation of the medicine's uptake, accumulation, and translocation. Consequently, up to this date, several studies have demonstrated that different veterinary antibiotics can be absorbed, accumulated, and translocated by crops grown in contaminated media and several factors of which the physicochemical properties of the antibiotics determine its uptake ability by the crop. Also, the uptake mechanism through which ionic antibiotics are absorbed, accumulated, and translocated remains weakly understood and further studies on persistent and ionizable compounds should be performed.

Lastly, in spite of the numerous studies performed till now, the knowledge of scientists on the accumulation of antibiotics in plants remains inaccurate as it has mainly been scrutinized under laboratory conditions and not under realistic field conditions. The scarcity of this kind of information renders us with poor estimation on the possible risks, in agriculture, of treated crops with wastewater and bio-solids consumed by humans and animals.

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Chapter 9
Uptake of Gentamicin, Oxytetracycline, and Tylosin by Lettuce and Radish Plants

Sandra A. Youssef, Isam I. Bashour, Yusuf A. Abou Jawdeh, Mohamad T. Farran, and Nadim Farajalla

Abstract Many farmers use antibiotics in livestock and poultry production. The aim of this study was to evaluate the ability of lettuce and radish to absorb these antibiotics from two growth media (soil amended with 5% manure and soil without manure) both spiked with antibiotics (0, 2.5, 5, and 10 mg/kg). A factorial completely randomized pot experiment was conducted in the greenhouse of the American University of Beirut, Lebanon. The antibiotic analysis was done by ELISA. The results showed that gentamicin accumulated in lettuce roots (12.7 ng/g) and leaves (17.7 ng/g) at approximately equimolar ratio (1:1 ratio) whereas in radish it accumulated in the leaves (31.5 ng/g) at a higher concentration than in the roots (16.4 ng/g) (2:1 ratio, leaves:roots). Tylosin was absorbed only at the highest concentration treatment (10 mg/kg) by lettuce roots (11.2 ng/g) with a limited translocation to the leaves (3.58 ng/g), whereas in radish a higher absorption and accumulation of tylosin was observed in both the roots (56.6 ng/g) and leaves (62.9 ng/g). Oxytetracycline was not absorbed by lettuce but it accumulated in radish at low levels (1.83–3.98 ng/g) in the roots and 4.85–6.69 ng/g in the leaves. The addition of manure to the soil enhanced the uptake of gentamicin and tylosin but not oxytetracycline. The results indicated also that increasing the concentrations of antibiotics in the growing media did not always lead to a significant increase in the concentrations of antibiotics in plant tissues.

Keywords Antibiotic uptake · Pot experiment · Radish · Lettuce

S. A. Youssef · I. I. Bashour (✉) · Y. A. Abou Jawdeh · M. T. Farran
Faculty of Agricultural and Food Sciences, American University of Beirut, Beirut, Lebanon
e-mail: ib02@aub.edu.lb

N. Farajalla
Issam Fares Institute for Public Policy and International Affairs, Beirut, Lebanon

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9.1 Introduction

Worldwide, veterinary antibiotics are not only used therapeutically to treat infectious diseases but also at subtherapeutic levels to promote growth of food-producing animals mainly cattle, poultry, swine, and fish. With the increase of large-scale animal feeding operations the usage of antibiotics in animal feed increased tremendously (Phillips et al. 2004). Veterinary antibiotics once given to these animals are partially absorbed and subsequently excreted either as parent compound or as their metabolites. Kumar et al. (2005a, b) stated that approximately 90% of antibiotics are excreted as parent compounds in manure. Manure, a source of soil enhancement, is applied to agricultural lands to provide nutrients for plants and improve production. When manure containing antibiotics is applied to soil, the antibiotics it contains can be transported to surface water or groundwater or dissolved in soil solution to become available for plant uptake (Kuchta and Cessna 2009; Michelini et al. 2012; Bassil et al. 2013; Chowdhury et al. 2016).

The antibiotic contamination by plants has led to the emergence of antibiotic-resistant bacteria (Babic et al. 2006). According to Chowdhury et al. (2016) frequently consumed vegetables, containing low levels of antibiotics, may contribute to the development of bacterial antibiotic resistance. Moreover, Kong et al. (2007) stated that there is a potential risk that plants are capable of spreading antibiotics from the soil into the food chain. It has been stated by the World Health Organization (WHO) in 2015, that such resistant bacteria can be communicated from food animals to humans, mainly through food such as meat, eggs, milk, or plants. Accordingly, the food chain is one of the leading means of antibiotic resistance transmission.

A limited survey conducted in Lebanon in 2008 to assess antibiotic usage in several Lebanese farms showed that the top five most used antibiotics are streptomycin, penicillin, gentamicin, oxytetracycline, and tylosin (Choueiri 2008). The last three antibiotics were included in the study because they belong to different chemical groups but have the same mode of action through inhibition of protein synthesis.

This study was conducted to investigate the uptake by lettuce and radish plants of these three antibiotics, gentamicin (aminoglycoside, formula weight: 477.6), tylosin (macrolide, formula weight: 460.4), and oxytetracycline (tetracycline, formula weight: 916.1), and also to study the effect of soil-manuring on the uptake of these antibiotics by lettuce and radish.

9.2 Materials and Methods

A factorial randomized complete design pot experiment (4 × 3 × 2) was conducted in the green house of the American University of Beirut, Lebanon. The soil was collected from virgin land in Beirut region, and the antibiotic-free manure was collected

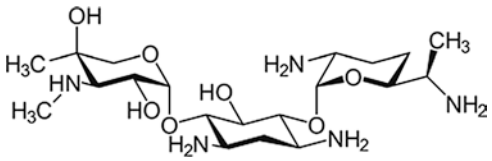

Fig. 9.1 Structural formula of gentamicin (Class: aminoglycoside—FW: 477.596 g/mol)

from dairy cows which had not received any antibiotic treatment for more than 6 months at the Agricultural Research and Education Center (AREC) of the American University of Beirut in Beqaa valley, Lebanon.

Two freshly consumed vegetables were selected to be used in the experiment (lettuce and radish). Radish seeds were sown (five radish seeds per pot), whereas the lettuce seedlings were obtained from the nursery and transplanted (one lettuce seedling per pot). The pots were watered with distilled water as needed.

Three antibiotics widely used in animal production in Lebanon having the same mode of action (inhibition of protein synthesis) but belonging to different chemical groups were used in the experiment. Two antibiotics, gentamicin and oxytetracycline, are of a similar, small molecular weight, and the third tylosin is larger, about twice their molecular weight (Fig. 9.1).

The antibiotics gentamicin (Fig. 9.1), oxytetracycline (Fig. 9.2), and tylosin (Fig. 9.3) were obtained from a vet pharmacy.

9.2.1 Pot Experiment

Four levels of antibiotics (0, 2.5, 5, and 10 mg/kg of soil) were tested and replicated three times in two growing media (soil amended with 5% manure and soil without manure).

For the soil with manure treatments, pots were filled with 5 kg of soil (sieved in 10 mm sieves) mixed with 0.25 kg manure making 5.25 kg/pot. For the soil without manure treatments, the pots were filled with 5.25 kg of sieved soil. The required amount of each antibiotic was diluted in 100 mL of water containing 0.5 g of fertilizer (20-20-20 + Trace elements) and mixed well in the growing media inside a clean plastic bag and placed in the pot to secure no leaching of antibiotics would take place.

9.2.2 Antibiotic Analysis in Plant Tissues

After 45 days, the plants (lettuce and radish) were harvested as a whole (roots and leaves). Antibiotic analyses were done on both leaves and roots by enzyme-linked immunosorbent assay (ELISA) kits (Abraxis, Warminster, PA, USA).

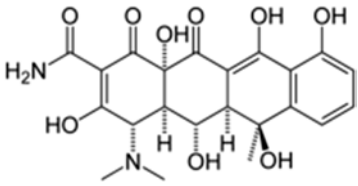

Fig. 9.2 Structural formula of oxytetracycline (Class: tetracycline—FW: 460.434 g/mol)

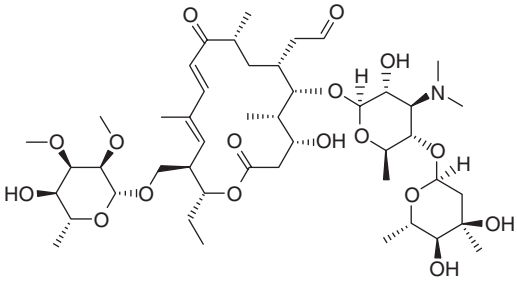

Fig. 9.3 Structural formula of tylosin (Class: macrolide—FW: 916.1 g/mol)

After thorough washing of the roots and leaves and as recommended by the manufacturer of the ELSA-Kits, gentamicin and tylosin were extracted with distilled water at a ratio of 1:3 (1 g fresh plant tissue to 3 mL distilled water) and oxytetracycline was extracted by the McIlvaine buffer provided by the ELISA kit manufacturer (0.2 M sodium dibasic solution, 0.1 M citric acid, pH adjusted to 7.0 using 6 N sodium hydroxide, and diluted 1:1 with methanol) at a ratio of 1:3 (1 g fresh plant tissue to 3 mL McIlvaine buffer). The indirect ELISA protocol was followed as recommended by the manufacturers. The antibiotics concentrations in plant tissues are reported on a fresh weight basis.

9.2.3 Statistical Analysis

In this trial, a 2 × 4 factorial arrangement of treatments with interactions in a complete randomized design was used for each crop. The factors being soil manure level (0 and 5%) and antibiotic levels (0, 2.5, 5, and 10 mg/kg soil). Data were analyzed using the General Linear Models (GLM) procedure and treatment means compared with Student-Newman-Keuls Test (SNK) where applicable (SAS Institute 2008). Whenever, interactions were significant, data were analyzed as one-way ANOVA. Statistical significance for all procedures was tested at a probability level of 0.05.

9.3 Results and Discussion

9.3.1 Lettuce

All interactions tested in this trial were significant; therefore, the data were analyzed by one-way ANOVA and the means of the treatments are presented in bar graphs.

9.3.1.1 Gentamicin in Lettuce Leaves and Roots

The concentrations of gentamicin in lettuce leaves and roots are shown in Figs. 9.4 and 9.5, respectively.

The results of analysis of lettuce leaves (Fig. 9.4) indicate that there is a significant difference between the control (0 mg/kg) and the three other levels of gentamicin (2.5, 5, and 10 mg/kg) in the manure-amended soil. However, no significant differences existed between the control and the three gentamicin treatments in the soil without manure amendment. This indicates that manuring the soil enhanced gentamicin absorption by lettuce. This result agrees with Sukul et al. (2008) stating that the presence of manure increased the sorption tendency of antibiotics significantly and with Dolliver and Kumar (2007) stating that sulfamethazine concentrations in plant tissue increased with corresponding increase of sulfamethazine in manure.

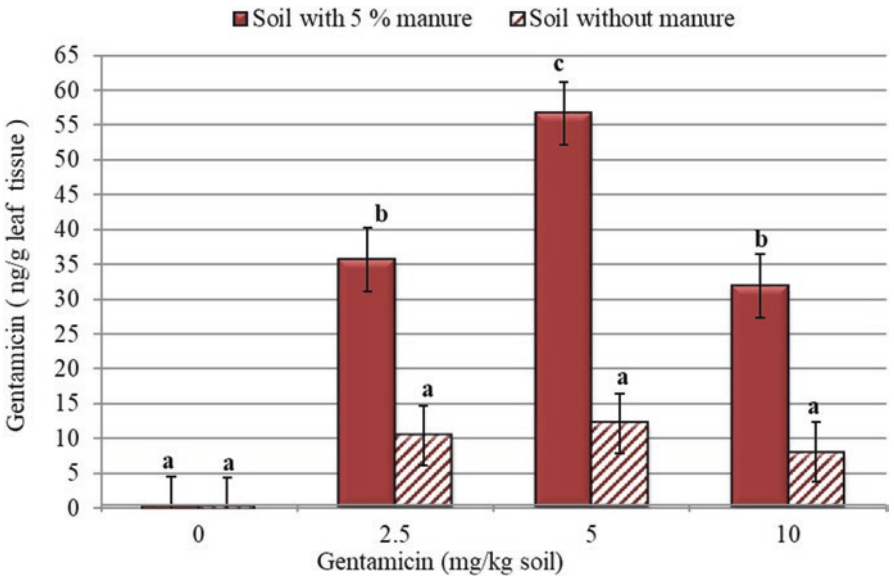

Fig. 9.4 Concentrations of gentamicin in lettuce leaves. Error bars show SD of the means. Different letters indicate statistical significance at 0.05 probability

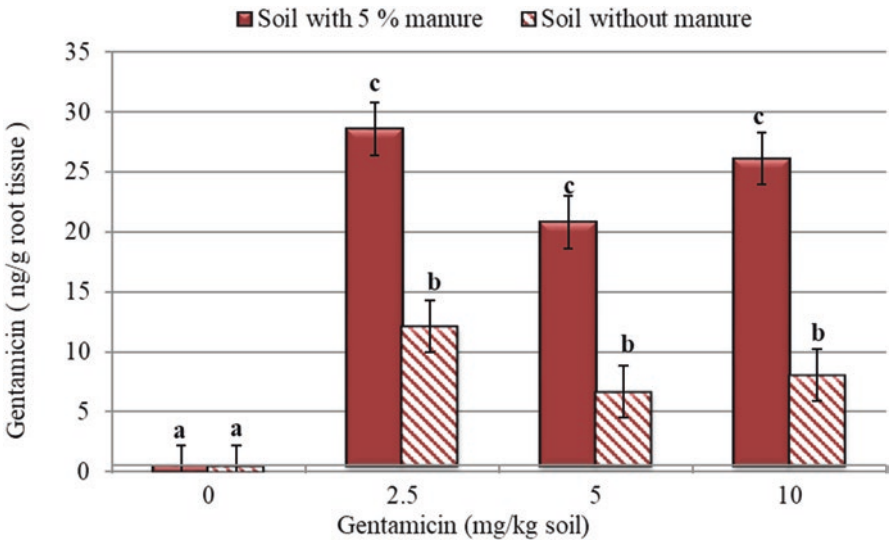

Fig. 9.5 Concentrations of gentamicin in lettuce roots. Error bars show SD of the means. Different letters indicate statistical significance at 0.05 probability

The data also indicate that increasing the gentamicin level in the growing media did not lead to a significant increase in gentamicin concentrations in lettuce leaves. This result disagrees with Ahmed et al. (2015) stating that the total amount of antibiotics accumulated in the plant tissues significantly increased as the level of their additions increased. However, our results generally agree with the findings of Azanu et al. (2016) reporting that plants have a limited sorption capacity for organic contaminant from contaminated irrigation water.

The results of analysis of the lettuce roots (Fig. 9.5) show a clear accumulation of gentamicin with a significant difference between the control and all other treatments. No significant differences were obtained among the three gentamicin levels (2.5, 5, and 10 mg/kg). This indicates that the lettuce root accumulated gentamicin and translocated it to the leaves at concentrations equal or slightly higher than those in the roots making the partitioning coefficient of roots:leaves to be about 1:1.

Similar to the leaves, the addition of manure facilitated the accumulation of gentamicin in the lettuce roots. These results are in agreement with the reported findings of Sukul et al. (2008) and Dolliver and Kumar (2007) and also with the findings of Bassil et al. (2013) in Lebanon who stated that gentamicin uptake in lettuce took place from manure-amended soil which was spiked with low rates of gentamicin (0.5 and 1.0 mg/kg), and no significant differences were obtained between the tested rates.

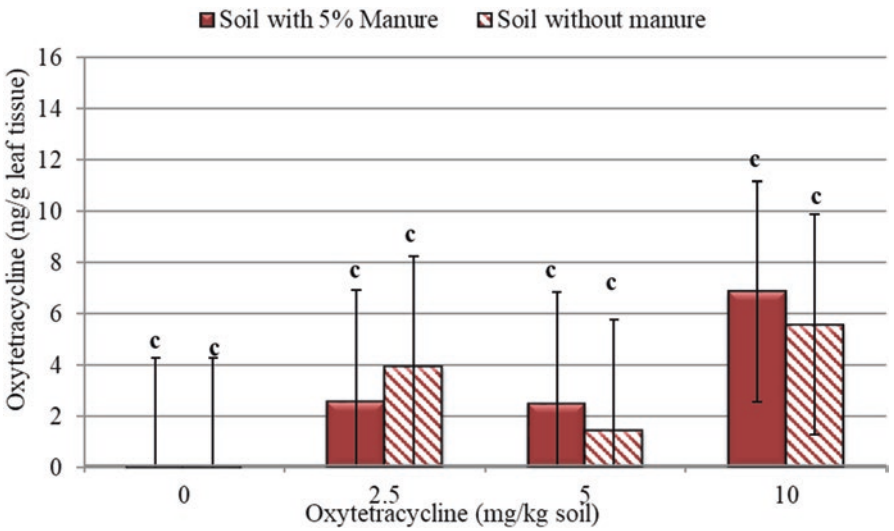

Fig. 9.6 Concentrations of oxytetracycline in lettuce leaves. Error bars show SD of the means. Different letters indicate statistical significance at 0.05 probability

9.3.1.2 Oxytetracycline in Lettuce Leaves and Roots

The concentrations of oxytetracycline in lettuce leaves and roots are shown in Figs. 9.6 and 9.7, respectively.

The data (Figs. 9.6 and 9.7) show that there is no significant difference in the concentrations of oxytetracycline between the control and any of the treatments in the leaves or roots of lettuce. Therefore, it can be speculated that lettuce plants did not absorb oxytetracycline from the soil with or without manure amendment. This result agrees with the findings of Chitescu et al. (2013) on grass and watercress plants.

Although oxytetracycline is known to persist in the soil for a long time, more than 150 days (Boxall et al. 2006), it can be tightly adsorbed onto the soil particles and hardly be desorbed into the soil solution (Rabølle and Spliid 2000), thus making it not available for plant uptake.

9.3.1.3 Tylosin in Lettuce Leaves and Roots

The concentration of tylosin in lettuce leaves and roots are present in Figs. 9.8 and 9.9, respectively.

The results of the analysis of lettuce leaves (Fig. 9.8) indicate that there was a significant difference in tylosin concentration only between the control and the highest level (10 mg/kg) in the manured soil.

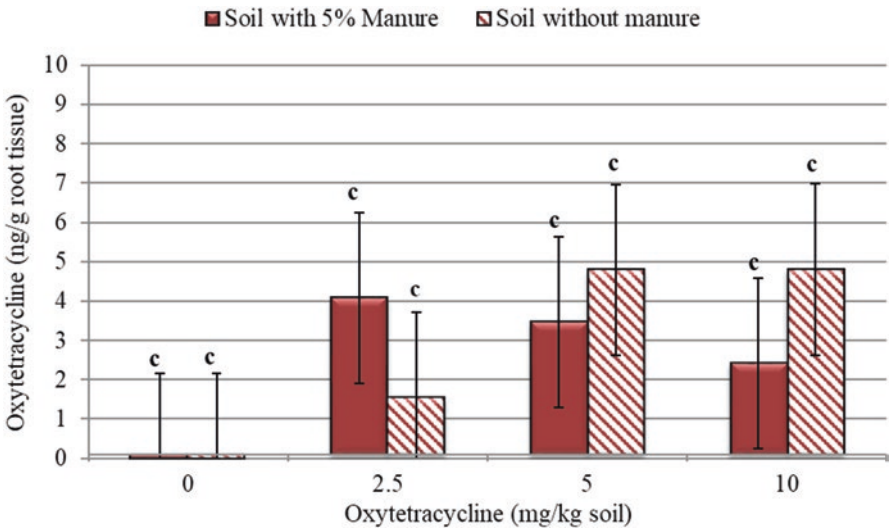

Fig. 9.7 Concentrations of oxytetracycline in lettuce roots. Error bars show SD of the means. Different letters indicate statistical significance at 0.05 probability

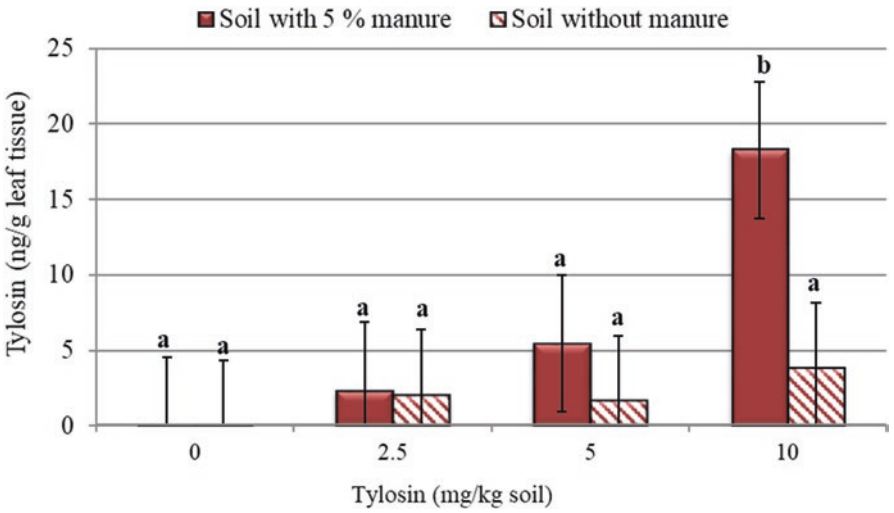

Fig. 9.8 Concentrations of tylosin in lettuce leaves. Error bars show SD of the means. Different letters indicate statistical significance at 0.05 probability

The concentrations of tylosin in lettuce roots (Fig. 9.9) grown in the manure-amended soil at 5 and 10 mg/kg levels were significantly higher than those of the control. No significant differences existed between the control and any of the treatments of lettuce grown in the soil without manure.

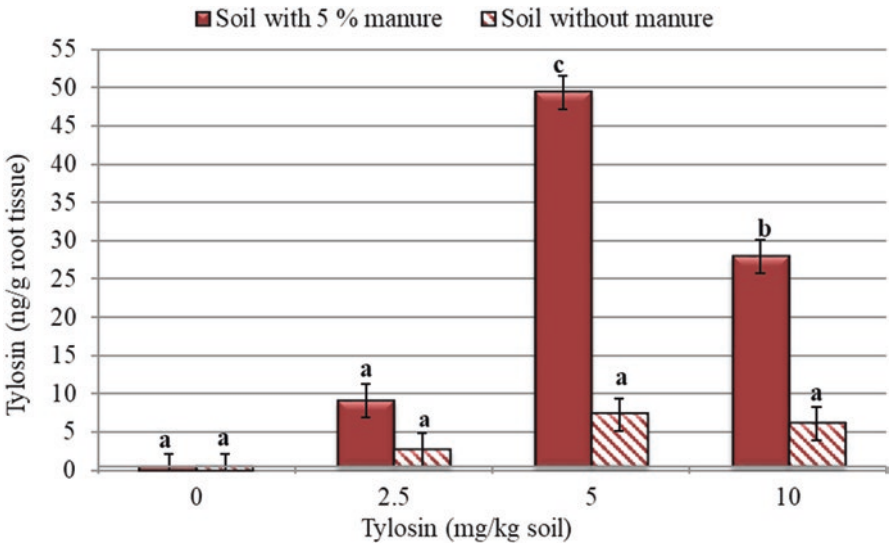

Fig. 9.9 Concentrations of tylosin in lettuce roots. Error bars show SD of the means. Different letters indicate statistical significance at 0.05 probability

Again, this indicates that manure facilitated the tylosin absorption by lettuce roots and its translocation to the leaves was significant at the highest concentration (10 mg/kg). This agrees with the findings of Kang et al. (2013) who mentioned that almost all vegetables took some antibiotics from manure treatment. Also, Kumar et al. (2005a, b) stated that the more the antibiotic present in manure, the higher its concentration in plant tissue.

9.3.1.4 Radish

All interactions tested in this trial were significant; therefore the data were analyzed by one-way ANOVA and the means of the treatments are presented in bar graphs.

9.3.1.5 Gentamicin in Radish Roots and Leaves

The concentrations of gentamicin in radish roots and leaves are shown in Figs. 12.10 and 12.11, respectively.

The results of analysis of radish roots (Fig. 9.10) show that there is a significant difference in the concentration of gentamicin between the control and the other three treatments. The accumulations in radish roots were significantly higher in the manure-amended soil than in the soil without manure amendment. In the manured soil there is no significant difference between the treatments 2.5 and 5 mg/kg whereas both levels were significantly higher than that of 10 mg/kg. On the other

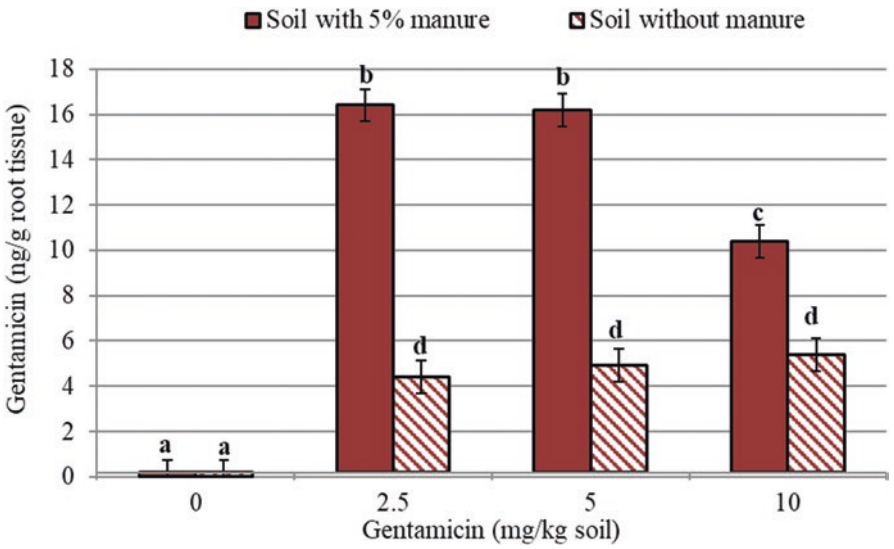

Fig. 9.10 Concentrations of gentamicin in radish roots. Error bars show SD of the means. Different letters indicate statistical significance at 0.05 probability

hand, for the soil without manure treatment, the 2.5, 5, and 10 mg/kg levels were not significantly different from each other but relatively higher than the control and lower than the manured treatments. This agrees with the findings of Bassil et al. (2013) testing the uptake of gentamicin at lower concentrations (0, 0.5, and 1 mg/kg). They reported that there was a significant increase in the concentrations of gentamicin between the 0 and both of 0.5 and 1.0 mg/kg treatments, but no significant difference between the 0.5 and 1.0 mg/kg.

The results of the analysis of radish leaves (Fig. 9.11) show that gentamicin concentrations in leaves of plants grown in the soil with 5% manure were significantly higher than the plant grown in the soil without manure.

9.3.1.6 Oxytetracycline in Radish Roots and Leaves

The concentrations of oxytetracycline in radish roots and leaves are shown in Figs. 9.12 and 9.13, respectively.

The result of analysis of radish roots (Fig. 9.12) show that there is a significant increase in oxytetracycline concentrations in radish roots between the control and all the other treatments (2.5, 5, and 10 mg/kg) in both growing media.

The accumulation of oxytetracycline in the leaves of radish was significant at 5 and 10 mg/kg levels (Fig. 9.13). Almost all the absorbed oxytetracycline from treatment 2.5 mg/kg was accumulated in the roots (edible part) but at levels 5 and 10 mg/kg the partitioning ratio of roots:leaves was about 1:1.5. This partially agrees with Xu and Zhang (2014) results who stated that the radish plant can absorb

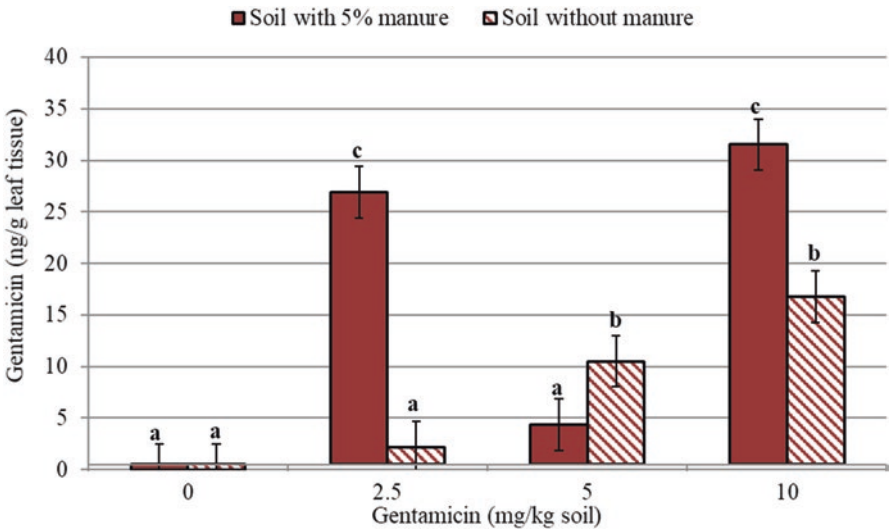

Fig. 9.11 Concentrations of gentamicin in radish leaves. Error bars show SD of the means. Different letters indicate statistical significance at 0.05 probability

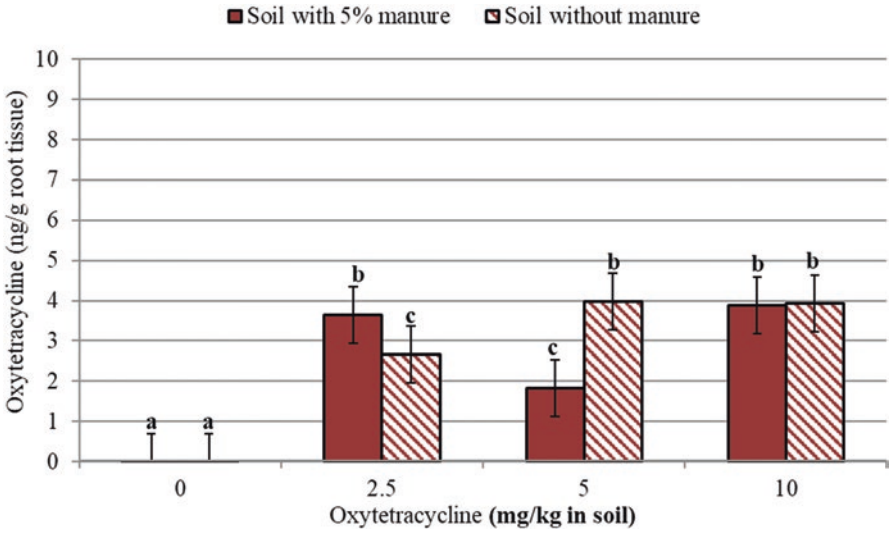

Fig. 9.12 Concentrations of oxytetracycline in radish roots. Error bars show SD of the means. Different letters indicate statistical significance at 0.05 probability

oxytetracycline from the soil (amended with pig manure and spiked with six oxytetracycline levels 0, 2, 5, 10, 25, and 50 mg/kg of soil). They also mentioned that the amounts of oxytetracycline absorbed by plants increased with increasing the antibiotic level in the soil.

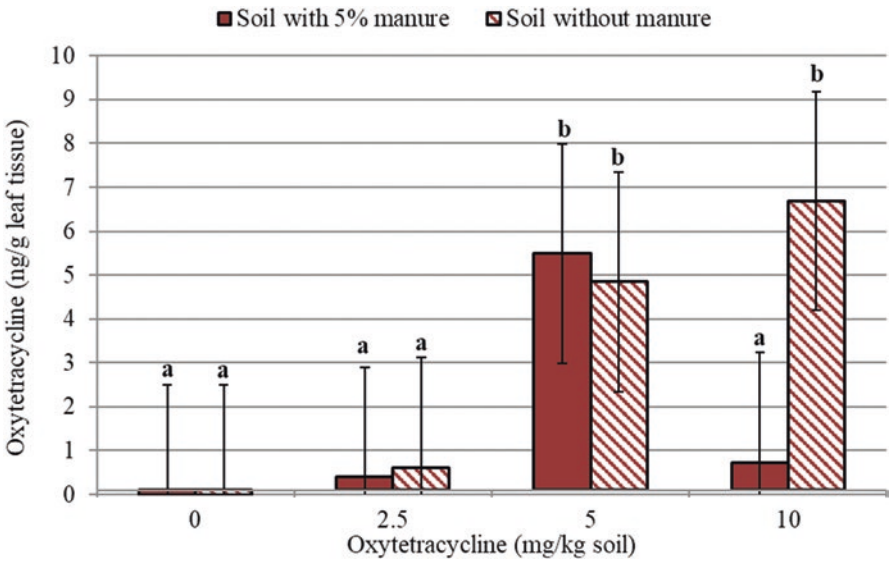

Fig. 9.13 Concentrations of oxytetracycline in radish leaves. Error bars show SD of the means. Different letters indicate statistical significance at 0.05 probability

9.3.1.7 Tylosin in Radish Roots and Leaves

The results of tylosin concentration in radish roots and leaves grown in manure-amended soils are present in Fig. 9.14.

The data show that tylosin accumulated in radish roots and was translocated to the leaves where it accumulated at almost the same concentrations (partitioning ratio root to leaves was about 1:1). There was significant difference between the control and all other treatments of tylosin in roots and leaf tissues. Increasing the rate of tylosin in the growing media did not lead to an increase in the concentration of tylosin in radish tissues.

Similar to gentamicin, the translocation of tylosin from the roots to the leaves took place at all levels. Thus, whenever tylosin is present in the growing media it will be absorbed by radish.

9.3.1.8 Comparison of the Concentration of Gentamicin, Oxytetracycline, and Tylosin in Lettuce and Radish

In Lettuce

The average concentrations of the three antibiotics in lettuce leaves and roots are shown in Fig. 9.15.

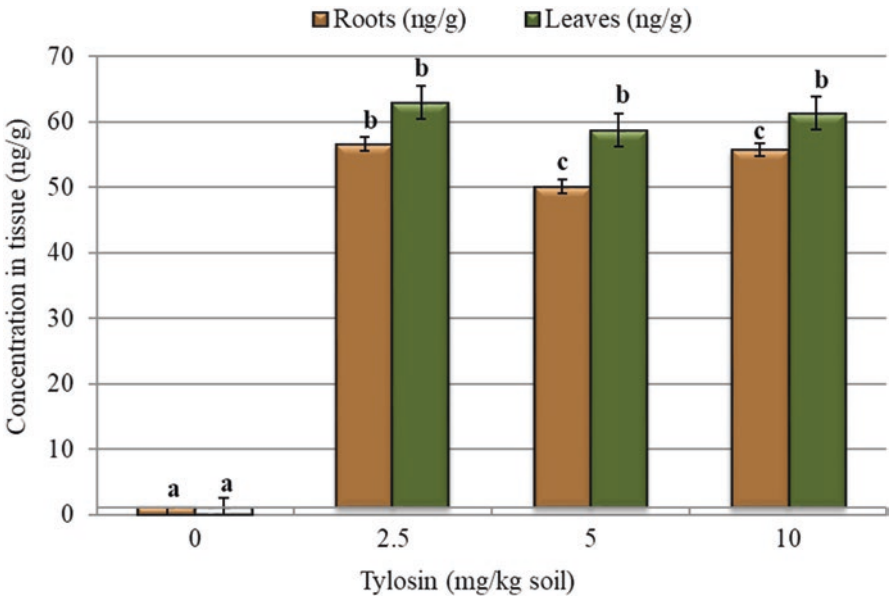

Fig. 9.14 Concentrations of tylosin in radish roots and leaves. Error bars show SD of the means. Different letters indicate statistical significance at 0.05 probability

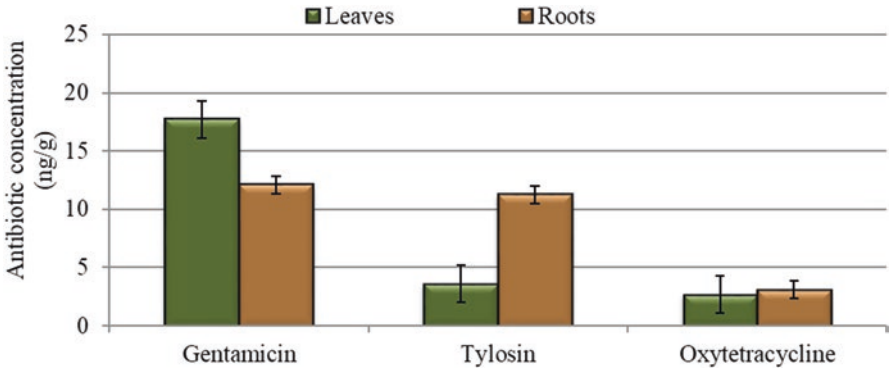

Fig. 9.15 Concentrations of gentamicin, tylosin, and oxytetracycline in lettuce leaves and roots. Error bars shows SD of the means

The levels were gentamicin > tylosin > oxytetracycline. Oxytetracycline was not absorbed by lettuce plants.

Gentamicin was absorbed by the roots of lettuce (12.7 ng/g) and translocated to the leaves at an average value of 17.7 ng/g. Tylosin accumulated mostly in the roots and partial translocation to the leaves took place only at the highest level (10 mg/kg).

In Radish

The results (Fig. 9.16) indicate that the concentrations of antibiotics in radish leaves and roots were as follows: tylosin > gentamicin > oxytetracycline.

Tylosin accumulated in the roots of radish at an average of 40.6 ng/g and translocated to the leaves at similar concentrations. Gentamicin was absorbed by the roots of radish at an average rate of 10.8 ng/g and translocated to the leaves at a higher rate (16.8 ng/g). However, oxytetracycline was accumulated in the radish roots at low concentrations (2.6 ng/g) and very low amount (2.4 ng/g) was translocated to the leaves, only at the highest level in soil (10 mg/kg).

The concentration of tylosin in radish root was significantly higher than that of gentamicin and oxytetracycline. These results are different from the findings of Kumar et al. (2005a, b) on corn, green onions, and cabbage who reported that these crops took up chlortetracycline but not tylosin. This indicates that plants differ in their uptake of antibiotics from soil solution and also differ in their accumulation sites and partitioning ratios (roots: leaves).

It was reported by Kumar et al. (2005a, b) that the molecular size of the antibiotic determines its availability to plants. They reported that green onions and cabbage plants took up chlortetracycline (small molecule) but not tylosin (large molecule). They interpreted their results by suggesting that tylosin molecular mass is almost double the mass of chlortetracycline molecule and thus could not be taken up as easily by plants, both in mass flow and active uptake processes. Also, Bassil et al. (2013) suggested that the molecular size of the antibiotic may be a determining factor for antibiotic availability for plant uptake. Our results contradict the findings of Bassil et al. (2013) and Kumar et al. (2005a, b) in which tylosin (large molecule) at high concentration (10 mg/kg) was taken up by lettuce roots and translocated to the leaves at a value of 3.58 ng/g (very low concentrations if it was not taken) whereas in radish the average concentration of tylosin in the roots was 56.6 ng/g and in the leaves 62.9 ng/g. Moreover, working with three different antibiotics, gentamicin

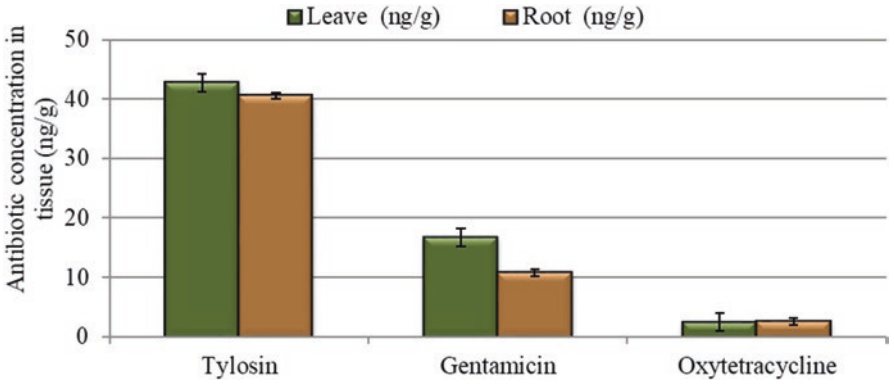

Fig. 9.16 Concentrations of tylosin, gentamicin, and oxytetracycline in radish roots and leaves. Error bars shows SD of the means

(477.596 g/mol), tylosin (916.1 g/mol), and oxytetracycline (460.434 g/mol) belonging to three different classes (aminoglycoside, macrolide, and tetracycline respectively) it can be speculated that in addition to plant type, the chemistry (formula and charge) of the antibiotic is a major factor in determining its solubility and availability in soil solution, uptake, and accumulation in plant tissues. Comparing gentamicin and oxytetracycline, two antibiotics with similar molecular weight, we may conclude that uptake by plant is not depending on the antibiotic's molecular size only but also on their chemical formula, charge, solubility in soil solution, and plant type.

9.4 Conclusion

The accumulation of three broad spectrum antibiotics (gentamicin, oxytetracycline, and tylosin follow the same order in naming the antibiotics) in lettuce and radish tissues was studied in a greenhouse pot experiment using ELISA technique for antibiotics analysis.

In lettuce leaves (edible part) for treatments of 0, 2.5, 5, and 10 mg antibiotic/kg soil, the average concentration of gentamicin in the manured soil were 0, 35.7, 56.7, and 31.9 ng/g fresh weight, respectively. But, the increase in tylosin was significant only at the 10 mg/kg manured soil (18.27 ng/g leaf tissue). There was no significant increase in the oxytetracycline concentration in lettuce leaves.

In radish roots (edible part), the average increase in gentamicin concentration was significant between the control and the other treatments in the manure-amended soils and reached 16.4, 16.2, and 10.4 ng/g fresh weight for the 2.5, 5, and 10 mg/kg soil, respectively. The concentrations of tylosin in radish roots were 0, 56.6, 50.1, and 55.7 ng/g in manure-amended soil. Moreover, the average increase of the concentration of oxytetracycline was significant between the control and the other treatments in the manure-amended soils at accumulation values that are much lower than those of gentamicin and tylosin of 0, 3.65, 1.83, and 3.89 ng/g for the 0, 2.5, 5, and 10 mg/kg treatments, respectively.

From the above results it can be concluded that the application of manure to the soil increased the uptake of the three tested antibiotics by lettuce and radish plants. The absorptivity of lettuce and radish of gentamicin and tylosin was higher than their absorption of oxytetracycline.

More research is needed to evaluate the effect of other antibiotics on crop growth and the influence of crop type on antibiotic uptake and accumulation in the edible parts. The effect of consuming vegetables with low levels of antibiotics on human health should also be investigated.

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Chapter 10
Bioavailability of Antibiotics and Their Toxicity

Izhar Ullah, Essa Ali, and Fakhar-ud-Din

Abstract Antimicrobial agents are the gift of science against pathogenic microorganisms causing infectious diseases. Antibacterial drugs are specifically used against bacteria and are of two types, i.e., bacteriostatic that can inhibit bacterial growth and bactericidal that can cause death of bacteria. Antibacterial mainly target either cell wall synthesis (like beta-lactams, vancomycin), bacterial protein synthesis (like tetracycline, clindamycin, streptogramins, chloramphenicol, aminoglycosides, and linezolid), or nucleic acid metabolism of bacteria (like sulfonamides, trimethoprim, quinolones). Infectious diseases are the major reason of premature deaths. Mortality rate due to these ailments raised up to 50,000/day deaths in last decades. Over the past few years, optimization of the use of antibiotics has gained much concern owing to the alarming increase in bacterial resistance and lack of new antibiotic classes under development. For the optimum effect and low toxicity we prefer those antimicrobials having high oral bioavailability. Bioavailability is the portion of dose after administration by route that is bioavailable in systemic circulation without any change in characteristics for its therapeutic effect. It is one of the basic pharmacokinetic properties of drugs. Bioavailability is an important factor because it defines the dose of drug to be administered for its desired therapeutic effect. The more bioavailable a drug is, the less of its amount will be required to attain therapeutic effect and so lower will be the body exposure for high dose.

Keywords Bioavailability · Antibiotics · Toxicity

I. Ullah (✉)
Faculty of Medical and Health Sciences, Department of Pharmacy, University of the Poonch Rawalakot, Rawalakot, Azad Kashmir, Pakistan

E. Ali
Department of Food Science and Technology, Zhejiang University of Technology, Hangzhou, People's Republic of China

Fakhar-ud-Din
Department of Pharmacy, Quaid-i-Azam University, Islamabad, Pakistan

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10.1 Introduction

Antimicrobial drugs are the gift of science against infectious diseases. Antibacterial drugs are mainly classified as bacteriostatic that can inhibit bacterial growth and bactericidal that can cause death of bacteria. Antibacterial mainly target either cell wall synthesis (like Beta-lactams, vancomycin), bacterial protein synthesis (like tetracycline, clindamycin, streptogramins, chloramphenicol, aminoglycosides, and linezolid), or nucleic acid metabolism of bacteria (like sulfonamides, trimethoprim, quinolones) (Dasgupta 2012). Normal flora consists of microorganisms, mainly bacteria, which reside inside and outside the body without causing any infection. New microorganisms can also colonize to overcome individual's defense system. Whenever there is a decline in the body immune system either by diseases or by drugs (anticancer or immunosuppressant), the same microorganisms become pathogenic. If individual is immunocompromised, then these microbes may cause diseases very frequently (Hemaiswarya et al. 2008). Infectious diseases are the main reason of premature deaths. Mortality rate due to these diseases raised up to 50,000 deaths per day in last decades. These diseases are also a great danger for the cancer and immunocompromised patients (Resistant 2007). Bacteria are majorly classified as gram-positive and gram-negative bacteria. Gram-positive bacteria differ from gram-negative by their external structure, i.e., bacterial cell wall. Gram-positive bacteria have thicker layer of peptidoglycan above inner cytoplasmic membrane (Beveridge 1999). Gram-negative bacteria contain lipopolysaccharide which is absent in gram-positive bacteria.

Changes in society, technological innovations, and the pathogenic microorganisms are the factors contributing to the emergence of new diseases, re-emergence of the diseases once managed, and the development of resistance to antimicrobial drugs (Cohen 2000). Inappropriate use of antimicrobial drugs, inadequate diagnosis, comprehensive use of antibiotics in medical centers, and use of antimicrobials in animal feeds and agriculture are few of the main causes of resistance development in pathogenic microorganisms (Swartz 1997).

Over the past few years, optimization of the use of antibiotics has gained much concern owing to the alarming increase in bacterial resistance and lack of new antibiotic classes under development (Bosso 2005; Van Bambeke et al. 2006). In this regard, advancement in the field of anti-infective pharmacology has led to the emergence of a new discipline "(PK/PD) pharmacokinetics/pharmacodynamics of antibiotics", which is referred to as the "discipline that focuses to understand the relationships between drug concentrations and its desirable (antibacterial effect) and undesirable effects (e.g., side effects) (Pai et al. 2014).

Bioavailability is the portion of dose after administration by route that is bioavailable in systemic circulation without any change in characteristics for its therapeutic effect. It is one of the basic pharmacokinetic properties of drugs. Bioavailability is an important factor because it defines the dose of drug to be administered for its desired therapeutic effect. The more bioavailable a drug is, the less of its amount will be required to attain therapeutic effect. However, it is also worth mentioning that most of the newly discovered therapeutic agents have poor solubility and it thus

renders them less bioavailable (Siddiqui et al. 2017). One of the basic tools in pharmacokinetics is bioavailability, as it should be measured when calculating dosages for routes other than intravenous route of administration (Allam et al. 2011). For bioavailability we are mainly concerned when drug is administered orally as this route faces various barriers to reach the systemic circulation. Strongly lipophilic and hydrophilic drugs are not suitable for oral administration because of inability to permeate through GI mucosa and low solubility in aqueous medium of GI. Log *P* value (Partition coefficient) will affect the transport characteristics of active pharmaceutical ingredient; API (drug) with log *P* value (partition coefficient) above 03–01 will show poor transport characteristics because best passive absorption through lipid membrane is at log *P* value of 3–1 (Agarwal et al. 2014).

From recent past years drug bioavailability has become a subject of interest not only in drug development but also in early stages of drug discovery. It is due to the fact that most of the candidate drugs failed during clinical trials were because of problems in absorption, distribution, metabolism, excretion (ADME), and toxicological parameters, rather than lack of efficacy. Recent advances are being made in pharmaceutical industry to improve success rates by considering the pharmacokinetic parameters ADME and toxicological aspects in drug discovery in early stage. Therefore, the numbers of publications on drug bioavailability have been increasing steadily from recent past.

Oral drug delivery has many advantages such as patient compliance, low cost, and avoidance of problems related to parenteral administration, such as infection risk and pain (Ensign et al. 2012). However, bioavailability is the main factor that needs to be considered when designing formulations for oral administration because poor bioavailability of drug may lead to development of resistance in case of antibiotics which further leads to therapeutic failure. Various factors such as limited permeability, poor solubility, and high rate of drug degradation in GIT are mainly responsible for inadequate bioavailability of the drugs. To overcome these problems nanotechnology is a promising tool. Antibiotics can be endowed with new and improved properties when combined with nanotechnological approaches like high surface:volume area and better bioavailability (Sharma et al. 2012).

Nanotechnology offers wide range of approaches to overcome the problems associated with antibiotics like their poor solubility and low bioavailability. Among these, solid lipid nanoparticles (SLNs) are the most suitable tool as they are made up of generally regarded as safe excipients like biodegradable and biocompatible lipids. These nanoparticles efficiently improve the bioavailability of poorly soluble drugs without any cytotoxicity against the mammalian cells. They enhance the bioavailability either by improving the solubility of drugs or by prolonging their release and residence time. They also offer protection to the drugs from excessive degradation in the GIT, thus increasing the concentration of drug in plasma (Lin et al. 2017). Mesoporous silica nanoparticles (MSNs) are another recently developed approach investigated for bioavailability enhancement of poorly soluble drugs. These nanoparticles like the SLNs impart stability to the drug molecules against the harsh conditions of GIT like low pH (Hata et al. 1999). Moreover, their high surface:volume ratio and large pore volume facilitate the delivery of higher concentrations of drug molecules to the target tissues and organs (Florek et al. 2017).

According to the European Medicines Evaluation Agency (EMEA), bioavailability is "The rate and extent to which an active moiety is absorbed from a pharmaceutical form and becomes available in the systemic circulation." There are two types of bioavailability:

1. Absolute bioavailability is referred to as the fraction/amount of dose from the extra vascular route, e.g., oral dose in unchanged form that reaches the systemic circulation in reference to dose given by an intravenous route.

   It can be measured by calculating the respective AUC after oral administration and intravenous administration as depicted in Eq. (10.1). /Dose_{po} and/ Dose_{test} in equation 10.1 and 10.2, respectively should be in line with their respective equations and not in second line which make equations incorrect.

   Therefore to avoid the effect of nonlinearity, the plasma concentrations following both intravenous and oral dosing should be similar.

$$\text{Absolute bioavailability} = \text{AUC}_{po} / \text{AUC}_{iv} \times \text{Dose}_{iv} / \text{Dose}_{po} \tag{10.1}$$

2. Relative bioavailability is referred to the fraction of a dose of drug reaching the systemic circulation relative to a reference product. Calculated as given by Eq. (10.2).

$$\text{Relative bioavailability} = \text{AUC}_{test} / \text{AUC}_{ref} \times \text{Dose}_{ref} / \text{Dose}_{test} \tag{10.2}$$

   Oral bioavailability is measured by the fraction of given dose absorbed in the GIT (f_a) and fraction that is not metabolized in liver (f_h) and the intestinal tract (f_g) as in Eq. (10.3) (El-Kattan and Lee 2017).

$$F = f_a \cdot f_g \cdot f_h \tag{10.3}$$

   Area under the curve (AUC) is probably the most single determinant of bioavailability (Spyker et al. 1977).

There are two major factors effecting bioavailability of a drug, i.e., product oriented like drug solubility, the rate of in vivo dissolution, and permeability, and secondly by patient factors such as physiological status, the integrity of the gastrointestinal tract, site of drug absorption, presystemic drug metabolism (intrinsic variables), membrane transporters, and extrinsic variables such as the effect of food or concomitant medication (Martinez and Amidon 2002). The fraction of drug absorbed is mostly considered the bioavailable fraction (Bioavailability) that joins the systemic circulation (Musser and Anderson 2001). In case of antibiotics while dealing with infectious diseases we are much concerned with the concentration. The effectiveness of an antibiotic can be predicted by a number of pharmacokinetic and pharmacodynamic principles. One of the starting point for predicting a drug's efficacy and maintenance of serum concentration is detecting the serum concentration of drug and its MIC for target pathogen. Achieving the MIC for a pathogen has become general guideline for conventional antimicrobial therapy (MacGregor and Graziani 1997; Pillai et al. 2010). Suboptimal target site concentrations have major clinical

implications, as they may contribute to therapeutic failures (Brunner et al. 2000; Joukhadar et al. 2001) particularly for bacteria for which in vitro MICs are higher. Furthermore, it can be conceived that bacterial resistance is triggered by subinhibitory concentrations in tissue. Therefore, according to the recommendations of standard reference texts on current medical treatment, impaired target site distribution is considered particularly when there is inconsistency between susceptibility testing and clinical response. Important clinical data can be extracted by data on tissue penetration of drugs as many studies have demonstrated that target site concentration profile is an important indicative of clinical outcome and in this respect it is more predictive than the plasma drug concentration (Pai et al. 2014).

10.2 Penicillin

Penicillins are β-lactam antibiotics and are cell wall synthesis inhibitors. Due to cell wall synthesis inhibiting action these antibacterial are bactericidal and kill the bacteria at particular concentration reached to the site of infection. Penicillins are classified into following five major groups; description is given in Table 10.1 (Nathwani and Wood 1993).

10.2.1 Bioavailability and Toxicity

For bioavailability of any drug we are mainly concerned with other than intravenous route of administration as this route provides 100% bioavailability. Penicillins differ markedly in their oral absorption. Some acid labile compounds like penicillin G, antipseudomonal penicillins, and methicillin are poorly absorbed through GIT while acid-stable compounds can have high oral absorption pattern differences.

Table 10.1 Classification of penicillins

Classes of penicillins	Members
Natural penicillins	Penicillins G
	Penicillin V
Penicillinase resistant penicillins	Methicillin,
	Nafcillin
	Isoxazolyl penicillin
Aminopenicillins	Ampicillin
	Amoxicillin
Carboxypenicillins	Carbencillin
	Ticarcillin
Acylureidopenicillins	Azlocillin
	Mezlocillin
	Pipracillin

Amoxicillin is absorbed highly (74–80%) after oral administration and food does not affect its absorption while ampicillin absorption is decreased by food and has lesser oral absorption (33–54%) compared to amoxicillin (Bennett et al. 2014). In 1977 a study demonstrated that there is no significant difference of absorption extent and AUC when amoxicillin was administered orally and through intramuscular rout. This study showed more than 80% of amoxicillin absorption through both routes of administration (Spyker et al. 1977). While dealing with penicillin G and penicillin V the oral absorption is higher for penicillin V (60%) and food interact with the absorption of penicillin-G while penicillin V absorption is not affected. Total drug concentration after oral administration of 500 mg dose for penicillin G and penicillin V is about 2 and 3.5 μg/mL, respectively, taken fasting. Oxacillin, dicloxacillin, flucloxacillin, and cloxacillin have 33%, 37%, 44%, and 49% absorption after oral dose while these drugs interact with food and their absorption is decreased while nafcillin has very low oral absorption and also interact with food. Ticarcillin and piperacillin is not absorbed through GIT (Barza and Weinstein 1976; Humbert et al. 1979; Josefsson and Bergan 1982; Klein and Finland 1963; Libke et al. 1975; Lode et al. 1984; Meyers et al. 1980; Nauta and Mattie 1975).

Jose Alexander invented a novel method for enhancing the bioavailability of poorly absorbed orally administered drugs including the penicillin antibiotics like amoxicillin, oxacillin, nafcillin, cloxacillin, dicloxacillin, ticaricillin, penicillin G, penicillin V, methicillin, and nafcillin. The method utilizes the acylcarnitines absorption enhancing agents. These compounds used as bioavailability enhancers are pharmaceutically acceptable salts which are more potent than other absorption promoting agents and pose less risk of tissue damage at the concentrations used for absorption enhancement (Alexander and Fix 1985).

10.2.2 Adverse Effects and Toxicity

Penicillins show hypersensitivity reactions as the most important adverse effects which range from rash to anaphylaxis in severity. When penicillins are administered, they can act as haptens and combine to the human proteins. Penicillins allergy is mainly provoked by the two derivatives, i.e., penicilloyl and penicillanic acid (Yates 2008). Very common reaction to penicillins is serum sickness which is characterized by urticaria, fever, angioneurotic edema, and joint pain. Stevens-Johnson syndrome and exfoliative dermatitis are rarely occurring allergic reactions to penicillins. Penicillins cause neutropenia uncommonly but this reaction recovers if causative agent is discontinued (Kerr et al. 1972). Penicillins cause interstitial nephritis (Appel and Neu 1977), which more commonly occurred with methicillin. Hypokalemia is another adverse effect of penicillins when administered in high doses, particularly of ticarcillin. High doses of penicillin G can provoke seizures as CNS toxicity (Barrons et al. 1992). Gastrointestinal disturbances may occur using oral dose of all penicillins but frequency with ampicillin is high (Maraqa et al. 2002).

10.3 Cephalosporins

Cephalosporins are also β-lactam antibiotics and their discovery was reported in 1945 (Bo 2000). Their mechanism of antibacterial action is same to the other β-lactam drugs. They target the peptidoglycan cross linkage and thus inhibit the synthesis of cell wall and are considered as bactericidal (Vogelman and Craig 1986; Wise and Park 1965). Cephalosporin is classified into four classes (Marshall and Blair 1999) as given below in Table 10.2.

10.3.1 Absorption and Bioavailability

Cephalosporins have very variable bioavailability. Cefazolin is parentally administered drug and is available in both IV and IM formulations. Cefazolin was studied in animal model and was observed to have very good absorption after IM injection and showed 78.4 ± 18.8% bioavailability (Sams and Ruoff 1985) and its intraperitoneal (IP) administration also showed almost same bioavailability (77.9 ± 3.1%) (Low et al. 2000) (Fig. 10.1).

Cephalothin and cephapirin are also parentally administered drugs of first generation and their mean systemic bioavailability given IM are 65.0 ± 20.5% and

Table 10.2 Classification of cephalosporin is given in this table

Classes of cephalosporin's	Members
First generation	Cefazolin
	Cephalothin
	Cephapirin
	Cephradine
	Cefadroxil
	Cephalexin
Second generation	Cefamandole
	Cefonicid
	Cefaclor
	Cefprozil
	Cefuroxime
Third generation	Cefoperazone
	Cefotaxime
	Ceftazidime
	Ceftizoxime
	Ceftriaxone
	Cefdinir
	Cefditoren
	Cefixime
Fourth generation	Cefepime

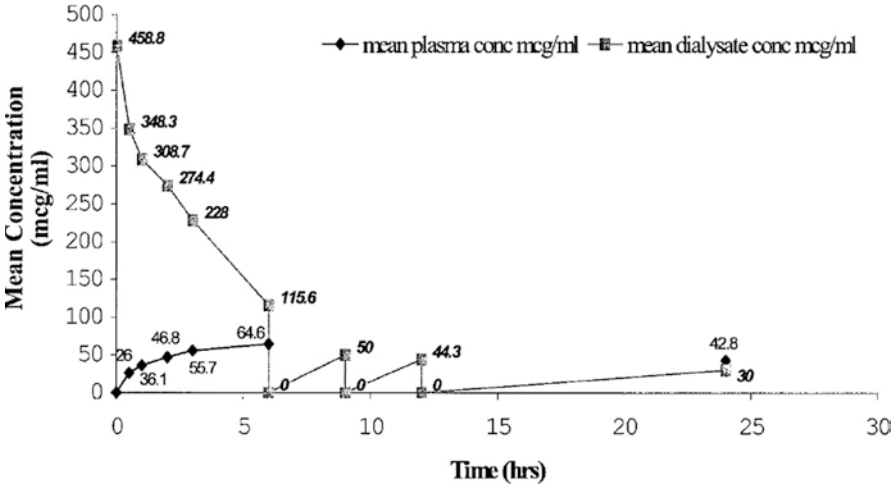

Fig. 10.1 Concentration–time profile of plasma and dialysate after intraperitoneal administration of cefazolin. Adopted from" Pharmacokinetics and bioavailability of cefazolin in horses" (Sams et al. 1985) with permission

67.89%, respectively (El-Komy 1995; Ruoff and Sams 1985). Cephradine of first-generation cephalosporin is found in both oral and parental dosage forms and its relative bioavailability is approximately 94% and its absorption was almost complete relative to IV dosing. Cefadroxil was found in a study to have bioavailability ranged from 90% to 100% estimated from plasma levels or from the amounts of drug excreted in the urine (García-Carbonell et al. 1993) (Fig. 10.2).

Cephalexin after oral administration showed rapid and complete absorption with peak levels reached within 1 h in suspension form; cephalexin appeared in serum within 9 min while in the form of capsule it takes 28 min. The oral absorption (Bioavailability) parameter varies if cephalexin is taken with food (Griffith and Black 1970; Nightingale et al. 1975). Cefprozil is a second-generation cephalosporin and is available in oral dosage form. Cefprozil exhibits linear pharmacokinetics and is essentially completely absorbed after oral administration and plasma as well as urine data show about 90% bioavailability (Shyu et al. 1992). Cefuroxime by itself is not absorbed orally and is given in the form of cefuroxime-axetil which showed absolute bioavailability of 35–45% in various studies while food increased its absorption (Williams and Harding 1984). Cefdinir belongs to third-generation cephalosporin and is available in oral dosage form having bioavailability of 16–21% in capsule dosage form and 21% in suspension form. Food exerts no clinically significant effect on cefdinir bioavailability (Williams and Harding 1984). Cefexime is another member of third-generation cephalosporins. The absolute bioavailability of this drug was determined through assays to be 52.3% and 47% after administration of 200 mg of oral solution and capsule, respectively (Faulkner et al. 1988). The fourth-generation drug cefepime is available only in parenteral dosage form.

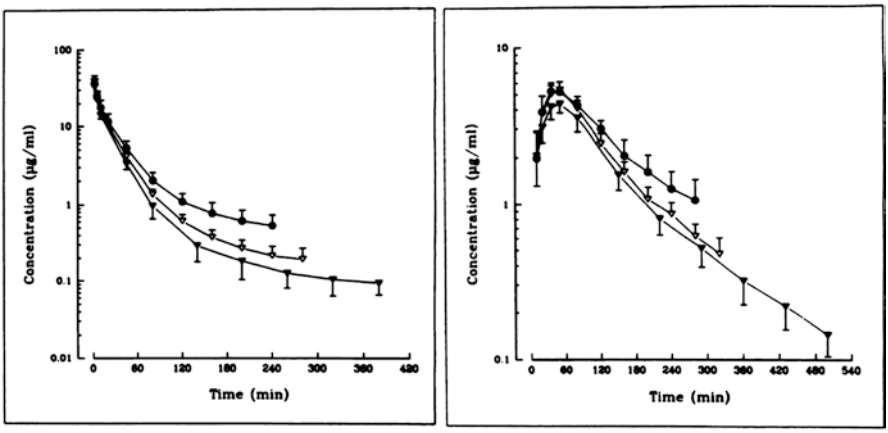

FIG. 2A *Mean plasma levels and standard deviations of cefadroxil after intravenous administration of 2.5 (●), 10 (▽), and 15 mg (▼).*

FIG. 2B *Mean plasma levels and standard deviations of cefadroxil after oral administration of 2.5 (●), 10 (▽), and 15 mg (▼).*

Fig. 10.2 Mean plasma levels and standard deviations of cefadroxil after intravenous administration (**a**) and oral administration (**b**). Adopted from "Pharmacokinetics and bioavailability of cephalothin in horse mares" (Ruoff et al. 1985) with permission

Krutika K et al. prepared cefdinir nanosuspension to increase its oral bioavailability. The particle size of resultant nanosuspension was 224.2 ± 2.7 nm while the zeta potential was found to be −15.7 ± 1.9 mV. Upon in vivo evaluation, a threefold increase in oral bioavailability was revealed as compared to the marketed formulation (Adcef) (Sawant et al. 2016).

10.3.2 Adverse Effects and Toxicity

The adverse effects profile of all the four generations can vary to some extent but remain same within single class. Like other β-lactam antibiotics hypersensitivity reactions are also the adverse effects caused by cephalosporins (Kelkar and Li 2001), but the frequency of hypersensitivity reactions occurrence is lower than penicillins. Cutaneous rashes accompanied with eosinophilia and sometimes fever occur in 7% or less individuals taking these drugs (Norrby 1987). Other severe reactions like anaphylaxis, serum sickness, or angioedema occur less commonly. These reactions are mediated by immunoglobulin E (IgE) and can occur in less than 1 patient out of 100,000 (Romano et al. 2002). In children and with the use of cefaclor the frequency of serum sickness may increase (Hebert et al. 1991). Cephalosporins can cause other adverse reactions like GIT disturbances (diarrhea, nausea, vomiting, biliary sludge, and transient transaminase elevation) and interstitial nephritis. Some hematologic reactions are eosinophilia, neutropenia, thrombocytopenia, impaired platelet aggregation, and hemolytic anemia. They can affect CNS very rarely by inducing seizures and encephalopathy in less than 1% patients taking these drugs.

Other rare adverse reactions are drug fever, disulfiram-like reaction, and phlebitis (Barza et al. 1986; Fainstein et al. 1983; Foster et al. 1980; Ingalls and Freimer 1992; Shearer et al. 1988).

10.4 Carbapenems

Ertapenem, meropenem, imipenem, and doripenem are the drugs occurring in this group. Carbapenems are β-lactam antibiotics and are bactericidal by inhibiting cell wall synthesis. All the abovementioned members of this group are poorly absorbed through GIT; therefore they are formulated in parenteral dosage form. Bioavailability of ertapenem after IM administration is approximately 90% (Keating and Perry 2005). Meropenem administered through IV, IM, or subcutaneous route has average bioavailability of 93–99% while imipenem has 89% bioavailability after IM administration (Albarellos et al. 2016; Craig 1997; Signs et al. 1992). Oral bioavailability of meropenem is low because it is BCS class 4 drug with low permeability and low solubility. Nanosuspension of meropenem was prepared to enhance is dissolution and solubility which will lead to an increased bioavailability. The in vitro evaluation suggested that the solubility and dissolution of meropenem was significantly enhanced as compared to pure drug (Chirumamilla et al. 2017).

10.4.1 Adverse Effects and Toxicity

Generally carbapenems are well-tolerated drugs. Some major adverse effects are like coagulation abnormalities, Clostridium difficile associated colitis, hepatotoxicity, or nephrotoxicity. Some commonly occurring adverse reactions are diarrhea, nausea, vomiting, phlebitis, and headache. All these drugs are believed to cause seizures due to their structural similarity with γ-aminobutyric acid (GABA) and seizures frequency is increased with imipenem compared to other members (Miller et al. 2011; Mori et al. 2007).

10.5 Monobactams

Monobactams are monocyclic β-lactam antibiotics having a 2-oxoazetidine-1-sulfonic acid moiety. The only member of this group is aztreonam (Sykes and Bonner 1985). This drug is administered through i.v. or i.m. route and has very poor bioavailability through oral route (Hopefl 1985; Swabb et al. 1983). Aztreonam is well tolerated but can cause local reactions like phlebitis in nearly 2% patients. Nausea, vomiting, diarrhea, and rashes can occur in less than 1% patients (Squibb and Sons n.d.).

10.6 Aminoglycosides

Aminoglycosides are very important part of the antibacterial family. Streptomycin produced by Streptomyces species and was the first member of this aminoglycoside family. Names of this family members that are derived from Streptomyces spp. end with "mycin" while those derived from Micromonospora spp. end with "micin." They are the protein synthesis inhibitors by binding to the 30s subunit of prokaryotic ribosomes causing misleading the protein synthesis which leads to accumulate nonfunctional proteins in bacteria (François et al. 2005; Lynch and Puglisi 2001; Rando 2001). Different classes of aminoglycosides are given as below in Table 10.3.

10.6.1 Absorption and Bioavailability

As discussed earlier, aminoglycosides are available mostly in parenteral dosage form. Streptomycin was studied for its pharmacokinetic profile and showed 88% bioavailability after i.m. administration (Zhu et al. 2001). Complete bioavailability occurs while administered through i.m. and subcutaneous (SC) routes in gouts (Uppal et al. 1997). Dibekacin, tobramycin, kanamycin, gentamicin, and isepamicin were studied for their pharmacokinetic profile and they all showed complete absorption and 100% bioavailability after intramuscular injection (Driessen et al. 1978; Radwanski et al. 1997; Segal et al. 1988; Verbist et al. 1982). Netilmicin and sisomicin also have bioavailability greater than 90% after intramuscular administration (Chung et al. 1981; Humbert et al. 1978). Framycetin and Neomycin are not absorbed through GIT and are mostly used topically (Breen et al. 1972). Paromomycin shows very poor oral absorption (Bissuel et al. 1994; Hens et al. 2014). However, like other

Table 10.3 Classification of aminoglycosides

Classes of aminoglycosides	Members
Streptomycin	Streptomycin
Kanamycin	Amikacin
	Arbekacin
	Tobramycin
	Dibekacin
	Kanamycin
Gentamicin	Gentamicin
	Sisomicin
	Isepamicin
	Netilmicin
Neomycin	Framycetin
	Paromomycin
	Neomycin
Spectinomycin	Spectinomycin

aminoglycosides, its absorption is nearly 100% from IM injection (Kip et al. 2018). Bioavailability of spectinomycin after i.m., sc and oral administration was 136.1% and 128.8%, respectively. The oral bioavailability was 11.8% and 26.4% after 50 and 100 mg/kg body weight, respectively. Previous studies showed that tobramycin exhibit poor oral absorption due to the increased efflux via the P-gp efflux pump. Encapsulating tobramycin in solid lipid nanoparticles (SLNs) inhibits the efflux of the drug, thus increasing its absorption and bioavailability (Bargoni et al. 2001).

10.6.2 Adverse Effects and Toxicity

A common and frequent potential of causing nephrotoxicity exist among all the aminoglycosides but neomycin is the most and streptomycin is the least toxic drug (Denamur et al. 2008; Sandoval et al. 2006). Various drugs like vancomycin and teicoplanin can increase nephrotoxic risk of aminoglycosides (Fabre et al. 1976). Clinical trial data show that many days of treatment is needed for aminoglycosides to cause nephrotoxicity (Buchholtz et al. 2009). Aminoglycosides can cause vestibular and cochlear damage showed by in-vivo studies (Xie et al. 2011). Hearing loss and dizziness can be caused by streptomycin (Hinshaw and Feldman 1945). Aminoglycosides can cause neuromuscular blockade in very rare cases but is a lethal toxic effect of this group of drugs. This toxic effect was determined through various experiments for streptomycin, neomycin, kanamycin, gentamicin, tobramycin, netilmicin, and amikacin (Nordström et al. 1990; Pittinger and Adamson 1972). Isepamicin can induce oto-toxicity, nephrotoxicity, and vestibulotoxicity. However, animal and clinical studies show that isepamicin is one of the less toxic aminoglycosides (Tod et al. 2000).

10.7 Tetracyclines

Chlortetracycline, the first tetracycline, was discovered by Benjamin M. Duggar in Duggar 1948 and since the time of discovery this class remained an important part of antibiotics (Duggar 1948). They have wide range of activity and are broad-spectrum bacteriostatic drugs. They cause antibacterial effect by inhibiting protein synthesis of bacteria by binding to the 30S bacterial ribosomal subunit reversibly (Craven et al. 1969). Classification of tetracycline are given in Table 10.4.

10.7.1 Absorption and Bioavailability

Tetracyclines are primarily absorbed in the GIT parts, stomach, and proximal small bowel. Oxytetracycline is the most least absorbtion (60%) after oral administration (Singh et al. 2005). Tetracycline has bioavailability in the range of 77–88% (Wood

Table 10.4 Classification of tetracycline (Fuoco 2012)

Classes of tetracyclines	Members
First generation	Tetracycline, chlortetracycline, oxytetracycline, demeclocycline
Second generation	Doxycycline, lymecycline, meclocycline, methacycline, minocycline, rolitetracycline
Third generation	Tigecycline

et al. 1975). Demeclocycline is 66% absorbed when administered orally while chlortetracycline has 30% absorption through GIT (Agwuh and MacGowan 2006). Doxycycline and minocycline are almost completely absorbed with a bioavailability of more than 80% and with an average of 95% (Chopra 2011; Saivin and Houin 1988). Tigecycline has limited oral absorption and is administered through IV route (Agwuh and MacGowan 2006). Multivalent cations like calcium, iron, aluminum, and magnesium decrease absorption of tetracyclines by 50–90% due to chelate formation.

10.7.2 Adverse Effects and Toxicity

Generally as class tetracyclines are well tolerated but a French review showed that minocycline has more serious and frequent adverse effects compared to other members of the class (Smith and Leyden 2005). This class of antibiotics affects GIT and cause diarrhea, nausea, vomiting, epigastric pain, and heart burn. These effects are more common with doxycycline (Hey et al. 1982; Winckler 1981). The hypersensitivity reactions (facial edema, anaphylaxis, urticaria) are rarely occurred due to tetracyclines but more frequent with minocycline. Tetracycline users can face photosensitive rashes when exposed to sun (Smith and Leyden 2005). This reaction is caused by drug accumulation in the skin and can associate with papules, oncholysis, vesiculations, and edema (Bethell 1977). Tetracyclines adverse reaction of hyperpigmentation is also well reported and commonly caused by minocycline (Smith and Leyden 2005). As a result of chelation with calcium, the tetracyclines deposit in bones and teeth and as a result teeth can become stained (Demers et al. 1968; Moffitt et al. 1974). Doxycycline has a lower bones and teeth deposition potential compared to other tetracyclines (Chiu et al. 1998). In early life when children receive tetracyclines the deposition in deciduous teeth occurs. This deposition can also occur in developing fetus if the mother takes tetracyclines during late pregnancy (Madison 1963). Tetracyclines deposition can also decrease the growth of bones in infants (Cohlan 1963). These drugs can also cause fatal hepatotoxicity and is more frequent with tetracycline intravenous administration in high dose (Schultz et al. 1963). Doxycycline appeared to be safe regarding liver toxicity (Vial et al. 1997). Other toxic effect is nephrotoxicity. Tetracyclines can exaggerate renal impairment by inhibiting protein synthesis and cause hyperphosphatemia, azote-

mia, and acidosis (Shils 1963). Minocycline also affects central nervous system by causing reversible vertigo, dizziness, lack of concentration, and tinnitus. Women are more exposed to the vestibular adverse effects compared to men (Fanning et al. 1977). Tetracycline, minocycline, and doxycycline are also noticed to cause idiopathic intracranial hypertension (Lochhead and Elston 2003).

10.8 Chloramphenicol

Chloramphenicol was isolated from soil organism *Streptomyces venezuelae* and since 1949 it is in clinical use as a broad-spectrum antibiotic (Ehrlich et al. 1947). It remained and inexpensive drug that is broad spectrum and can target many gram-positive, gram-negative, anaerobic, and atypical organisms but due to aplastic anemia risk this drug is no more drug of choice for any infection (Rich et al. 1950). Chloramphenicol is a protein synthesis inhibitor and binds to the 50S subunit of the bacterial ribosome (Green et al. 1975). Bioavailability of chloramphenicol is approximately 80% when administered in capsule form and is rapidly absorbed in intestinal tract (Ambrose 1984; Pestka 1971; Smith and Weber 1983).

10.8.1 Adverse Effects and Toxicity

The most significant toxic effect of this drug is its toxicity towards bone marrow. Chloramphenical suppresses the bone marrow reversibly which is due to its direct pharmacological effect. As a result, any combination of reticulocytopenia, leukemia, anemia, or thrombocytopenia may occur (Manyan et al. 1972; Yunis 1973). Chloramphenicol can also cause hemolytic anemia with glucose-6-phosphate dehydrogenase deficiency (Mccaffrey et al. 1971). The other hemolytic toxicity is the uncommon but mostly fatal aplastic anemia. Due to this toxic effect chloramphenicol use is now very limited (Balbi 2004). A circulatory collapse called gray baby syndrome occur in newborn and premature infants which is associated to high concentration of chloramphenicol (Sutherland 1959). Gray baby syndrome is characterized by abdominal distension, vomiting, cyanosis, flaccidity, gray color, circulatory collapse, and ultimately death (Suarez and Ow 1992; Werner et al. 1985). Prolong therapy with chloramphenicol can cause optic atrophy and blindness. These symptoms are mostly reversible but blindness can be permanent (Fung et al. 2011; Woolf 1965). It can cause Jarish-Herxheimer reactions and can induce bleeding if used orally for prolonged duration (Cahill 1962). Chloramphenicol can disturb immunity development during active immunization (Ambrose and Coons 1963).

10.9 Macrolides

Macrolides antibiotics class has various members like erythromycin, clarithromycin, and azithromycin, and erythromycin is the first member of this class of antibiotics and was derived in 1952 from a strain of *Saccharopolyspora erythraea.* They cause their antibacterial effect by binding to 50S ribosomal subunit and so inhibiting RNA-dependent protein synthesis (Edelstein 2004; Leclercq and Courvalin 2002).

10.9.1 Absorption and Bioavailability

The absolute bioavailability of erythromycin was determined through in vivo experiment which was 32 ± 7% for enteric coated 250 mg capsule and for the 250 mg duodenal solution was 43 ± 14% (Somogyi et al. 1995). Clarithromycin has good absorption potential after oral administration and has almost 50% bioavailability (Piscitelli et al. 1992). Azithromycine has 37% bioavailability after oral administration in 500 mg single dose (Schentag and Ballow 1991). Azithromycin has low bioavailability owing to its poor solubility. Chen Dong Hou et al. prepared azithromycin nanosuspension to increase its solubility that will further enhance the bioavailability of the drug. Results of in vitro release and solubility studies showed an obvious enhancement in the solubility and dissolution rate as compared to the raw drug. Similarly nanoparticles of azithromycin were prepared solvent/antisolvent precipitation method to achieve an increase in the solubility and oral bioavailability of drug due to reduction in particle size that offers larger surface area. The particle size ranged from 200 to 400 nm. These nanoparticles of azithromycin offered a 2.93-fold increase in the dissolution as compared to the raw drug (Hou et al. 2012; Pouretedal 2014).

SLNs loaded with clarithromycin were prepared to increase its oral bioavailability. Results of pharmacokinetic studies in rats revealed a 2.3-fold increase in $C_{max,}$ twofold increase in $T_{max,}$ 1.4-fold increase in mean residence time, and fivefold enhancement in the relative oral bioavailability of clarithromycin on oral administration of clarithromycin loaded SLNs (Sharma et al. 2016).

10.9.2 Adverse Effects and Toxicity

Except for *C. difficile* colitis and ventricular arrhythmias the other unwanted events caused by erythromycin are not life threatening. Erythromycin causes irritative reactions including abdominal cramps, diarrhea, nausea, vomiting, and gas more commonly (Ellsworth et al. 1990). High concentration given through IV route can cause thrombophlebitis which can be decreased by its dilution. Allergic reactions include fever, skin rash, and eosinophilia. Cholestatic hepatitis can occur with the use of erythromycin rarely (Inman and Rawson 1983). Reversible hepatotoxicity

including jaundice has occurred with the use of erythromycin stearate salt and also with the ethylsuccinate ester of erythromycin (Carson et al. 1993). When used in high concentration through IV route, erythromycin lactobionate or oral dose of erythromycin can cause transient hearing loss (Eckman et al. 1975; Karmody and Weinstein 1977). Other adverse effects include polymorphic ventricular tachycardia, superinfection, and infantile hypertrophic pyloric stenosis (Cooper et al. 2002; Katapadi et al. 1997; Ray et al. 2004; SanFilippo 1976).

At usual doses clarithromycin and azithromycin have very low adverse effects potential. The most commonly occurring adverse events are GIT disturbances like nausea, vomiting, diarrhea, and abdominal pain (Bahal and Nahata 1992; Piscitelli et al. 1992). In few patients acute phychosis or "mania" has been reported taking clarithromycin (Katapadi et al. 1997). Clarithromycin can cause teratogenic effects and is discouraged to take in pregnancy (Turner and Aziz 2002). Hepatic toxicity, dizziness, tinnitus, and reversible hearing loss are the some events reported with the use of azithromycin (Kolkman et al. 2002; Longo et al. 1997; Wallace Jr et al. 1993). Torsades de pointes (Polymorphic tachycardia) cases also increased when risk factors like increasing age, concomitant drug use (like cisapride), and female gender occur (Shaffer et al. 2002).

10.10 Glycopeptides

10.10.1 Vancomycin

Vancomycin was isolated from *Amycolatopsis orientalis* and is the first glycopeptides antibiotic developed in the mid-1950s. The glycopeptides inhibit the late stages of cell wall synthesis in multiplying bacteria (Fraser et al. 2005; Lipsky et al. 1999). Vancomycin is not absorbed orally and is mostly administered intravenously (Shively and Thompson 1995). Clinical uses of vancomycin showed sever ototoxicity in six patients using 1–2 g daily dose. After measuring the vancomycin level it showed 80 and 100 μg/mL concentration which induced ototoxicity but this adverse effect was later on studied and was concluded to be very rare adverse effect of vancomycin (Geeaci et al. 1958). Since the start of vancomycin clinical use, nephrotoxicity has been associated which was thought to be related to impurities related to the early preparations of vancomycin (Elyasi et al. 2012). Some factors are like high dose (≥4 g/day), weight of patient (≥101.4 kg), critically ill patients, and decreased creatinine clearance (<86.6 mL/min) and the most common side effects with vancomycin are infusion-related reactions. Rapidly developing pruritus or erythematous rash affects face, neck, upper trunk, and head and can be associated with hypotension and angioedema. These reactions are commonly known as red neck of red man syndrome reported during vancomycin infusion (Myers et al. 2012). Neutropenia can also be observed due to long-term vancomycin administration in frequency up

to 13%. Thrombocytopenia is very rarely reported with the use of vancomycin. Cases of more severe reaction than maculopapular or erythematous rash such as toxic epidermal necrolysis, Steven-Johnson syndrome, and linear IgA bullous dermatosis have also been reported (Blumenthal et al. 2012; Von Drygalski et al. 2007).

10.11 Sulfonamides

Sulfonamides are clinically important antimicrobials derived from sulfanilamide, which is structurally similar to para-aminobenzoic acid (PABA), required factor for the synthesis of folic acid. Sulfonamides are bacteriostatic and inhibit bacterial growth by hindering with the folic acid synthesis of microorganisms (Eyster 1943). Sulfonamides are classified in Table 10.5 given below.

10.11.1 Absorption and Bioavailability

Most of the short-acting and medium-acting sulfonamides are absorbed almost completely and rapidly from the stomach and small intestine. Sulfisoxazole is completely absorbed after intramuscular and oral administration and bioavailability ranges more than 97% (Kaplan et al. 1972; Suber et al. 1981). Mostly these sulfonamides are administered in combination with trimethoprim-like drugs. Sulfadiazine showed bioavailability in animal models in range of 80% (Abu-Basha et al. 2009; Baert et al. 2003). The absolute bioavailability of sulfaguanidine was studied in animal model which was 56% in neonates and is many times lower in adults (Mizuno et al. 1986).

Table 10.5 Classification of sulfonamides (Actor et al. 2000; Smith and Powell 2000)

Classes of sulfonamides	Members
Short acting or medium acting sulfonamides	Sulfisoxazole
	Sulfadiazine
	Sulfamethoxazole
Long acting sulfonamides	Sulfadoxine
Sulfonamides limited to GI tract	Sulfaguanidine
	Sulfasuxidine
	Sulfathalidine
Topical sulfonamides	Sulfacetamide
	Mafenide

10.11.2 Adverse Effects and Toxicity

Sulfonamides can cause diarrhea, nausea, vomiting, fever, rash, depression, jaundice, headache, hepatic necrosis, and drug-induced lupus (Price and Venables 1995). Excessively high dose of sulfadiazine is associated with tubular deposits of sulfonamide crystals and crystalluria. More toxic effects of sulfonamides may include aplastic anemia, acute hemolytic anemia, agranulocytosis, leukemia, and thrombocytopenia. Sulfonamides compete for bilirubin-binding sites on plasma albumin if administered during the last month of pregnancy and may cause increased unconjugated bilirubin in fetal blood, which increase the risk of kernicterus. Sulfonamides administered through any route can significantly increase hypersensitivity reactions. Erythema multiforme, erythema nodosum, vasculitis, fixed drug eruption, and anaphylaxis are the most important reactions (Wolkenstein et al. 1995).

10.12 Quinolones

Nalidixic acid is the first member of this class of antimicrobials which was identified in 1962 by Lesher and associates as by-product of chloroquine synthesis. Different quinolones like sparfloxacin, travofloxacin, temafloxacin, and gatifloxacin were identified and due to their severe toxicities they were removed from clinical use. In the 1970s, oxolinic acid as well as cinoxacin were also developed before identification of more potent and wide spectrum fluorine and piperazinyl-substituted derivatives. With good oral absorption, wide spectrum of activity, and good safety profile these newer fluoroquinolones resulted in extensive clinical use. Peprazinyl include norfloxacin, enoxacin, and ciprofloxacin, methyl-piperazinyl include perfloxacin, ofloxacin, lomefloxacin, fleroxacin, temafloxacin, levofloxacin, grepafloxacin, and gatifloxacin. Sparfloxacin is a dimethylpiperazinyl (Domagala 1994). The quinolones cause rapid bacterial cell death by inhibiting bacterial DNA synthesis (Aldred et al. 2014). Norfloxacin upon oral administration exhibits low bioavailability (40%) and poor permeability (Gips and Soback 1996). Zhao dong et al. prepared norfloxacin SLNs with the objective to improve its bioavailability. Pharmacokinetic studies in rats showed sustained release of norfloxacin while the relative bioavailability was enhanced by 12-fold without any cytotoxicity (Dong et al. 2011).

10.12.1 Absorption and Bioavailability

Most of the drugs in this class are well absorbed through GIT approaching from 50% to 100%. Peak concentration in serum is mostly attained in 1–3 h after administration. Their absorption is not affected by food or achlorhydria but food can delay

the time to achieve peak serum concentration (Sörgel and Kinzig 1993; Staib et al. 1989). Norfloxacin, ciprofloxacin, and gemifloxacin have 50%, 70%, and 71% bioavailability, respectively, while pefloxacin, ofloxacin, and levofloxcin have bioavailability more than 95%. Moxifloxacin has bioavailability in range of 86–100% (Bennett et al. 2014).

10.12.2 Adverse Effects and Toxicity

Sulfonamides can cause gastrointestinal adverse effects more frequently like nausea, vomiting, anorexia, and abdominal discomfort and less frequently can cause diarrhea (Kuhlmann et al. 2012; Owens Jr and Ambrose 2005). The next common adverse effects are of central nervous system like dizziness, headache, insomnia, and mood alteration. Sulfonamides can cause delirium, hallucinations, psychosis, and seizures (Tomé and Filipe 2011). Sulfonamides caused allergic and skin reactions in 0.4–2.8% of patients during clinical trials (Ball et al. 2004). Drug fever angioedema, serum sickness like syndromes, urticaria, vasculitis reactions are uncommon. Quinolones can inhibit potassium channels and so can delay repolarization in cardiac tissue and so can prolong QT interval on the ECG (Finch et al. 2002). Eosinophilia and leucopenia mostly occur in below than 1% of users (Davidson et al. 2002). Hypoglycemic events are also reported in sulfonamides users (Abramowicz 2003).

10.13 Antimycobacterial Agents

Antimycobacterial agents are commonly classified as first-line drugs with acceptable toxicity and better efficacy, and second-line drugs with greater toxicity and less efficacy. First-line agents include isoniazid (INH), ethambutol, rifampin, and pyrazinamide (PZA), while streptomycin, linezolid, quinolones, amikacin, capreomycin, para-aminosalicylic acid, kanamycin, and ethionamide are second-line drugs (Bennett et al. 2014; Blumberg et al. 2003; Iseman et al. 1993).

10.13.1 Absorption and Bioavailability

Isoniazid is completely and rapidly absorbed after oral and intramuscular administration (Weber and Hein 1979). Rifampicin oral bioavailability of single dose is about 93% which can be decreased to 68% after 3 weeks of chronic administration (Loos et al. 1985). Isoniazid due to its short plasma half-life (1–4 h) requires higher doses repeated to maintain the plasma drug levels. Bhandari et al. incorporated isoniazid in SLNs and its oral pharmacokinetics were evaluated in rat model.

The results showed that the relative bioavailability in plasma was enhanced by sixfold while a fourfold increase was found in brain as compared to the free drug (Bhandari and Kaur 2013).

10.13.2 Adverse Effects and Toxicity

The major and severe toxic effect of INH and rifampin is hepatitis while the most feared toxicity is fulminant hepatic failure (Mitchell et al. 1976). INH can cause neurotoxic effect, hypersensitivity reactions, metabolic acidosis, seizures, hyperglycemia, and coma. Rifampin can cause hypersensitivity with flushing, pruritus, fever, cutaneous vasculitis, thrombocytopenia eosinophilia, and hemolysis. Gastrointestinal disturbance is very frequent with the use of rifampin (Bennett et al. 2014). The most frequently occurring adverse effects of PZA are nausea and vomiting, and in nearly 15% users it may cause hepatotoxicity (Zierski and Bek 1980). Various adverse effects of PZA are rhabdomyolysis with myoglobinuric renal malfunctioning, interstitial nephritis, and photosensitivity (Namba et al. 1991; Sanwikarja et al. 1989; Zierski and Bek 1980). Neuropathy is the major toxic effect of ethambutol causing peripheral neuropathy (Chatterjee et al. 1986). Hyperuricemia, hypersensitivity, and gastrointestinal intolerance can occur infrequently with ethambutol.

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Chapter 11
Effect of Antibiotics on Plant Growth in a Water Culture

Lara J. El Gemayel, Isam I. Bashour, Yusuf A. Abou Jawdeh, Mohamad T. Farran, and Nadim Farajalla

Abstract The primary entrance pathways of antibiotics in the environment are through wastewater and spreading animal manure as a mean of irrigation and fertilization onto agricultural lands. The direct consequences of irrigating with wastewater on farm lands are surface runoff and leaching of veterinary antibiotics into deeper soil layers. Depending on their mobility in soils, these compounds represent a great threat to close rivers, groundwater, streams, and aquatic life, thus contaminating them and the crops grown around them. The aim of this study is to evaluate the uptake and accumulation sites of antibiotics in plants grown in nutrient solution and their effect on plant growth. Antibiotic analysis was done using the enzyme-linked immunosorbent assay (ELISA). Lettuce and radish were grown at 0, 5, and 10 mg/L of enrofloxacin, oxytetracycline, and tylosin. The results indicate that the three antibiotics were absorbed in radish bulbs at an average of 67.7, 30.98, and 407.45 ng/g, respectively. In lettuce leaves, enrofloxacin, oxytetracycline, and tylosin accumulated at an average of 59.39, 6.83, and 343.83 ng/g, respectively. Enrofloxacin and oxytetracycline reduced lettuce and radish growth by 70%, whereas tylosin had no significant effect on plant growth. The levels of antibiotics found in the edible crops fell in the acceptable maximum residual level, and thus may not cause health hazards to humans. Further research is needed on the uptake mechanism of antibiotics in plants and their fate in the environment.

Keywords Antibiotic absorption · Antibiotic effect · Enrofloxacin · Oxytetracycline · Tylosin · Lettuce · Radish · Nutrient solution

L. J. El Gemayel · I. I. Bashour (✉) · Y. A. Abou Jawdeh · M. T. Farran
Faculty of Agricultural and Food Sciences, American University of Beirut, Beirut, Lebanon
e-mail: ib02@aub.edu.lb

N. Farajalla
Issam Fares Institute for Public Policy and International Affairs, Beirut, Lebanon

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11.1 Introduction

In the past decade, the use of antibiotics in agriculture and animal industry has been booming; animals and humans consume antibiotics which have thousands of different chemicals (Pan et al. 2014). Veterinary drugs (hormonal growth promoters and antibacterial drugs) have been used in the fields of aquaculture, agro-industry, and animal husbandry as a mean to improve weight gain rate and feed efficiency or treat and prevent illnesses in food-producing animals (Beyene 2016). Eventually, after usage, these bioactive chemicals enter the environment as residues from either pharmaceutical manufacturing, wastewater, or manure (Pan et al. 2014). Many wastewater treatment plants do not completely remove pollutants; therefore, antibiotics survive the treatment and remain in biosolids or wastewater which are then applied as fertilizers or irrigation water on agricultural lands to improve soil quality (Ding et al. 2011; Gottschall et al. 2012).

Sometimes, urban greeneries and agricultural lands are irrigated with wastewater as well as replenishing the groundwater and surface water as a solution to the water shortage; result of climate change, pollution, population growth and regional drought (Pan et al. 2014). On a global level, around 20 million hectares of agricultural lands are irrigated with wastewater which was treated to different extents (Pan et al. 2014). Nevertheless, Carvalho, Basto, Almeida, and Brix (2014) stated that an addition of treatments to wastewater managements only removed 60% of most drugs; consequently wastewater treatments are not sufficient to remove all organic pollutants and some toxic organic and inorganic pathogens and pollutants, which are mainly biologically active, remain in it, thus creating a higher hazard when entering the environment. Even though there are developed guidelines tackling the quality criteria of irrigation water, they do not consider the possible risks from trace quantities of organic pollutants such as personal care products and pharmaceuticals in treated effluent and surface water from wastewater treatment plants. Low concentrations of antibiotics in irrigation water can be absorbed and accumulated by crops and/or accumulate in irrigated soils.

Veterinary antibiotics are relatively easy to adsorb to soil particles as they are organic compounds displaying a wide range of functional groups as well as they can be amphoteric, amphiphilic, or ionic. Tetracyclines and divalent metal cations are prone to bind and adsorb onto soils, organic matter, and minerals, and hence their extractability is reduced, which in turn reduces their accessibility for plant uptake (Zhang et al. 2016b). Youssef and Bashour (2017) studied the absorption of tylosin and oxytetracycline by radish and lettuce grown in soil. Tylosin remained in the roots of lettuce (2.38–18.27 ng/g) whereas in radish roots it had a partial translocation (55.7 ng/g). Oxytetracycline was not accumulated in neither of the crops. To confirm that their charge retained them from being absorbed by plants, these antibiotics were tested again by El Gemayel (2018) on lettuce and radish grown in a nutrient solution (hydroponic system). The objectives of this research were to (1) evaluate the uptake of enrofloxacin, tylosin, and oxytetracycline by lettuce and radish grown in a nutrient solution; three different antibiotics used as human and animal medicine as well as for animal feed, (2) to find major accumulation sites of these antibiotics

in lettuce and radish crops grown in a hydroponic media, and (3) to evaluate the effect of these antibiotics on lettuce and radish growth. To our knowledge, this study is among the few done hydroponically and tackling the effect of antibiotic charge on its uptake by plants. Findings of this study would facilitate a better understanding of the uptake of antibiotics from soil or wastewater as well as the persistence of antibiotics in soil.

11.2 Materials and Methods

As per El Gemayel (2018) study, target antibiotic compounds were selected based on their extensive use in livestock and poultry in Lebanon and their physicochemical properties. Tylosin, oxytetracycline, and enrofloxacin, which belong to three different antibiotic classes, were included in the study (Table 11.1). They were obtained from a veterinary store in Lebanon. Individual stock solutions were prepared at 100 mg/L in distilled water and stored in the fridge at 4 °C for 30 days before starting the experiment. Towards the end of the experiment, 40 days after planting, the reagents McIlvain buffer, PBS, and 80% methanol were prepared and used for the extraction of oxytetracycline, tylosin, and enrofloxacin, as per the instruction of the manufacturer of the ELISA kits (ABRAXIS); they were stored in the fridge at 4 °C. About 2 h prior to usage they were removed and brought up to room temperature.

11.2.1 Experimental Design

Lettuce (*Lactuca sativa*) and radish (*Raphanus raphanistrum*) were the test crops, as all their parts are widely consumed raw, especially in salads. The lettuce was chosen as a leafy green, whereas the radish was chosen as a root crop (bulb). Radish and lettuce seedlings were transplanted into white plastic containers (length: 35 cm, width: 26 cm, height: 13 cm) filled with 6 L capacity; Hoagland nutrient solution (Table 11.2) was used as the growing media and was spiked with antibiotics at 0, 5 and 10 mg/L rates. The pH of the solution was adjusted to 6.5. The rates of antibiotics used in the experiment were within the range of commonly detected levels in wastewater and animal manure in Lebanon (Choueiri 2008). Each treatment was replicated three times in a CRD experiment. The experiment was harvested 1 month after transplant and roots, leaves, and bulbs were separated, weighed, and analyzed fresh for their antibiotics content.

The setting of the experiment was done in the greenhouse area of AUB, on the 13th of October 2017 where the air-conditioning was maintained at a temperature of 24 °C and plastic containers, air perforated tubing, pumps, and tubes were purchased new and used clean. The containers preparation followed an official experimental scheme, with defined treatments, replications, and controls (3 treatments × 3 replications = 9 containers per antibiotic). For the aeration of the liquid

Table 11.1 The physicochemical characteristics of the three antibiotics used in the study

Antibiotic/class	Family	Formula/ molecular weight	Charge	Usage[1]	Release to environment
Enrofloxacin/ Fluoroquinolone	Fluoroquinolone	$C_{19}H_{22}FN_3O_3$/ 359.39 g/mol	Positive at pH < 7 Negative at pH > 7[2]	Therapeutic use in cattle	Ciprofloxacin[2]
Oxytetracycline/ tetracycline	Polyketide	$C_{22}H_{24}N_2O_9$/ 460.434 g/ mol	Positive at pH < 7 Negative at pH > 7[3]	Therapeutic use, disease prevention, feed efficiency, and weight gain in chickens, swine, cattle, and sheep Growth promotion in swine, chicken, and cattle	Oxytetracycline[4]
Tylosin/ macrolide	Macrolide	$C_{46}H_{77}NO_{17}$/ 916.1 g/mol	Positive[5]	Disease prevention in chicken, cattle, and swine Growth promotion, weight gain, and feed efficiency in swine	Tylosin B (desmycosin), Tylosin A-Aldol, Dihydro-desmycosin, Tylosin D (relomycin)[6]

Source: (El Gemayel 2018), (Brown et al. 2017)[1], (Ötker and Akmehmet-Balcıoğlu 2005)[2], (Bansal 2013)[3], (Tian et al. 2017)[4], (Zhang et al. 2016a)[5], (Wegst-Uhrich et al. 2014)[6]

media, in every container, an aeration perforated tubing connected to a small electrical pump was installed. A perforated tray was placed onto every container as a cover. Each container received 3 physiologically similar seedlings of each crop (total of 6 plants in each container: 3 lettuces and 3 radishes).

Before transplanting, the seedling roots were washed thoroughly with distilled water to remove all solid particles. The plants were wrapped with a piece of cheesecloth to protect and keep the seedlings straight in the hole. The peripheries of the containers were closed with masking tape to prevent sunlight from entering into the container and reduce evaporation. The air-conditioning was kept on during the experiment at temperature 24 °C. After harvesting, the extraction and analysis of antibiotics was done immediately on fresh tissue of roots and leaves of both crops, and on the radish bulbs using ELISA kits and following the procedure as described by the manufacturer.

Table 11.2 Modified Hoagland nutrient solution used in the experiment

Macronutrients						
Compound	**Concentration of stock solution (M)**	**Concentration of stock solution (g/L)**	**Element**	**Concentration of each element in the solution (mg/L)**	**Volume of stock solution (mL/L)**	**Volume of stock solution (mL/6 L)**
KNO_3	1.00	101.10	N	224	6.0	36.0
$Ca(NO_3)_2.4H_2O$	1.00	236.16	K	235	4.0	24.0
$NH_4H_2PO_4$	1.00	115.08	Ca	160	2.0	12.0
$MgSO_4.7H_2O$	0.50	123.245	P, S, mg	62, 32, 24	2.0	12.0
Micronutrients						
Compound	**Concentration of stock solution (mM)**	**Concentration of stock solution (g/L)**	**Element**	**Concentration of each element in the solution (mg/L)**	**Volume of stock solution (mL/L)**	**Volume of stock solution (mL/6 L)**
KCl	50	3.728	Cl	1.77	1.0	6.0
H_3BO_3	25	1.546	B	0.27		
$MnSO_4.H_2O$	2.0	0.338	Mn	0.11		
$ZnSO_4.7H_2O$	2.0	0.575	Zn	0.13		
$CuSO_4.5H_2O$	0.5	0.125	Cu	0.03		
$(NH_4)_6Mo_7O_{24}.4H_2O$	0.5	0.081	Mo	0.05		
Fe-EDDHSA	40	13.844	Fe	1.12	2.0	12.0

Source: El Gemayel (2018), Epstein and Bloom (2005)

11.2.2 Nutrient Solution

Hoagland nutrient solution was used for growing crops at pH value of 6.5. Its available nutrient content is represented in Table 11.2.

11.2.3 Extraction Methods

The target compounds were extracted and measured following a well-defined procedure for all treatments (El Gemayel 2018; Youssef 2016). Tissue extraction took place directly after harvest; each treatment with its respective level was placed into a labeled paper bag. The total weights of plants were taken and also the weights of roots, leaves, and bulbs were measured separately. The plants were washed well with distilled water and blotted to dry using clean tissue paper. The plant materials (roots, leaves, and bulbs) were chopped into small pieces using a knife and a representative sample (2 g roots, 2 g bulbs, and 6 g leaves) was extracted at a ratio of 1:5 (1 g plant tissue to 5 mL solvent; 6 times dilution). Using an ELISA grinder, until all the plant material was fully ground and formed a suspension, uniform grinding and mixing are trivial to get representative samples and accurate analytical results. In a 50-mL labeled falcon tube, the suspension was placed and vortexed for 2 min for homogenization. Each mixture was filtered into its respective volumetric flask using F40 Whatman filter papers. The filtrate was then poured into a new labeled falcon tube and placed in the refrigerator at a temperature 4 °C.

11.2.4 Tissue Analysis

According to the procedure of the manufacturer, all the extracted samples were analyzed fresh for enrofloxacin, tylosin, and oxytetracycline using ELISA test kits brought from ABRAXIS: enrofloxacin (product no. 522511), tetracycline (product no. 52254BA), and tylosin (product no. 52256B). The procedure adapted is described by the kit manual. The kits include 96 wells coated plate with enrofloxacin, tylosin, or oxytetracycline and the standards were supplied by the manufacturer.

11.2.5 Statistical Analysis

Data was pooled and analyzed using the General Linear Model (GLM) procedure of the Statistical Analysis System (SAS) version 9, year 2008. Means were compared with Student-Newman-Keuls Test (SNK) where applicable. The design was a com-

plete randomized design with three treatments consisting of antibiotic concentrations in nutrient solution (0, 5 and 10 mg/Kg). Each treatment was replicated three times where three distinctive plants of lettuces and radishes were transplanted into the same container. Also, measurement of plant weights (total, roots, leaves, and bulbs) were recorded.

11.3 Results and Discussion

11.3.1 Enrofloxacin Uptake

The results (Fig. 11.1) show that in both crops there is a significant difference at $P < 0.05$ between the control and the two rates of enrofloxacin (5 and 10 mg/kg), but there is no significant difference between enrofloxacin concentrations in radish and lettuce edible parts at 5 and 10 mg/kg (distribution ratio of root: bulb: leaf is about 1:1:1).

Liu et al. (2013) grew *Phragmites australis* in water and reported a greater amount of ciprofloxacin in tissue at higher levels of the drug. The accumulated concentration of enrofloxacin at 5 and 10 mg/kg nutrient solution in lettuce leaves was 48.99 and 69.79 µg/kg and in radish bulbs 63.04 and 72.31 µg/kg, respectively (Fig. 11.1). Referring to the Maximum Residue Level (MRL) range 15.4–50 µg/kg (Yu et al. 2018), in lettuce leaves at 5 mg/Kg of nutrient solution, enrofloxacin falls within the range whereas at 5 mg/Kg of nutrient solution in radish and at higher antibiotic level for both crops, its accumulation level is greater than the upper MRL

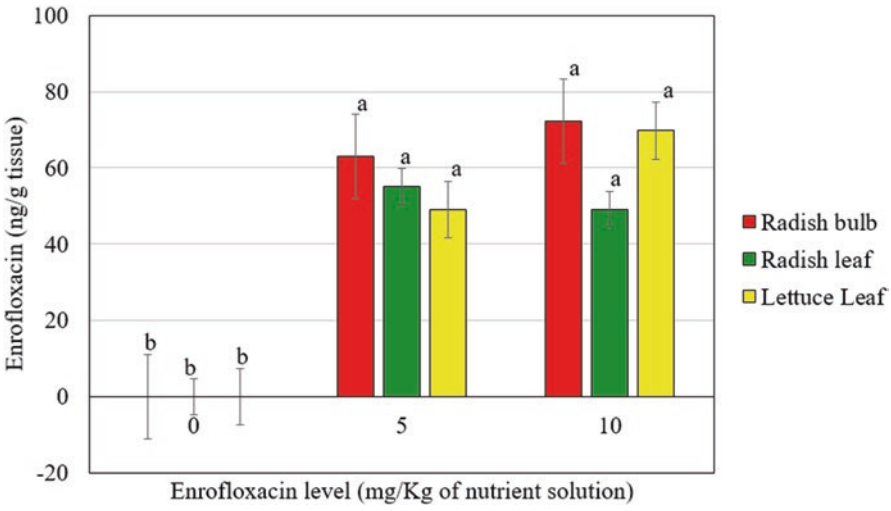

Fig. 11.1 Concentration of enrofloxacin in radish and lettuce edible parts. Mean values followed by different superscripts differ significantly at $P < 0.05$. *Source*: Modified from El Gemayel (2018)

level of Yu et al. (2018). Thus, contamination of nutrient solution with 5 mg/kg or higher of enrofloxacin is bad to human consumption and may reduce growth of lettuce and radish too.

11.3.2 Tylosin Uptake

The results (Fig. 11.2) show that there is a significant difference between the control and the other two levels of tylosin (5 and 10 mg/kg). Despite the mild increase in the drug accumulation in roots, leaves, and bulbs at 10 mg/kg, no significant increase was observed between the two tylosin concentrations. Therefore, as the concentration of tylosin increased from 5 to 10 mg/kg in the nutrient solution, its accumulation did not significantly increase in tissue. Tylosin was present in high levels in lettuce and radish tissues, and it accumulated the most in the edible parts: bulb of radish and leaves of lettuce, varying from 350 to 410 µg/kg at 5 and 10 mg/kg rates, respectively (Fig. 11.2). Therefore, regardless of the tylosin level in the nutrient solution, its accumulation in radish edible parts greatly exceed the MRL range 15.4–50 µg/kg (Yu et al. 2018). Similarly, this is in accordance with our results of lettuce grown in a nutrient solution spiked with tylosin; both lettuce roots and leaves accumulated tylosin at concentrations greater than 300 ng/g (El Gemayel 2018) (Fig. 11.3).

In lettuce leaves, significant increase in the uptake and accumulation of tylosin was observed between the control and the other two treatments. Nevertheless, the accumulated concentration of tylosin at 10 mg/kg in leaves is lower than that at

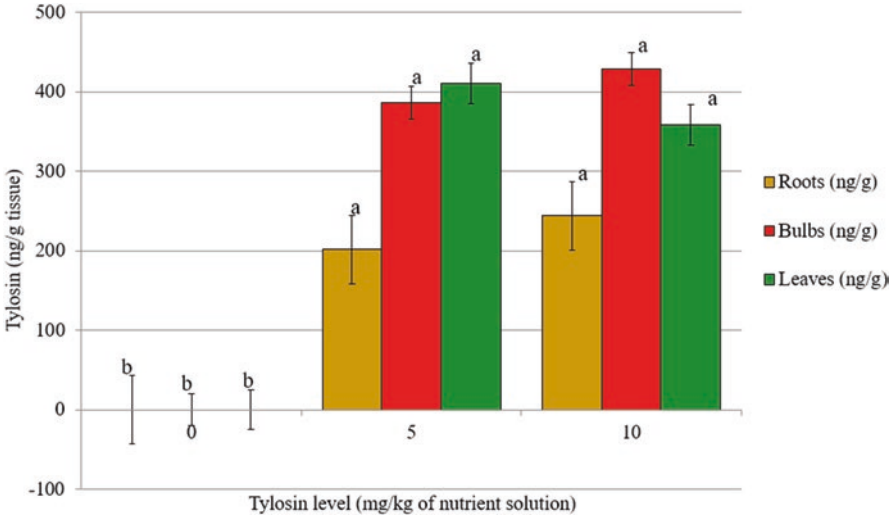

Fig. 11.2 Concentration of tylosin in radish grown hydroponically. Mean values followed by different superscripts differ significantly at $P < 0.05$. *Source*: (El Gemayel 2018)

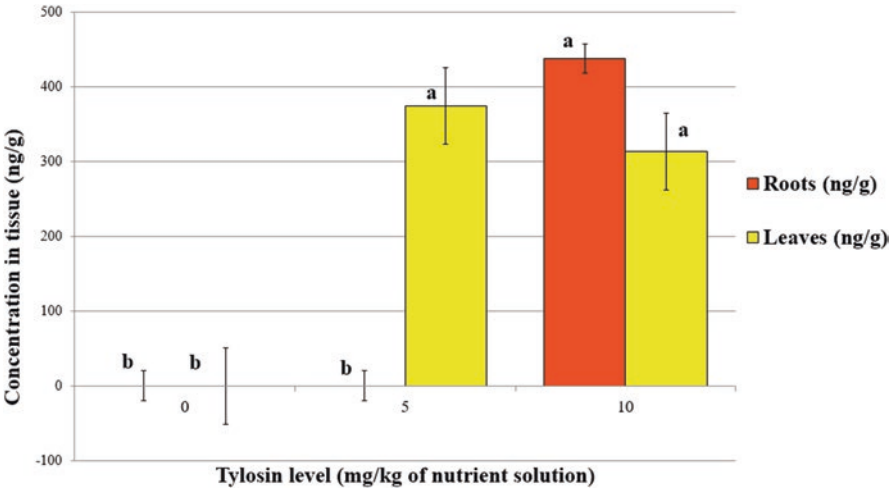

Fig. 11.3 Concentration of tylosin in lettuce grown hydroponically. Mean values followed by different superscripts differ significantly at $P < 0.05$. *Source*: (El Gemayel 2018)

5 mg/kg, suggesting that at a higher antibiotic concentration tylosin is not translocated to the leaves. A previous study done by Youssef and Bashour (2017) revealed that the accumulation of tylosin in lettuce grown in soil with manure was not significant at levels 0, 2.5, 5, and 10 mg/kg, and in radish, the translocation of tylosin from roots to leaves readily occurred at all levels. Kang et al. (2013) justifies the absence of tylosin absorption by onions, cabbage, and corn grown in soil to be due to the large molecular size of tylosin. Our results demonstrate that both lettuce and radish grown in nutrient solution accumulated tylosin: 60 and 6 times more than in soil, respectively. Consequently, it can be deduced that the lack of tylosin accumulation in crops grown in soil media could be due to its positive charge rather than large molecular size; so it will be held over the surface of the negatively charged clay particles.

The data in Table 11.1 shows that the lowest accumulated concentration of tylosin in radish and lettuce plant materials was 200 ng/g; hence, at both levels, the accumulated concentration is much higher than the MRL upper limit (15.4–50 µg/kg; Yu et al. 2018).

11.3.3 Oxytetracycline Uptake

The results (Figs. 11.4 and 11.5) show that, in the edible parts, leaves of lettuce and bulbs of radish, there is a significant difference in the concentration of oxytetracycline between the control, 5 and 10 mg/kg levels. This indicates that irrespective of the concentration, oxytetracycline was absorbed and accumulated in lettuce and radish plants (20–48 ng/g) at relatively much higher concentrations than when these

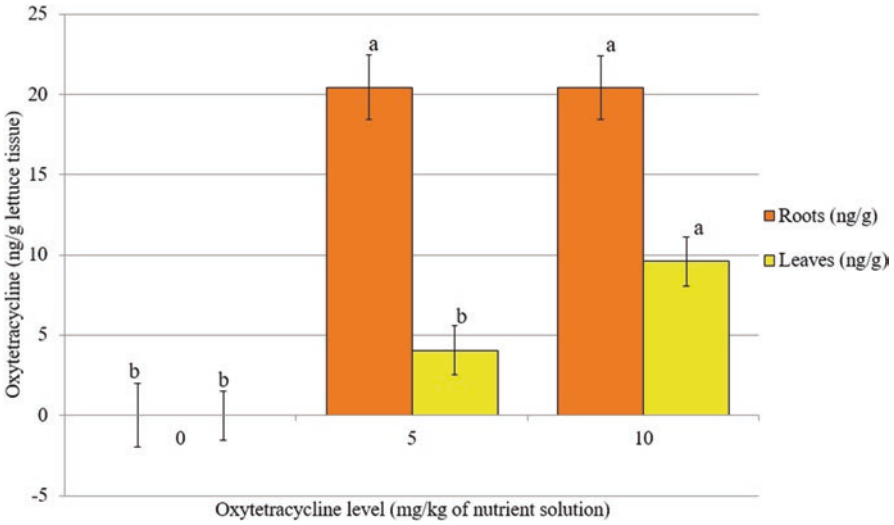

Fig. 11.4 Concentration of oxytetracycline in lettuce roots and leaves grown hydroponically. Mean values followed by different superscripts differ significantly at $P < 0.05$. *Source*: Modified from El Gemayel (2018)

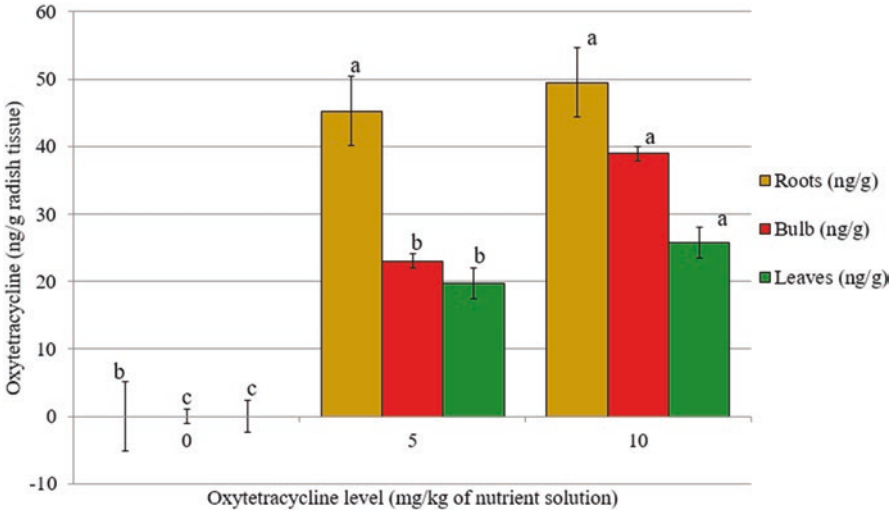

Fig. 11.5 Concentration of oxytetracycline in radish roots, bulbs, and leaves grown hydroponically. Mean values followed by different superscripts differ significantly at $P < 0.05$. *Source*: (El Gemayel 2018)

plants were grown in soil spiked with similar levels of oxytetracycline (4–6 ng/g of tissue). This could be due to its adsorption to the surfaces of the negatively charged soil particles such as soil oxides, organic matter, and clays (Youssef 2016). Compared to leaves and bulbs, roots were the ones that accumulated the highest levels of oxytetracycline in both lettuce and radish crops (Figs. 11.4 and 11.5). Liu et al. (2013) reported that roots stored the highest amount of antibiotics, followed by leaves and stems. The sequence of accumulation of oxytetracycline in radish was as follows: roots > bulbs > leaves (Fig. 11.5). The highest accumulation of oxytetracycline in roots can be explained by the fact that it was adsorbed to the charge within the roots, hence not translocated easily to the leaves.

In lettuce and radish plants, the concentrations of oxytetracycline in the tissue at 10 mg/kg were higher than that of 5 mg/kg, but the increase was not always significant. The increase of oxytetracycline in roots was not significant, whereas leaves and bulbs significantly accumulated more of the drug at 10 mg/kg. This demonstrates that as the antibiotic concentration increases in the nutrient solution, its uptake and accumulation increases as well. In their study, Liu et al. (2013) indicated that there is a positive correlation between the antibiotic level and its accumulated concentration: in *Phragmites australis*, the total stored content of oxytetracycline in the crop at 1000 μg/L was 6901 ng/g dry weight whereas at 0.1 μg/L it was 165 ng/g dry weight. Liu et al. (2013) also demonstrated that high antibiotic levels (greater than 10 μg/L) exerted a toxic effect on root activity. They also reported that the exposure of the crop to higher sulfamethazine, ciprofloxacin, and oxytetracycline concentrations led to a greater amount in the crops grown hydroponically.

Additionally, in all lettuce and radish plant parts, the highest storage of oxytetracycline is below 50 μg/kg (Fig. 11.5), hence falling in the interval of the estimated acceptable MRL limits (15.4–50 μg/kg) (Yu et al. 2018).

11.3.4 Crop Biomass and Phytotoxicity

The results of analysis (Figs. 11.6 & 11.7) show that the three antibiotics led to a decrease in the yield of both crops, but not all reductions were at the same rate. Enrofloxacin and oxytetracycline have similar significant negative impacts on lettuce and radish growth; both affected their growth the most. In lettuce, compared to the control, enrofloxacin caused a 71.36% decrease, oxytetracycline 65.97%, and tylosin 17.89%; tylosin only had a negative nonsignificant impact on lettuce roots. Referring to the accumulation of antibiotics, the highest concentration was in lettuce roots; hence the lowest decrease in growth was observed in leaves, 68.66% enrofloxacin, 64.81% oxytetracycline, and 14.28% tylosin, and 84.54% enrofloxacin, 72.03% oxytetracycline, and 37.29% tylosin in roots. This agrees with the statement of Nickell and Finlay (1954) that other antibiotics such as neomycin, netropsin, and polymyxin inhibited growth depending on the concentration applied, the duration of the growth period, and the experimental conditions. At four different levels

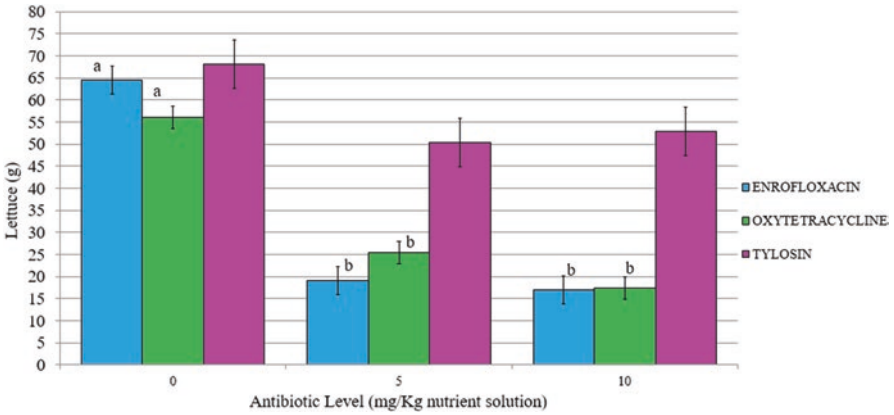

Fig. 11.6 Effect of enrofloxacin, oxytetracycline, and tylosin on lettuce grown hydroponically. Mean values followed by different superscripts differ significantly at $P < 0.05$. *Source*: Modified from El Gemayel (2018)

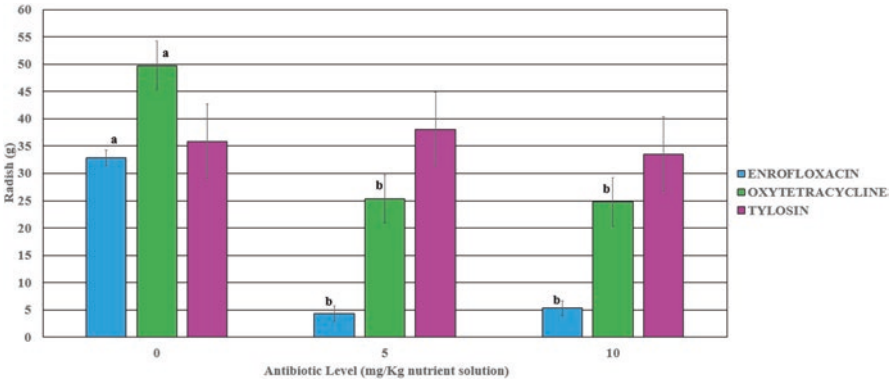

Fig. 11.7 Effect of enrofloxacin, oxytetracycline, and tylosin on radish grown hydroponically. Mean values followed by different superscripts differ significantly at $P < 0.05$. *Source*: Modified from El Gemayel (2018)

(1, 5, 10, and 20 mg/kg), neomycin decreased plant weight by 10, 85, 90, and 95%, respectively.

Similar to lettuce, the radish results of growth showed that tylosin caused a slight nonsignificant decrease in weight, followed by oxytetracycline and enrofloxacin which caused a drastic effect on radish growth. In Fig. 11.7, compared to the control's average plant weight, radishes grown in nutrient solution spiked with tylosin, oxytetracycline, and enrofloxacin decreased by 9%, 37%, and 88%, respectively. Moreover, the bulb and the root of the radish were the most affected parts by the three antibiotics: a decrease of 93%, 58%, and 26% with enrofloxacin, oxytetracycline, and tylosin treatments, respectively. Azanu et al. (2016) asserts that antibiotics primarily accumulate in roots, thus affecting their growth. Nevertheless, leaves

were the least affected parts. Compared to the control, tylosin caused a decrease of 19%, oxytetracycline 33%, and enrofloxacin 87%. Consequently, the decreasing order in which these antibiotics affect radish weight negatively is: enrofloxacin > oxytetracycline > tylosin (El Gemayel 2018). Ahmed et al. (2015) findings show that tetracycline added to soils at 5, 10, and 20 mg/kg inhibited the growth of cucumber, lettuce, and tomato.

Visually, enrofloxacin appeared to have the greatest phytotoxic effect on both radishes and lettuces growth. Growth was weak, roots short, and leaves thin, chlorotic, and small. As the concentration of enrofloxacin and length period increased, the phytotoxicity was more pronounced (El Gemayel 2018). The results of Migliore et al. (2003) were similar to our results, stating that at 100 μg/L of enrofloxacin caused a toxic and hermetic effect; the toxic effect increased with time and was observed on the leaves of bean and radish as well as in the primary roots of lettuce and cucumber. They also suggested that crops grown in a high enrofloxacin concentration (5000 μg/L) partially metabolize the latter into ciprofloxacin.

In the case of oxytetracycline and tylosin, even though lettuce grown at 5 mg/Kg of tylosin looked smaller, on the scale, the difference was not significant, whereas in oxytetracycline it is. This agrees with Liu et al. (2009) who stated that among tetracyclines and tylosin, tylosin was the one which detained the lowest toxicity, specifically on cucumber and rice seeds.

11.4 Conclusion

Lettuce and radish crops were grown hydroponically and administered with enrofloxacin, tylosin, and oxytetracycline, three antibiotics widely used in livestock and poultry production in Lebanon. The accumulation, effect, and physicochemical characteristics of these antibiotics were studied using ELISA analysis. The results of this study clearly demonstrated that crops grown in a nutrient solution containing antibiotics are capable of absorbing different types of antibiotics even if the latter was not absorbed when grown in soil. Enrofloxacin accumulated in lettuce and radish edible parts (leaves and bulbs) at an average of 50 ng/g of tissue and translocated uniformly all over the plant a ratio of root to bulbs to leaves at 1:1:1. Compared to plants grown in soil, both tylosin and oxytetracycline were absorbed at higher rates by lettuce and radish. Tylosin is the antibiotic that accumulated the most in both crops at an average of 344 ng/g of lettuce leaves, 384 ng/g and 407 ng/g in radish leaves and bulbs, respectively. Oxytetracycline was absorbed by lettuce and radish grown in nutrient solution, implying that its charge is a main reason why it was not absorbed by the same plants grown in soil. It accumulated the most in lettuce roots at an average of 20 ng/g and then translocated to leaves at an average of 7 ng/g. In radish bulbs and leaves, it accumulated at an average of 31 and 23 ng/g, respectively.

For growth, enrofloxacin reduced lettuce crop weight by 72% and the radish overall weight by 88%. Visually, as the enrofloxacin level in the nutrient solution increased, the phytotoxicity was more pronounced on both crops. Oxytetracycline

caused an overall growth decrease of 66% on lettuces and 37% on radishes. On the contrary to enrofloxacin and oxytetracycline, tylosin lowered the growth, but the decrease was not significant in the concentration applied in the study.

More research should tackle the effect of the antibiotics physicochemical properties on their absorption and effect on plants. The mechanism of antibiotics absorption and translocation in plants should also be assessed thoroughly as well as their effect on human health.

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Chapter 12
Epidemiological, Ecological, and Public Health Effects of Antibiotics and AMR/ARGs

Sadia Shakoor, Zahra Hasan, and Rumina Hasan

Abstract Worldwide morbidity and mortality caused by infectious diseases is high, mandating high rates of antibiotic use among humans and animals. Antibiotics of anthropogenic origin often contaminate the environment. The arising ecological pressure results in alteration of bacterial "biomes," high resistance rates in environmental microorganisms, and increase in the gene pool which contributes to antibiotic resistance. A number of such antibiotic resistance genes are carried on mobile genetic elements that can easily be exchanged between bacteria. The ecological net effect is an expanding population of resistant organisms contributing to spread of antibiotic resistance in both the clinical and the nonclinical environments. In nonclinical environments, antibiotics upset the natural symbiotic balance between microorganism and macroorganism communities. In clinical environments, while therapeutic antibiotic adverse effects are easily observed, the, impact of subinhibitory concentrations of antimicrobials on human health are less apparent and require investigations. In summary, impact of antimicrobial resistance is extensive, threatening not just health and food safety but also our environment. Actions are thus required to both safeguard efficacies of antimicrobial agents, and also to protect the environment from damage by them.

S. Shakoor
Department of Pathology & Laboratory Medicine, Department of Pediatrics & Child Health, Aga Khan University, Karachi, Pakistan
e-mail: sadia.shakoor@aku.edu

Z. Hasan
Department of Pathology & Laboratory Medicine, Aga Khan University, Karachi, Pakistan
e-mail: zahra.hasan@aku.edu

R. Hasan (✉)
Department of Pathology & Laboratory Medicine, Aga Khan University, Karachi, Pakistan

Faculty of Infectious and Tropical Diseases, London School of Hygiene and Tropical Medicine, London, UK
e-mail: rumina.hasan@aku.edu

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The development of antimicrobials changed the course of medicine. With the availability of antimicrobials many infectious diseases previously associated with considerable morbidity and mortality could be overcome. Modern medicine progressed by leaps and bounds secure in the knowledge that infections would be managed by antimicrobial agents. However, this confidence was challenged by two things: slowing down of the pipeline for new antimicrobials (Koulenti et al. 2019; Durand et al. 2019) and the development of antimicrobial resistance.

Of the currently known antibiotics a number are synthetic compounds, but most are natural products of microorganisms. As such antimicrobials, and indeed resistance to them, are both part of normal microbial defense mechanisms (Durand et al. 2019). Antimicrobial resistance however, is not a new phenomenon. Phylogenetic of OXA genes (that encode beta-lactamases) have shown that much of the diversity in these genes is the result of ancient events, and that the OXA genes were mobilized to plasmids from chromosomes millions of years ago (Barlow and Hall 2002). Genes that confer antimicrobial resistance have been identified in samples of permafrost dating back thousands of years (D'Costa et al. 2011; Kashuba et al. 2017). Environmental sample of Lechuguilla cave in New Mexico, isolated for more than four million years, yielded 93 bacterial strains of which several species were resistant in vitro to three or more antibiotics classes (Bhullar et al. 2012). Antimicrobial resistant genes (ARGs) have been isolated from gut microbiomes of an ancient mummy (Lugli et al. 2017) and from antibiotic naïve glaciers in the Antarctic (Van Goethem et al. 2018). In recent times though, antimicrobial resistance has come to be viewed as one of the biggest health threats faced by mankind (O'Neill 2016).

12.1 Epidemiological Impact of Antibiotics and ARGs

12.1.1 Pharmacoepidemiology and Evolution of Antibiotic Use

The first antimicrobials to be made available commercially included arsphenamine, a chemical compound discovered by Paul Ehrlich marketed in 1911 as Salvarsan@ and Mapharsen@ for the treatment of syphilis (Gensini et al. 2007). This was followed by sulphanilamide marketed as Protonsil@ in 1935 (Lewis 2013). Penicillin though discovered by Fleming in 1928 was made commercially available in 1940 (Durand et al. 2019). Since then several synthetic or naturally derived molecules were explored and a number added to the antimicrobial armamentarium. The global expansion of pharmaceutical industry, reduced cost of production in particular

following expiry of drug patents, together with increasing demands from health care providers and patients, allowed for an exponential increase in global availability of antimicrobials. In many parts of the world with limited access to health care antimicrobials came to be used as first-line agents for a number of conditions, and in countries with weak health systems, made available over the counter. Microorganisms develop antimicrobial resistance either by de novo mutations under clinical antibiotic selection or through acquisition of mobile genes from other bacteria that have evolved resistance following exposure to antimicrobials at some earlier point in time. The increasing and unregulated exposure to antimicrobials (which in many parts of the world included poor quality drugs) therefore selected for organisms that were resistant to them. Patients treated with antibiotics became colonized with resistant organisms carrying them as part of their microbiome. Inadequate infection control measures and poor access to hygiene allowed the spread of these resistant bacteria initially within health care facilities, but ultimately within the communities as well.

In parallel to human use, antimicrobials also came to be recognized as being valuable in protecting farmed animals against infections leading to increased productivity; antifungals were found to be useful in protecting crops seeds and bulbs safeguarding the farmers' economic interest and ensuring affordable protein and food safety for many. To the extent that currently agricultural antibiotic use exceeds human consumption (Van Boeckel et al. 2015), excessive antimicrobial use in food production contributed not only to selection of antimicrobial resistance in the environment, but to its spread through the food chain (Kirchhelle 2018).

When exploring the spread of antimicrobial resistance another important link to be considered is the environment. Approximately 50–90% of antibiotics administered to humans and animals are reportedly excreted via urine and feces, as a mixture of parent drug and metabolite forms (Kümmerer 2009). Pharmaceutical industry too discharges waste (which includes waste containing antimicrobial agents) into sewage. The antimicrobial compounds thus discharged may associate with sewage sludge, or be released to rivers. Sludge-associated drugs will enter agricultural systems when the sludge is used as a fertilizer, or when wastewaters are used for irrigation (Kinney et al. 2006). Thus, significant levels of active drugs end up in the environment, where they may persist in soil and aquatic ecosystems (Kümmerer 2009; Wellington et al. 2013) contributing to generation of antimicrobial resistance among the environmental microbiota (including amongst microbiota of health care facilities), which in turn passes back to humans and to animals and into the food chain (Chamosa et al. 2017; Ekwanzala et al. 2018). Additionally, human/animal waste contains microbiome of those exposed to antimicrobials, antimicrobial resistant bacteria, and/or antimicrobial resistance genes, the resistome (Van Schaik 2015; Proia et al. 2018). Wastewater containing such resistomes and used for either agriculture or indeed for drinking too represents a significant risk for spreading bacteria and antimicrobial resistance among both humans and animals (Lamba et al. 2018; Bougnom et al. 2019).

12.1.2 Economic Epidemiology

The significance of and risk from AMR is enormous. It is estimated that by 2050, ten million lives a year and a cumulative 100 trillion USD of economic output are at risk due to the rise of drug-resistant infections (O'Neill 2016). A recent report from the Organization for Economic Co-operation and Development (OECD) predicts that 2.4 million people in Europe, North America, and Australia will die from infections with resistant microorganisms in the next 30 years and that such infections could cost up to US$3.5 billion per year (OECD 2018). Analysis of European Antimicrobial Resistance Surveillance Network (EARS-Net) 2015 data suggests that the burden of infections due to antibiotic resistance organisms has increased between 2007 and 2015 and was similar to the cumulative burden of influenza, tuberculosis, and HIV (Cassini et al. 2019). Yet antimicrobial usage continues to increase. A recent study analyzing the trends of antibiotic consumption in 76 countries from 2000 to 2015 reports that antibiotic consumption, expressed in defined daily doses (DDD), increased 65% (21.1–34.8 billion DDDs), and the antibiotic consumption rate increased 39% (11.3–15.7 DDDs per 1000 inhabitants per day). The study further found that the increase was in particular driven by low- and middle-income countries (LMICs), where rising consumption was correlated with gross domestic product per capita (GDPPC) growth (Klein et al. 2018).

In response to these concerns at its sixty-eight World Health Assembly in 2015 World Health Organization endorsed a global action plan to tackle antimicrobial resistance (World Health Organization 2015) One year later the United Nations General Assembly (UNGA) termed antimicrobial resistance as one of the biggest threats to global health endangering major priorities including human development (World Health Organization 2016). In response to these concerns countries were called upon to develop their national AMR action plans encompassing a One Health agenda. The challenge remains in prioritizing implementation and balancing cost benefit of each component. The Organisation for Economic Cooperation and Development (OECD) report recommends five simple measures to reduce AMR: hand and environmental hygiene, antibiotic stewardship, rapid testing to distinguish bacterial from viral infections, delayed antibiotic prescriptions, and mass media campaigns. The report further estimates that in OECD countries this could be achieved by investing as little as US$2 per person per year and could avoid 75% of deaths caused by infections with resistant microorganisms (OECD 2018; Hofer 2019). In contrast, a multivariate analysis of 2008–2014 data from 103 countries by Collignon et al. (2018) conclude that given the importance of resistant bacteria and of resistance genes in spreading AMR, reducing antibiotic consumption alone would not be sufficient for the problem and call for improved sanitation, clean water, good governance, increased investment in public health care, and the importance of regulating the private health sector (Collignon et al. 2018; Hofer 2019). Equally, curbing antimicrobial use in the animal and agricultural sector remains a challenge. A number of wealthy countries like Korea, Japan, the USA, and EU

member states have managed to stall decades of increasing antibiotic use and establish surveillance systems. However, in the absence of long-term funding commitments and international controls, antibiotic stewardship remains patchy in middle- and low-income countries. As such regulating food supply chains and reducing antibiotic consumption in the farm and agricultural industry across the world will require global solutions that are subject to transparent evaluation (Kirchhelle 2018).

12.2 Ecological Impact of Antibiotics and ARGs

Ecosystems are complex structures with significant heterogeneity with respect to geography, time, and components of the environment and animate species involved. It follows therefore that ecological impact of antibiotics and ARGs should vary within different ecosystems. With what little knowledge and evidence is available however, it can be surmised that antibiotics have a significant impact on micro- and macroecosystems and should be treated as ecotoxins.

Despite its ubiquity, microbial ecology is understudied. Since much was unknown about microbial ecology and its predictors before the beginning of this decade, relatively few planned studies have evaluated the impacts of antibiotics, antibiotic residues, and ARGs on various ecosystems.

12.2.1 Impact on Natural Ecosystems

12.2.1.1 Importance of Microbial Diversity in Terrestrial and Aquatic Ecosystems

Microbial diversity in the environment affects biogeochemical cycles (Panizzon et al. 2015), which in turn are critical to the sustainability of macroecosystems. Although very little is known about diversity of microbes owing, in part, to the inherent difficulty of studying non-cultivable microbes, it is well accepted that microbial abundance and diversity in natural ecosystems is essential to their productivity (Prosser et al. 2007).

Antibiotics from anthropogenic (man-made) sources in the environment affect bacterial diversity by inducing a selective pressure, causing a shift in the natural competition toward those microbes that have the ability to withstand this selective pressure. This change in the structural and/or functional composition of microbial communities can affect nitrogen cycles, nutrient cycles, biodegradation in nature (through affecting the proportion and function of anaerobic digesters), and other natural processes that are crucial for optimal functioning of other ecosystems (Blaser et al. 2016). Any effect on microorganism communities therefore extends to indirect impact on larger ecosystems.

12.2.1.2 Impact on Agroecosystems

Ecological risk assessment of the impact of pesticides on plant communities, alluvial soil, and related aquatic environments is a prime example of the effect of toxins on these larger ecosystems (Khetan and Collins 2007). Since antibiotics affect bacterial communities which in turn affect plant rhizospheres etc., a similar conceptual framework applied to antibiotics as plant toxins has been favored by Brandt et al. (2015) and Grenni et al. (2018). Several studies have demonstrated the effect of small sub-inhibitory concentrations of antibiotics and antibiotic residues on bacterial community and rhizosphere structure which can potentially lead to low productivity of agroecosystems with significant agronomic impact (Revellin et al. 2018; Topp et al. 2017). Studies on plants have also identified measured environmental concentrations (MECs) and the predicted no-effect concentrations (PNECs)/non-observed effect concentrations (NOECs) of major antibiotics (Park and Choi 2008; Santos et al. 2010) as toxicological endpoints. Further research into more antibiotic classes and similar limits for different ecosystems is warranted.

ARGs present in soil and agroecosystems impact constituent microbial populations through transmission into different bacterial species. It has been proposed that ARGs in bovine manure can make way into the farm environment with subsequent risk of transmission to consumers via farm produce (Doyle et al. 2017).

12.2.1.3 Impact on Aquatic Ecosystems

Through direct and indirect means, anthropogenic (human and veterinary use) antibiotics enter the natural aquatic environment. Presence of antibiotics and their residues in these aquatic microcosms, wastewaters, as well as in natural marine and freshwaters impacts microbial diversity as well as aquatic animal species and their functions at different trophic levels (Kümmerer 2009).

Aquatic algal populations are affected by the presence of macrolides, tetracyclines, sulfonamides, and quinolones in the order of micrograms per liter (Santos et al. 2010). Both acute and chronic toxic effects have been observed. Macrolides and tetracyclines also impact photosynthesis in cyanobacteria and also disrupt the balance between these beneficial bacteria and toxic weeds, increasing populations of the latter (Pomati et al. 2004). Crustaceans also exhibit chronic toxicity as a result of macrolide and quinolone accumulation (Yamashita et al. 2006). Although studies show no observable effects on fish populations (Isidori et al. 2005), an effect on algal species is thought to eventually affect fish by impacting the aquatic food chain.

Water reuse is also impacted by the presence of antibiotics, antibiotic residues, and ARGs. Microbial water quality is dependent on the presence and quantitation of microbes and absence of pathogenic bacteria. As antibiotics directly affect bacterial compositions, their presence in water ultimately impacts water reusability (Liu et al. 2016).

Aquatic environments are ideal for horizontal gene transfer from one bacterial species to another, thus facilitating spread of antibiotic resistance. Presence of antibiotic concentrations favors survival of organisms carrying ARGs and also perhaps the presence, dispersal, and transmittance of mobile genetic elements (MGEs) (Martínez et al. 2015).

12.2.1.4 Impact on Human and Animal Microbiomes

Evidence on the role of the human microbial ecosystem—the human microbiome—in human health and disease is vast. Diversity and composition of the human microbiome are currently of great interest (Costello et al. 2012). Antibiotic use by humans, whether therapeutic or prophylactic, or resulting from contamination of food, water, or presence in other products, affect the microbiome through modification of this diversity and composition. Microbiomes are present on the human skin, epithelialized orifices, the respiratory tract, the gastrointestinal tract, and the genitourinary tract. Although normal gastrointestinal microbiota are sufficiently resilient to counter major ecological shifts in species, repeated courses of antibiotics are thought to lead to major and perhaps more persistent changes in an individual's microbiome (Jakobsson et al. 2010). Changes thereof can potentially lead to a shift in the microbiome toward less resilient bacteria more easily overcome by pathogens, and also putatively a larger microbial resistome.

Understanding of the human-microbial symbiosis has now evolved to an extent that ecological paradigms have been applied to clinical situations. Transplantation of feces from a healthy donor (therefore with a "healthy" microbiome) into patients with *Clostridium difficile* colitis has not only revolutionized the management of this disease, but has also impacted the understanding of the role of microbiome in human health (Bakken et al. 2011).

Antibiotics are common stressors also of the animal microbiome, inducing a so-called "dysbiotic" state (Zaneveld et al. 2017) leading to possible animal health effects. Presence of therapeutic and subtherapeutic (through indirect exposure) concentrations of antibiotics in animal gut has been shown to affect quantities of ARGs (Field and Hershberg 2015).

The presence of resistance genes against trimethoprim, a synthetic antibiotic in human, mammalian, and farm soil microbiomes, alike suggests that some exchange interface exists between these ecosystems, suggesting a much wider impact (Fitzpatrick and Walsh 2016). As applications of metagenomics, metabolomics, and proteomics enable more accurate studies on composition and diversity of the human and animal microbiome, the ecological impact of antibiotics, possible toxicological endpoints, and the resulting overarching health effects will be better understood.

12.2.2 Impact on Artificial Ecosystems

Man-made environments have their own unique ecosystems, and individuals and surfaces within these built environments have their own microbial ecosystems. Metagenomic approaches to some of these ecosystems have been applied recently, and although antibiotic concentrations have not yet been studied widely, evidence from natural ecosystems suggests that antibiotics within these environments also affect microbial ecosystems and constituent ARGs. Among these ecosystems which affect human life significantly and have their own microbiomes are food production facilities (e.g., food manufacturing/packaging plants) (Doyle et al. 2017), aquaculture ecosystems, intensive urban farming, horticulture, and hospitals. The recently initiated Hospital Microbiome Project (Westwood et al. 2014) is likely to reveal further aspects of the built hospital environment and surfaces which can lead to improved understanding of microbial ecology therein. It has been postulated that the presence of antibiotic-impregnated surfaces in hospitals can lead to increase in resistance among resident microbial flora (Strachan et al. 1991; Caselli et al. 2016). Furthermore, patients within hospitals may be considered individual microbial ecosystems where antibiotics significantly impact the microbiome and ARGs (Lofgren et al. 2016).

12.3 Public Health Impact of Antibiotics and ARGs

Antibiotics have revolutionized the treatment of infectious diseases. While this has tremendously impacted the health of those suffering from disease, there are other consequences of using antibiotic that are fast becoming clear and relevant in recent years.

Consequences of antibiotic use may be direct, resulting from intentional therapeutic or prophylactic use, or indirect, due to the presence of subtherapeutic concentrations in the environment, food, and water. Both acute and chronic effects of antibiotic use have been observed at the individual and population level in humans. These result from three main mechanisms or drivers: an increasing resistance gene pool or "resistome," alterations in microbiomes, and direct tissue toxicity (Fig. 12.1).

12.3.1 Consequences of Expanding Resistome

The hidden resistome in the animate and inanimate environment is a direct public health threat due to its causal association with clinical AMR. Spread of environmental antibiotic resistance genes has resulted in very high clinical resistance among pathogens, severely restricting treatment options for life-threatening infections. Attributable mortality resulting from infections due to resistant pathogens is high in critically ill patients (De Kraker et al. 2011), and also contributes to high health care

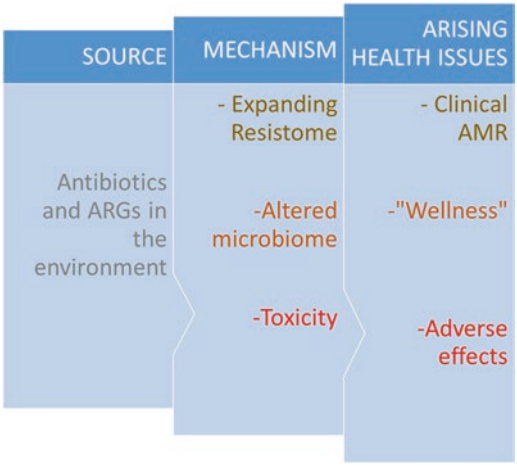

Fig. 12.1 Mechanisms and health effects of antibiotic and ARG contamination in the environment

costs due to prolonged hospital stays alone (De Kraker et al. 2011). By 2050, it is predicted that AMR will cause around 4 million deaths in Asia alone, and 10 million deaths per annum globally (O'Neill 2016).

AMR has also forced older antibiotics and some repurposed drugs as antibiotics into clinical usage. Repurposed drugs and older antibiotics such as polymyxins have uncertain dosages pharmacokinetics, and adverse effect profiles (Palomino and Martin 2012). Clinical use of these drugs therefore often results in toxicities that require further medical interventions, burdening the individual, health care facilities, and health systems.

Intensive management of drug-resistant infections also necessitates investment in diagnosis and treatment of AMR pathogens. This increases health expenditure, and competes with investments in preventive health programs especially in resource limited settings.

12.3.2 Consequences of an Altered Microbiome

As highlighted previously, environmental antibiotics impact microbiomes, which differ remarkably between healthy and diseased humans. Deviations in population structure of microorganisms constituting the "healthy microbiome" affect the health of individuals and populations. This functional impact of the microbiome has been observed in skin disease (Byrd et al. 2018), bowel disease (Heeney et al. 2018), and liver disease (Adolph et al. 2018), and it is expected that further research into this domain will uncover more systemic relationships. Microbiomes are also thought to have a bearing on the nervous system and behavior (Ghaisas et al. 2016).

Alteration of the microbiome in pregnancy is associated with gestational diabetes and adverse fetal outcomes such as preterm birth (Bassols et al. 2016; Prince

et al. 2016). In utero exposure to antibiotics can potentially alter the developmental microbiome of the fetus, which modulates the infant immune system (Stiemsma and Michels 2018). Although causality has not been proven, exposure to antibiotics prenatally and in infancy is thought to lead to noncommunicable diseases such as asthma (Hoskin-Parr et al. 2013) or inflammatory bowel disease (Hviid et al. 2011) in later life. The recent obesity epidemic has also been associated with antibiotic exposure and microbiome modification in early childhood (Leong et al. 2018). Antibiotics induce microbial dysbiosis even at subinhibitory concentrations (Berendonk et al. 2015). The association of subinhibitory exposure with dysbiosis and disease needs to be determined through further research.

Gastrointestinal microbiome structure affects tumor formation, as suggested by murine studies (Zackular et al. 2013). In a recent study, long-term antibiotic use in adults was associated with colorectal adenomas; however, independent association with antibiotics remains to be elucidated (Cao et al. 2018). Breast cancer may result from a disturbance of the "estrobolome"—the collective enteric bacterial engine that can metabolize estrogens (Kwa et al. 2016).

12.3.3 Consequences of Antibiotic Toxicity

Data on direct toxic effects of subtherapeutic antibiotics on humans are limited. However, recognition of antimicrobials as toxins in food and water is increasing (Hanekamp and Bast 2015), and no-effect antibiotic concentrations in food have been proposed and revised (Barton 2000). Despite this, comprehensive information on risk assessment and Thresholds of Toxicological Concern (TTC) are lacking for antibiotic classes (Hanekamp and Bast 2015). TTC studies at a population level are also critical to understanding of the various types of anti-infective side effects potentially caused by subtherapeutic concentrations of antibiotics (Barton 2000). Three categories of antibiotic toxicities merit special mention. These are tumorigenesis, allergic and pseudoallergic reactions, and idiosyncratic immune reactions.

Antibiotics such as quinolones and tetracyclines are potentially genotoxic and cytotoxic to mammalian cells (Smart and Lynch 2011; Çelik and Eke 2011). While these antibiotics are monitored in food and biosolids, other mutagenic antibiotics such as furazolidones (Magee et al. 2018) are often overlooked and metabolites of which may contribute to side effects (Hoogenboom et al. 2002).

Allergenic potential of antibiotics is well known; however, the allergenic concentrations and potentials at subtherapeutic levels present in food and water are poorly understood. Veterinary antibiotics also have several toxicological effects observed at high concentrations, and occasional occupational exposure to these can lead to serious side effects (Joint FAO/WHO Expert Committee 2009). Penicillin residues in milk have been documented to trigger a Type I hypersensitivity reaction (Dewdney et al. 1991) rarely. However, reports of other antibiotics triggering allergic reactions are often unsubstantiated and thought to be insignificant (Dayan 1993).

The most well-known example of an immune-mediated reaction to small amounts of antibiotic contamination in food is that of chloramphenicol. This antibiotic is known to cause a dose-independent aplastic anemia (Settepani 1984) through exposure in food. Other reported instances of smaller concentrations of antibiotics causing adverse effects in humans are rare; however, this may be due to the inherent difficulty of monitoring for such effects at the population level. The overall health impact and spectrum of toxicities of contaminant antibiotics may be much wider and hitherto unrecognized.

Assessing public health impact of any intervention is central to influencing policy decisions. The adverse impact of clinical AMR on human health and economy is indisputable. The projected future impact on morbidity and mortality as predicted by the O'Neill report makes it essential to revise current policies in light of emerging evidence to not only safeguard efficacy of antimicrobial agents, but also to protect the environment from damage by them.

The interdependence of environments, humans, animals, and microbiota is evident in the manner in which changes at epidemiological, ecological, and the public health level are impacted by the presence of antibiotics and spread of ARGs through the ecosystem. As these environments are interlinked, it is necessary that risk assessment and solutions to prevent antimicrobial resistance encompass all sectors where the antibiotics and ARGs interface with the animate world.

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Chapter 13
Surveillance and Environmental Risk Assessment of Antibiotics and AMR/ARGs Related with MRSA: One Health Perspective

Vanessa Silva, Susana Correia, José Eduardo Pereira, Gilberto Igrejas, and Patrícia Poeta

V. Silva · S. Correia
Microbiology and Antibiotic Resistance Team (MicroART), Veterinary Sciences Department, University of Trás-os-Montes and Alto Douro (UTAD), Vila Real, Portugal

Associated Laboratory for Green Chemistry (LAQV-REQUIMTE), University NOVA of Lisboa, Caparica, Portugal

Department of Genetics and Biotechnology, University of Trás-os-Montes and Alto Douro (UTAD), Vila Real, Portugal

Functional Genomics and Proteomics Unit, University of Tras-os-Montes and Alto Douro (UTAD), Vila Real, Portugal
e-mail: vanessasilva@utad.pt

J. E. Pereira
Microbiology and Antibiotic Resistance Team (MicroART), Veterinary Sciences Department, University of Trás-os-Montes and Alto Douro (UTAD), Vila Real, Portugal

G. Igrejas
Associated Laboratory for Green Chemistry (LAQV-REQUIMTE), University NOVA of Lisboa, Caparica, Portugal

Department of Genetics and Biotechnology, University of Trás-os-Montes and Alto Douro (UTAD), Vila Real, Portugal

Functional Genomics and Proteomics Unit, University of Tras-os-Montes and Alto Douro (UTAD), Vila Real, Portugal
e-mail: gigrejas@utad.pt

P. Poeta (✉)
Microbiology and Antibiotic Resistance Team (MicroART), Veterinary Sciences Department, University of Trás-os-Montes and Alto Douro (UTAD), Vila Real, Portugal

Associated Laboratory for Green Chemistry (LAQV-REQUIMTE), University NOVA of Lisboa, Caparica, Portugal
e-mail: ppoeta@utad.pt

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Abstract According to the World Health Organization (WHO), infections with antimicrobial resistant bacteria result in an estimated number of 700,000 human deaths globally per year. In the 2016 United Nations General Assembly, world leaders acknowledged the link between antimicrobial resistance (AMR) and the misuse of antibiotics in public health, animal, food, agriculture, and aquaculture sectors, committing to a concerted One Health approach to AMR involving the various sectors and actors in defense of human, animal, and environmental health. The use of antibiotics promotes the development of AMR and influences co-selection processes in bacterial communities leading to the dissemination of antibiotics, AMR bacteria, and antibiotic resistance genes (ARGs) among humans, pets, livestock, wild animals, and the natural environment. Considering the promiscuity of bacterial gene transfer systems, the presence of ARGs in the environment is increasingly beheld as an ecological problem. Hence, there is an urgent need to understand the dynamics of AMR and a One Health approach is essential to evaluate the origin, spread, and flow mechanisms of AMR and ARGs. Some AMR bacteria and ARGs are spreading more rapidly than others, becoming pandemic. Methicillin-resistant *Staphylococcus aureus* (MRSA) is an important pathogen with serious morbidity and mortality. MRSA strains used to be associated with nosocomial infections but have now disseminated to the community, animals, and environment. Besides, MRSA strains are constantly changing with new different clones emerging in different geographical areas. Thus, it is globally recognized the urgency to monitor and better understand the characteristics and transmission routes of AMR and ARGs in MRSA strains.

Keywords Surveillance · Environmental risk assessment · Antibiotics · AMR/ARGs · MRSA

13.1 Introduction

The discovery of penicillin marked a new era. Antibiotics were able to cure infections once considered incurable and often cause of death. From there, antibiotics became the support of modern medicine since they play a crucial role on the extension of expected life spans and are essential for invasive surgery and treatments such as chemotherapy (Fair and Tor 2014). However, antimicrobial resistance (AMR) has been increasing worldwide, becoming a public health concern. New resistance mechanisms are emerging and spreading worldwide, threatening the treatment of common infectious diseases which might result in chronic infections, disability, or even death (Asokan and Asokan 2016). Resistance to common antimicrobial agents by clinically relevant bacteria is increasing at an alarming rate. Furthermore, the global dissemination of antimicrobial resistance genes (ARGs) and their transference between different species of bacteria are associated with longer hospital stays and increased mortality. Over the past few years, epidemiologic studies have

revealed that there has been a dramatic increase in bacterial resistance to multiple antibiotics (Ventola 2015). Data from the World Health Organization and several reports on AMR show that if we do not discover alternatives to antibiotics by 2050, antibiotic resistance will kill more people than cancer with about ten million deaths per year (de Kraker et al. 2016). AMR occurs naturally overtime, usually through genetic changes and selective pressure; however, the misuse and overuse of antimicrobial agents are accelerating this process. Studies have demonstrated a direct correlation between the emergence of antimicrobial resistant bacteria and antibiotic consumption (Ranucci et al. 2017). Antimicrobials are still used as growth promoters and to prevent infection in livestock worldwide. These antimicrobials are released along with manure contaminating the environment and posing a selective pressure on soil bacteria leading to the development of AMR (Singer et al. 2016). Furthermore, the antibiotics used in livestock are ingested by humans when consuming the meat. Antibiotics, resistant bacteria, and antibiotic resistance genes can be disseminated through the environment and, therefore, AMR can be transmitted among humans, pets, livestock, wild animals, and the natural environment. Hence, there is an urgent need to understand the dynamics of AMR.

13.2 Acquisition and Dissemination of Antimicrobial Resistance

AMR can be acquired by several mechanisms, such as mutation of chromosomal genes or acquisition of external resistant genes. Thus, AMR can be divided into intrinsic and acquired resistance. Intrinsic resistance is a primitive resistance that occurs naturally in the absence of selective antimicrobial pressure, based on genes present in the chromosomes which are not usually transferable. This type of resistance occurs in all bacteria of the same species or genus, regardless of their place of isolation, and may result from the modification of antibiotic target sites, presence of a permeability barrier, or induction of repressed genes in the presence of antibiotics. Intrinsic resistance is mostly acquired by genetic recombination or mutation leading to changes in the DNA sequence which may result in new or altered proteins or changes in the amount of proteins (Munita and Arias 2016). In addition to the mutations in bacterial chromosomes that may confer resistance to antimicrobials, mobile genetic elements are responsible for most of resistance gene dissemination. Acquired resistance results from mutation and/or horizontal gene transfer (HGT) leading to the acquisition of one or more resistance mechanisms (von Wintersdorff et al. 2016). This type of resistance can be temporary, when the bacteria needs to adapt to certain conditions, or permanent. The acquisition of ARGs, and eventually their dissemination, is accomplished through mobile genetic elements that can either move between bacterial cells, such as plasmids, transposons, and bacteriophages, or move from one genetic location to another within the same cell, such as integrons (Bennett 2008). These mobile elements may carry several ARGs that confer resistance to

different classes of antimicrobials. Most importantly, there are three fundamentally distinct mechanisms by which acquisition of resistance genes through HGT can occur: transformation, transduction, and conjugation.

Plasmids are circular, double-stranded extrachromosomal DNA molecules with a self-replicating capacity that perpetuate independently of the bacterial chromosome. These genetic elements are not essential for the bacterial cell but may confer new functions that can be advantageous under certain environmental conditions, which facilitates the survival and propagation of the species. Besides, they also have genes for replication, metabolism, fertility, resistance to bacteriocins, and/or bacteriophage genes (Kado 1998). Bacteria can survive under the selective pressure of antimicrobials when containing plasmids carrying ARGs to those antimicrobials. Nevertheless, as plasmids are not essential for bacteria, many of them are lost in the absence of selective pressure (Buckner et al. 2018). Plasmids may be present in two or more copies in the cell and may carry several ARGs and virulence genes. These elements can be found in a wide diversity of bacteria since they can be transmitted between bacteria of the same species or bacteria from different species; there are plasmids that replicate only in bacterial strains of related genera while others have a broader spectrum of bacterial groups where they can circulate (Davies and Davies 2010). Resistance genes encoded in plasmids are often located within segments called transposons. Transposons are linear DNA sequences present on chromosomes or plasmids that are able to translocate from one site to another of a replicon, or between replicons. Transposons carry an enzyme, called transposase, that is responsible for transposition, promoting the transfer of antibiotic resistance genes. Plasmids can carry several transposons which makes it possible to exchange several resistance genes in a single transfer between bacterial cells (Hinnebusch and Tilly 1993). ARGs are often further clustered within mobile elements called integrons, which are frequently found within transposons and plasmids. Integrons are mobile genetic elements that have the genetic information to encode a protein capable of integrating or releasing motile elements such as ARGs (Domingues et al. 2012). Integrons are very similar to transposons; however, they lack the repetitive terminal sequences and proteins necessary for their transposition. These elements may capture gene cassettes encoding genes mostly related to antibiotic resistance, building modular multiresistance units (Hall 2001). Through this type of mechanism, several resistance gene modules can be assembled in the same plasmid, which can be transferred to other bacteria in only one conjugative event. These processes turn bacteria into a versatile microorganism, capable of adapting quickly to selective pressure by antibiotics.

13.3 One Health Approach

AMR is spreading globally and is threatening public health. It is important to recognize that AMR is a problem involving not only humans and human health but also animals and the environment. These three domains are clearly linked to each other,

being the principle recognized globally as One Health. Antibiotics used in livestock are creating ideal conditions for the development of antibiotic-resistant bacteria not only in animals but also in the soil, water, and farm environment. The selective pressure of antibiotics exerted on commensal bacteria of those animals selects resistant bacteria and forces the development of resistance. Livestock presenting antibiotic-resistant bacteria are a source of ARGs that can be transmitted to farm workers, veterinaries, and other animals living in the farm. Besides, food animals carrying AMR and ARGs can transmit them to humans and animals through food consumption. The urine and feces of livestock contain large concentrations of not fully metabolized antibiotics, resistant bacteria, and ARGs. Manure application on agricultural fields is a common practice which leads to the spread of antibiotics and ARGs and further contamination of soil and surface and groundwater. ARGs are transferred to soil and water bacteria by HGT and antibiotics present in manure exert selective pressure on soil and water bacteria. Furthermore, the on-farm wastewater and sewage treatment plants are also responsible for the spread of ARGs since the amount of ARGs remains unaltered despite the reduction in antibiotic concentration after wastewater treatment. These ARGs are thus released into the environment and most end up on soil, water, and rivers. The uptake of ARGs present in soil by food-plants may also represent a source of resistance that can be transmitted to human and animals by its consumption. Wild animals living near the farm environment are also at risk of acquiring resistant bacteria and ARGs through the contact with farm animals and farm environment. Drinking water is usually produced from surface water which, once contaminated with antibiotic-resistant bacteria and ARGs, may transmit them to humans, companion and wild animals and livestock. ARGs and resistant bacteria can also be transmitted among wild animals through the food chain and to humans through the consumption of wild game meat. Therefore, when assessing the risk of AMR and ARGs dissemination it is extremely necessary to take into account the three components: humans, animals, and environment, which are interconnected and cannot be regarded as individual elements.

13.4 Antibiotics, AMR, and ARGs in the Environment

The dissemination of antibiotics, AMR, and ARGs beyond clinical settings includes many routes, such as agricultural and environmental microbial communities (Singer et al. 2016). Antibiotics are widely used in agricultural practices worldwide to promote animal growth and to prevent infections in those animals. Therefore, contamination of the environment with veterinary antibiotics is an increasing concern. Antibiotics consumed by humans and animals are excreted by feces and urine as a high percentage of antibiotics is not metabolized in the organism, and so antibiotics in their unchanged and active forms are released to the environment (Kumar et al. 2005). These unmetabolized antibiotics are introduced in effluent and sludge from domestic wastewater treatment plants, hospitals, and livestock farms leading to their release in the environment and further contamination of soil and surface and

groundwater (Zhang et al. 2015). Besides the fact that residues of antibiotics may have an ecotoxic effect, the development of antimicrobial resistant bacterial strains is the main concern. The occurrence of antibiotics and ARGs in different environmental compartments, such as soils, groundwater, river water, wastewater treatment plants, and livestock farms, has been extensively reported. However, a WHO, FAO, and OIE tripartite report recently developed to monitor country progress in addressing AMR in the human, animal, plant, food safety, and environmental sectors revealed an insufficient level of regulation to protect the environment from the hazards of antimicrobial production, with only 78 countries having regulations to prevent AMR environmental contamination, of which only 10 have regulations to restrict antimicrobial residue discharge into the environment.

13.4.1 Soil Environment

The presence of antibiotics in soil and surface water has been well documented over the years and the subjects go from antibiotic transport (Blackwell et al. 2007; Bailey et al. 2015; McEachran et al. 2015) to sorption (Boxall et al. 2002; Lin and Gan 2011; Zhang et al. 2014; Pan and Chu 2016) and leaching processes (Aust et al. 2010; Pan and Chu 2017). The soil resistome is influenced by various factors, such as the loading of ARGs, HGT, selective pressure, and shifts in soil organic matter content (Nesme and Simonet 2015). Nevertheless, manure seems to be the main source of antibiotics and ARGs input in soil since many studies have reported the increase and persistence of ARGs following manure application (Xie et al. 2016). Even in cases in which the farm animals have never been treated with antibiotics, manure still adds AMR bacteria carrying ARGs to soil (Heuer et al. 2011). A recent study conducted in Spain investigated the availability and persistence of various antibiotics in soil and soil amended with manure and found that in the soil aqueous phase there was a high prevalence of sulfamethoxazole, followed by sulfamethazine and lincomycin, and in soils amended with manure the values increased between 6% and 53% (Albero et al. 2018). Chen et al. (2018) reported that antibiotics contained in manure can persist in soils at low concentrations for long periods of time which can lead to an antibiotic accumulation in soils with repeated input of manure congaing antibiotics. Furthermore, the dissipation of antibiotics was significantly faster in soils amended with raw manure than compost manure. Soil samples from 11 dairy farms across the United States were investigated for the presence of ARGs. Soils emended with manure from animals treated with antibiotics are more likely to have a higher frequency of ARGs, as well as altered microbial community composition (Wepking et al. 2017). Another study conducted with dairy cows compared the soil amended with cow manure to soil that received inorganic fertilizer and reported that the application of dairy manure increased the abundance of ARGs in soil when compared with the inorganic fertilizer. AMR in soils is not only originated by antibiotic contamination either by manure application or wastewater disposal, but also due to other contaminants which are responsible for promoting dissemination of

ARGs and mobile genetic elements by cross-resistance and co-resistance processes (Ashbolt et al. 2013). Metal pollution promotes stress response on bacterial communities. Several studies have reported a relationship between metals and AMR proliferation (Knapp et al. 2011; Li et al. 2015; Su et al. 2015; Abella et al. 2015). Ninety garden soil samples from Australia were analyzed to evaluate the occurrence of ARGs and compared with metal concentrations present in those soil samples. Relationships between metals and ARGs were found, and even low concentrations of metals influenced the occurrence of ARGs (Knapp et al. 2017). Furthermore, metals are usually added to livestock feed to promote growth and prevent infections.

13.4.2 Aquatic Environment and Wastewater Treatment Plants

Pathogenic bacteria and ARGs are constantly released with wastewater into the water environment. Due to the Council Directive 91/271/EEC of 21 May 1991 concerning urban wastewater treatment, sea disposal from wastewater treatment plants was banned. Antibiotics and ARGs present in sewage and manure are only partly eliminated in wastewater treatment processes; the remaining antibiotics and ARGs go to effluents and then they finally reach the aquatic environment (Giger et al. 2003). Studies reported that around 56 different antibiotics belonging to six different classes have been detected in domestic wastes. Furthermore, the antibiotic discharge rate of municipal wastewater treatment plants are between 3 and 710 g/day (Wu et al. 2017). Surface water, groundwater, and rivers constitute a mode of antibiotic and ARG dissemination. Drinking water is produced from surface water which can lead to the spread of ARGs among human and animal populations by its consumption, thus representing a potential hazard to human and animal health (Djenadi 2017). Although in the last years several studies have investigated the presence of antibiotics and ARGs in surface waters, only a few have considered groundwater. Burke et al. (2016) studied the presence of 26 veterinary antibiotics in surface waters and groundwater. Trimethoprim was the most prevalent antibiotic found in groundwater being detected in 11 out of 15 shallow groundwater samples. Despite being in low concentrations (5–12 ng/L), trimethoprim was found to be very persistent since its occurrence was detected in groundwater with an age of approximately 10 years. A study conducted by Chen et al. (2018) reported the detection of 35 antibiotics belonging to six different classes, namely chloramphenicol, lincosamides, marcrolides, quinolones, sulfonamides, and tetracyclines, in 74 shallow groundwater wells in China. From the 35 antibiotics investigated, 34 were detected in a frequency of 4–70% and at a concentration between 0.3 and 1199.7 ng/L. Furthermore, 73 of the 74 wells investigated contained traces of at least one antibiotic. Another recent study by Kivits et al. (2018) reported the presence of antibiotics in groundwater in an intensive livestock farming in the Netherlands. Water from ten multilevel wells was analyzed for the presence of 22 antibiotics from several different classes. Four antibiotics were detected above the quantification limits with a con-

centration ranging from 0.30 to 18 ng/L. Furthermore, sulfamethoxazole and sulfamethazine were the most frequently detected. The presence of antibiotics in aquatic environments can be attenuated by physical, chemical, and/or biological processes leading to rapid removal of some antibiotics, such as macrolides, quinolones, and tetracyclines; nevertheless, the attenuation of antibiotics in river water differs between rivers (Luo et al. 2011; Aymerich et al. 2016). Hanamoto et al. (2018) studied the natural attenuation of four antibiotics during transport along the Thames River in the United Kingdom and the Katsura River in Japan. The attenuation of sulfamethoxazole, which is one of the antibiotics most commonly found in water environment, was low in both rivers. Attenuation of several antibiotics investigated in this study was due to the sediment sorption capacity in the rivers. Xu et al. (2015) investigated the presence of antibiotics and ARGs in a sewage treatment plant and their preservation in the effluent receiving river water in China. The antibiotics studied were partially eliminated during sewage treatment procedure unlike the ARGs of which the concentrations were kept relatively constant. However, the concentrations of antibiotics and ARGs were comparably lower in the river. A study conducted by He et al. (2016) examined the presence of 18 antibiotics belonging to the sulfonamide, tetracycline, chloramphenicol, and macrolide classes and 22 ARGs conferring resistance to these antibiotics in the receiving water and soil from three swine feedlots. In all swine farms, the majority of ARGs and antibiotics persisted after the on-farm waste treatment procedure. Besides, a high variety and abundance of ARGs was still detected in the receiving soil and river water. Treatment of wastewater and sewage cannot eliminate completely pharmaceutical compounds such as antibiotics due to their variable physicochemical properties (Voulvoulis and Lester 2005). Therefore, water treatment facilities have been considered reservoirs for numerous ARGs. The occurrence of antibiotics and ARGs in wastewater and sewage treatment plants, as well as in their effluents, have been investigated in several studies worldwide. Hultman et al. (2018) investigated the presence of ARGs in two different municipal treatment plants in Finland. The four genes studied were identified in different bacteria in both treatment plants; however, these genes were detected in the influent and effluent. An interesting study conducted by Caucci et al. (2016) investigated the seasonal relationship of antibiotic prescriptions and the occurrence of ARGs in wastewater. They found a clear seasonal pattern for the presence of ARGs with the higher rates of antibiotics prescribed in autumn and winter coinciding with high levels of AGRs conferring resistance to those antibiotics. Besides, the treatment of wastewater did not influence the relative level of ARGs. Another study investigated the ARGs and horizontal gene transfer potential at an urban wastewater treatment plant during four seasons. The transposases and most of the AGRs studied were found in wastewater, being the relative abundance higher in the influent than the effluent (Karkman et al. 2016).

13.4.3 Animal Farm Environment

Levels of resistance in livestock need to be determined in order to measure any threat to public health (Woolhouse et al. 2015). Despite the ban of growth promoters in Europe since 2006 (EC Regulation No. 1831/2003), in several other countries the use of growth promoters is unmonitored, which often leads to high usage (Zhu et al. 2013). A study conducted in eastern China revealed that livestock farms are a massive environmental reservoir of ARGs (Cheng et al. 2013). Another study conducted in China investigated the potential risks of ARGs conferencing resistance to chloramphenicol in swine feedlots and their surrounding environment. A correlation made between ARGs and chloramphenicol residues demonstrated that environmental antibiotic residues might rise the occurrence of ARGs. Furthermore, swine feedlot are likely a source of ARG dissemination since the same chloramphenicol resistance genes were found in fields adjacent to swine feedlots (Li et al. 2013). Karczmarczyk et al. (2011) investigated the genotypic characteristics of multidrug-resistant strains recovered from cattle and the farm environment in Ireland and concluded that cattle can act as a reservoir of ARGs frequently associated with mobile genetic elements and co-transfer of ARGs is a common mechanism. A study conducted by de Been et al. (2014) examined the AMR in bacterial isolates recovered from farmers and their pigs. Their findings strongly suggested the transmission of AMR between pigs and pig farmers which can occur both through direct contact and aerosols. McEachran et al. (2015) studied the capability of antibiotics, ARGs, and cattle-associated bacteria to aerially disperse in particulate matter from beef cattle pasture land and found out that there is significant potential for their widespread distribution via airborne particulate matter.

13.4.4 Wild Animals

Some bacteria are part of human natural microbial flora and can also be found in animals, wildlife, and the environment. AMR found in humans, pets, and farm animals may result from direct contact with antibiotics. However, wild animals are not excepted to be in contact with antibiotics, and so it is necessary to investigate the source of AMR in bacteria of those animals (Allen et al. 2011). Still, wild animals can act as reservoirs of AMR bacteria and ARGs and are potential sources of dissemination of these elements. Furthermore, the proximity of wild animals with human activities may influence the bacterial profiles of those animals. Several reports have demonstrated that wild animals carry ARGs and bacteria; therefore, a One Health investigation of their role in AMR and ARGs dynamics is necessary (Vittecoq et al. 2016). Nevertheless, little attention has been given to wildlife. Wild game meat is an important reservoir of ARGs since they can spread AMR through

the food chain to human and other animals. Antibiotic-resistant bacteria, often resistant to multiple antibiotics, were found in game meat from several animals, such as roe deer (Lillehaug et al. 2005), wild boar (Poeta et al. 2009; Zottola et al. 2013), wild rabbit (Silva et al. 2010a; Santos et al. 2013), pheasant (Kandričáková et al. 2015), wild pigeon (Veldman et al. 2013), and wild partridges (Silva et al. 2018a). Wild birds, particularly migratory birds that travel long distances, may act as transporters and effective disseminators of AMR (Silva et al. 2012; Klibi et al. 2015). Hernandez et al. (2013) showed that the detection rate of AMR strains isolated from the fecal flora of wild birds was higher than from local humans. AMR and ARGs have the potential to be transferred from wild birds to humans even where the rate of AMR strains is low (Bonnedahl et al. 2015). A study conducted by Rose et al. (2009) revealed that the prevalence of AMR bacteria was higher in seabirds than marine mammals from the same coastal area. Nevertheless, studies have reported that AMR bacteria is absent from wild birds living in remote places which indicates that anthropogenic inputs into the local environment may be responsible for the development and spread of AMR (Thaller et al. 2010; Ramey et al. 2018). The food chain may also contribute to the spread of AMR. AMR was not found in any fecal samples recovered from song thrushes from Portugal, possibly due to their diet based mainly on invertebrates and soft fruit and berries (Silva et al. 2010b). AMR has been detected in other wild animals, such as small mammals (Literak et al. 2010), wolfs (Gonçalves et al. 2011), and foxes (Carson et al. 2012; Radhouani et al. 2013). Wild animals living close to livestock farms and waste, and to anthropogenic activities, are more likely to be colonized by resistant bacteria. Rodents living close to farm environments showed a high prevalence of resistant bacteria (Literak et al. 2009). Also, a study conducted by Blanco et al. (2009) showed that red-billed chough living near the farm environment presented AMR profiles resembling to those found in manure used in the area. All these environments can be a source of AMR and ARGs and each one can contribute to the development and spread of this hazard. Among the bacteria most pathogenic to human health, *Staphylococcus aureus*, particularly methicillin-resistant *Staphylococcus aureus* (MRSA), is becoming one of the major threats.

13.5 Methicillin-Resistant *Staphylococcus aureus*

S. aureus is a spherical Gram-positive bacterium forming grape-like clusters which is usually found in skin, skin glands, and mucous membranes, including the nose and mouth of humans and some animals. This bacterium is one of the first described pathogens and is still responsible for a great part of nosocomial and community-associated infection that can result in serious complications. *S. aureus* are able to colonize skin and soft tissues, bloodstream and urinary and respiratory tract, and in systemic infections, it can cause osteomyelitis, mastitis, septicemia, and chronic wound infection (Köck et al. 2010). *S. aureus* can also be responsible for implant-

associated infections due to its ability to produce biofilm on abiotic surfaces. Furthermore, *S. aureus* is a toxin producer and is equipped with a wide range of virulence factors which have the ability to induce toxic and lytic effects on host cells, being associated with several toxin-mediated conditions, such as foodborne infections and toxic shock syndrome (Víquez-Molina et al. 2018). Nevertheless, one of the greatest challenges in the treatment of *S. aureus* infections is its aptitude to acquire resistance to several classes of antibiotics. Shortly after the introduction of penicillin, *S. aureus* strains carrying resistance to this antibiotic were reported (Rammelkamp and Maxon 1942; Kirby 1944). Furthermore, the first methicillin-resistant *S. aureus* (MRSA) strain was isolated approximately 10 years after the introduction of methicillin to the market (Jevons 1961). Resistance to β-lactam antibiotics in MRSA strains is determined by β-lactamases and penicillin-binding proteins which confer resistance to all β-lactam antibiotics. Several different penicillin-binding proteins have been reported, including PBP2a which is encoded by the *mec*A gene. *S. aureus* under antibiotic selective pressure acquire the *mec*A gene, which is carried by transposons or plasmids and is the most prevalent gene conferring resistance to β-lactams (Miao et al. 2017). Moreover, *mec*A is integrated in the Staphylococcal Cassette Chromosome (SCC) *mec,* which is a vital mobile genetic element in MRSA. The SCC*mec* typing became a technique widely used to investigate and classify the origin of MRSA strains. So far, the *mec*A and its homologs *mec*B, *mec*C, and *mec*D are enclosed within 13 SCC*mec* (Lakhundi and Zhang 2018). The *mec*C gene was first reported in 2011 and has been widely identified in wild animals. The gene may be responsible for mistakes in MRSA infection diagnosis since it is very rare in humans and produce a distinctive antibiotic susceptibility profile compared to *mec*A MRSA (Paterson et al. 2014). The *mec*C gene is associated with the new SCC*mec* XI. However, the most common SCC*mec* type found in humans are from I to V, being SCC*mec* types I, II, and III frequently associated with nosocomial MRSA strains whereas types IV and V are usually found in community acquired MRSA strains (Monecke et al. 2011). To investigate the clonal lineages of *S. aureus* strains, typing of multilocus sequence (MLST) and *spa* are performed. MRSA strains are often resistant to several classes of antibiotics including penicillins, cephalosporins, aminoglycosides, macrolides, and lincosamides; hence, treatment options of MRSA infections are limited to few antibiotics, such as vancomycin and linezolid (Mahmood et al. 2010). MRSA emerged rapidly within a short time as a dangerous pathogen causing nosocomial infections and these kind of infections have long been the classical presentation of MRSA infections (Köck et al. 2010). However, in the past decades MRSA has emerged as an agent associated with infections in community with serious morbidity and mortality, and in humans without prior healthcare contact (Ray 2017). MRSA dissemination and colonization have increased from the hospital to the community and further to animals and the environment. Apart from humans, MRSA colonization has been detected in animals and the environment, including pets, farm animals, wildlife, aquatic species, soil, and water.

13.5.1 MRSA in Soil and Water Environment

The occurrence of MRSA in manure was recently reviewed and several studies have reported correlations between manure application and the occurrence of resistance genes in soil and farm environment (Friese et al. 2013). However, very little attention has been given to the prevalence of MRSA in soils unrelated to livestock. One MRSA strain was isolated from soil unrelated to a slaughterhouse in South Korea. This MRSA strain is associated with community-acquired (CA) MRSA since it belonged to SCC*mec* IV. The strain did not present multiresistance which is another feature typical of CA-MRSA isolated in South Korea (Ko et al. 2019). Another study investigated the prevalence and antibiotic resistance of *S. aureus* and MRSA strains isolated from soil samples collected from residential (25 samples) and hospital (25 samples) areas in India. Four and five *S. aureus* were isolated from residential and hospital areas, respectively, being the isolates from the residential area susceptible to almost all antibiotics tested. On the opposite, *S. aureus* isolates from the hospital area were resistant to a wide range of the tested antibiotics (Sudha 2016). Schulz et al. (2012) investigated the occurrence of MRSA on soil surfaces near to pig holdings. The soils were not emended with manure; however, livestock-associated MRSA was detected in 73% of the soil surface samples. All soil MRSA strains belonged to *spa* types t011 and t034 that are specifically associated with the livestock MRSA. Soil contamination with MRSA strains may occur due to the activity of human carriers or other animals like rodents. Furthermore, it is likely that MRSA can survive for longer periods on soil surfaces since it has been showed that MRSA strains can survive on hard surfaces for weeks (Makison and Swan 2006). Another study compared the presence of MRSA in manure and in soil amended and non-amended with manure from the same animals. Although MRSA strains were found in manure, MRSA was not found in any soil samples. Nevertheless, high prevalence of other ARGs was found in soil amended with manure (Esperón et al. 2018). Manure can enter water through inadvertent mechanisms. MRSA in surface and groundwater and rivers is often associated with livestock manure and human sewage. However, studies regarding the occurrence of MRSA strains in groundwater, surface water, and river water are still very scarce. Antibiotics present in water, particularly β-lactam antibiotics, can also be responsible for AMR and the spread of methicillin resistance in the environment. β-Lactam antibiotics have been detected in surface waters (Watkinson et al. 2009). Water can be a source and a vector of transference of ARGs between humans, animal, and the natural environment. Moreover, hospital and community-acquired MRSA strains survive in sea and river water for at least 14 days postinoculation. Still, the survival of livestock-associated MRSA is not fully understood (Wan and Chou 2014). The *mec*A gene was detected in rivers in Australia (Barker-Reid et al. 2010) and its *mec*C homolog was also found in river water in Spain (Porrero et al. 2014). This gene was typed by MLST and showed similarity to those detected in wild animals in the same geographic region which may highlight the potential of river water to carry, transport, and disseminate methicillin resistance related ARGs. MRSA strains have been detected in

wastewater and sewage treatment plants in hospital and municipal wastewater which suggests that wastewater treatment plants might be a potential source for dissemination and development of MRSA strains. Moreover, wastewater environments can represent an unrecognized health threat since Börjesson et al. (2010) showed that wastewater treatment reduced the amount of MRSA strains but selected for strains presenting more resistance. Additionally, in the same study, new *spa* types of MRSA strains recovered from wastewaters were found. The occurrence of the *mec*A gene in swine wastewater is usually low when compared to other resistance genes, such as *tet*A and *tet*B (conferring resistance to tetracycline) and *erm*A and *erm*F (conferring resistance to erythromycin) (Brooks et al. 2014). The prevalence of tetracycline resistance genes is expected to be high since tetracyclines, followed by penicillins and sulfonamides, are the most consumed antibiotics by food-producing animals in Europe (Silva et al. 2018b). Besides, pig farms do not use methicillin or closely related derivatives, and so co-resistance is likely to occur as a result of using other antibiotics (Monnet et al. 2004). In a study conducted by Wan and Chou (2014), the presence of MRSA and *mec*A gene was evaluated in municipal and swine slaughterhouse wastewaters. Both wastewater sources contained the *mec*A gene and high levels of this gene were found in the effluents of both wastewater treatment plants. Furthermore, each source had MRSA strains with own unique characteristics. A higher prevalence of the *mec*A gene and MRSA strains was found in swine wastewaters and those strains were classified as multiresistant. *S. aureus* naturally existing in the several environmental compartments may become MRSA through horizontal gene transfer due to continuous discharge of swine wastewater carrying the *mec*A gene.

13.5.2 MRSA in Livestock

The use of antimicrobial agents in animal husbandry has significantly contributed to the spread of MRSA strains and the *mec*A gene among livestock. Farm animals, in particular, pigs, can comprise a distinct MRSA reservoir since a rapidly emerging type of MRSA, CC398, seems to be associated with these animals (Lewis et al. 2008). Initially, MRSA CC398 strains, including several *spa*-types, were isolated from pigs, but later were also isolated from veal calves (Graveland et al. 2010), poultry (Monecke et al. 2013), and humans (Graveland et al. 2011). Studies have shown that livestock-associated MRSA represents an increasing source of infections in humans (Köck et al. 2013). Virulent factors seem to be lacking in MRSA CC398 but these strains tend to exhibit increased ability to acquire mobile genetic elements (Schijffelen et al. 2010). Transmission of MRSA CC398 and other livestock-associated MRSA strains from livestock to humans have been widely reported. Furthermore, several studies have shown that people working or living near animal farms are particularly susceptible to be colonized by livestock-associated MRSA (Carfora et al. 2016; Locatelli et al. 2017). A study conducted in Australia investigated the carriage of MRSA isolated from pigs and pig farmers and

found two predominant strains: MRSA ST398 and the Australian ST93. Furthermore, they confirmed the occurrence of both anthropozoonotic and zoonotic transmission (Sahibzada et al. 2017). Locatelli et al. (2017) investigated the association of MRSA strains in pig farms and revealed that livestock-associated MRSA strains were transmitted between pigs, pig barns, milk, and farm workers confirming the zoonotic potential of these strains. Other MRSA clones have been isolated from pigs rather than ST398. For example, despite being a human-associated clone, ST8 MRSA clone has been recovered from swine (Sunde et al. 2011; O'Brien et al. 2012). A study conducted in Italy in slaughter pigs and workers showed that, although the most frequently found MRSA clone was ST398, the ST8 clone was also recovered from the pigs. Moreover, the nares of about 8% of the slaughterhouse workers were colonized with MRSA strains belonging to ST398, ST8, and others, and the isolates recovered both from pigs and humans were indistinguishable (Normanno et al. 2015). Handling food products contaminated with MRSA or the ingestion of these products impose a public health concern. The professionals working with animals colonized by MRSA are at constant risk as it has been shown that with several hours per week in direct contact with these animals there is a high risk of being colonized (Moodley et al. 2008; Denis et al. 2009; Normanno et al. 2015). Not only farm workers and other professionals working closely with MRSA-carrying animals are at risk of being colonized. Reynaga et al. (2018) conducted a study in residents of nursing homes in Spain which were close to pig farms. They isolated MRSA strains from 32 out of 204 residents and MRSA CC398 was identified in 15.6% of MRSA-positive residents. People working in contact with other livestock carrying MRSA, rather than pigs, are also at risk of colonization. Veal calves are known livestock-associated MRSA carriers and people in close contact with these animals have a highly elevated risk of MRSA carriage. The prevalence of MRSA strains found in humans living and working on a veal calf farm was much higher than the general population (Graveland et al. 2010). Lim et al. (2013) investigated the occurrence of MRSA in a dairy cattle farm in South Korea, and 6.3% of milk samples, 4.7% of hand and nose samples from farmers, and 1.2% of farm environment samples were positive for MRSA. Nevertheless, the majority of MRSA were ST72, which is a community-associated clone and the most predominating in that country. *S. aureus* is often isolated from dairy mastitis and is considered one of the principal pathogens causing this infection (Nam et al. 2010). Although methicillin is not used to treat mastitis in dairy cows, MRSA strains have been widely recovered from milk (Margariti et al. 2014; Klibi et al. 2018). Thus, MRSA present in farm environments and farm workers carrying MRSA may be one of the sources of MRSA infection in these animals (Lim et al. 2013). Ronco et al. (2018) conducted a study investigating the presence of *S. aureus* and MRSA in bulk tank milk and dairy cows with clinical mastitis. Although the prevalence of MRSA was low, one MRSA ST398 clone was recovered from a dairy cow with clinical mastitis.

13.5.3 MRSA in Wildlife

MRSA has been found in livestock and pets in studies conducted in recent years in which these animals are described as MRSA carriers with zoonotic potential. Wild animals have also been described as MRSA carriers; nevertheless, less attention has been given to these animals. Several studies investigated the presence of MRSA strains in wild boars. Sousa et al. (2017) analyzed samples from mouth and nose of 45 healthy wild boars and one MRSA strain was recovered. The MRSA strain belonged to ST398 which is the same ST usually found in livestock, particularly in swine. Despite the contact of wild boars with anthropogenic sources of those bacteria, the MRSA-carrying wild boar was hunted in an area where there are no pig farms. Nevertheless, the strain harbored the *scn* gene (IEC-type B system), which indicates that it is from human origin. In a recent study conducted in Spain, among 371 nasal samples of wild boars, one MRSA strain was recovered and also belonged to ST398. MRSA ST398 have also been isolated from European brown hare (Monecke et al. 2016) and in urban wastewater (Gómez et al. 2016), which suggests that this clone has spread from livestock farms to the environment. Another study has reported the presence Panton-Valentine leucocidin (PVL)-positive MRSA strains recovered from wild boars. This is a concern to public health since PVL is one of the major exotoxins of *S. aureus* causing severe disease (Kraushaar and Fetsch 2014). Other studies have found *S. aureus* as a colonizer of wild boars, but no MRSA strains were found (Meemken et al. 2013; Seinige et al. 2017). Studies reporting the presence of MRSA or the *mec*A gene in other wild animals, such as foxes, deer, and birds, are scarce. However, the *mec*A gene was detected in coagulase-negative *Staphylococcus* spp. In foxes living in rural and semirural areas (Carson et al. 2012), MRSA strains were recovered from red deer and vultures in Spain (Porrero et al. 2013) and one *S. epidermidis* carrying the *mec*A gene was isolated from birds of prey in Portugal (Sousa et al. 2014). Several studies focusing in wild animals have found that the *mec*C gene was widely distributed among these animals. This gene was first described in 2011 in MRSA strains from the UK, Denmark, and Ireland (Shore et al. 2011; García-Álvarez et al. 2011). This gene is quite similar to *mec*A but is associated with SCC*mec* XI and CC130. Gómez et al. (2014) examined the two MRSA strains isolated from 101 samples from small wild animals. Both strains harbored the *mec*C gene and belonged to C130. *mec*C-positive MRSA strains can be transmitted to humans and other animals and be spread through the environment with negative consequences for public health. Several other studies have reported the presence of the *mec*C gene in wild rodents (Mrochen et al. 2018), wild hare (Feßler et al. 2018), wild hedgehogs (Bengtsson et al. 2017), and white stocks (Gómez et al. 2015),

13.6 Conclusions

The potential hazard posed by the continued dissemination of AMR and ARGs is threatening humans, animal, and environment health and should be considered a high-risk matter. Although AMR occurs naturally, the use of antimicrobials might be promoting the development of AMR and influencing co-selection processes in bacterial communities. The presence of ARGs in the environment are increasingly seen as an ecological problem. Hence, the One Health approach is essential to evaluate the origin, spread, and flow mechanisms of AMR and ARGs and to make an action plan to tackle antimicrobial resistance. Some bacteria and ARGs are spreading more rapidly than others and are becoming pandemic. MRSA strains, which used to be associated with nosocomial infections, have disseminated to the community, animals, and environment. Furthermore, MRSA strains are constantly changing, and new different clones are emerging in different geographical areas. Thus, it is extremely necessary and urgent to monitor the characteristics and transmission routes of MRSA strains.

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Chapter 14
Fate of Antibiotics and AMR/ARGs in the Environment

Zdzisław Markiewicz and Magdalena Popowska

Abstract This chapter presents a concise review of antibiotics and microbiocides in the natural soil environment, both naturally occurring and, more importantly, those entering the environment as a result of human ventures and the fate of the antibiotics entering various environments is discussed. Also, different environments contain a multitude of various microorganisms, including pathogenic and potentially pathogenic ones, as well as bacteria and fungi entering them via various routes related to farming, industrial activities, healthcare, and numerous other aspects of everyday human activity. Some of the microorganisms in the environment can carry genes coding the synthesis of various antibiotics. Also present in the environmental microbiome as well as in free DNA (eDNA) are numerous genes (ARGs), collectively called the mobilome, whose products confer resistance to various classes of antibiotics and other microbiocides. The mechanisms of various types of bacterial resistance to these compounds are also concisely dealt with. The antibiotic pressure in the environment, coupled with various modes of horizontal gene transfer (HGT) and physical and chemical conditions in the soil, can favor the formation of a pool of bacteria resistant to different antibiotics, including multiresistant strains, some of which can be dangerous pathogens or opportunistic disease-causing bacteria.

Keywords Fate · Antibiotics · ARGs · AMR · Environment

Z. Markiewicz (✉)
Institute of Microbiology, Faculty of Biology, University of Warsaw, Warsaw, Poland

Spectrum Dermatology, Seattle, WA, USA
e-mail: zd.markiewicz@uw.edu.pl

M. Popowska
Institute of Microbiology, Faculty of Biology, University of Warsaw, Warsaw, Poland
e-mail: magdapop@biol.uw.edu.pl

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14.1 Introduction

Antibiotic molecules synthesized by various microbes have been produced in the natural soil environment practically since the beginning of life on our planet, long before they were discovered and their usefulness in treating bacterial infections was realized. It is only natural that organisms that produce antibiotics should have also developed mechanisms protecting against their own antibiotics. In addition, the coexistence of antibiotic producing and nonproducing bacteria would have resulted in the coevolution of resistance mechanisms in nonproducing environmental bacteria as well. Since the mid-1940s, antibiotics began to be used and produced on a mass scale and many synthetic and semisynthetic derivatives have been introduced into clinical and veterinary use. Large amounts of antibiotics have been and still are, in spite of certain restrictions imposed by different countries, being used in animal husbandry as well as in the breeding of poultry and fish. A large proportion of the antibiotics in everyday use, or the metabolic breakdown products of these molecules, find their way to the soil and aqueous environments, and in a much smaller amount also to the air, as described in an accompanying chapter in this volume (Chap. 2; Krzeminski et al. 2020). Moreover, dangerous pathogenic bacteria may also reach these environments, including the air, in which the antibiotic selection pressure may bring about the uptake of resistance factors from the environmental resistome (Wright 2007, Li et al. 2018). Li's group carried out a survey of 30 ARGs across 19 cities around the world. The studied ARGs are resistant to seven common classes of antibiotics: quinolones, β-lactams, macrolides, tetracyclines, sulfonamides, aminoglycosides, and vancomycins. The most diverse group of airborne ARGs, with 18 different subtypes detected, was found in Beijing, China, while San Francisco had the highest overall level of airborne ARGs. Genes resistant to β-lactams and quinolones were the two most abundant types of ARGs in all the cities studied. Low levels of ARGs resistant to vancomycin, an antibiotic of last resort for MRSA treatment, were also found in several air samples (Li et al. 2018). In view of these observations, the environment is constantly and increasingly being focused on for the role it plays in the ubiquitous spread of clinically relevant antimicrobial resistance (AMR) to antibiotics.

The enormous amounts of antibiotics that reach the various environments under a host of varying and often changing conditions have been mentioned in Chap. 7 (Zalewska and Popowska 2020) of this volume and there is no need to expand on the topic herein because it could easily consist a separate chapter on its own. Of importance is not only the amounts of antibiotics that reach the environment but the persistence of these antibiotics in the soil and how they are degraded, if at all. Since this is a very broad topic of necessity, the focus will be mostly, but not exclusively, on the soil environment.

The concentrations of antibiotics persisting in the soil are below their minimum inhibitory concentrations (MICs) but it is such concentrations and the pressure that they exert on the soil microbiome that results in the appearance of strains resistant to antibiotics (Popowska et al. 2012; Krawczyk-Balska and Markiewicz 2016;

Markiewicz and Kwiatkowski 2018). The phenomenon of the appearance of resistance to various antibiotics as well as the mechanism by which intra- and interspecies resistance is spread will also be briefly dealt with in this chapter.

14.2 Presence and Fate of Antibiotics in the Environment

Antibiotics are naturally present in the soil in varying amounts, depending on the physico-chemical properties of the soil and, more importantly, on the nature and number of the soil microorganisms producing them, that is on the microbiome of a particular soil or soils, which may have strongly varying degree of complexity. One gram of soil can contain thousands of individual microbial taxa, as well as viruses and members of all three domains of life (Woese and Fox 1977; Fierer et al. 2014; Fierer 2017). However, in addition to antibiotics naturally produced by soil microorganisms, increasingly larger amounts of these compounds have been reaching the soil since the late 1940s by various means, in the very beginning through dusts and sprays used on agricultural crops to protect against plant diseases (Siminoff and Gottlieb 1951, Martin and Gottlieb 1995) and in more recent history indirectly with manure or liquid waste, as a result of antibiotics used to supplement the fodder of farm animals. Moreover, antibiotics used in the animal husbandry reach the environment by a number of other pathways, including the drug production process itself, the disposal of unused or expired drugs, and through the disposal of waste material carrying drugs (Buchberger 2007). Antibiotics excreted by animals are usually concentrated in the solid phase as a result of sorption dynamics.

Attempts to follow the fate of antibiotics entering the environment were made quite early (Pramer 1958). However, the topic became the subject of much greater focus when larger and larger amounts of antibiotics and their metabolites began reaching the soil as a result of the intensive use of these compounds in animal husbandry, veterinary medicine, human medicine, and other areas, and the role of the antibiotics reaching the environment in the increase and spread of antimicrobial drug resistance (AMR) was determined.

The presence of antibiotics in the soil and their persistence depend on very many factors, the most important of which are whether a soil was amended with manure or wastewater and if so, with what kind, with what frequency, over what range of time, etc. Also, of importance in the presence and persistence of antibiotics are such factors as the physical and chemical characteristics of the soil, which may be different in the rhizosphere and bulk soil, and meteorological factors such as precipitation, humidity, or temperature. These factors as well as others such as pH, soil texture, and organic compound content play an important role in the behavior of antibiotics in the soil. The fate of antibiotics is also strongly affected by sorption properties. Sorption can determine, for instance, whether an antibiotic molecule is leached to surface or ground or surface waters and thus determines its availability for biodegradation (Wegst-Uhrich et al. 2014). Many antibiotics have functional groups, such as hydroxyls, amines, or carboxyls. Depending on the pH values in

different soils, the protonation or deprotonation of these groups that can take place results in the production of negative or positive charges, and the charged molecules can bind to the soil matrix through electrostatic attraction or cation exchange. Last, but not least, as mentioned above, an important role is played by the abundance of microorganisms in any particular soil (Hermans et al. 2016).

An interesting recent study by Pan and Chu (2016) followed the adsorption and degradation of five antibiotics—tetracycline, erythromycin, sulfamethazine, norfloxacin, and chloramphenicol added to sterilized and non-sterilized agricultural soils under aerobic and anaerobic conditions. All of these antibiotics were susceptible to degradation under aerobic conditions, with half-lives ranging from 2.9 to 43.3 days in non-sterilized soil and 40.8 to 86.6 days in sterilized soil. Degradation occurred at a higher rate under aerobic conditions but was relatively persistent under anaerobic conditions. For all the antibiotics, a higher initial concentration was found to slow down degradation and prolong their persistence in the soil, thus affecting their fate. The degradation behavior of the antibiotics varied in relation to their physicochemical properties as well as the microbial activities and aeration of the recipient soil.

Numerous studies have been made to determine the amounts of antibiotics or their metabolites used in animal livestock production for growth promotion and improvement of feed efficiency in untreated (raw) or composted manure that agricultural soil is amended with, usually in liquid form. Antibiotics are poorly absorbed in the gut of animals. As a consequence, a large part of the initial compound is excreted. From the application to livestock to the "end product," which is manure, the range of excreted antibiotic residues lies between 17% and 90% (Jjemba 2002). Part of these residues enters the environment through direct application on a field or through run off from the storage. After excretion, part of these residues can also be re-transformed to the initial compound, and thus become active again (Sarmah et al. 2006). In a very interesting and in depth study by Chee-Sanford et al. (2009) it has been estimated that approximately 75% of antibiotics are not absorbed by animals and excreted in waste. Many such studies have been carried out and the numbers can differ greatly depending on a number of various factors but the general rule is that a significant percentage of antibiotics used can pass from the original site of application to the environment. The rate of antibiotic decomposition depends on a number of environmental factors and also on the chemical nature of the antibiotic itself. Quite a number of antibiotics are difficult to be degraded due to their particular biological, physical, and chemical properties. Among these are the tetracyclines and erythromycin. Bansal (2012) studied the fate of tetracyclines in manure and found that their degradation is very low and follows first-order kinetics. The rate of degradation increases with increased moisture content, temperature, and amount of nitrogen and decreases with increasing antibiotic concentration. In general, tetracyclines are very stable and persist in the environment for extended periods of time.

On the other hand, beta-lactam antibiotics are a class of compounds that are highly susceptible to hydrolysis under mild acidic and basic conditions (Livermore 1995). Hydrolysis of the beta-lactam ring yields a metabolically inactive product. A study of surface water, soils, and liquid manure by Christian (2003) found very low

concentrations of beta-lactam antibiotics in examined samples. Similar results for various water samples were obtained by Cha et al. (2006). However, there may be various reasons for this. A recent study in China showed the high prevalence of beta-lactamase genes in farmlands, which could affect the amount of beta-lactams found (Yang et al. 2019). There are also quite numerous reports showing different results. Subbiah et al. (2011) tested 10 different antibiotics added to three soil-water slurries (silt-loam, sand-loam, and sand; 20% soil [wt/vol]) and incubated the mixtures for 24 h at room temperature. The antibiotic activity of the resultant supernatant was assessed by culturing a sensitive *Escherichia coli* strain in the filter-sterilized supernatant supplemented with Luria-Bertani broth. Large differences in the abilities of the supernatants to suppress growth of *E. coli* were observed. Ampicillin, cephalothin, cefoxitin, and ceftiofur supernatants completely inhibited growth. So did the supernatant from the samples supplemented with florfenicol. Growth of the test bacteria was completely uninhibited in the supernatants from soil samples to which neomycin, tetracycline, and ciprofloxacin had been added. High-performance liquid chromatography (HPLC) analysis demonstrated that cefoxitin and florfenicol were almost completely retained in the supernatants, whereas tetracycline and ciprofloxacin were mostly removed. Antibiotic dissipation in soil, presumably dominated by adsorption mechanisms, was sufficient to neutralize 200 ppm of tetracycline; this concentration is considerably higher than reported contamination levels. Soil pellets from the tetracycline slurries were resuspended in a minimal volume of medium to maximize the interaction between bacteria and soil particles, but sensitive bacteria were still unaffected by tetracycline ($P = 0.6$). Thus, residual antibiotics in soil do not necessarily exert a selective pressure, and the degree to which the pharmaceutical remains bioactive depends on the antibiotic. In general, it can be concluded that the fate of antibiotics in soil depends to a large degree on the properties of the soil itself (Cycon et al. (2019).

When liquid manure, sewage, or sludge is added to the soil, of importance is whether that material has been previously composted or not. Composting has been shown to significantly affect the antibiotic concentration, which is of importance because one of the major consequences of the discharge of antibiotics into the environment could be the formation of bacterial resistance to a specific antibiotic. In a study by Ezzariai et al. (2018), four groups of antibiotics (tetracyclines, fluoroquinolones, macrolides, and sulfonamides) were focused on because of their widespread prevalence in sludge and manure and because of their possible effects on several target and nontarget species. The concentrations of these antibiotics range between 1 and 136,000 μg kg^{-1} of dry matter in sludge and manure, representing a potential risk for human health as well as the environment. During the composting of sludge or manure, the removal of these antibiotics ranged from 17% to 100%. The calculated half-lives ranged for most of them, in turn, from 1 to 105 days (Ezzariai et al. 2018).

The availability and persistence of six veterinary antibiotics, a lincosamide, a fluoroquinolone, two tetracyclines, and two sulfonamides, and also ciprofloxacin in soil as well as soil amended with composted poultry manure was studied (Albero et al. 2018). The incubation assays were conducted at a controlled temperature of

25 °C with different water regimes (e.g., 80% of water holding capacity) and drying-rewetting cycles. The studied antibiotics were determined in soil and soil aqueous phase samples by LC-MS/MS using internal standards. The results indicated that the highest levels found in the soil aqueous phase were for sulfamethoxazole, followed by sulfamethazine and lincomycin, whereas the levels of chlortetracycline, doxycycline, ciprofloxacin, and enrofloxacin were very low (≤1.8%) (Albero et al. 2018). A positive correlation was observed between the antibiotic concentrations and the content of the dissolved organic carbon in soil aqueous phase with the incubation time. An increase in the apparent sorption coefficients of these antibiotics, except chlortetracycline and lincomycin, was observed when the soil was amended with composted manure. Except for the fluoroquinolones, when following the fate of all the other antibiotics, a fast dissipation of antibiotics of around 70% after 90 days of incubation was observed, with half-lives ranging from 8 to 27 days. These values increased between 6% and 53% in manure amended soil; nevertheless, half-lives remained short (9 and 27 days for lincomycin and sulfamethazine, respectively). The results obtained showed that the route of entry of antibiotics into the soil, through recycled water or manure, may have an important effect on their fate, particularly regarding their availability in soil. In general, composting of manure strongly affects the concentration of antibiotics that it may contain. It was also reported that the half-lives of antibiotics belonging to different classes in manure were shorter than the planned storage periods, suggesting that significant degradation of the antibiotic molecules might take place prior to soil amendment. Quinolones and tetracyclines were the most persistent of the antibiotics studied, with half-lives close to 100 days. Aeration of manure sludges was found to strongly negatively affect the fate (persistence) of antibiotics. A broad laboratory study by Gavalchin and Katz (1994) embraced seven antibiotics in soil-feces sludges and found that the order of persistence was:

$$\text{chlortetracycline} \rightarrow \text{bacitracin} \rightarrow \text{erythromycin} \rightarrow \text{streptomycin} \rightarrow \text{bambermycin} \rightarrow \text{tylosin} \rightarrow \text{penicillin}$$

Similar results have been reported by others in more recent research.

A large number of studies have been carried out on the presence and fate of antibiotics in the soil, both in the natural environment and under laboratory conditions in which the persistence of antibiotics added to soil samples was studied. In one such laboratory study 12 different soil types were enriched with clindamycin, sulfamethoxazole, and trimethoprim and the fate of these antibiotics over time was studied. The antibiotics were found to yield different derivatives of the antibiotics, such as clindamycin sulfoxide, hydroxy clindamycin sulfoxide, N-demethyl clindamycin, N4-acetylsulfamethoxazole, and hydroxy trimethoprim (Koba et al. 2018).

The fate of oxytetracycline introduced into the soil with manure was investigated under field conditions by Aga et al. (2005). Soil cores were collected approximately once a month for over a period of 2 years and subsampled at various depth increments. The samples were analyzed by ELISA (enzyme-linked immunosorbent

assay) and/or by liquid chromatography-mass spectrometry (LC-MS). Whereas LC-MS showed that oxytetracycline declined to <50% of its initial soil concentration after 3 weeks, ELISA showed that total tetracyclines did not decline significantly 5 months following the application of manure. The differences between ELISA and LC-MS results are attributed to the broad cross-reactivity of the antibodies employed, which detect many structurally related tetracyclines, including their isomers and degradation products. Only trace amounts (≤1.0 microg/kg) of oxytetracycline were observed in the subsurface soil, and no oxytetracycline was detected in water samples from field lysimeters, suggesting that the antibiotic has low mobility in soil (Aga et al. 2005).

Antibiotics in soil and manure can be degraded in a variety of abiotic processes, some of which have been mentioned above, as well as in biotic ones. Obviously, the biotic breakdown of antibiotics is carried out by the microbial populations that comprise the soil microbiome.

Beta-lactams, macrolides, and sulfonamides appear to be the most susceptible classes of antibiotics to hydrolysis (Gajewska et al. 2004). Penicillin G was found to be readily biodegraded, as was amoxicillin (Gartiser et al. 2007). In the same study imipenem and nystatin showed a certain degree of biodegradation and could be regarded as partially biodegradable with the formation of stable metabolites. An interesting case is that of the aminoglycosides. In spite of their intensive use in animal husbandry, several studies have shown extremely low concentrations of these antibiotics, if any, in animal feces or manure, and consequently also in soil amended with natural fertilizer.

14.3 The Antibiotic Resistance-Inducing Effects of Microbicides in the Environment

The presence of bacteria resistant to antibiotics, including multidrug (multiple)-resistant bacteria (MDR), also known as "superbugs," has become a grave phenomenon with dire future consequences as these bacteria readily spread and may become ubiquitous. This is particularly true in view of the lack, as yet, of easily applicable and widespread alternative methods for combating bacteria. Moreover, there is a stark lack of new drugs in the development pipeline—every antibiotic available today is derived from a class discovered before 1984 (Markiewicz and Kwiatkowski 2018). At best, they are still in their infant stages. The results of a very recent study led by prof. J. A. Roberts in which high-throughput qPCR and geochemistry were used to quantify the abundance and diversity of antibiotic resistance genes (ARGs) and selected mobile genetic elements (MGEs) in samples of soil taken at eight locations in the remote High Arctic of Norway (McCann et al. 2019) revealed the presence of very many antibiotic resistance genes (ARGs), though with highly varied levels depending on location. These included the so-called New Delhi metallo-beta-lactamase 1 (NDM-1) coding gene which inactivates carbapenems, which are

considered a last-resort antibiotic for certain untreatable conditions. One of the aims of this study was to prove that in such a remote area there would be no drug-resistant genes. These genes could have found their way to where they were detected with human waste, in spite of the distance to the main research base, or with bird excrements or carried by small mammals. A similar study involved Arctic/sub-Arctic (polar) sediments that were screened for the abundance and diversity of 30 ARGs against sulfonamide, tetracycline, aminoglycoside, quinolone, macrolide, and β-lactam antibiotics (Tan et al. 2017). Polar sediment ARGs were detected by qPCR at relatively low levels (10^{-9} to 10^{-5} copies/16S rRNA gene copies) compared to the reference sites, which were heavily impacted regions of China (10^{-8} to 10^{-2} copies/16S rRNA gene copies) (Sui et al. 2016). Phylogenetic analyses of resistance sequences from both the Arctic marine sediments as well as of a major database of human pathogens indicated that the ARGs in the polar region were the result of a mix of human influence and natural origins. These studies and others provide significant information on the global reach of antibiotic resistance associated with anthropogenic activities.

Considering the above it is not at all surprising that AMR is becoming so prevalent in our everyday environment in which antibiotics are used in enormous amounts, frequently unnecessarily, mistakenly, or both. The ubiquitous presence of drug-resistant bacteria, according to some estimates, accounts for over 700 hundred thousand deaths worldwide every year, including 230,000 people who die from multidrug-resistant tuberculosis (The Review on Antimicrobial Resistance 2015). A study published by the World Bank in March 2017 estimated that AMR would exert a drag on global GDP of between 1.1% and 3.8% points between now and 2050, which could cost over US$100 trillion in this span of time (World Bank 2017).

It is now obvious that the selection pressure from antibiotics in the environment brings about, accelerates, and disseminates antibiotic-resistant pathogenic bacteria (Bengtsson-Palme and Larsson 2016). As shown in the accompanying chap. 7 of this book, antibiotics reach the environment in enormous amounts. Those that are more frequent in sludges are usually less water soluble, such as norfloxacins, trimethoprim, sulfamethoxazole, and doxycycline. A survey of biocides in sewage sludges in the USA revealed the most abundant were triclocarban and triclosan, followed by a variety of antibiotics. The latter, in the order of decreasing concentration, were:

$$\text{ciprofloxacin} \rightarrow \text{ofloxacin} \rightarrow \text{epitetracycline} \rightarrow \text{tetracycline} \rightarrow \text{minocycline} \rightarrow \text{doxycycline} \rightarrow \text{azithromycin}.$$

Similar results have been obtained by Chinese researchers (e.g., Zhao et al. 2019).

Another group of compounds contributing to selection for antibiotic resistance are various biocides besides those mentioned above. These include chlorhexidine (HXC), quaternary ammonium compounds (QACs), benzalkonium chloride, cetrimide, and didecyldimethylammonium chloride (DDAC), but also, for instance, formaldehyde, methanol, or ethanol. Many of these biocides have very low MIC values against a range of different bacteria, including *Pseudomonas aeruginosa,*

Escherichia coli, and *Staphylococcus aureus,* and have been shown to select for mutations that can confer clinically relevant antibiotic resistance. Under laboratory conditions it has been demonstrated that isolated CHX mutants of *Pseudomonas stutzeri* demonstrated variable levels of increase in resistance to polymyxin B, gentamicin, nalidixic acid, erythromycin, and the beta-lactam antibiotic ampicillin (Russell et al. 1998). A good example of co-resistance in the environment is that of plasmid pSAJ1 from a methicillin-resistant strain of *S. aureus* that also conferred resistance to chlorhexidine and benzalkonium bromide, as well as resistance to several other antibiotics—kanamycin, amikacin, tobramycin, and gentamicin (Yamamoto et al. 1988). Similarly, acquired bacterial resistance to heavy metals that are released in large quantities to the environment may lead to increased resistance of the clones to antibiotics.

Bacterial resistance to antibiotics can be mediated by a number of major mechanisms. These are: (1) alteration (inactivation) of a drug into an inactive derivative by specific enzymes, (2) inhibition of the transport of a drug into the cell as a result of changes in the permeability of the cell envelope, (3) modification or blocking of the target of a drug, (4) removal of a drug from the cell via transportation systems, that is pumps, (5) formation of an alternative metabolic pathway that bypasses the site of action of a drug, or (6) changes in the functioning of regulatory systems that are not directly related to the mechanism of drug action (Dever and Dermody 1991; Munita and Arias 2016; Khan et al. 2018; Markiewicz and Kwiatkowski 2018). Another less frequently encountered mechanism is sequestration of the drug (Naik and Dubey 2013). Bacterial resistance to a heavy metal can also translate to altered susceptibility of the cell to antibiotics. This is observed in the case of reduced membrane permeability, which affects such metals as As, Co, Mn, or Zn and the antibiotics ciprofloxacin, tetracycline, chloramphenicol, and beta-lactams. Similarly, resistance being the result of efflux from the cell can affect susceptibility to several metals such as Cd, Cu, Co, As, or Zn as well as the antibiotics tetracycline, chloramphenicol, and penicillin. Sequestration can apply to the metals Cd, Cu, or Zn and the antibiotic coumermycin A (Baker-Austin et al. 2006). In other words, in some cases mutations that result in the enhanced resistance of bacteria to metals may to some extent alter their susceptibility to antibiotics and vice versa (Markiewicz and Kwiatkowski 2018).

Contaminants contributing to the formation of antibiotic resistance, whether these are heavy metals, other biocides or antibiotics, or the products of their breakdown, enter the environment with various effluents or as a more or less concentrated sludge or slurry, i.e., suspension of solids. The concentrations of these compounds are highly variable. Also entering the environment via the same routes are microorganisms or fragments of microbial cells created, e.g., through self-induced lysis or broken down by other means. Therefore, large amounts of DNA, meaning genes, also enter the environment. In soil and sediments, approximately 1 μg extracellular DNA can be recovered per g material (Ogram et al. 1987), and in fresh and marine waters approximately 0.03–88 μg dissolved DNA per liter can be found (DeFlaun and Paul 1989). The environment has, in fact, been pointed out by many authors as a soil resistome, and thus a reservoir for resistance genes that can be picked up

and disseminated by various bacteria, including pathogens (D'Costa et al. 2007; D'Costa et al. 2011). More about this further down.

Going back to antibiotics, the extent to which these metabolites contribute to the development of AMR is not very well established. It has been well established under laboratory conditions that sub-MIC concentrations can and do select for resistant bacteria (Andersson and Hughes 2014). However, laboratory conditions strongly differ from those in nature and can be variable and the established in vitro MIC values, defined as the lowest concentration of drug that inhibits visible growth of a target bacterial population, are more typical of a clinical setting. Andersson and Hughes (2014) defined a minimum selective concentration (MSC), which represents the lowest concentration of an antimicrobial that give resistant strains a competitive advantage based on growth rates. The MSC represents the point at which the benefit in growth exceeds the fitness cost of carrying the resistance trait vs. a nonresistant strain (Gullberg et al. 2011), and as such, there is a competitive advantage for having the resistance trait at concentrations greater than MSC (Sandegren 2014). Moreover, another study indicated that sublethal concentrations of disinfectants could result in the selection of resistant bacterial populations, and that MSCs would be a more sensitive indicator of selective pressure, especially in environmental systems.

This better reflects enrichment possibilities for resistant bacteria in environments where low levels of antimicrobial are present, for example, in soils and drinking water sources. Lundström et al. (2016) studied whether antibiotics released into the environment could enrich for antibiotic resistance genes and antibiotic-resistant bacteria by selection pressure and thereby increase the risk of their transmission to humans and animals. Their elegant model used tetracyclines which are commonly detected in natural environments. To see if tetracycline pollution in aquatic environments promotes development of resistance, they determined MSCs in biofilms of complex aquatic bacterial communities using both phenotypic and genotypic assays. Tetracycline significantly increased the relative abundance of resistant bacteria at 10 μg/L, while specific *tet* genes (*tetA* and *tetG*) increased significantly at the lowest concentration tested (1 μg/L). Moreover, the taxonomic composition of the biofilm communities changed with increasing tetracycline concentrations. Metagenomic analysis revealed a concurrent increase of several *tet* genes and a range of other genes providing resistance to different classes of antibiotics (e.g., *cmlA, floR, sul1,* and *mphA*), indicating potential for co-selection. Consequently, MSCs for the *tet* genes equal to or less than 1 μg/L suggest that current exposure levels in the environment could be sufficient to promote resistance. These observations concur with the results of several studies on low concentrations of antibiotics which have shown them to have other effects besides being selectors of resistance. They also work as generators of genetic and phenotypic variability by increasing the rate of adaptive evolution, including resistance development, and as signaling molecules influencing various physiological activities, including virulence and gene expression (Lorian 1975; Andersson and Hughes 2014). A recent large study was aimed at estimating the concentrations of 111 commonly used antibiotics reaching the environment and embraced 170 species of bacteria belonging to many different taxa. This work

provided estimated upper boundaries for selective concentrations (lowest MICs) and Predicted No Effect Concentrations (PNECs) for resistance selection for all these antibiotics. The PNECs determined ranged from 8 ng/L to 64 μg/L, and in most cases, PNECs for selection of resistance were below available PNECs for ecotoxicological effects (Bengtsson-Palme and Larsson 2016). The values obtained by these authors and similar future studies may guide the implementation of compound-specific emission limits.

14.4 The Genesis of Antimicrobial Resistance (AMR) in the Environment and Its Dissemination

As mentioned above, the release of antibiotics and other antibacterials into the environment is accompanied by the frequent simultaneous release of bacterial cells and their fragments, which include DNA. Many genera of bacteria release DNA from their cells during active growth. These include, but are not limited to, *Alcaligenes, Acinetobacter, Azotobacter, Bacillus, Escherichia, Flavobacterium, Micrococcus, Neisseria,* and *Pseudomonas* (Lorenz et al. 1991; Matsui et al. 2001). In fact, most bacteria examined to date release DNA during growth in vitro (Lorenz and Wackernagel 1994, Ibáñez de Aldecoa et al. 2017) and frequently this increase is mediated by quorum sensing (Deep et al. 2011). Some studies have suggested extracellular DNA is an important component of bacterial biofilms (Steinberger and Holden 2005). The release of DNA is not limited to bacteria. Other microorganisms such as archaea and fungi are the source of extracellular DNA (eDNA) as well. Besides the natural release of DNA, large amounts of eDNA are found in the environment as a result of the death of cells that can be the result of lysis by bacteriophages, autolysis brought about by own cell enzymes, or the effect of bactericidal agents. Extracellular DNA should not be confused with environmental DNA, also referred to as eDNA (Taberlet et al. 2012), which refers to the total DNA that can be extracted from an environmental sample. eDNA appears to be quite stable once in the environment, where nucleolytic enzymes are strongly diluted and usually in conditions not favoring their activity. Andersen et al. (2001) showed that DNA in a biomass that was subjected to heat treatment at 90C and adjusted to pH 11 survived these procedures and fragments of close to 2000 base pairs could be amplified using PCR. The stability of eDNA in soil has not been very well studied, one of the reasons being sorption. Both chromosomal and plasmid DNA sorb to sand and clay, especially at lower pH values and in the presence of metals. Binding of eDNA has been shown to depend on the physical and chemical properties of the soil constituents and can occur via weak electrostatic forces, ligand exchange, or hydrogen bonding (Cai et al. 2006). DNA studies are less difficult in aqueous environments, such as efflux from water purification plants or sewage. Modern-day techniques allow the identification of known genes coding products that confer antibiotic resistance. These techniques, based on the use of gene probes and polymerase chain

reaction (PCR), provide rapid and sensitive detection without the need for bacterial growth and isolation, which is extremely difficult in view of the large numbers of frequently unknown bacterial species. Detection and identification of genes using microarray technology is also very helpful in the study of various specimens, including environmental samples. Some examples of genes conferring resistance to various antibiotics are presented in a different part of this volume (Zalewska and Popowska 2020). The most common seem to be genes conferring resistance to tetracycline resistance, in particular efflux genes, as well as ribosomal protection genes (Chee-Sanford et al. 2001). Molecular techniques have also shown agricultural soils to be rich in genes closely related to the *vanA* gene in enterococci, which have been shown to confer glycopeptide resistance (Guardabassi and Agerso 2006). Many antibiotic resistance genes coexist on self-transmissible genetic elements with other genes that can determine resistances to heavy metal or other biocides. Antibiotic and mercury resistance genes in enterobacterial strains were found to coexist on transposon *Tn21*, which encodes a mercury-resistance operon, transposition functions as well as resistance to certain aminoglycosides and sulfonamides. Interestingly, it has been shown that some DNA damaging antibiotics, such as the fluoroquinolones, induce a major (global) stress response mechanism known as SOS that results, among others, in a dramatic increase in the rate of horizontal transfer of antibiotic resistance genes (Beaber et al. 2004). The term SOS response refers to a set of co-regulated genes that are induced in response to DNA damage. The system is widespread in bacteria and promotes cell survival by repairing damaged genomes. In *E. coli*, for example, the SOS system consists of more than 40 genes.

It is obvious from the above as well as from other chapters of this book that there are many ways ARGs can enter the environment, whether soil, water, or air, and persist in it. Composting of manure, heat treatment of biomass, anaerobic digestion, sludge digestion, sequencing batch reactors, and other methods have been employed to reduce the amount of antibiotics as well as ARGs, as well reviewed by Waseem et al. (2017). ARGs and antibiotic-resistant bacteria were removed the best by chemical treatments such as ozonation, chlorination, and peracetic acid treatment of urban wastewater. Treatment of sewage sludge, domestic sewage, or chicken manure in which ARGs had been determined using biological treatment technologies gave much worse results. The amount of ARGs in wastewaters can often actually increase as a result of such treatments as anaerobic digestion. This is because bacterial cells that are killed can release their intracellular contents. A study by Farkas et al. (2016) showed that the number of enterobacteria decreasing as a result of treatment was accompanied by a concomitant increase in AMR and multidrug-resistant bacteria carrying gene *intl1*, part of an integron (see below).

To sum up, the soil or water environment in areas of human activity is rich in diverse microorganisms as well as nucleic acids, which translates to multiple genes coding a broad array of different products. Antibiotics present in the environment can exert, especially at sublethal concentrations, a pressure that can result in the appearance of drug resistance among various types of bacteria. The acquired resistance can be passed down from one generation to the next, in what is known as vertical transfer. In such cases resistance is mostly the result of a mutation or mutations

occurring in DNA. A mutation alters the genotype of the cell and may or may not alter its phenotype. If a mutation changes the susceptibility of a bacterium to an antibiotic, for instance, then we observe a change of phenotype to resistance to it.

Three main types of genes preexisting in the genome of wild-type susceptible bacterial cells are relevant for the emergence of antibiotic-resistant mutants. These are (a) genes involved in the synthesis and cell positioning of the antibiotic target; (b) genes involved in the access of the antibiotic to the target; and (c) genes whose products protect the target from the drug (Markiewicz and Kwiatkowski 2018). These may include antibiotic-modifying enzymes or, for instance, proteins involved in the removal (transport) of antibacterial compounds to outside the cell. In the case of antibiotic resistance, the mutation rate is frequently defined as the frequency at which detectable mutants arise in a bacterial population in the presence of a given antibiotic concentration. If the bacterial population is not killed effectively by a given concentration of antibiotic, that is, at sub-MIC values, the cells are under stress, which may increase the mutation rate. The frequency of a mutation in any particular gene is about 10^{-7} per generation but this frequency can be greatly increased in the presence of such stress. Mutated genes conferring resistance to an antibiotic, as well as various DNA elements, can be transferred to other cells of the same species or of other, even unrelated, ones in processes collectively termed horizontal gene transfer (HGT) but sometimes also referred to as lateral gene transfer. The acquisition of foreign DNA through HGT is one of the most important drivers of bacterial evolution and is also frequently behind the development of resistance to antimicrobial agents.

The main events in HGT are conjugation, transduction, and transformation, briefly mentioned in accompanying Chap. 7 of this volume (Zalewska and Popowska 2020). The role of transformation, that is, the uptake of exogenous DNA by bacterial cells, is probably limited in the environment, even though the gene pool in it can be very rich (see above), since the recipient cells must be in a state of competence. This state can be induced in a limited number of both gram-negative and gram-positive species in the laboratory, and many human pathogenic bacteria, including representatives of the genera *Campylobacter, Haemophilus, Neisseria, Pseudomonas, Staphylococcus,* and *Streptococcus*, are naturally transformable (Lorenz and Wackernagel 1994). In the environment competence can arise as a result of, for instance, starvation but is rather limited (Johnston et al. 2014).

Transduction is the bacteriophage-mediated exchange of external genetic material. Two types can be distinguished, generalized and specialized transduction (Goh 2016). In the former, any random portion of the bacterial host genome is packed inside the mature virion. In the latter, DNA from a specific region of the host genome is picked up and incorporated into the virion. Transduction occurs in many different bacteria, including such pathogens as representatives of the genera *Staphylococcus, Escherichia, Salmonella,* or *Pseudomonas.* A bacteriophage, usually double-stranded DNA, can infect a bacterium without killing it, establishing instead a stable relationship. The phage integrates into the bacterial DNA, replicating with it, and is passed to the progeny of the bacterium. The virus is now called a prophage, and the host cell, a lysogen (Batinovic et al. 2019). However, in some cases lysogeny can

turn into a lytic cycle, depending, as in the case of phage lambda, on the levels of two repressor proteins. The one that accumulates to a greater degree controls the outcome of infection, that is, lysis versus lysogeny. When lysis occurs, the newly formed virions can package several genes of the host, with relatively low fidelity of the process, and subsequently transfer them to a different host cell in the process of transduction. In this event, the transferred bacterial DNA can integrate into the recipient bacterium's genome through homologous recombination or recircularize into a replicating plasmid (Novick et al. 2010). Pathogenicity islands are known to horizontally transfer to new strains using this mechanism (Novick 2003).

Conjugation is a type of horizontal gene transfer that requires direct contact between mating cells of the same taxon but can also occur between cells of different genera. The process of conjugation requires a donor cell and a recipient cell. As a rule, conjugation involves the participation of MGEs as vehicles to share genetic information, though direct transfer from one genome to another is not uncommon (Thomas and Nielsen 2005). An example is the F plasmid of *E. coli* which mobilizes the transfer of the donor genome. The most important MGEs are plasmids and transposons, which play a key role in the development and dissemination of antimicrobial resistance (Munita and Arias 2016).

These processes involve the activity of many constituents of the mobilome, which is a pool of genes located within all mobile genetic elements (MGE) of a cell, that is, genomic/pathogenicity islands, integron-associated gene cassettes, insertion sequences (IS elements), plasmids, prophages mentioned above, and transposons (Piotrowska and Popowska 2015). These genes are often referred to as "flexible" and may encode virulence factors, toxic compounds, as well as resistances to various compounds, including antibiotics. Bacteria are able to acquire antibiotic resistance genes that provide protection against most known antibiotics. The acquisition and dissemination of such genes by horizontal gene transfer events has led to the rapid emergence and dissemination of antibiotic resistance among bacteria (Carattoli 2013; Di Conza and Gutkind 2010).

Plasmids are mostly double-stranded and circular replicons of independent extrachromosomal DNA. They cover a variety of sizes from small, often cryptic plasmids to large megaplasmids with many features allowing them to adapt to various conditions in the environment. The vast majority of plasmids carry a number of different transposons (e.g., Tn*3*, Tn*21*, Tn*1213*, Tn*1721*, Tn*4401*), integrons of the first, second, or third class, and IS elements. IS are the most simple transposable elements (TE) that average about 0.5–3 kb and very often are flanked by short sequences of inverted repeats (IR). A transposase gene, encoding the transposition of an IS, is usually located between IRs (Piotrowska and Popowska 2015) and is responsible for transposition events. Recombination among IS elements generates chromosomal events, such as deletions, inversions, or translocations, leading to diversity. Pathogenic bacteria as a rule harbor more IS elements than other bacteria. Transposable elements (transposons, Tn) are similar to IS elements, but have a more complex structure than IS because in addition to the transposase enzyme, they also harbor various genes responsible for specific phenotypes (Oliver et al. 2013). A transposon is a DNA sequence that can change its position within a genome, with

the ability to create or reverse mutations or alter genome size. Tns are larger than ISs, though both these elements have inverted repeats at their termini and a transposase gene. The genes carried by transposons differ widely, and frequently in the laboratory various antibiotic resistance genes are introduced into the Tn structure as selectable markers that allow to follow the fate of the transposon (Alekshun and Levy 2007). This can also occur naturally in the environment. There are two known types of transposition: conservative and replicative. In the former a Tn is excised from one location and is inserted at a second. Subsequently, there is only one Tn both at the beginning of the event and at the end. In replicative transposition, a copy of the original Tn is produced and inserted at a different location (Chandler et al. 2015).

There are very numerous examples of the role of MGEs in the environment. Isolates of the clonal complex 398 livestock-associated methicillin-resistant *Staphylococcus aureus* (MRSA) isolates, very frequently identified in Europe and North America (Cuny et al. 2013), are often resistant to a number of antimicrobial agents. Studies on the genetic basis of antimicrobial resistance in these bacteria identified SCC*mec* cassettes, various transposons, and plasmids of different sizes that harbor antimicrobial resistance genes. While large plasmids that carry multiple antimicrobial resistance genes – occasionally together with heavy metal resistance genes and/or virulence genes – are frequently seen in livestock-associated MRSA, certain resistance genes are also associated with small plasmids of up to 15 kb in size. These small resistance plasmids usually carry only one, but in rare cases also two or three, antimicrobial resistance genes (Feßler et al. 2018).

Members of the highly heterogeneous family *Pasteurellaceae* cause a wide variety of diseases in both humans and animals. However, mutations, as well as the acquisition and dissemination of resistance genes, strongly reduce the efficacy of antimicrobial agents, with the emergence of strains resistant to a number of different antibiotics, such as aminoglycosides, beta-lactams, macrolides, tetracyclines, trimethoprim, and others. Molecular analysis of isolates of *Pasteurella*, *Actinobacillus,* and *Haemophilus* revealed that, in many cases, antimicrobial resistance genes are associated with plasmids. Most of the resistance plasmids identified are less than 15 kb in size, and are non-conjugative, though many carry *mob* genes and have been shown to be mobilizable. For these plasmids to be horizontally transferred by conjugation, the genes encoding the conjugation machinery must be supplied and the only conjugative elements so far identified in members of the *Pasteurellaceae* are ICEs, including those recently described in *P. multocida* and *A. pleuropneumoniae* (Klima et al. 2014). These ICEs may be the source of the genes required for conjugal transfer of the smaller mobilizable plasmids. Some of the resistance genes seem to be more common among members of the *Pasteurellaceae* whereas others have been detected in a wide range of bacteria. This may be due to the fact that streptomycin resistance genes *strA-strB*, for example, have been found to be harbored on broad range plasmids (Scholz et al. 1989) and are also associated with transposon *Tn*5393 (Chiou and Jones 1993). This may explain their widespread occurrence, being the result of HGT since the location of resistance genes on mobile genetic

elements allows their spread into bacteria across both various species and genera (Michael et al. 2018).

Integrons were recognized at the end of the 1980s as a system that carries genetic determinants of the components of a site-specific recombination system that recognizes and captures mobile gene cassettes (Cambray et al. 2010). They play a significant role in the acquisition and spread of ARGs. The essential part of an integron is composed of three key elements: the *intl* gene encoding a site-specific recombinase, an adjacent site, *att*, that is recognized by an integrase and is the receptor site for the gene cassette and a promoter for the expression of the cassette (Hall and Collis 1995, Hall et al. 1999). Integrons are not able to transfer by themselves but can associate with IS present in transposons or conjugative plasmids that serve as a vehicle for their intra- or inter-species transmission (Waldor 2010). Gene cassettes are small mobile elements that generally consist of a single gene and a downstream 59-base element, which is a recombination site. Cassettes differ from most other known mobile elements in that they do not encode the enzymatic machinery responsible for their movement; this is supplied by an integron.

A recent study investigated the persistence and distribution of ARGs in a cattle husbandry environment 2 years after cessation of operations (Agga et al. 2019). Metagenomic DNA was extracted from soil samples and total bacterial population (16S rRNA). Total *Enterococcus* species and class 1 integrons harboring integrase (*int*1) genes, as well as erythromycin (*erm*B and *erm*F), sulfonamide (*sul*1 and *sul*2), and tetracycline (*tet*O, *tet*W and *tet*Q) resistance genes, were quantified and found to be still high 2 years after cattle removal suggesting a lasting effect of confined beef cattle production system on the persistence of bacteria and ARGs in the soil (Agga et al. 2019).

14.5 Concluding Remarks

The discovery and subsequent use of antibiotics has saved the lives of numerous people in the past 70+ years. However, the frequent misuse and unnecessary use of antibiotics has resulted in some dire consequences, the most important of which is the appearance and dissemination of antibiotic-resistant strains of various bacteria, including pathogenic ones. The problem is not new, the first antibiotic-resistant mutants were identified soon after the discovery and implementation of the drugs, the first resistant bacterium being penicillin-resistant *Staphylococcus aureus*, identified in 1947 (CDC 2017). Since that time the number of pathogens resistant to antibiotics has avalanched and now strains belonging to all major pathogenic species have acquired some form of resistance. The development of antibiotic resistance should be viewed as a "normal" adaptive response and a clear manifestation of Darwinian's principles of evolution. Many of the bacterial pathogens associated with epidemics have evolved into multidrug-resistant (MDR) forms. For example, MDR *M. tuberculosis* is currently a major pathogen found in both developing and industrialized nations. Other serious infections include those caused by *Acinetobacter*

baumannii, *Campylobacter jejuni*, *Citrobacter freundii*, *Clostridium difficile*, *Enterobacter* spp., *Enterococcus faecium*, *Enterococcus faecalis*, *Escherichia coli*, *Haemophilus influenzae*, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Salmonella* spp., *Serratia* spp., *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Stenotrophomonas maltophilia*, and *Streptococcus pneumoniae* (Davies and Davies 2010).

The term "superbugs" refers to microbes with enhanced morbidity and mortality due to multiple mutations endowing high levels of resistance to the antibiotic classes specifically recommended for their treatment (Bravo et al. 2018). Each year in the USA, at least two million people are infected with antibiotic-resistant bacteria, and at least 23,000 people die as a result (CDC 2017) and antibiotic resistance has rapidly evolved in the last decades to become now one of the greatest public health threats of the twenty-first century. In fact, infections that are untreatable due to multidrug resistance of the infecting organism have become unsettlingly common in clinical settings (Bravo et al. 2018). This phenomenon is of global proportions and consists a grave threat to public health. Antimicrobial resistance is progressing very dynamically. The appearance of increasingly greater numbers of resistant strains is caused above all by the excessive or improper use of antibacterial in many different areas: medicine, veterinary medicine, animal production, agriculture, food industry, and protection of the environment. The effects of the improper use of antibiotics in one area affect all the other ones. A consequence of the common use of these compounds, especially in preventive treatment and animal production, was an increase in the incidence of AMR among bacterial strains isolated from animals. One example is resistance to glycopeptide antibiotics, including vancomycin, which until quite recently was considered the drug of last resort. One of the reasons indicated as the cause for the appearance of *Enterococcus* spp. strains resistant to vancomycin was the addition of avoparcin to cattle fodder (Różańska et al. 2013). Taking into account the threat to public health related to the increased resistance of strains isolated from animals in 2006 the European Union banned the use of any antibiotics or chemotherapeutics in animal husbandry, except for therapeutic usage: Regulation (EC) No 1831/2003 of the European Parliament and of the Council of 22 September 2003 on additives for use in animal nutrition. This is an important step but only the very first one. The significance of the problem of increasing bacterial resistance and its relation to the use of antibacterial agents as well as the threat to human health have led to the intensification of studies on the clinical aspects of resistance and the monitoring of the phenomenon in zoonotic bacteria – both pathogens (directly threatening human health) and commensals (being a potential reservoir of resistance genes and an indicator of the use of antibacterial agents). In spite of the growing awareness of the threats, illustrated by the hugely increasing number of papers published on the topic and the discussion of the problem in mass media, monitoring of antibiotic resistance among microbes in food and knowledge of the importance of this phenomenon for medicine and veterinary medicine are still inadequate. There is a lack of uniform international guidelines which would enable studying this phenomenon on a global scale. A multi-sectorial approach to fighting AMR requires coordinated action with regard to resistance among bacteria of food, animal, or

environmental origin and their penetration into the direct vicinity of humans. An integrated system for monitoring AMR should enable the comparison of data on the ubiquity of drug resistance in animal husbandry, among microorganisms in food and in the human body. Moreover, strong efforts, accompanied by strict regulations and guidelines (Larsson et al. 2018) and involving technological, social, economic, and behavioral endeavors should be made to limit the amount of antibiotics used and to put an end to their contaminating the environment.

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Chapter 15
Bioremediation Strategies for Removing Antibiotics from the Environment

Umesh B. Jagtap

Abstract Antibiotics are used to treat/prevent infections in humans and animals, but their overuse causes soil, water, and environmental pollution. Additionally, this will lead to the rise of antibiotic-resistant bacteria and antibiotics resistance genes in environment causing serious health problems in human and animals. Bioremediation is an inexpensive and eco-friendly process that utilizes the living organisms (bacteria, fungi, algae, plants, and animals) to remove or detoxify pollutants within a given environment. This chapter summarizes recent scientific reports on the use of bioremediation strategies to remove antibiotics from the environment.

Keywords Bioremediation · Strategies · Antibiotics · Environment

15.1 Introduction

Antibiotics are major pharmaceutical compounds extensively used to treat/prevent human infections and increase feed conversion in animal pharming (Bunce and Hellyer 2018; Kirchhelle 2018). This results in world over increase in demand and production of antibiotics (Van Boeckel et al. 2014, 2015). The antibiotics were released into the environment through various anthropogenic activities like manuring and overthrown antibiotics ensuing soil, water, and environmental pollution. The antibiotics are persistent in the environment (Ezzariai et al. 2018; Tasho and Cho 2016) (Fig. 15.1).The selective pressures that are imposed by antibiotic compounds on bacterial population in the environment promote emergence of antibiotic-resistant bacteria (ARB) carrying antibiotic resistance genes (ARGs). The spread and acquisition of ARGs by clinically relevant bacteria led to develop serious problem for health of human and animals (Berendonk et al. 2015; Hinchliffe et al. 2018). The

U. B. Jagtap (✉)
Department of Botany, Government Vidarbha Institute of Science and Humanities, Amravati, Maharashtra, India

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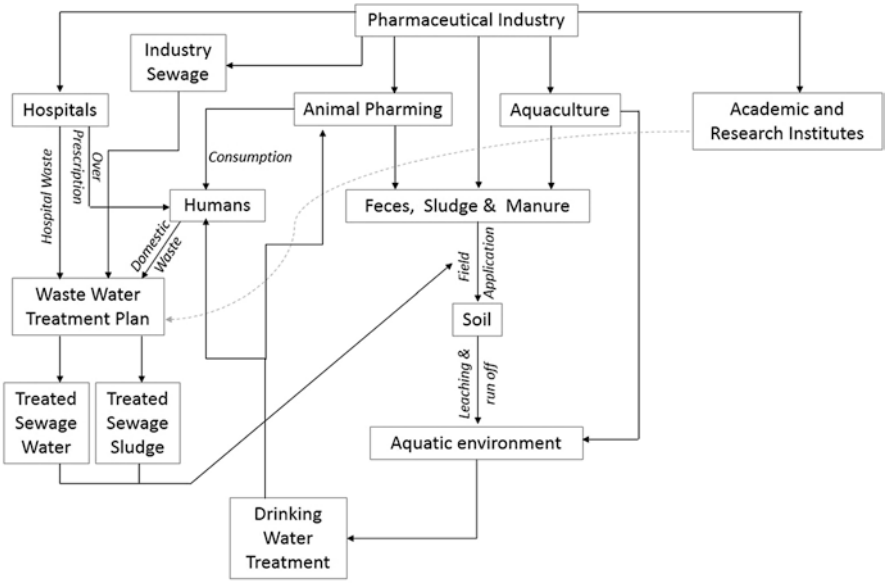

Fig. 15.1 Potential sources, pathways, and different entry routes of antibiotics in environment. (Du and Liu 2012; Li 2014; Tasho and Cho 2017)

large number of deaths are caused by antimicrobial resistance and that number is likely to rise to 300 million by 2050 if the problem is left unaddressed (Bunce and Hellyer 2018; O'Neill 2016). This chapter summarizes recent scientific reports on the use of bioremediation strategies to remove antibiotics from the environment.

15.2 Bioremediation

Bioremediation is a process that utilizes the living organisms (bacteria, fungi, algae, plants, and animals) to remove or detoxify pollutants within a given environment. Bioremediation approaches are generally classified as in situ or ex situ. In situ bioremediation involves treating the polluted material at the site while ex situ involves the removal of the polluted material to be treated elsewhere (Azubuike et al. 2016). The different types and strategies of bioremediation are shown in Fig. 15.2.

15.2.1 Bacterial Remediation: Removal of Antibiotics by Bacteria

The bacteria are most common group of organisms used for the bioremediation. Bacterial remediation can play an important role and offers inexpensive and eco-friendly option for removal of antibiotics from environment. The bacteria which is

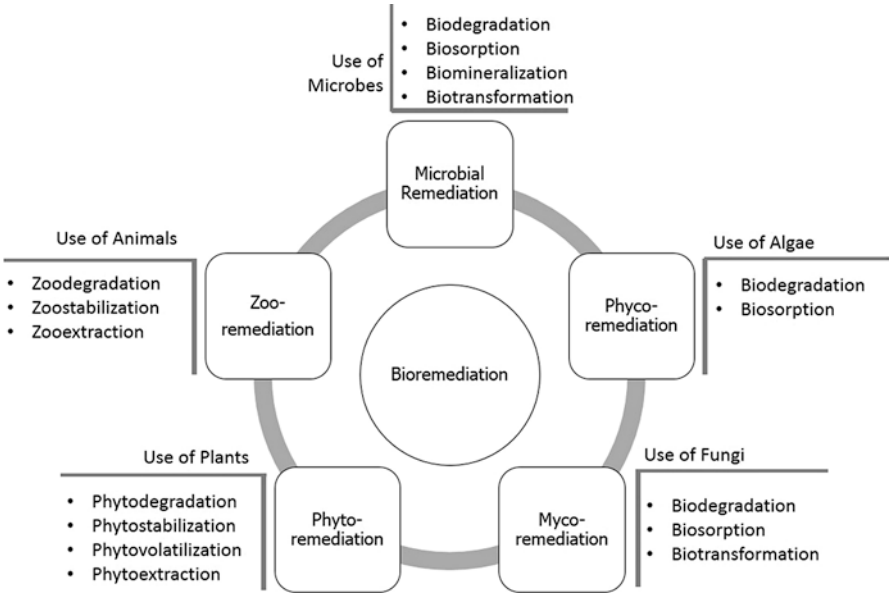

Fig. 15.2 Bioremediation techniques and strategies

used for bioremediation process should possess certain characteristics such as the ability to survive under extreme conditions (e.g., redox, moisture, nutrient, osmotic factor, and pH) and compete with indigenous microbial populations (Morikawa 2006). The exploitation of the bacterial methods such as biosorption and enzymatic biodegradation processes for the removal of heavy metals and antibiotics has been reviewed by Al-Gheethi et al. (2015).

The increased loads and persistent presence of antibiotics in the environment can cause selective pressure for bacteria that leads to the development of antibiotic resistance/or tolerance in bacteria. Antibiotic resistance and antibiotic tolerance do not seem to be equal. "Resistance" is used to describe the inherited ability of microorganisms to grow at high concentrations of an antibiotic, irrespective of the duration of treatment, and is quantified by the minimum inhibitory concentration (MIC) of the particular antibiotic, whereas "tolerance" is more generally used to describe the ability, whether inherited or not, of microorganisms to survive transient exposure to high concentrations of an antibiotic without a change in the MIC, which is often achieved by slowing down an essential bacterial process (Brauner et al. 2016).

A microbial bioremediation approach may involve both biosorption and biodegradation processes. Diverse strains of algae have been effectively employed for the removal of antibiotics (Table 15.1). Al-Gheethi et al. (2014) studied the biosorption of heavy metal ions and the antibiotic cephalexin from secondary effluents by the cell biomass of tolerant bacterial strains isolated from a secondary effluents generated by sewage treatment plants at Penang, Malaysia. The maximum biosorptive capacity of cephalexin was observed in mixed living cell biomass (60 mg g^{-1}). For

Table 15.1 Some antibiotic degrading bacterial species

Organism	Strain	Source	Biodegradation condition	Biomass	Removal	Time	References
Shewanella oneidensis	MR-1	Soil Microbiology Laboratory of Northwest Agriculture and Forestry University (Shanxi, China).	LB + 10 mg/L SPY or SMX, 30 °C	10^{-7} CFU/mL	SPY (23.91%); SMX (59.88%)	5 days	Mao et al. (2018)
Shewanella sp.	MR-4	Soil Microbiology Laboratory of Northwest Agriculture and Forestry University (Shanxi, China).	LB + 10 mg/L SPY or SMX, 30 °C	10^{-7} CFU/mL	SPY (23.43%); SMX (63.89%)	5 days	Mao et al. (2018)
Alcaligenes faecalis	CGMCC 1.767	Pure culture	MMSM + 1 g/L acetate + 1 g/L glucose + 50 mg/L SMX + 100 ppm vitamin C; 30 °C	OD540 = 0.20	80–90% SMX	16 h	Zhang et al. (2016)
Rhodococcus rhodochrous	ATCC 13808	Pure culture	MMSM + 3 g/L glucose + 31.6 mg/L SMX or 43.4 mg/L SMZ, 26 °C	–	SMX (20%); SMZ (14%)	SMX 36 days; SMZ (12 days)	Gauthier et al. (2010)

Rhodococcus equi	ATCC 13557	Pure culture	MMSM + 6 mg/L SMX + 0.5 g/L glucose,26 °C	_	SMX (15–29%)	120 h	Larcher and Yargeau (2011)
Microbacterium sp.	BR1	Activated sludge (AS)	MSM + 127 mg/L SMX	OD600 = 1.0	SMX (24–44%) mineralization	16 days	Bouju et al. (2012)
			MMSM + 0.5 mg/L yeast extract + 0.5 mg/L vitamins + 127 mg/L SMX, 28 °C	OD600 = 1.0	SMX (100%)	56 h	Birkigt et al. (2015)
Bacillus subtilis	1556WTNC	Sewage treatment plants (STPs) at Penang, Malaysia	Sterilized treated sewage effluents + 0.2 to 5 mg/L amoxicillin or ampicillin or cephalexin orcefuroxime or ciprofloxacin, 35 °C	5.98 log10 CFU mL^{-1}/50 mL sewage effluent	Amoxicillin (25.03% at 1 mg/mL); ampicillin (15.59% at 0.8 mg/mL); cephalexin (22.59% at 1 mg /mL); cefuroxime (10.62% at 1 mg/mL); ciprofloxacin (2.45% at 0.6 mg/mL)	12 days	Al-Gheethi and Norli (2014)
Achromobacter denitrificans	PR1	AS	MSM + 152 ppm SMX	OD600 = 1.00	100%	14 days	Reis et al. (2014)
			MSM + 152 ppm SMX + 590 ppm succinate	OD600 = 1.00	100%	1.8 days	
			MSM + 25 ppm SDZ	OD600 = 1.00	98%	2.3 days	
			MSM + 31 ppm SDM	OD600 = 1.00	48%	2.3 days	
			MSM + 28 ppm SMT	OD600 = 1.00	98%	2.3 days	
			MSM + 25 ppm SPY	OD600 = 1.00	100%	2.3 days	
Methylobacterium sp.	SDZ-W2-SJ40	AS	AMS + 0.04% yeast extract + 5 mg/L SDZ	OD600 = ~1.00	SDZ (55.2%)	12 days	Mulla et al. (2016)

(continued)

Table 15.1 (continued)

Organism	Strain	Source	Biodegradation condition	Biomass	Removal	Time	References
Arthrobacter sp.	D2	AS	MSM + 50 mg/L SDZ + 2 g/L glucose, 37 °C	OD595 = 0.30	SDZ (99%) degradation; SDZ (82.5%) mineralization	53 h	Deng et al. (2016)
Arthrobacter sp.	D4	AS	MSM + 50 mg/L SDZ + 2 g/L glucose, 37 °C	OD595 = 0.30	SDZ (99.8%) degradation; SDZ (34.4%)mineralization	11 days	Deng et al. (2016)
Geobacillus thermoleovorans	S-07	AS	MMSM + 0.5 g/L glucose + 10 mg/L sulfonamides; 70 °C	OD600 = 2.00	95% SM2 degradation; 72.96–85.96% other sulfonamide degradation	24 h	Pan et al. (2017)
Acinetobacter sp.	W1	AS	MSM + 5–240 mg/L SMX + 5 mg/L DO, 25 °C	OD600 = 0.2	100% SMX degradation; 95–100% SMX mineralization	24 h	Wang and Wang (2018)
Paracoccus sp.	SDZ-PM2-BSH30	Pig manure	AMS + 0.04% yeast extract + 5 mg/L SDZ; 30 °C	OD600 = ~0.8	50.0% SDZ degradation	12 days	Mulla et al. (2016)
Kribbella sp.	SDZ-3S-SCL47	Sediment	AMS + 0.04% yeast extract + 5 mg/L SDZ; 30 °C	OD600 = ~1.1	60.6% SDZ degradation	12 days	Mulla et al. (2016)

SDZ sulfadiazine; *SDM* sulfadimethoxine, *SMZ* sulfamethazine; *SMT* sulfamethizole; *SPY* sulfapyridine; *SMX* sulfamethoxazole; *MMSM* minimum mineral salt medium, *MSM* mineral salts medium, *LB* Luria-Bertani medium, *AMS* ammonium mineral salts

living cells, Gram-positive bacteria had a higher biosorptive capacity than Gram-negative ones (50.91 vs. 40.44 mg g^{-1}). For dead cells, Gram-negative bacteria had a higher biosorptive capacity (25.11 vs. 15.99 mg g^{-1}). Among all individual bacterial strains, the highest biosorptive capacities were observed in living cell biomass of *B. subtilis* 1612WTNC (35.02 mg g^{-1}) and dead cell biomass of *B. cepacia* 103WTNC (40.74 mg g^{-1}). Furthermore, the authors evaluated the biosorption of cephalexin by bacterial biomass (living and dead cells) in aqueous solutions contaminated with the heavy metals Ni^{2+} (1 mg L^{-1}), Cu^{2+} (1 mg L^{-1}), Zn^{2+} (2 mg L^{-1}), Pb^{2+} (0.5 mg L^{-1}), and Cd^{2+} (0.1 mg L^{-1}). The efficiency of cephalexin biosorption was reduced by more than 40.83 and 82.88% (living and dead cells, respectively) in the presence of 1 mg L^{-1} Ni^{2+} ions compared with the control, whereas no biosorption by dead cell biomass was recorded in aqueous solutions contaminated with cadmium, zinc, copper, and lead ions.

In another report, Al-Gheethi and Norli (2014) have investigated the biodegradation of antibiotics (cephalexin, cefuroxime, ampicillin, and amoxicillin) in sewage-treated effluents by b-lactamase produced from *B. subtilis* 1556WTNC. The biodegradation process was performed at the optimal conditions for b-lactams production (5.9 log10 CFU mL^{-1}; pH 6.5; temperature 35 °C for 12 days). They revealed that the maximum biodegradation was 25.03% at 1 mg mL^{-1} for amoxicillin, 15.59% at 0.8 mg mL^{-1} of ampicillin, 22.59% at 1 mg mL^{-1} of cephalexin, 10.62% at 1 mg mL^{-1} of cefuroxime, while it was 2.45% at 0.6 mg mL^{-1} of ciprofloxacin.

15.2.2 Phytoremediation: Removal of Antibiotics by Algae

Phytoremediation is a part of bioremediation where algae are being used for the removal or biotransformation of pollutants, including nutrients, xenobiotic, and CO_2. Algae are aquatic and photoautotrophic organisms offering cost-effective, nonintrusive, and safe cleanup technology for removal of antibiotics from environment. The algae offer several advantages as follows: (1) The blue-green alga (cyanobacteria) uses light energy source and CO_2 for its growth and survival. This way it helps in carbon sequestration and mitigation of global warming. (2) They are capable of not only photosynthesis but also fixing up atmospheric nitrogen, and they can survive better under the nutrient-limited conditions. (3) Microalgae have the greatest abundance of plant biomass in aquatic environments. Microalgae cultures can be cultivated in open ponds or in large-scale water reservoirs. At the same time, the algal growth under laboratory conditions provides reliable and consistent supply of biomass. (4) As the nontarget organism, green algae have higher tolerance to antibiotics than bacteria. (5) They have the potential to treat sites polluted with more than one type of pollutant. (6) They are economically more viable and an eco-friendly tool. (7) They generate lesser volume of chemical and/or biological sludge to be disposed of (Dixit and Singh 2015).

Guo and Chen (2015) have applied alga-activated sludge combined system as a novel treatment to remove cephalosporins. The green alga *C. pyrenoidosa* performed excellent removal capacity for the four target antibiotics (cefradine, cefalexin, ceftazidime, and cefixime). In addition, the green alga has high tolerance to the impact of the antibiotics. A satisfactory growth ability of *C. pyrenoidosa* was observed during the treatment and the algal cell size increased with the removal process. Cefradine could be partly removed by the acclimated activated sludge after a long time adaptation, while an excellent removal efficiency was obtained based on the un-acclimated green alga and un-acclimated activated sludge directly in the combined system.

However, most of the studies focused on the removal capability of algae, which grown in an unpolluted environment before the treatment and ignored whether the feedback of alga to the toxic stress influenced the removal capability in a subsequent treatment batch. Algal tolerance of contaminants plays a decisive role in continuous pollution treatment processes. It is possible that the sensitivity or tolerance of algae changes after the first treatment and therefore causes feedbacks during continuous treatment that influences the final removal efficiency. Therefore, in another study, Chen et al. (2015) investigated and compared algal feedback and removal efficiency of *C. pyrenoidosa* in a sequencing batch reactor algae process (SBAR) to remove cefradine. The results revealed that during the first treatment batch, the antibiotic cefradine influenced the biomass of the green algae *C. pyrenoidosa*. Meanwhile, the "toxic background" of the algae also produced a physiological response and degraded the antibiotic in the subsequent treatment batch. However the maximum population inhibition rate was observed 96 h after the second treatment batch for all tested concentrations. The result indicated that the green algae were also able to adapt to varied pollution loads in different treatment batches.

The use of lipid-accumulating microalgae to remove antibiotics from wastewater has offered additional benefit of biofuel production. Guo et al. (2016) observed that the use of microalgal strains (namely, *Chlorella* sp. Cha-01, *Chlamydomonas* sp. Tai-03, and *Mychonastes* sp. YL-02) improves removal of cephalosporin antibiotics 7-amino cephalosporanic acid (7-ACA) by hydrolysis and photolysis reactions without affecting microalgal lipid accumulation ability. However, 7-ACA had slight inhibition effects on the microalgal growth (9.6–12%). Thus, the current approach of the use of microalgal strains is to establish the best conditions for simultaneous removal of 7-ACA in real wastewater and production of lipid-rich microalgal biomass for subsequent biofuels generation seems to be a cost-effective and bio-safe technology.

C. pyrenoidosa algae was evaluated in the elimination of antibiotic ceftazidime and its basic parent structure 7-ACA with removal rates of 92.70% and 96.07%, respectively (Yu et al. 2017). The algal removal mainly involved a rapid adsorption, a slow cell wall transmission, and the final biodegradation. The LC-MS analysis revealed that Δ-3 ceftazidime and trans-ceftazidime were regarded as the metabolites of ceftazidime and the metabolite of 7-ACA was regarded as a compound which shared the similar structure with 4-chlorocinnamic acid. This study

demonstrates that using green algae to treat antibiotic is promising for the application due to the potential of high removal efficiency and low environmental impact (Yu et al. 2017).

Numerous microalgae species in a single, natural habitat interact with each other synergistically or antagonistically and may compete for nutrients and/or light. Therefore, multispecies tests are expected to provide a more realistic appraisal of the response of microalgae to the exposure of toxic compounds. This fact represents a motivation for the study of ecotoxicity and removal of a fluoroquinolone antibiotic enrofloxacin (ENR) by five individual microalgae species (*Scenedesmus obliquus*, *Chlamydomonas mexicana*, *Chlorella vulgaris*, *Ourococcus multisporus*, *Micractinium resseri*) and their consortium (Xiong et al. 2017). The authors have found that the microalgae consortium showed a higher sensitivity toward ENR than the individual microalgae species. However, ENR removal efficiency of the constructed microalgae consortium was comparable to that of the most effective microalgal species.

A phycoremediation approach may also involve biosorption, where the biomolecule binds to the algal wall (i.e., biosorbent). The high surface area to volume ratio (*S*/*V* ratio) of the algae and functional groups (amino, carboxyl, hydroxyl, and carbonyl groups) on the surface of algal biomass makes algae an attractive choice for biosorption. Santaeufemia et al. (2016) showed that, the living biomass of the microalga *Phaeodactylum tricornutum* is a useful tool for oxytetracycline (OTC) phycoremediation. The use of living biomass was much more effective and efficient than the same amount of dead biomass. A culture of *Phaeodactylum tricornutum* microalga (equivalent to 0.4 g of dry biomass L^{-1}) eliminated 97% of 2.5 mg L^{-1} of OTC in 11 h. The highest sorption capacity was 29.18 mg g^{-1}. The culture conditions of this microalga allowed to combine bioremediation with photodegradation. Thus, the results obtained in this study demonstrated that living biomass of this microalga was a promising low-cost and an eco-friendly alternative to be used in the OTC removal from seawater solutions. Similarly, algae have been investigated as a biosorbent for the removal of antibiotics such as tetracycline (de Godos et al. 2012), norfloxacin (Zhang et al. 2012), and spiramycin (Liu et al. 2012).

15.2.3 Mycoremediation: Removal of Antibiotics by Fungi

Mycoremediation is a part of bioremediation where fungi are being used for the removal or biotransformation of pollutants. Recently, excellent reviews have been published describing the role of fungi in biodegradation of pharmaceutical compounds (Olicón-Hernández et al. 2017) and pesticides (Spina et al. 2018). The unique characteristics of fungi such as the ability to form extended mycelial networks, the low specificity of their catabolic enzymes, and their independence from using pollutants as a growth substrate make these fungi well suited for bioremediation processes (Harms et al. 2011).

Currently, fungi have been proven to be effective in degrading and mineralizing recalcitrant antibiotics due to their powerful enzymatic machinery (extracellular ligninolytic enzyme system), robust morphology, and diverse metabolic capacity (Čvančarová et al. 2015). A number of fungi that are antibiotic degraders belong to the phyla Ascomycota and Basidiomycota followed by the sub-phylum Mucoromycotina (Table 15.2).

Fungi have a variety of strategies to counteract with a myriad of toxic compounds such as recalcitrant polycyclic aromatic hydrocarbons (PAHs), pesticides, and antibiotics. These strategies include nonenzymatic process such bioadsorption, biomineralization (bio-precipitation) as well as biotransformation and biodegradation mediated by enzymatic systems (Olicón-Hernández et al. 2017). The role of various fungal species in remediation of antibiotics were summarized in Table 15.2

15.2.4 Phytoremediation: Removal of Antibiotics by Plants

The usage of natural or genetically modified plants and their associated rhizospheric microbes to remediate contaminated soil, sediments, and water is known as phytoremediation. The fate and effect of pharmaceutical compounds in the environment and their uptake and remediation by plants have been well reviewed in previous publications/review articles (Carvalho et al. 2014; Jagtap 2017; Tasho and Cho 2016).

Phytoremediation has recently been receiving attention as a promising, cost-effective, and eco-friendly method to remove active pharmaceutical ingredients from contaminated soil and water as compared to conventional methods. However, the phytoremediation technologies are less utilized for the removal of antibiotics from soil (Jagtap 2017).

Pteris vittata (L.) was evaluated for the removal of tetracycline (TC) antibiotics from water. The results showed that more than half of the TCs could be removed from the water solution (with the starting concentration of TCs about 1.0 mg kg^{-1}) after 1 day of treatment. No TCs (less than 0.01 mg kg^{-1}) were detected in the solution after 5 days of treatment. Accumulation of TCs was very low in both the roots and the pinnae of *Pteris vittata*, which indicates that accumulation in the fronds is not the main removal mechanism. The main removal mechanism was plant uptake and/or degradation in the fronds (Li et al. 2015). Present results provide a feasible method for removal of TCs from livestock-polluted wastewater. However, more research work should be done before any real-world application is made.

Preliminary results by Gahlawat and Gauba (2016) demonstrated that *Brassica juncea* could remove 71% tetracycline after 24 days in in vitro conditions. However, as initial tetracycline concentrations were increased in the media, the remediation rate also improved. However, at higher concentrations, the plants showed phytotoxicity as depicted by the decrease in shoot length of the germinated seeds (Gahlawat and Gauba 2016).

Table 15.2 Some antibiotic degrading fungi

Division (fungal taxon)	Name of organisms (fungal organism)	Antibiotic	Metabolites	Comments	Detection method	Focus of study	References
Basidiomycota	*Pleurotus ostreatus*	Ciprofloxacin	–	(1) maximum enzyme production at the highest concentration of CIP (500 ppm). (2) degraded products exhibited a decreased antimicrobial activity	Titrimetric and spectrophotometric assays, HPLC	Biodegradation	Singh et al. (2017)
Basidiomycota	*Irpex lacteus*[b], *Trametes versicolor*[a]	Ciprofloxacin	Desethylene-N-ciprofloxacin; 7-amino-1-cyclopropyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid; N-acetylciprofloxacin[a]; Desethylene-N-acetylciprofloxacin; N-formylciprofloxacin; desethylene-N-formylciprofloxacin; hydroxymethyl-N-ciprofloxacin	(1) *Irpex lacteus* and *Trametes versicolor* degraded fluoroquinolones most efficiently. (2) the compounds are attacked at the piperazinyl moiety: Substitution or decomposition.	HPLC-MS/MS	Biodegradation	Čvančarová et al. (2015)

(continued)

Table 15.2 (continued)

Division (fungal taxon)	Name of organisms (fungal organism)	Antibiotic	Metabolites	Comments	Detection method	Focus of study	References
				(3) Only *I. lacteus* removed the antibiotic activity during the degradation. (4)manganese peroxidase might participate in the degradation			
		Ofloxacin	9-Fluoro-10-[(2-formamidoethyl) amino]-3-methyl-7-oxo-2,3-dihydro-7H-[1,4] oxazino[2,3,4-Ij]quinoline-6-carboxylic acid[b]; 9-fluoro-10-(4-formylpiperazin-1-yl)-3-methyl-7-oxo-2,3-dihydro-7H-[1,4] oxazino[2,3,4-Ij]quinoline-6-carboxylic acid; desethylene-Norfloxacin; 10-[(2-acetamidoethyl) amino]-9-fluoro-3-methyl-7-oxo-2,3-dihydro-7H-[1,4] oxazino[2,3,4-Ij] quinoline-6-carboxylic				

			Acid; 10-amino-9-fluoro-3-methyl-7-oxo-2,3-dihydro-7H-[1,4] oxazino[2,3,4-Ij]quinoline-6-carboxylic acid; 10-(4-acetylpiperazin-1-yl)-9-fluoro-3-methyl-7-oxo-2,3-dihydro-7H-[1,4] oxazino[2,3,4-Ij]quinoline-6-carboxylic acid[a]; desmethyl-N-ofloxacin[a]				
		Norfloxacin	Desethylene-N-norfloxacin[a]; 7-amino-1-ethyl-6-fluoro-4-oxo-1, 4-dihydroquinoline-3-carboxylic acid; desethylene-N-acetylnorfloxacin; N-formylnorfloxacin;				
Basidiomycota	*Trametes versicolor*	Ofloxacin	–	–	–	–	Gros et al. (2014)
Ascomycota	*Aspergillus terreus FZC3*	Gentamicin	–	(1) the gentamicin removal efficiency exceeded 95% by day 7 under optimized culture Conditions	–	Biosorption and biodegradation	Liu et al. (2016)

(continued)

Table 15.2 (continued)

Division (fungal taxon)	Name of organisms (fungal organism)	Antibiotic	Metabolites	Comments	Detection method	Focus of study	References
Basidiomycota	*Gloeophyllum striatum*	Ciprofloxacin, Enrofloxacin	–	–	–	Biodegradation and metabolite identification	Wetzstein et al. (1997, 1999)
Zygomycota	*Mucor ramannianus*	Enrofloxacin	–	–	–	Biotransformation	Parshikov et al. (2000)
Ascomycota	*Pestalotiopsis guepini*	Ciprofloxacin and norfloxacin	–	–	–	Biotransformation	Parshikov et al. (2001)
Basidiomycota	*Trametes versicolor*	Norfloxacin and ciprofloxacin	–	–	–	Biodegradation	Prieto et al. (2011)
Ascomycota	*Trichoderma viride*	Ciprofloxacin and norfloxacin	4-Hydroxy-3-oxo-4-vinylcyclopent-1-enyl ciprofloxacin; 4-hydroxy-3-oxo-4-vinylcyclopent-1-enyl norfloxacin.	Metabolites found to be optically active	HPLC; LC/ESI MS; NMR	Identification of degraded products	Parshikov et al. (2002)
Mucoromycotina	*Cunninghamella elegans*	Flumequine	–	–	–	Biotransformation	Williams et al. (2007)

[a,b]Indicates metabolites formed by respective fungal organism after antibiotic degradation

HPLC high performance liquid chromatography, *MS* mass spectrometry, *NMR* nuclear magnetic resonance, *LC/ESI-MS* liquid chromatography electrospray ionization mass spectrometry

Sulfamethazine (SMN) was taken up and translocated by alfalfa (*Medicago sativa*) grass grown hydroponically in a commercially available nutrient solution supplemented with 10 mg L^{-1} of SMN antibiotic. Analysis of alfalfa sap, root zone, middle one-third, and top portion of the foliage showed varying uptake rate and translocation of SMN. The highest average amount of SMN (8.58 μg kg^{-1}) was detected in the root zone, followed by the top portion (1.89 μg kg^{-1}), middle one-third (1.30 μg kg^{-1}), and sap (0.38 μg kg^{-1}) samples, indicating a clear distribution of SMN within the sampled regions. The ultraviolet spectra of parent SMN and translocated SMN identified in different parts of the plant present the possibility of metabolization during the uptake process. Uptake of SMN using alfalfa grown under hydroponic conditions has potential as a promising remediation technology for removal of similar antibiotics from wastewater lagoons (Kurwadkar et al. 2017).

In another study Singh et al. (2018) studied the phytotoxicity pertaining to growth, oxidative stress, and biochemical traits as well as degradation of amoxicillin antibiotics in the duckweed *Spirodela polyrhiza*. The results showed that the high dose (1 mg L^{-1}) of amoxicillin caused a significant ($p < 0.05$) decrease in photopigments, protein, starch, and lipid content and an increase in carotenoids/total Chlorophyll and Chlorophyll a/Chlorophyll b ratios in fronds of *Spirodela polyrhiza*. The results showed a shift in biomarkers: a decrease in frond growth and relative growth rate (16.2–53.8%) and an increase in the activities (mmol mg $protein^{-1}$) of catalase (0.021–0.041), ascorbate peroxidases (0.84–2.49), and superoxide dismutase (0.12–0.23) in fronds. The significantly ($p < 0.05$) greater reduction in amoxicillin content in duckweed setups (84.6–100%) than in the control (62.1–73%) suggested that phytodegradation is an important mechanism in removing antibiotics from water, apart from hydrolysis and photodegradation, which occur in control setups. Overall, the results suggested a toxic effect of amoxicillin on *Spirodela polyrhiza*, even at low concentrations, and nonetheless, the duckweed contributed directly to the degradation of antibiotics in the water and throughout the phytoremediation process.

15.3 Conclusion and Future Perspectives

The use of antibiotics also plays a vital role in medical treatment, veterinary, and agriculture farms to cure or prevent bacterial infections in humans, to increase feed efficiency as well as growth performance in animals and plants, respectively. However, due to its intensive use, antibiotic pollution has emerged as an urgent issue. Furthermore, widespread antimicrobial resistance poses a threat to public and animal health. The inveterate undesirable effects of antibiotics on the environment should attract considerable attention to remove antibiotics from the contaminated environments.

Bioremediation in the broad sense offers a powerful technology for the removal of various contaminants from environment. Hitherto, varieties of organisms have been characterized to degrade/remove antibiotics from the environment. However, genetics and biochemistry of the highly efficient/desired organism have yet not been properly explored. This will lead to the identification of the functional genes and enzymes and their role in the antibiotic degradation. Moreover, the advances in genetic engineering and synthetic biology tool box will also be helpful in order to develop large-scale applications of antibiotic degrading organisms for bioremediation.

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Chapter 16
Trend of Antibiotics and Resistance Genes in Water Resources and Wastewater Treatment Plants

Toqeer Ahmed, Muhammad Zeeshan Hyder, Irfan Liaqat, Rabbia Murtaza, and Gloria I. B. Obioh

Abstract Antibiotics usage and development of resistance genes in water resources is one of the global issues which needs to be addressed on priority. Antibiotics have been used as safeguard against diseases and to protect public health, but excessive and unwise use is unsafe to both human and environment. Development of resistance bacteria and their antibiotic-resistant genes (ARGs) in water resources, especially in the wastewater treatment plant, and transfer of water and soil bacteria into human pathogens is alarming. Different treatment options and effective strategies like use of UV, ozone, nanofiltration, etc. can be effective ways to reduce antibiotic-resistant bacteria (ARB). In this chapter, ARB and ARGs in water resources, methods for analyzing ARGs and ARBs in water resources of Pakistan, regulations and policy implications to conserve water resources, and advanced treatment option to reduce ARGs in wastewater plants have been discussed. The studies showed are encouraging but more work on advanced treatments could be able to limit the ARGs spread in the environment.

T. Ahmed (✉) · R. Murtaza
Centre for Climate Research and Development (CCRD), COMSATS University Islamabad, Islamabad, Pakistan
e-mail: toqeer.ahmed@comsats.edu.pk

M. Z. Hyder
Department of Biosciences, COMSATS University Islamabad, Islamabad, Pakistan
e-mail: zeeshan_hyder@comsats.edu.pk

I. Liaqat
Centre for Climate Research and Development (CCRD), COMSATS University Islamabad, Islamabad, Pakistan

Department of Biosciences, COMSATS University Islamabad, Islamabad, Pakistan

G. I. B. Obioh
Environmental Biotechnology & Bioconservation Department, National Biotechnology Development Agency (NABDA), Abuja, Nigeria

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Keywords Antibiotics · Resistance genes · Water resources · Wastewater treatment plants

16.1 Introduction

Antibiotics have been used as safeguard to save public health since its discovery in 1928 by Alexander Fleming but their extensive use, misuse, and unwise use are alarming and unsafe to both humans and environment. Proclamation of antibiotics in water resources and soil is increasing and water distribution system is providing habitat for the growth of antibiotic resistance bacteria (ARB) and this might be a reason of increasing antibiotics resistance genes (ARGs) in water resources (Xi et al. 2009; Manaia et al. 2018). Wastewater treatment plants (WWTPs) which are important component of modern urban settings, designed to efficiently use water resources, bear huge potential of unintended dispersal of ARGs. These plants receive water from multiple sources including domestic sanitary drainage, poultry and animal farms, industrial effluents, and hospital drainage to mention few. The waste from these diverse sources is often rich in organic matter, minerals, and particulate matter, thereby offering conducive environment for the growth of variety of bacteria (Baquero et al. 2008; Rizzo et al. 2013; Guo et al. 2013; Michael et al. 2013; Hembach et al. 2017). After various treatments in the WWTPs, the organic matter is broken down and maximum waster recovered and cleaned. However, the effluents which are released to the environment have high concentration of ARB, treated organic matter, and minerals, providing favorable conditions where ARBs containing ARGs may thrive and acquire ARGs through horizontal gene transfer and other mechanisms (Turolla et al. 2018).

Wastewater has been observed as a main reserve of antibiotics and ARB. Resistance to antibiotics has been observed in treatment process and tap water along with wastewater plants (Zhang 2016; Xi et al. 2009). Occurrence of metabolites or their residue in human and animal waste and leaching of these into soil and water contaminating both soil and water resources and their persistent stay in the water environment may increase risk of antibiotic resistance genes (WHO 2014). Empirical studies have confirmed that ARBs originating from human and animal feces do enter and contaminate water bodies. It has also been reported that about 90% of all antibiotics excreted through human feces and urine passes into water resources and or found in wastewater treatment plants (SIWI 2018). Along with human and animal interventions, many industries contribute in the antibiotics contamination of water resources which potentially alter the whole water ecosystem. Mixing of heavy metals and disinfectants along with antibiotics may damage the water ecology and antibiotic resistance can occur (Baquero et al. 2008).

Climate play a significant role in predicting the presence of ARB in water resources, especially surface water, as wet season has more antibiotics resistance than dry season (Sanderson et al. 2016) (Fig. 16.1).

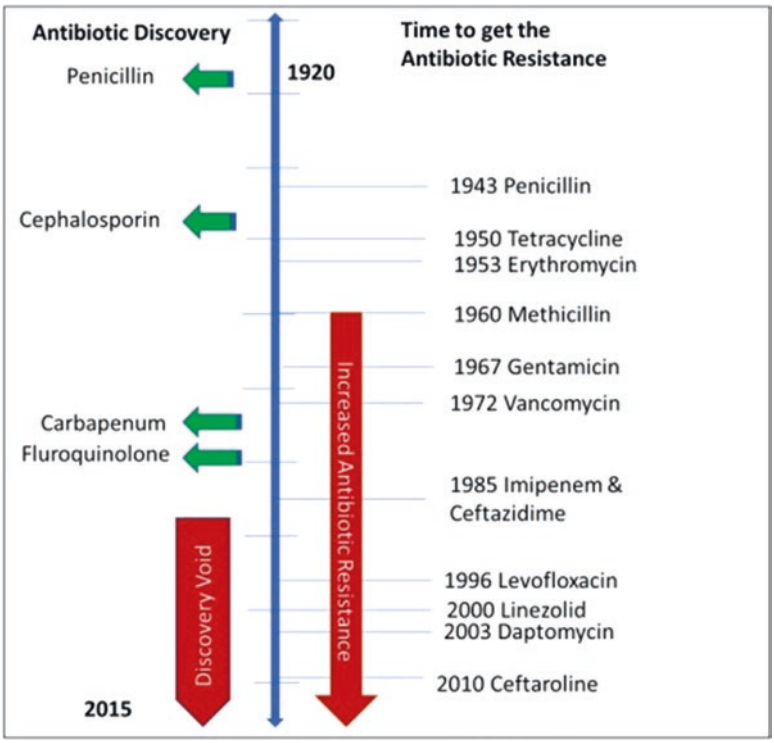

Fig. 16.1 Antibiotic resistance versus time to develop AR (Adopted form Zaman et al. 2017)

Extracellular ARGs are more persistent and play crucial role in horizontal gene transfer through alteration. Coagulation and filtration method were developed for the removal of eARGs from wastewater. The studies have shown that the combined processes efficiently lessened the absolute load of total ARGs and eARGs from effluent. However, co-rejection and precoagulation during subsequent microfiltration can enhance the overall performance for the removal of ARGs. It has been analyzed that the integrated processes show good results for the removal of phosphate and dissolved organic carbon. This shows the advanced treatment technologies when used in combination show desirable results to ensure the water reuse (Li et al. 2019).

A study described the removal of eight antibiotics like azithromycin, erythromycin, and tetracycline from urban wastewater via the process of ozonation performed at different hydraulic retention times and ozone doses. Also, total cultivable *E. coli* was found inactive at 40 min HRT and 1/4 gO_3 g DOC^{-1}. High ozone doses are essential to completely remove and prevent the regrowth of ARGs from wastewater but, on the other hand, mineralization was extremely low in terms of total organic carbon when the high ozone doses were functional. It hence increases the phytotoxicity for some plant species (Iakovides et al. 2019).

The source and reservoir of antibiotics resistance genes (ARGs) are the wastewater treatment plants. In order to explain the abundance, diversity, and expression and hosts of ARGs from three wastewater treatment plants in Taiwan, a metatranscriptomic and metagenomics methods were applied. The results showed that around 65.8% were present and actively copied while their transcript abundance was positively linked with ARGs profusion ($p < 0.001$). On the other hand, plasmid-associated ARGs had larger likelihood of transcription as compared to the ARGs encrypted by chromosomes. This study offers a detailed insight of the prevalence, host, and expression of ARGs in activated sludge. It also clarifies the dissemination and distribution of ARGs in treatment plants which is good for risk assessment and management of ARGs and ARBs in the environment (Liu et al. 2019).

From last few decades, environmental pollutants together with antibiotics have turned out to be an increasingly public health risk. Antibiotics overdose and misuse have directed to the development of antibiotic-resistant genes (ARGs) that denote a health risk. Furthermore, wide range of antibiotic-resistant genes of bacterial species originate from places like water bodies and reservoirs due to the selective pressure from the existence of antibiotic-resistant bacteria deposits. In this way, graphene oxide (GO) exploited in numerous fields along with remediation of the environment. In this chapter, we present a short overview of antibiotic resistance bacteria, their frequently used resistant genes, their occurrence, and their behavior in the environment (Bytesnikova et al. 2019).

In order to explore the effectiveness of TiO_2-photocatalytic treatment through UVA-LEDs, the ancillary urban wastewater samples were jagged with sulfamethoxazole (SMX), ofloxacin (OFL), azithromycin (AZT), and trimethoprim (TMP) at 100 mu g L^{-1}. Multiple effective parameters were analyzed like the catalyst load, irradiation settings, radical scavenger, and methanol use as solvent carrier. In order to treat the urban wastewater, 1.00 g L^{-1} catalyst and symmetrically distributed LEDs were evaluated for the elimination of antibiotics at real concentrations, along with the inactivation and regrowth of bacterial colonies. Clindamycin (CLI) embattled when SMX was not perceived. To decrease the analyzed antibiotics below the bacterial weight and detection limits, 1-h treatment was enough.

For total heterotrophs, bacterial regrowth was detected. The opposition percentage of antibiotics is comparable to or lower than that of secondary urban wastewater. Thus, in this case the circumstances can be adjusted to reduce microbial regrowth (Biancullo et al. 2019). One more study described that antibiotics use on a larger level produces antibiotic resistance bacteria, and they might not be entirely removed by the customary wastewater treatment processes. The beneficial process for disinfection is to avoid the discharge of ARB in the surface water. Graphitic carbon nitride (g-C_3N_4) is a metal-free photocatalyst that is very good agent for wastewater treatment because of its photoactivity with visible light and low cytotoxicity. The use of g-C_3N_4-mediated photocatalysis at >300 and > 400 nm on ARBs improved their inactivation to 0.64–1.26 log and 0.31–0.41 log, respectively. This study lays the base for further research on photocatalysis use for wastewater treatment (Ding et al. 2019).

16.2 ARB and ARGs in Water Resources and Wastewater Treatment Plants

Treatment plants accumulate wastewater from diverse sources and apply multistep procedure for the treatment of wastewater. Mixing of different wastes and bacteria from different origins become a source of emerging ARB. Urban wastewater treatment plants (UWTPs) are known to be one of the significant ways for proliferation of antibiotics resistance from humans to the environment. The sewage inflowing the UWTPs combines the discharged and remnants formed in the region. Consequently, it is estimated that the UWTP influents reflect the characteristics of the microbiome of the human residents assisted as well as the occurrence of resistance genes, resistant bacteria, and their related mobile genetic elements (Manaia, 2017; Rizzo et al. 2013; Berendonk et al. 2015). The most important forces for the conservation and propagation of resistant bacteria and their associated genes in wastewater can be the manifestation of antimicrobial remnants, which in addition to choosing resistant phenotypes also disrupt the native bacterial populations (Novo et al. 2013). Based on retrospective evidences, Merlin et al. (2011) defined the transmission of the antibiotic-resistant plasmid pB10 in urban wastewater slush that is kept in microcosms on exposure to low concentrations of amoxicillin and sulfamethoxazole. Enterococci are among the utmost examined bacteria in urban wastewater treatment. Numerous authors by means of different methodologies showed high resistance rates of about 20–44% for erythromycin, quinolones, and tetracycline and substantially lower values of about 1–7% for sulfonamides and aminopenicillins (Ferreira da Silva et al. 2006; Martins da Costa et al. 2006; Łuczkiewicz et al. 2010).

Another group of bacteria, *Escherichia coli*, is extensively investigated. Perhaps due to distinct ecology and biology, the antibiotics that are studied for high resistance rates were slightly varied from those experimented in enterococci. Higher resistance rates were seen for tetracycline, sulfonamides, and aminopenicillins of about 10–40%, while relatively lower rates are stated for gentamycin or quinolones of less than 10% (Ferreira da Silva et al. 2007; Galvin et al. 2010). In these collections, for *E. coli* high resistance values were detected for resistance prevalence with extensive antiquity of antibiotic use like tetracyclines, sulfonamides and aminopenicillins or tetracycline and erythromycin for enterococci (Partridge 2011; Manaia et al. 2012).

Despite the limited evidence concerning antibiotic resistance in clean water, still it may have bacteria, belonging to genera Sphingomonas, Sphingobium, Acinetobacter, and Pseudomonas or nonfecal Enterobacteriaceae; that have the ability to resist several antibiotics (Faria et al. 2009; Xi et al. 2009; Vaz-Moreira et al. 2011, 2012). However, different studies reported that wastewater treatment processes reduce the ARB population, thus entailing rigorous investigations regarding spread of ARB and ARGs in wastewater treatment plants (Hultman et al. 2018; Karkman et al. 2018). A study performed to test the presence of ARB in different environments including dairy farms, hospitals, and suburban garden, which was enriched by using animal manure, found 70, 75, and 77% frequencies of ARB,

respectively (Esiobu et al. 2002). Another study done by Xi et al. (2009) on the frequency of antibiotics resistance in distribution and drinking water treatment system reported that ARBs were slightly higher in the potable water than treatment plant (product) water. Xi et al. concluded that the supply system serves as source for the distribution of antibiotics resistance to other pathogens. Marathe et al. (2013) worked on treatment plant receiving wastewater from drug manufacturer as source for multidrug resistance bacteria and reported that human activity contributes to concentration of ARGs and wastewater treatment plants provide habitat for ARB. ARG's incidence and transmission in UWTPs have also been examined. These resistance genes are positioned on mobile genetic elements like transposons, integrons, and plasmids (Auerbach et al. 2007; Guillaume et al. 2000; Schwartz et al. 2003). The ARGs that have been detected in UWTPs and their mechanism of action are illustrated in Table 16.1.

Antibiotics RB and ARGs in gray water along with effectiveness of chlorination was tested and found tetracycline-resistant genes while they found chlorination as an effective method for the reduction of ARB in gray water (Troiano et al. 2018). Selection pressure is a significant problem in the prevalence and distribution of ARGs in wastewater which is now recognized as even low concentration of antibiotics can impact in the selection of ARGs and directed the overgrow of sensitive bacteria in water (Andersson and Hughes 2014) (Fig. 16.2).

Wastewater is known as the richest water habitat for antibiotic resistance genes. Antibiotic resistance genes (ARGs), encoding each recognized mechanism of resistance, i.e., reduced permeability/efflux, modification of drug, target protection, or target modification, are identified all over the water cycle. These resistance genes have been distinguished either in total genomic DNA samples or in isolates of bacteria (Munita and Arias 2016) where a characteristic sign of resistance encoding genes to former antibiotics such as aminoglycosides, beta-lactams, tetracyclines, and sulfonamides can be found (Vaz-Moreira et al. 2014) (Table 16.2).

Most of the resistant genes are found in plasmids while few integron gene cassettes can be transferred between bacteria (Garcillan-Barcia et al. 2011). Lately, Zhang et al. (2011) confirmed the plasmids primarily protected by gram-negative bacteria of alpha, beta, and gamma classes and some members of Mycobacterium genera, Nocardiopsis, and Bacillus that are rich in wastewater surroundings are suitable carriers of multidrug resistance genes, tetracycline, and macrolide in those environmental habitats (Zhang et al. 2011) (Fig. 16.3).

16.3 ARB in Water Resources: The Pakistan Experience

In Pakistan, both water availability and quality issues prevail and overuse of antibiotic along with unprescribed sale of antibiotics is increasing ARB in the community and water resources. A study was conducted in one of the cities of Pakistan to test the prevalence of ARB against Kanamycin and Ampicillin in drinking water and found 42.5% and 57.5% ARB, respectively, and it was concluded that poor sanitary

Table 16.1 Antibiotic-resistant genes detected in UWTPs and their mechanism of action (Adopted from Rizzo et al. 2013)

Class	Genes	Mechanism of action	References
Aminoglycosides	aad A1	Modification by adenylylation (DM)	Araujo et al. (2010); Barlow et al. (2009)
	Str (A, B); aph (A, A-3, A-6, 2) StrB	Modification by phosphorylation (DM)	Boczek et al. (2007); Barraud & Ploy (2011)
Beta-lactams	Class A: GES, CTX, NPS, SHV, PER, TLA, TEM, VEB Class B: VIM, IMP Class C: CMY, ampC, Class D: OXA	Cleavage of beta lactam ring (production of beta lactamase) (DM)	APHA (2005); Auerbach et al. (2007); Bönemann et al. (2006); Boczek et al. (2007)
	mecA	Penicillin binding protein (TP)	Batt et al. (2006); Andrews (2009)
Glycopeptides	vanA	Modified peptidoglycan pentapeptide (TM)	Alexandrino et al. (2007) Batt et al. (2006)
Macrolides	mel	Macrolide-efflux protein (DE)	Boczek et al. (2007)
	ereA2	Erythromycin inactivation (DM)	Boczek et al. (2007)
	ermB erm(B, F)	Modification by 23S rRNA Methylation (TP)	Alexandrino et al. (2007) Boczek et al. (2007)
	mph(A, B)	Macrolide phosphotransferase (DM)	Boczek et al. (2007)
Quinolones	aacA6-ib-cr	Modification by acetylation (DM)	Armstrong et al. (1982)
	qnr (A3, B1, B2, B4, B5, S2) qnrS; qnrVC	DNA gyrase protection (TP)	Boczek et al. (2007) Bönemann et al. (2006)
Sulfonamides	sul2 sul(1, 2, 3)	Modified dihydropteroate synthase (TM)	Barraud & Ploy (2011) Boczek et al. (2007)
Trimethoprim	dfr(A1, A12, 18) dfr (II, V, VII, XII, 13, 16, 17, A19, B2, D); dhfr (I, VIII, XV)	Dihydrofolate reductase (DM)	Bönemann et al. (2006); Barlow et al. (2009)

(continued)

Table 16.1 (continued)

Class	Genes	Mechanism of action	References
Multidrug	acr (B, D), mex (B, D, F, I, Y)	Multidrug resistance efflux pump (DE)	Boczek et al. (2007)
Tetracyclines	tetU	Other	Boczek et al. (2007)
	tetA tet(A, B, D, G, H, Y, 31, 35, 36, 39)	Tetracycline efflux pump (DE)	Barraud & Ploy (2011) Andrews (2009); Baquero et al. (2008)
	tetM tet(M, S)	Ribosomal protection protein (TP)	Alexandrino et al. (2007) Boczek et al. (2007)
	tetX	Tetracycline modification (DM)	Boczek et al. (2007)

DE drug efflux, *DM* drug modification, *TP* target protection, *TM* target modification

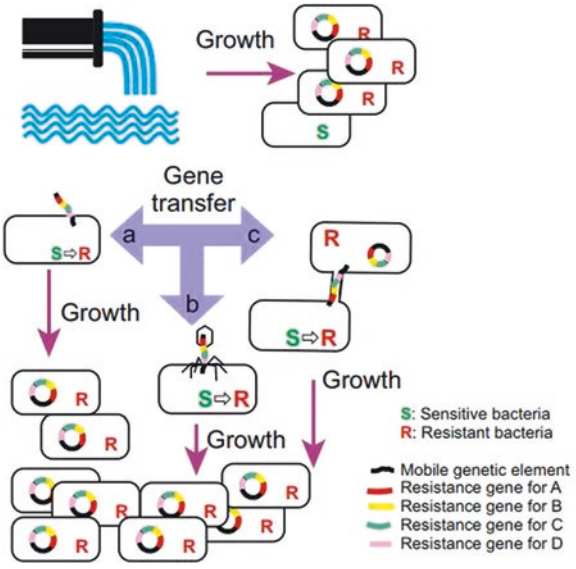

Fig. 16.2 The sensitive bacteria by attaining a resistance gene become resistant through (a) transformation, (b) transduction, (c) conjugation (Adopted from Karkman et al. 2018)

conditions might be the reason of contaminated drinking water resources (Samra et al. 2009). Another study conducted in fish farming environment both in Tanzania and Pakistan reported the antibiotics resistance gene in pools due to integrated fish farming practices including animal waste and antibiotic residue. Antibiotic resistance and high heterotrophic counts were studied in per-urban areas of Karachi and reported 96% unsafe drinking water with 29.2% with beta lactamase spectrum in fecal indicator bacteria. Enteric bacteria were found resistant to ciprofloxacin, sulfamethoxazole, and ceftazidime (Shakoor et al. 2018). Similarly, another study from Karachi city reported the ARB in drinking water. They reported 50% ARB

Table 16.2 Antibiotic-resistant genes in water environments (Adopted from Zhang et al. 2009)

Categories	Gene	Biological source	Environmental source
Tetracycline efflux protein	tetA	*Salmonella, Escherichia*, *Pseudomonas, Listeria, Aeromonas, Alcaligenes, Arthrobacter*, *Vibrio,* and *Comamonas*; plasmids pRSB101, pB10, and pTB11	AS, EW, DW, SD, SW, NW & US
	tetB	*Afipia, Arthrobacter, Alcaligenes, Escherichia, Burkholderia, Serratia, Pseudomonas, Vibrio,* and *Staphylococcus*	AS, EW, DW, SW NW, US
	tetC	*Aeromonas, Alcaligenes, Arthrobacter, Brevibacterium*, and *Pseudomonas*	AS, EW, SW & US
	tetD	*Escherichia; Aeromonas*, microbial community	AS, DW, SW, EW & US
	tetE	Aeromonas, Vibrio and Pseudoalteromonas,	AS, SW, SD, EW & US
Aminoglycoside resistance genes	aacA4	Plasmid pTB11	AS & NW
	aacA29b	Plasmid pTB11	AS
	aadA1	Plasmid pTB11; *Citrobacter, Aeromonas*, and *Shigella*	AS, NW, EW, SW & US
	aadA2	Plasmids pB2, pB3 and pTB11; Escherichia, Aeromonas, and Vibrio	AS, SW, NW, SD, & US
	aadA4	Plasmid pB8	AS
	aadA5	Plasmid pTB11; Vibrio and Escherichia	AS & NW
Macrolide resistance genes	ermA	*Enterococcus*	EW & SW
	ermB	*Enterococcus* and *Bacillus*	EW & SW
	ermC	Microbial community	EW& SW
	ermE	Microbial community	SW
	ermF	Microbial community	EW& SW
Chloramphenicol resistance genes	cmlA1	Plasmid pB2 and pB3	AS
	cmlA5	Plasmid pTB11	AS
	catB2	Plasmid pTB11	AS
	catB3	Aeromonas	SW
	flo_R	*Listeria, Salmonella Pseudoalteromonas,* and *Vibrio*	DW, SW & NW
Vancomycin resistance genes	vanA	*Staphylococci* and *Enterococcus*	NW, SW, DW, EW & UW
	vanB	*Enterococcus*	EW, UW & NW
Sulphonamide resistance genes	dfrA1	*Escherichia, Salmonella,* and *Aeromonas*	SW & NW
	dfrA5	*Escherichia*	NW
	dfrA7	*Escherichia*	NW
	dfrA12	*Escherichia, Salmonella,* and *Aeromonas*	SW, DW & NW
	dfrA15	*Vibrio*	EW & NW
	dfrA17	*Salmonella* and *Escherichia*	NW & DW
	dfr18	*Vibrio*	NW

(continued)

Table 16.2 (continued)

Categories	Gene	Biological source	Environmental source
Trimethoprim resistance genes	sulI	Plasmids pB10, pB8, pB3, and pB2; Aeromonas, Escherichia and Listeria	NW, DW, AS, SW & SD
	sulII	*Salmonella, Escherichia*, *Acinetobacter*, and *Vibrio*	SD, DW, SW & NW
	sulIII	*Escherichia*	SD & NW
	sulA	Microbial community	SD
β-Lactam resistance genes	ampC	*Enterobacter, Salmonella*	DW, SW, NW & US
	bla_{PSE-1}	*Salmonella, Aeromonas*, and *Vibrio*	EW, SW, SD & US
	bla_{TEM-1}	*Escherichia*	DW
	bla_{OXA-1}	Plasmid pTB11	AS
	bla_{OXA-2}	Plasmids pB8, pB10, and pTB11; Aeromonas	AS, SW & EW
	bla_{OXA-10}	Plasmid pTB11	AS
	bla_{OXA-30}	*Salmonella*	SW
Penicillin resistance genes	mecA	*Staphylococcus*	DW, NW & US
	penA	*Listeria*	DW & SW

AS activated sludge from sewage treatment plant, *SW* wastewater from aquaculture, hospital, and production area of animal, *EW* effluent water of sewage treatment plants, *US* untreated sewage, *SD* sediments, *NW* natural water, *DW* drinking water

from the tested isolated were resistant to streptomycin, ampicillin, metronidazole, rifampicin, and erythromycin (Ansari et al. 2014). Both water availability and quality issues exist in city and per-urban areas of Karachi. Hospital wastewater (Ahmad et al. 2012) and drug formulation facilities (Khan et al. 2013) are also important source of antibiotics and hence leads to ARB in wastewater in Pakistan. It was suggested to adopt preventive measure along with enough resources allocation to save human health.

Pakistan in collaboration with WHO has made commitment as global challenge to address the AR issue and has made national action plan to address the AR challenge (WHO 2018). In Pakistan, lack of knowledge on the patient side, improper usage and not taking complete dosage by the patient, and self-medication are directly linked with the AR. There is dire need of community-based interventions, awareness raising on the subject, and trainings (Shaikh 2017). According to recent study, antibiotic consumption in Pakistan has increased 65% between 2000 and 2015 (Klein et al. 2018). ARGs in the selected samples like rivers, dams, and drug formulation site from Northern Pakistan were tested and found unpolluted, but in the downstream near Lahore city were found high level of antibiotic especially in the drug formulation sites and intI1 genes near the densely populated area due to anthropogenic activities (Khan et al. 2013).

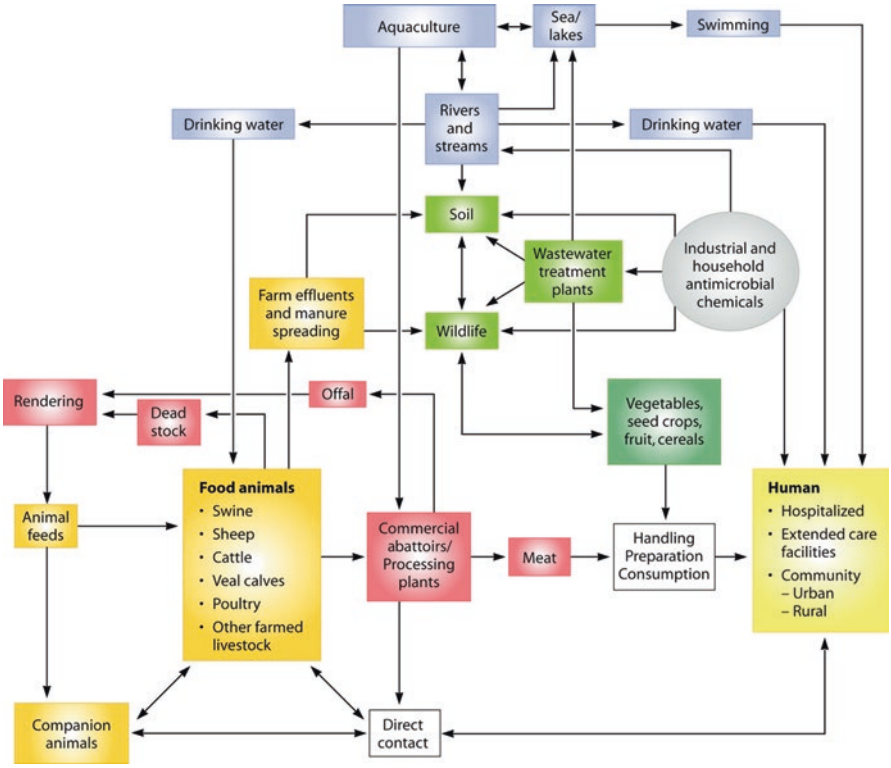

Fig. 16.3 Propagation of antibiotics and antibiotics resistance in various ecological habitats such as hospital, agriculture, community, and wastewater treatment plants (Adopted from Davies and Davies 2010)

Similarly, another study reported both antibiotics and metals resistance in five major rivers of Pakistan. *E. coli* and Klebsiella were most frequent among the tested strains resistant to Ampicillin (Sair and Khan 2017).

16.4 Regulations, Policy Implications, and Ways to Conserve Water Resource from Antibiotics Resistance

It is highly important and recommended that mankind saves water resources from antibiotics resistances. Many studies have reported the development of cost-effective, efficient methods for detection of antibiotics and ARGs and sources, and how to disinfect resources from resistance and mixing of human, animal, and environmental biota (Baquero et al. 2008). Industrial and hospital waste must be properly treated before releasing into water environment. Raising awareness about antibiotic resistance and its spread into water environment along with reducing

water pollution may help in regulating antibiotic resistance in water and soil environment (Rath and Patra 2018). Another study reported that regulations are not mostly followed and its implementation in different regions and countries is difficult. It is important to implement regulations. Secondly, prevention of transfer of environmental bacterial resistances to human pathogens is highly recommended in the management point of view (Larsson 2014). There is a prerequisite for more statistics on the environmental role of microbiomes in the increase of antibiotics resistance, to obtain complete compensation of therapeutic applications of antibiotics (Davies and Davies 2010). ESRC suggested the limited use of antimicrobials against infections. They further suggested the early diagnosis, hygienic practices, and raising awareness to the problems of antimicrobial resistance (ESRC 2015). Centre for Diseases Control and Prevention department (CDC) called it economic burden and action plan included reduction in inappropriate use of drugs, reduction in spread of ARB in different sectors, development of new therapies and effective products, and basic research (Friedren 2010). In order to reduce resistance in water resources and decrease risk related to public health, best management practices, wide-ranging monitoring strategies, and cost-effective treatment methods should be adopted (Hong et al. 2013).

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Chapter 17
Electrochemical Treatment of Antibiotics in Wastewater

Sajjad Hussain, Saad Ullah Khan, and Saima Gul

Abstract Antibiotics are vital for the healthcare of human and animals; these products are used to prevent, alleviate and cure disease and give a better way of living. However, after administration a large fraction of these products are excreted unchanged into water bodies, and nobody knows about the ultimate destination of these chemical compounds. Similarly the possible adverse effects due to the presence of these chemical to environment and human health are unknown due to lack of information. Most of the antibiotics products are reached to water bodies unaltered and known as active pharmaceutical ingredient. These products are transformed into metabolites and even into some other compounds through natural process which occurs in aquatic environment. Currently different technologies such as physical, chemical and biological or advance oxidation processes are used to treat the antibiotics. Some of these technologies are time consuming, ineffective and non-adequate for the emerging contaminants; furthermore few methods like AOPs (Advance Oxidation Processes) are innovative and efficient but they are expensive, need high energy and produced reactive or unstable oxidant which are not able to remove refractory contaminants. Therefore it needs an innovative green technologies which enable to decontaminate the water containing antibiotics. The electrochemical technologies offer an alternative way to treat these pollutants; the major process are electro-oxidation, electro-reduction, electrocoagulation, electro-Fenton, photoelectron-Fenton, sono-electrochemical, etc. The electrochemical technologies have some advantages over the other oxidation processes, like easily operation, high

S. Hussain (✉)
Faculty of Materials & Chemical Engineering, GIK Institute of Engineering Sciences & Technology, Topi, KP, Pakistan

Faculty of Engineering, Architecture, Urbanism and Geography, Federal University of Mato Grosso do Sul, CEP, Campo Grande - MS, Brazil
e-mail: sajjad.hussain@giki.edu.pk

S. Ullah Khan
Faculty of Materials & Chemical Engineering, GIK Institute of Engineering Sciences & Technology, Topi, KP, Pakistan

S. Gul
Department of Chemistry, Islamia College Peshawar, Peshawar, KP, Pakistan

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efficiency, coupling with other process, and low temperature required for its operation; moreover it can be powered with the help of solar panel to decrease the energy consumption. However still exist some challenges to overcome like designing and cost of electrode, improving the basic scientific understanding and in some cases the production of toxic intermediates. This study explores the electrochemical technologies and their application towards treatment of antibiotics.

Keywords Electrochemical oxidation · Antibiotics · Emerging contaminants · Electro-Fenton

17.1 Introduction

All living organisms depend on water and the terrestrial life, in particular depends on fresh water resources. However, more than 97% of total water reservoir is saline, which does not support life on land. 75% of fresh water are confined in ice caps and glaciers. Spring, stream, pond, lakes and rivers are the major sources of potable water, comprising 0.01% of the total water (Baird 1999). Recently the global climate is drastically changed which put a high tension on water supplies. Heretofore, some regions are facing water scarcity and Pakistan is among top 15 countries with serious water dearth (https://www.un.org.pk/water-insecurity/). As life on earth completely depends on water, therefore the quality and quantity should be managed properly. At the same time, the fast industrialization and urbanization have resulted in serious surface water pollution with organic compounds such as dyes, pesticides, herbicides, cleansing agents, foot stabilizer, their metabolites and transformation products.

Recently, the focus of environmental scientist is diverting from conventional organic pollutants and is mainly focused on the micro-organic pollutants, which are known as emerging contaminants. These chemicals are found at level of ($\mu g\ L^{-1}$) in the water bodies and are determined with the help of more sophisticated analytical instrumentations (Reemtsma and Jekel 2006; Barceló and Petrovic 2008). The recent advances in ultra-performance chromatography coupled with mass allow the detection of such emerging pollutants like pharmaceuticals, metabolites, and transformation (Petrovic and Barceló 2006). There is no common description not a comprehensive inventory that could be clearly included under the term "emerging contaminant". Many of these chemicals, in ppb levels or below, are responsible for contamination of aquatic environment. Because of such lower concentration they are often called micro-pollutants. Many of these have been found to form intermediates often more complex and toxic than the parent compound because they pass through various chemical, photolytic, hydrological, and microbial transformations in the aquatic environment (Fig. 17.1).

These contaminants might also be emerging because it discover from a novel source or it may reach to human through new route (Fig. 17.1) or it may be due to its sensing and decontamination methods (U.S. Department of Defense (DoD) 2006). Emerging pollutants can be classified into some broad categories like phar-

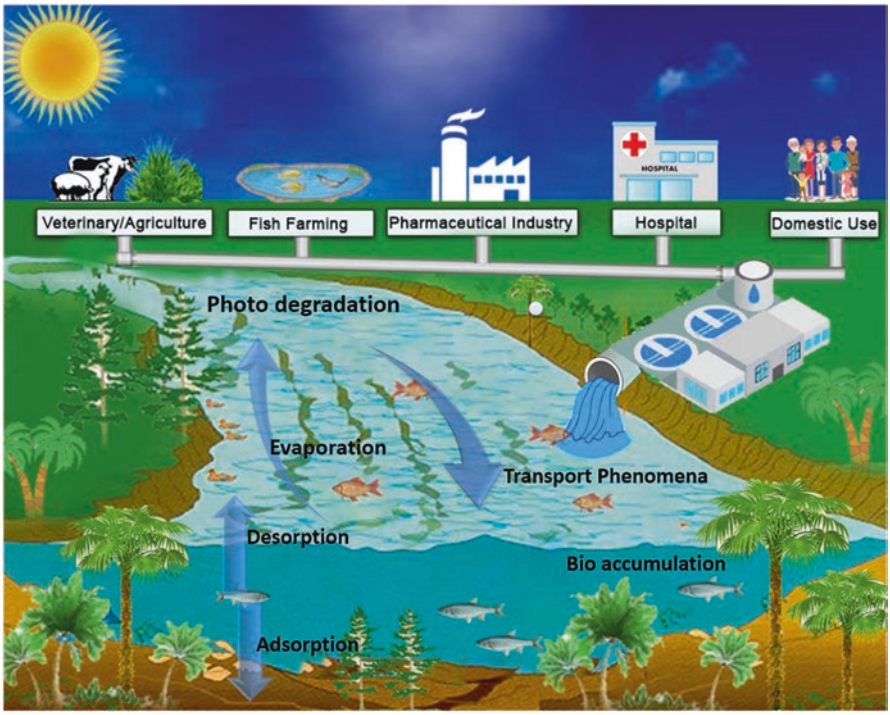

Fig. 17.1 Possible sources of pharmaceuticals distribution aquatic environment and their fate

maceuticals and personal care products (PPCPs), endocrine disruptor chemicals (EDCs), perfluorochemicals (PFCs), polychlorinated naphthalenes (PCNs), polydimethylsiloxanes (PDMSs), bisphenol A (BPA), polychlorinated alkanes (PCAs) quaternary ammonium compounds (QACs), triclosan (TCS), triclocarban (TCC), pesticides, benzothiazoles, benzotriazoles, and engineered nano-materials (Lapworth et al. 2012). Figure 17.2 represents simple classification of ECs and its properties.

The data-related presence, risk assessment and ecotoxicological are not available, so it is hard to explore adverse health or environmental consequences (Barceló 2003). In many countries these contaminants are in the process of legislation; however still their synergistic effects are unknown because of the lack of information. Some of these emerging contaminants have been discussed in detail below.

17.2 Endocrine Disrupting Chemicals (EDCs)

Among the emerging pollutants, endocrine disrupters affect animal reproduction (Ghiselli and Jardim 2007; Bila and Dezotti 2007). These substances may have synthetic (Xenoestrogens) or natural origins (phytoestrogens) and can disturb the

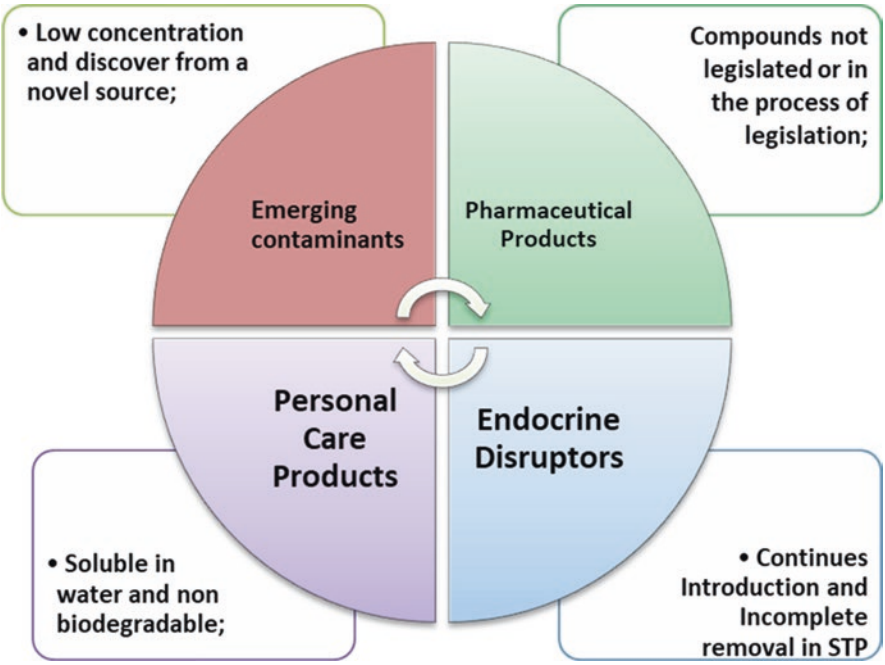

Fig. 17.2 Different types of emerging contaminants and their characteristics

endocrine system, even at low concentrations. The effects of these substances have been found in various situations such as feminization of fish, reduced hatching of eggs of various species of animals (Grover et al. 2009), cancer in humans, and disorder in the reproductive system. The endocrine system is one of the vital systems of the animal body consisting of glands, hormones and receptors found in various parts of the body. The endocrine system is important because hormones have an important regulatory function in various organs such as testis, ovary, pancreas, adrenals, thyroid, parathyroid, pituitary and thalamus glands. Some features are directly controlled by hormones, as the activities of organs, levels of salts, sugars and lipids in the blood, storage of energy, sexual characteristics, etc. There are various possible sources of EDCs; it can be synthesized or can come as by-products of emissions from pulp and paper industries, steel foundries and motor vehicles and incineration of products, especially those containing chlorine like PVC. However, there are several other products including cosmetics, sun blocks, fragrances, contraceptive pills, solvents, surfactants, pesticides, herbicides, plastics, etc. that may contain endocrine disrupters. Several substances classified (Maia and Dezotti 2007) as endocrine disrupters include phthalates (Haarstad and Borch 2004), flame retardant (Wensing et al. 2005), alkylphenols, organochlorine compounds, bisphenol (Abdelmalek et al. 2008; Alonso-Magdalena et al. 2006), polycyclic aromatic hydrocarbons, pesticides (Africa et al. 2006; Birkhøj et al. 2004), biphenyls compounds, drugs, phytoestrogens, natural estrogens and heavy metals. The endocrine disrupters are

accumulated in fatty tissues in infected animal's body. The combination of EDCs with another chemical may have a synergistic effect and the toxicity is often higher than that of each substance separately. In case of herbicides, there are cases where the sub-product is more hazardous than original substance and the mixture between the two make them even more toxic (Richard et al. 2005; Yousef et al. 1995).

Pesticides are also considered endocrine disruptors and have been found to cause infertility, cancer of the testis, prostate, breast and ovary, etc. The estrogenic activity of several pesticides has been a concern. Some organochlorines pesticides such as DDT and its metabolites, the methoxychlor, dieldrin and mirex have been found to have this disrupting action (Meyer et al. 1999).

Pharmaceutical products are the important class of chemical serving and improving the standards of human life. It can be categorized according to the biological action and purport such as the use of antibiotics to treat bacterial infections, analgesic to reduce pain, and antineoplastic in anticancer therapy. The antibiotics have potential towards bacteria resistance and that is why they are of specific interest; after administration these pharmaceuticals undergo structural changes in human and animals bodies and get converted into metabolites which still acts as API (active pharmaceutical ingredient).

This variation seldom complete and a specific proportion of the parent compound API is normally discharged alongside the metabolite. For example, few antibiotics are broken down to 95%, while other antibiotics are only 5%. An investigation of the intake and throughput rates of APIs demonstrated that 75% of the antibiotics utilized in Germany were discharged unaltered, i.e. still active APIs (Kümmerer and Henninger 2003). From way back, the manufacturing and use of chemicals and pharmaceuticals have caused severe environmental pollution and severe health effects. The APIs used in medicine and additives can enter the environment via various non-fixed sources, pharmaceutical industries, sewage treatment plant effluents and domestic wastes. Pharmaceuticals is utilized in veterinary for treatment of diseases, as growth promoter, and a huge amount is discharged as waste and ultimately enter the water bodies (Kümmerer 2010). Figure 17.1 shows the possible routes of such pharmaceutical products. If they become part of soil components, they can reach groundwater and some can also be transported from surplus to surface water in case of heavy rain.

17.3 Antibiotics

Antibiotics are an important group of pharmaceuticals. It is used for the bacterial infection. They are widely used as a medicine for human, animals as well as for marine life. The basic purpose of antibiotics is the prevention and treatment of microbial contagion, whereas it is also used for promotion of animal growth. The standard definition of an antibiotic is a chemical that microorganism produce to disturb the growth of other organism. It may be classified on the basis of chemical structure or the way of microorganism prevention. It is widely used in medicine,

veterinary, agriculture and marine inhibits for the obviation and treatment of diseases. Some antibiotics like streptomycines are utilized in fruit crops, while others are utilized in bee keeping (Kümmerer 2008).

Antibiotic is the class of pharmaceuticals that can be subgrouped into β-lactams, quinolones, tetracycline, macrolides, sulphonamides and others. Active compound may be neutral, ionic, or dipolar ions and come under the category of complex molecule with various functions. Physical, chemical and biological characteristic may vary with pH due to various functionalities in single molecule (Kümmerer 2008). Pharmaceuticals active compound, in particular antibiotics, are mostly complex molecules relative to the chemicals of industrial and environmental concern (Trivedi and Vasudevan 2007). Antibiotics may be metabolized more or less broadly by humans and animals. Human antibiotics are defecated in metabolic waste and can reach the sewage treatment plant (STP). As a still active compound, the non-metabolized fraction is excreted. From the survey of all compounds, approximately 75% of antibiotics consumed in Germany are unchanged (Kümmerer and Henninger 2003). In STP, antibiotics are only incompletely removed, and if it is not completely removed, it may reach the environment and mostly the water bodies. Its some amount may also touch the surface water or ground water. Liquid manure may also loose active substance during the raining from the top of soil.

Sulphonamides and trimethoprim are bacteriostatic agents that synergistically target and inhibit two pathway steps in bacterial folic acid synthesis (Masters et al. 2003; Skold 2001). Both these are very effective toward various potential bacterial infections. Sulphonamides are not entirely utilized and may excrete in parts into sewage in the form of source compounds and metabolites (Gobel et al. 2005; Hirsch et al. 1999). N4-acetylated is the main metabolite of sulphonamides, a biologically inactive product, that transform back to the active parent compound in period of sewage handling (Gobel et al. 2005). So, this may lead to inefficient subtraction of sulphonamides, especially sulphamethoxazole amid the biological treatment of waste (Gobel et al. 2007).

Sulphamethoxazole is one of the most commonly observed municipal sewage sulphamides (Brown et al. 2006; Kim et al. 2007; Gobel et al. 2007; Levine et al. 2006; Yang et al. 2005). Sulphamethoxazole, for instance, was noted to have concentration of up to 7.91 mg L^{-1} in sewage impacts in China, and this is among the 15 best selling medicine (Peng et al. 2006). Metabolites of some antibiotic like SMX (acetylated, N4 acetyle SMX) have been found in more concentration than parent antibiotic SMX in wastewater treatment plant.

SMX partially destroyed in conventional wastewater plants and ends up in water bodies with the possibility to unfavourably effect both marine and terrain organisms. Sulphamethoxazole in range of (0–1000 μg L^{-1}) has been found and can cause adverse effects on the plant kingdom (Brain et al. 2004). Adverse effects to aquatic bacteria (Vibrio fischeri), freshwater invertebrates (*Daphnia magna*) and Japanese medaka fish (*Oryzias latipes*) have been reported for sulphamethoxazole (Kim et al. 2007). As mentioned above, conventional techniques, e.g. biotreatment and physicochemical treatment, including coagulation, volatilization, adsorption, sedimentation and filtration, cannot completely remove the SMX in STPs and also show the

need of efficient removal of antibiotics and their metabolites from the water bodies to prevent its adverse effects on the environmental life (Jones et al. 2005; Rahman et al. 2009; Suárez et al. 2008).

The most important environmental problem in the present scenario is to safeguard the integrity of our water resources from the dangerous concentration of SMX. Today, the development of innovative and economical wastewater technologies aimed at reducing the daily amounts of antibiotics released into the environment or transforming them into less toxic or more biodegradable intermediates is increasingly important. A wide range of chemical and physical methods for the degradation of antibiotics in waste water are available. The methods of treatment included in this work are described in detail here.

17.4 Electrochemical Technologies for Wastewater Treatment

Electrochemical treatment is one of oxidation processes that can mineralize organic materials into water, carbon dioxide and inorganic molecules. The electrochemical oxidation process has proved fruitful in recent years for the treatment of wastewater, mainly because of its efficiency and ease of operation. The use of electrochemistry for environmental protection was the subject of a number of books and reviews. Somewhere else (Ibanez et al. 1994) the main features of electrochemical technologies are explained in detail. The electrochemical technologies are *flexible* and should be applied directly or indirectly and could be easily scale up from microliter to millions of litres, and it can be easily coupled with other processes like photochemical, sonochemical, bio-degradation or photo catalysis. The process is also an *energetically efficient* and work on minimum temperature and pressure and there is less power wastage during operation. Similarly the process is *easy to operate* and automate and only electrically controlled variables are used in the process. Along with all these characteristic the electrochemical process is also *environmentally compatible* and produce only electrons during the process, which are high potential particles whose reactivity can be adjusted by selecting an appropriate electro catalyst to avoid unwanted metabolites from being produced. Furthermore, it is *cost effective* in term of operation, space and labours; however some electrode materials are expensive which increase the capital cost of the process. Therefore intensive research aims to discover more efficient electrode materials, electro-catalyst and optimized parameters for electrochemical applications. In the last two decades, electrochemical technologies have become a focus of scientist around the world. Not only are the electrochemical technologies cost comparable to other technologies, they are sometimes more efficient and compact.

Electrochemical technologies offer a versatile way to treat the wastewater. Broadly speaking, it can be used to separate the pollutant from wastewater such as electrocoagulation and electro-filtration and it used to decompose and destroy the

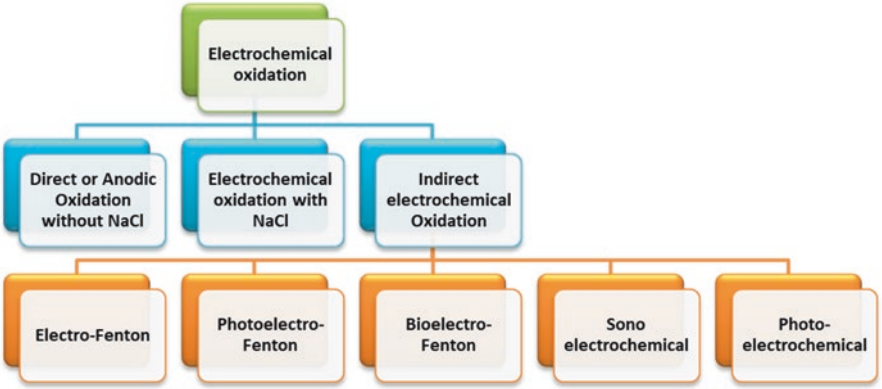

Fig. 17.3 Different electrochemical processes used for the degradation of antibiotics

organic pollutant into harmless product in water. The degradation of pollutants may be done by direct electrochemical process or indirect electrochemical process by some oxidant species generating at electrode surface. The electrochemical processes which produced (•OH) are called electrochemical advanced oxidation processes (EAOPs). As we already discussed, electrochemical processes are versatile and may be coupled with some other advanced oxidation processes. Some of the most common electrochemical processes used for the treatment of antibiotic are shown in Fig. 17.3.

17.5 Electrochemical Oxidation

17.5.1 Electrochemical Oxidation without NaCl

The application of electrochemical oxidation for wastewater effluent is not new; it has root back in the nineteenth centuries. Initially Kuhn studies oxidative degradation of some cyanides, thiocyanates and phenol compounds using both anodic and chlorine oxidizing (Kuhn 1971). After that, Nilsson investigated the oxidation of simple, monohydric phenols at a PbO_2 anode in aqueous sulphuric acid (Nilsson et al. 1973). Afterwards some others extensively investigated the synthetic wastewater containing phenol and aniline. However in the 1990s Feng and Johnson moved forward to explore the mechanism of electrochemical oxidation (Feng and Johnson 1990).

They reveal that upon the discharge of H_2O on the electrode surface (S) produced hydroxyl radicals (•OH) which adsorbed on electrode surface represent as S[OH] (Eq. 17.1). It is further proposed that these S[OH] may be shifted to oxidation product through oxygen transfer mechanisms (Eq. 17.2) or oxidize the organic compound (R) into (RO) as shown in equation (Eq. 17.3).

$$S[\ ] + H_2O \rightarrow S[^{\bullet}OH] + H^+ + e \tag{17.1}$$

$$S[^{\bullet}OH] + H_2O \rightarrow S[\ ] + O_2 + 3H^+ + 3e \tag{17.2}$$

$$S[^{\bullet}OH] + R \rightarrow S[\ ] + RO + H^+ + e \tag{17.3}$$

Furthermore, in the 1990s Comninellis elaborated a simplified mechanism for electrochemical oxidation of organic compound using different electrode materials (Pt, Ti/lrO_2, Ti/SnO_2). He proposed that oxide anodes (MO_x) forming the so-called higher oxide MO_{x+1} and degradation occurs with electrodes at the surface where hydroxyl radical accumulated (Comninellis 1994). Comninellis thoroughly investigated the electrochemical conversion mechanism of organic compound using metal and metal oxide electrodes and proposed that water molecule has adsorbed on the electrode surface and generate adsorbed hydroxyl radicals as in Eq. (17.4).

$$MO_x + H_2O \rightarrow MO_x\left(OH^{\cdot}\right) + H^+ + e^- \tag{17.4}$$

However it is strongly depend on anode materials and hence we differentiate them into two classes, i.e. active and nonactive anodes.

1. In case of active anode the Eq. (17.4) followed by interaction of oxygen of oxide electrode with MO_x ($HO^{\bullet}$) and transformed it into higher oxide state as followed by Eq. (17.5).

$$MO_x\left(OH^{\cdot}\right) + R \rightarrow MO_{x+1} + H^+ + e^-. \tag{17.5}$$

   The activation of surface redox pair MO_{x+1}/MO_x, often referred as active oxygen which may chemisorb active oxygen (MO_{x+1}). If there is no organic species in the premises, the physisorbed MO_x ($HO^{\bullet}$) and chemisorbed MO_{x+1} converted into dioxygen following (Eqs. 17.6 and 17.7)

$$MO_{x+1} \rightarrow MO_x + \frac{1}{2}O_2 \tag{17.6}$$

$$MO_x\left(OH^{\cdot}\right) \rightarrow MO_x + \frac{1}{2}O_2 + H^+ + e^- + MO_x \tag{17.7}$$

   However if organic compound are present, then the chemisorbed oxygen act as mediator for the selective conversion of pollutants on electrode surface.

$$MO_{x+1} + R \rightarrow MO_x + RO \tag{17.8}$$

2. In case of nonactive anode no higher oxide is formed and adsorbed hydroxyl radicals non-selective degraded the organic pollutants into CO_2 as represented in Eq. 17.8.

$$MO_x\left(OH^{\cdot}\right)+R \rightarrow MO_x+mCO_2+H^{+}+e^{-} \tag{17.9}$$

Nevertheless, "active oxygen" or "active electrode" by both chemisorbtion and physisorbtion also lead an aggressive side reaction such as oxygen production that results in reduction in the proficiency of the anodic process. Generally, anodes with low oxygen production potential such as carbon, graphite, IrO_2, RuO_2 or platinum have an "active" nature, and may only partially oxidize pollutants, while anodes with high oxygen production potential, such as antimony-doped tin oxide, lead dioxide or BDD, have "non-active" behaviour and leads to almost the total mineralization of organic pollutants. It is therefore considered ideal electrodes for the wastewater treatment (Comninellis 1994). These different types of electrode (anodes) with their oxidation potential are present in Table 17.1.

Organic pollutant can be degraded electrochemically by either direct or indirect oxidation. The mechanism of direct or indirect Ibanez in Fig. 17.4(1)) and (2).1 and oxidation mechanism has strongly depended on the electrode material construction, operating condition and supporting electrolyte (Panizza 2010) (Fig. 17.4).

The pollutant has been adsorbed on the electrode surface and then oxidized, the phenomenon is so-called direct oxidation (Fig. 17.4(1)) Low potential favours the direct electro-oxidation, but remediation rate is usually low and depending on the anode's electrocatalytic activity. High degradation has been reported by György et al. (1997) for the electrodes, namely Platinum and Palladium and metal oxide anodes like IrO_2, RuO_2–TiO_2 and Ir-TiO_2. The major problem associated with anodic electro-oxidation is decrease in catalytic activity at fixed potential before the evolution of oxygen. It is because of the development of organic film on the anode surface and commonly termed as the deactivation of electrodes. The proposal of *indirect oxidation* Fig. 17.4(2) is to prevent the fouling of the electrode by avoiding the exchange of direct electrons between the organic and the anode. Therefore, pol-

Table 17.1 Classes of electrode based on oxidation power and ability towards O_2 evolution reaction

Electrode type	Composition	Ability towards OER	Oxidation potential (V)	OER overpotential
Active anode	RuO_2-TiO_2 DSA-Cl_2	Good	1.4–1.7	0.18
	IrO_2-Ta_2O_5 DSA-O_2	Good	1.5–1.8	0.25
	Ti/Pt	Good	1.7–1.9	0.30
	Carbon/ graphite	Good	1.7	
Non-active anode	Ti/PbO_2	Poor	1.8–2.0	0.50
	Ti/SnO_2-Sb_2O_5	Poor	1.9–2.2	0.70
	p-Si/BDD	Poor	2.2–2.6	1.3

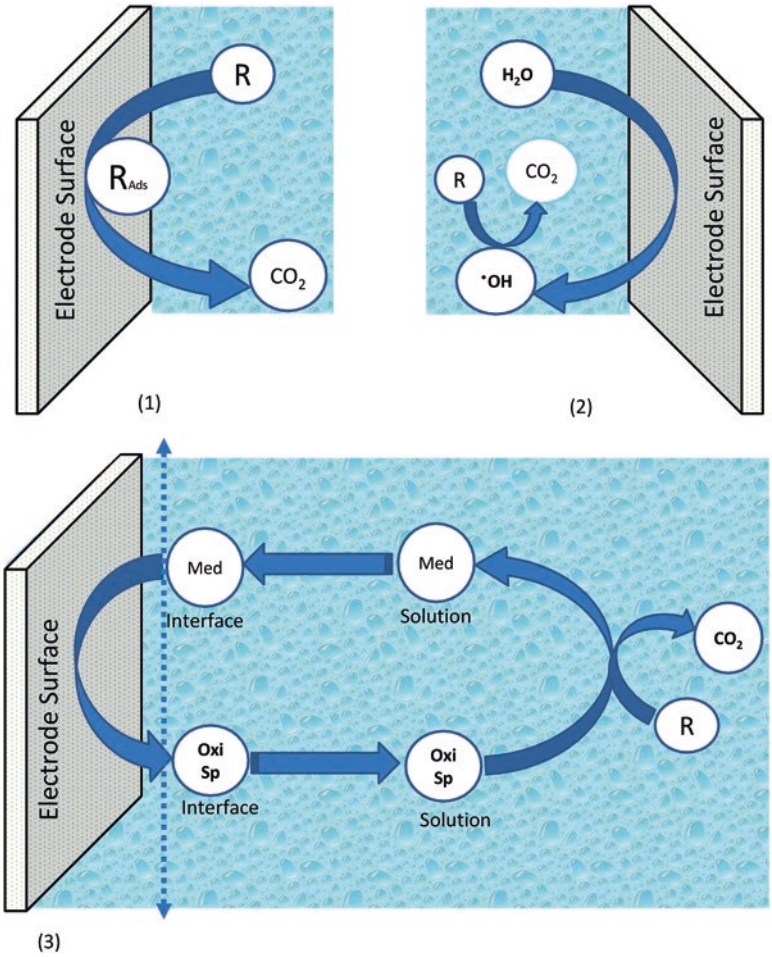

Fig. 17.4 Schematic diagram of electrochemical degradation mechanism of organic pollutant (R): (1) Direct electro-oxidation (2) by hydroxyl radical formation from water discharge and (3) by means of inorganic mediators

lutants are oxidized in indirect electrolysis through the generation of certain redox species (Fig. 17.4(3)), which provide a path for access of electron to the organics pollutants. Following are the key concerns for achieving high efficiency in indirect electrolytic processes:

- The potential of generated redox species should not be close to the potential for the evolution of oxygen.
- The generated redox species should be high.
- The reaction rate of the generated redox species and the contaminant should be higher than the competition rate.
- Adsorption of pollutants must be controlled.

Boron-doped diamond (BBD), a non-active electrode, has been examined broadly for the remediation of organic compounds in recent years. High O_2 over potential of BBD electrodes makes it better than metal oxide anodes and show efficient results for the direct oxidation of contaminants. Higher degradation activity towards organic compounds of BBD electrodes is due to the formation of weakly adsorbed ·OH radical from the electrolysis of water and has been presented in Eq. (17.10) (Comninellis 1994).

$$\mathrm{BBD} + \mathrm{H_2O} \rightarrow \mathrm{BBD}\left(\mathrm{\dot{O}H}\right) + \mathrm{H^{+1}} + 1e^{-1} \tag{17.10}$$

BBD anodes also generate many oxidizing species such as ozone, H_2O_2 and ferrate (Sáez et al. 2008; Sáez et al. 2008). BBD anodes can produce peroxydicarbonate ($C_2O_6^{2-}$), peroxydiphosphate ($P_2O_8^{4-}$) and peroxydisulphate ($S_2O_8^{2-}$), oxidants in the existence of ions, for example, CO_3^{-2}, PO_4^{3-} and SO_4^{-2}. Peroxydisulphate radical production occurs in two stages (Eqs. 17.11–17.14). First, the sulphate radical forms from the reaction of sulphate containing compound with electrogenerated ·OH, and in second stage, two sulphate radicals combine and produce peroxydisulphate (Serrano et al. 2002; Cañizares et al. 2009).

$$\mathrm{SO_4^{2-}} \rightarrow \mathrm{SO_4^{\cdot -}} + e^{-1} \tag{17.11}$$

$$\mathrm{HSO_4^{-}} + \mathrm{OH^{\cdot}} \rightarrow \mathrm{SO_4^{\cdot -}} + \mathrm{H_2O}(12) \tag{17.12}$$

$$\mathrm{H_2SO_4} + \mathrm{OH^{\cdot}} \rightarrow \mathrm{SO_4^{\cdot -}} + \mathrm{H_3O^{+}} \tag{17.13}$$

$$\mathrm{SO_4^{\cdot -}} + \mathrm{SO_4^{\cdot -}} \rightarrow \mathrm{S_2O_8^{2-}} \tag{17.14}$$

To strengthen the AO process efficiency, a great attention has been attracted towards the selection of suitable substrate (Wang et al. 2013). The criteria for suitable substrate is: good conductance of electricity, sufficient body strength, electrochemical inertness and easy development of protective films on the surface of the substratum through passivation (Chen et al. 2003). For this objective, various substrates have been studied for the BBD anode like Si, Ti, Nb, etc. Si substrate has generally been used for the BBD film deposition. But the delicate nature and variable conductivity due to experimental environment of Si create hurdles for the selection as a suitable substrate (Sun et al. 2011). Ti was found the suitable substrate for the BBD film deposition (Chen et al. 2003; (Sun et al. 2011; Chen 2004).

The removal and degradation of antibiotics has been studied by various researchers. Mora-Gomez et al. studied the degradation of Norfloxacin in a boron-doped diamond (BDD) or a novel Sb-doped SnO_2 ceramic anode (non-active anode). The process of degradation on both electrodes was found to be first order and BDD showed good oxidizing power towards antibiotic norfloxacin, and up to 92% TOC has been removed (Mora-Gomez et al. 2019). Kaur et al. reported the degradation

of antibiotic amoxicillin using active (Titanium coated ruthenium oxide) Ti/RuO_2 and investigated various operating parameters. They demonstrated that 60% amoxicillin degraded and 48% TOC has been removed and finally they also proposed the degradation pathway (Kaur et al. 2019).

17.5.2 Electrochemical Oxidation with NaCl

Electrochemical oxidation exhibits different behaviour in the presence of some active oxidizing species like chlorine, chlorine (Cl_2), hypochlorous acid (HOCl) and hypochlorite ions (OCl^{-1}). However, the mechanism of electro-oxidation using inorganic mediator is still not explored completely. Definitely, oxidation of organic molecules has been either accomplished at the electrode surface through adsorbed oxychloro species (i.e. chloro and oxychloro radicals) or in the main bulk solution through long-lasting oxidant (i.e. Chlorine, hypochlorous acid, or hypochlorite) by electro-oxidation of mediated chloride ion, referring reaction (Eq. 17.15–17.18):

$$2\,Cl^- \rightarrow Cl_2 + 2e^- \tag{17.15}$$

$$2H_2O \rightarrow 2OH^{\cdot} + 2H^+ + 2e^- \tag{17.16}$$

$$Cl_2 + 2H_2O \rightarrow HOCl + H^+ + Cl^- \tag{17.17}$$

$$HOCl \rightarrow H^+ + OCl^- \tag{17.18}$$

The formation of intermediacy active chlorine species and the mechanism of its reaction have been recommended by Bonfatti et al. (2000b) and Bonfatti et al. (2000a), (Fig. 17.5).

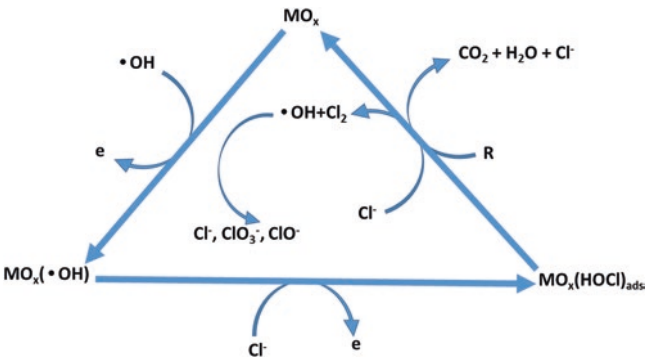

Fig. 17.5 Schematic diagram of chlorine-mediated electrochemical degradation mechanism of organic pollutant (adopted from (Bonfatti, De Battisti, et al. 2000))

They expanded the idea of Comninellis (1994) for the chlorine medium electro-oxidation process. They replaced mediated oxychloro species from chloride ions instead of hydroxyl radicals. The potential of anodic reactivity for the adsorbed chloride-oxychloride radicals increases in the chloride ion mediation by resisting the oxygen-evolution reaction. The widely used anode materials for onsite generation of active chloride oxidants depend on the platinum or on mixed metal oxides (such as, IrO_2, TiO_2, RuO_2,). These materials have high electro-catalytic property for chlorine production, and also long haul mechanical and chemical strength. Well-known dimensionally stable anodes DSA® has excellent capacity to generate electrochemically chlorine and hypochlorite by the electrolysis of NaCl which is widely used as a supporting electrolyte for the treatment of wastewater (Rajkumar and Kim 2006; Chen et al. 2011). The use of dimensionally stable anodes (DSA®) has been in the degradation of organic compounds such as atrazine (Malpass et al. 2010), carbaryl (Malpass et al. 2009) and dyes (Malpass et al. 2008), mainly due to their flexibility, electro-catalytic efficiency, chemical strength and good lifetime.

Traditionally strong oxidants species like chlorine (Cl_2), hypochlorous acid (HOCl) and hypochlorite ions (OCl^{-1}) were traditionally used for industrial wastewater treatment (Rajeshwar and 1997; Tarr 2003). These agents are also widely used for drinking water disinfection (Martínez-Huitle and Brillas 2008). Electrochemical technology provide a substitutive indirect electro-oxidation process for the treatment of organic pollutants with active chlorine species from direct chloride ion oxidation at appropriate anodes. This strategy contrasts from EO in the utilization of contaminated chloride containing solution amid electrolysis to

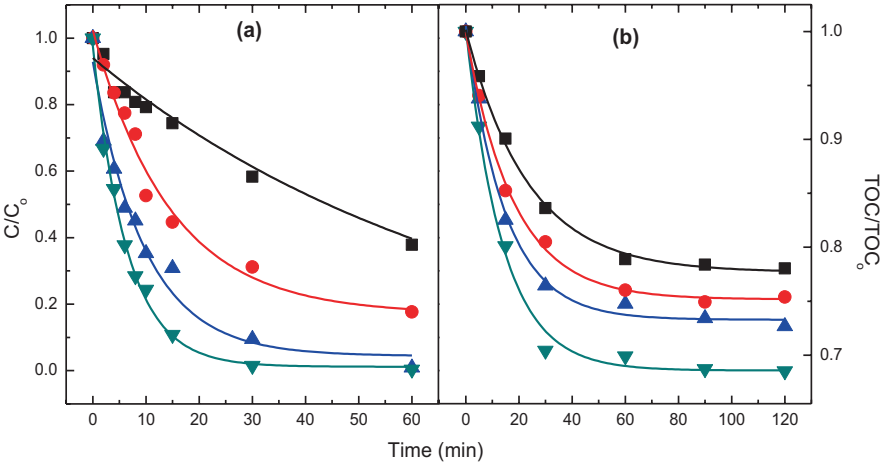

Fig. 17.6 Effect of NaCl (■ 0.02 M NaCl, ● 0.04 M NaCl, ▲ 0.08 M NaCl, ▼ 0.1 M NaCl) concentration on (**a**) degradation; (**b**) TOC removal of SMX (*condition:* pH = 3, temperature = 25 °C, initial concentration = 200 mg L^{-1}; current density = 40 mA cm^{-2}

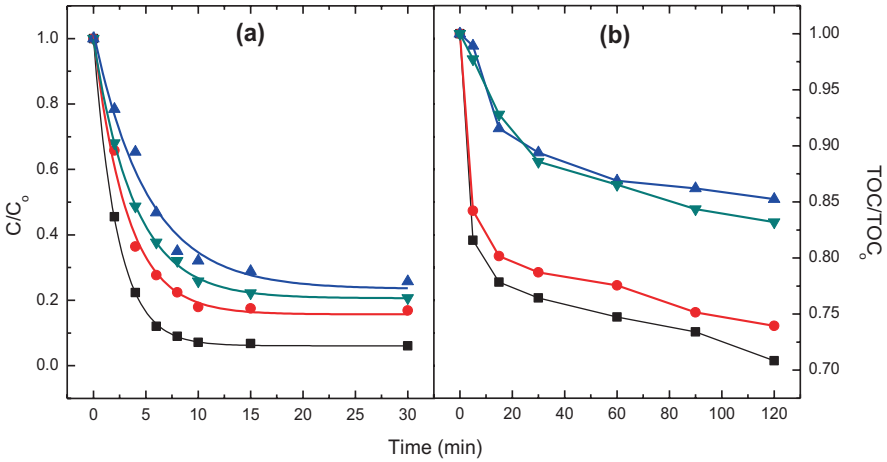

Fig. 17.7 Effect of pH (■ 2.5, ● 5, ▼ 7, ▲ 9) on (**a**) degradation; (**b**) TOC removal of SMX (*condition:* pH = 3, temperature = 25 °C, initial concentration = 200 mg L^{-1}; current density = 40 mA cm^{-2} (Hussain et al. 2015)

create active chlorine on site. However the major problem associated with indirect oxidation is the generation of chlorinated organic compound during the process, which subsequently increases the toxicity of wastewater. Panizza and Cerisola (2003) observed the chlorinated organic compound during the oxidation of 2-naphthol and surfaced to maximum at about 9 Ah dm^{-3}, which vanished during the electrolysis. On the other hand, it has also been reported that, by selecting suitable operating parameters, an organic compound, i.e. glucose, can be combusted in the chlorine medium without generation of organo-chlorine compounds (Bonfatti et al. 2000b; Bonfatti et al. 2000a).

Hussain et al. (2015) thoroughly elaborate an antibiotic sulphamethoxazole (SMX) using DSA electrode in sodium chloride medium. The work explain the kinetics and electrochemical degradation route under different experimental conditions. They also explain the NaCl, current density and pH have prominent effect on the degradation of sulphamethoxazole. Figure 17.6 reveals that mixed anode are capable of generating active chlorine species at different concentration of NaCl which efficiently degrade and mineralize antibiotic sulphamethoxazole.

The study also demonstrate the possible route of degradation of SMX and almost nine major intermediates of the reaction were identified by LC-ESI-QTOF- MS which contain some lower molecular weight organic intermediates like $C_6H_9NO_2S$ (m/z = 179), C_6H_4NOCl (m/z = 141), and C_6H_6O (m/z = 110)). The degradation of SMX proceeds via different routes involving rapture of oxazole and benzene rings by hydroxle radicals and active chlorine. Similarly the study also reveals and

depicted in Fig. 17.7 that degradation was pH dependent between 2.5 to 9 and reveal that acidic pH is more favourable for degradation, at this pH higher oxidation potential species like HOCl are responsible for the SMX degradation. Rajkumar et al. 2007), Figure 17.6 presents the effect of pH on the electrochemical degradation and mineralization of SMX.

The material of electrode plays a vital role in electrochemical degradation process, and lead oxide anode is an attractive, low-cost and better electrochemical activity towards organic oxidation (Chen et al. 2014; Dai et al. 2016). The electrocatalytic performance of PbO_2 anode can be enhanced by adding some internal layers like Sb-SnO_2, α-PbO_2, F^-, Co^{2+} and Ce^{3+}.

Dai et al. has successfully prepared rare earth Lanthanum (La) and Yttrium (Y), co-doped Ti/Sn-SbOx/PbO2 electrodes (La-Y-PbO2 electrode) that showed a high performance for the degradation and mineralization of aspirin (Dai et al. 2016). After that they utilized the modified PbO_2 anode for the levofloxacin and electrode showed high treatment efficiency and low cost (Xia and Dai 2018). The anodic degradation was dependent on current density 30 mA cm^{-2}, pH 3 and initial LFX (levofloxacin) concentration 800 mg L^{-1}. Different aromatic intermediate products of LFX degradation were found with different structure to the LFX molecule including pepiperazinyl hydroxylation, decarboxylation and defluorination. They also found some inorganic species like CO2, H2O, NH4+, $NO3^-$ and F^- during mineralization of levofloxacin.

17.6 Indirect Electrochemical Oxidation

17.6.1 Electro-Fenton Process

The Fenton process is a simple process for producing (•OH) through Eq. (17.19). The hydroxyl radicals perform an important role in electro-Fenton and Photo Electro-Fenton process, which degrade unselectively the organic compounds into carbon dioxide and water by hydroxylation and dehydrogenation reactions because of high reduction potential of (•OH) E^o = 2.8 V vs. SHE. Ferrous ion is vital for the Fenton process; however some other metal ions lik Cu^{2+} may also be used in Fenton like process to produce (•OH) from H_2O_2.

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^- + OH^{\cdot} \tag{17.19}$$

The H_2O_2 may also be decomposed into (•OH) by ultraviolet radiation in photo-Fenton process (Eq. 17.20), leading to some propagation (Eqs. 17.21–17.23) and termination reactions (Eqs. 17.24–17.26) (Bataineh et al. 2012; Keenan and Sedlak 2008; Lee et al. 2013; Liu et al. 2018).

$$H_2O_2 + h\nu \rightarrow 2OH^{\cdot} \tag{17.20}$$

$$OH^{\cdot} + H_2O_2 \rightarrow H_2O + HO_2^{\cdot} \tag{17.21}$$

$$HO_2^{\cdot} + H_2O_2 \rightarrow OH^{\cdot} + H_2O + O_2 \tag{17.22}$$

$$OH^{\cdot} + HO_2^{-} \rightarrow HO_2^{\cdot} + OH^{-} \tag{17.23}$$

$$2HO_2^{\cdot} \rightarrow H_2O_2 + OH^{-} \tag{17.24}$$

$$OH^{\cdot} + HO_2^{\cdot} \rightarrow H_2O + O_2 \tag{17.25}$$

$$2OH^{\cdot} \rightarrow H_2O_2 \tag{17.26}$$

$$Fe^{3+} + H_2O_2 \rightarrow Fe^{2+} + HO_2^{\cdot} + H^{+} \tag{17.27}$$

However the Fe^{3+} slow down the production of $^{\bullet}OH$ in Fenton process, and subsequently decrease the H_2O_2 degradation and generate unwanted hydroperoxyl radical ($HO_2^{\bullet}$) (Eq. 17.27).

The hydrogen peroxide is not only the way for producing $^{\bullet}OH$, likewise in electrochemical process these radicals are formed at the electrode solution interface from H_2O discharge reaction (Eq. 17.28) or in the bulk of solution by adding a suitable catalyst like Fe^{2+} and electro-generated hydroxyl radical (Eq. 17.29) (Brillas et al. 2009)

$$M + 2H_2O \rightarrow M\left(OH^{\cdot}\right) + H^{+} + e^{-} \tag{17.28}$$

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^{\cdot} + OH^{-} \tag{17.29}$$

The electro-Fenton process produce $^{\bullet}OH$ using electrochemical assisted Fenton process and H_2O_2 is regularly produced in bulk of solution by reduction of dissolved oxygen (Eq. 17.30) and small quantity of a catalyst like Fe^{2+} is added to produce $^{\bullet}OH$ in simple Fenton process (Eq. 17.19). Fe^{3+} is reduced straightforward to Fe^{2+} at cathode (Eq. 17.31) (Brillas et al. 2009).

$$O_2 + 2H^{+} + 2e^{-} \rightarrow H_2O_2 \tag{17.30}$$

$$Fe^{3+} + 2e^{-} \rightarrow Fe^{2+} \tag{17.31}$$

The electrolyte played an important role in any electrochemical process, and during EF process Na_2SO_4 is generally used as electrolyte due to its low activity, Though some other electrolytes like NaCl may also be used for EF process, NaCl produced Cl_2 and HOCl in EF process which start reaction with $^{\bullet}OH$ and consumed these strong oxidant (Eq. 17.32–17.37).

$$2Cl^- \rightarrow 2e^- + Cl_2 \tag{17.32}$$

$$Cl_2 + H_2O \rightarrow H^+ + Cl^- + HClO \tag{17.33}$$

$$OH^{\cdot} + Cl^- \rightarrow \dot{O}H\dot{C}l^- \tag{17.34}$$

$$\dot{O}H\dot{C}l^- + H^+ \rightarrow Cl^{\cdot} + H_2O \tag{17.35}$$

$$OH^{\cdot} + SO_4^{2-} \rightarrow O - OH^- + SO_4^{\cdot -} \tag{17.36}$$

$$SO_4^{\cdot -} + e^- \rightarrow SO_4^{\cdot -} \tag{17.37}$$

The electro-Fenton process generate electrochemically H_2O_2 using two or three electrodes in a single or separated cell. Generally graphite, carbon felt, boron doped diamond (BDD), Ti_4O_7 and gas diffusion electrode (GDE) are employed as cathode (Brillas and Sirés 2012) (Ganiyu et al. 2017) (Zhou et al. 2018) (Salazar et al. 2017). Sopaj et al. (2015) performed comparative study of different anode materials for degradation of sulphamethazine (SMT) in EF system. The author tested Pt, BBD, dimensionally stable anode (DSA, Ti/RuO_2-IrO_2) and graphite felt (GF). The author observed that GF is the most effective anode for the degradation of SMT at low applied current density. It cannot be used at higher current density as it burns for long operation time. The author concluded BBD as the best anode that achieved almost complete mineralization (98.5% TOC removal).

The use of EF process offers many advantages than simple Fenton process. In EF process, Fenton reagent is generated in situ and due to this in situ generation of Fenton reagents, the cost of transportation and storage and risk associated with it decreases. The reaction loss of Fenton regents also decreases; as in the EF process, a control amount of Fenton reagents is produced and consumed. So, as a result sludge formation also reduces. It provides a high rate of organic degradation due to Fe^{2+} electrochemical regeneration, that catalyses the reaction of Fenton (Sirés et al. 2014; Rodrigo et al. 2014; Oturan and Oturan 2018; Zhu et al. 2011).

There are a several working parameters engaged with the effectiveness of the EF process that have been examined for quite a while (Nidheesh and Gandhimathi 2012; Martínez-Huitle et al. 2015; Oturan and Aaron 2014; Brillas et al. 2009). The main parameters are: applied current density, type and concentration of catalyst, pH of contaminated water, distance between the electrode, supply of oxygen and more importantly the electrode materials (Liu et al. 2018).

The pH is an essential parameter and it is usually recommended to carry out the EF process at about pH 3 (Boye et al. 2003). H_2O_2 has been generated in the acidic medium as in (Eq. 17.28). However, pH value below 2 leads to conversion of H_2O_2 into peroxonium ($H_3O_2^+$) which is less reactive towards Fe^{2+} and less $^{\bullet}OH$ radical is produced via Fenton reaction (Mousset et al. 2017). H_2O_2 decomposes to water and oxygen at a high pH level. Fe^{3+} is precipitated as Fe $(OH)_3$ at pH above 4, and at pH below 1, Fe^{2+} forms H_2O_2 complexes (Pignatello 1992).

The voltage is also an essential factor in EF process because the rate of applied current promotes the production of •OH. This factor therefore considerably influences the oxidation and/or mineralization proficiency of the process (Oturan and Aaron 2014; (Sankara Narayanan et al. 2003). However, high current stimulates unnecessary side reaction (reduction or oxidation of water to H_2 or O_2) or wasting (recombination or oxidation reactions) in the electrolytic cell (Zhang et al. 2007; Brillas et al. 2009; Sirés et al. 2014).

The nature or concentration of the catalyst is another important parameter. Among the different catalyst tried for the EF process, iron (Fe^{2+}/Fe^{3+}) has been appeared to be extraordinary compared to others in light of the fact that it isn't unsafe, has low expenses and requires low concentration (Brillas et al. 2009). The optimal value of Fe^{2+} concentration depends on the cathode used. It has been reported that the optimal value of Fe^{2+} as a catalyst for carbon-felt cathode is in range of 0.1–0.2 mM, while for gas diffusion cathode, 0.5 mM has been reported in lots of literature (Brillas et al. 2009; Oturan and Aaron 2014; Sirés et al. 2014). In general, high Fe^{2+} concentrations are avoided as they promote the waste reaction as in (Eqs. 17.38 and 17.39) (Oturan and Aaron 2014; Panizza and Cerisola 2001; Babuponnusami and Muthukumar 2014).

$$Fe^{2+} + H_2O_2 \rightarrow Fe\left(OH\right)^{2+} \tag{17.38}$$

$$Fe^{2+} + OH^{\cdot} \rightarrow Fe^{3+} + OH^{-} \tag{17.39}$$

The Cu^{2+}/Cu^{+} pair was similarly examined as a catalyst in the EF process and conflicting results have reported. For a case, a current report has revealed that copper can be more effective and cost-proficient than iron with a greater rate of TOC reduction (Panizza and Cerisola 2001). Conversely, different analysts have accentuated that iron as a catalyst offers a superior mineralization productivity than Cu ion particles (Panizza and Cerisola 2001). Recently Zhou et al. (2018) studied the oxidation of levofloxacin and trimethoprim by EF process and compared the degradation of these two antibiotics in the presence of Pd-Fe_3O_4 and Fe_3O_4 as a catalyst. The author observed that levofloxacin shows high oxidation in the presence of Pd-Fe_3O_4 instead of Fe_3O_4. The rate constant increased from value of 0.156 to 0.243 min^{-1} by substituting Pd-Fe_3O_4 instead of Fe_3O_4 as a catalyst, while trimethoprim shows no improvement by changing catalyst.

Similarly, Barhoumi et al. (2017) also compared two different catalysts, i.e. chalcopyrite with conventional EF process for degradation of tetracycline. The author observed that the presence of chalcopyrite shows high degradation compared to conventional EF process. Almost complete mineralization (98% TOC removal) was achieved in the presence of chalcopyrite in 360 min of operation under optimum operating condition. Likewise, Chen et al. (2017) compared different catalytic degradation of antibiotic ciprofloxacin (CIP) in EF system. The author first studied the effect of Fe^{2+} and Fe^{3+} dosage for the oxidation and mineralization of CIP. Almost 73% and 72% TOC were removed by Fe^{2+} and Fe^{3+} at optimum condition. Then Fe^{3+}-CIP chelate was tested for the degradation of CIP. The author observed that

Fe^{3+}-CIP chelate had no significant effect on the degradation and mineralization of CIP in EF process.

Air supply and air diffuser are additionally critical parameters which impact oxygen exchange and hydrodynamic conditions in the reactors altogether. The low oxygen stream rate, the solution fed up unsaturated and oxygen exchange can turns into the controlling step, influencing the effectiveness of H_2O_2 production and consequently $^{\bullet}OH$ generation through Fenton's reaction.

The temperature has direct relation with the kinetics of reaction, and therefore it can be a crucial factor for EF process. It can also impact the mass transfer parameters (coefficient of diffusion, etc.). Temperature between 20 and 30 °C is commonly reported in the literature (Panizza and Cerisola 2001). As the temperature increase, it decreases the solubility of O_2 and enhances the decomposition rate of H_2O_2. Similarly, the type of electrolyte also affects the EF process. Ghoneim et al. (2011) conducted a study on the effect of nature of electrolyte. The author studied three different electrolytes, i.e. Na_2SO_4, NaCl and KCl. The author concluded that improved efficiency of SO_4^{-2} shows that SO_4^{-2} gives the higher conductivity compared to Cl^-. Similarly, Ghoneim et al. (2011) also compared the KCl and NaCl and the author concluded that higher degradation rate of KCl shows that K^+ gives higher conductivity compared to Na^+. It is also observed that sodium sulphate gives slower degradation rate of pollutant that $NaClO_4$ or $NaNO_3$ as a supporting electrolyte and it is due to formation of iron sulphate complexes in EF process. The presence of electrolyte in the solution decreases the ohmic potential drop and, as a result, the energy consumption of the process decreases. Furthermore, it has also been reported that too high concentration of electrolyte can also promote side reaction, and due to this the TOC reduction may decrease.

17.6.2 Photo-Electro-Fenton and Solar Photo-Electro-Fenton

Brillas' group broadly studied the phenomenon of EF treatment of organic compound assisting with irradiation by means of artificial UV radiation or solar radiation and termed as PEF or SPEF process (Brillas et al. 2009; Brillas et al. 1998; Boye et al. 2003; Flox et al. 2007; Flox et al. 2007; Garcia-Segura et al. 2014; Thiam et al. 2015). The Fe(III)-hydroxyl complexes formed in electro-Fenton process (Eq. 17.38) should be photo-catalytically reduced and should regenerate.OH and Fe^{2+} (Eq. 17.40) (Sun and Pignatello 1993), which result in the production of regeneration. Complexes are formed between Fe^{3+} and some organics due the direct photolysis according to the general (Eq. 17.41) (Vogler 1993; Zuo and Hoigne 1992; Faust and Zepp 1993). It allows the formation of weak oxidizing species such as superoxide anion radical, carbon dioxide anion radical and H_2O_2 and as a result Fe^{2+} regenerate in parallel.

$$\mathrm{FeOH}^{2+} + h\nu \rightarrow \mathrm{Fe}^{2+} + \mathrm{OH}^{\cdot} \tag{17.40}$$

$$\mathrm{Fe}\left(\mathrm{OOCR}\right)^{2+} + h\nu \rightarrow \mathrm{Fe}^{2+} + \mathrm{CO}_2 + \mathrm{R}^{\cdot} \tag{17.41}$$

PEF process utilizes the UVA of wavelength in range of 315–400 nm, UVB of wavelength in range of 280–315 nm and UVC wavelength in range of <280 nm radiation. Artificial lamps radiation may be utilized in PEF treatments. The wavelength and intensity of radiation source may affect the degradation mechanism. The degradation rate can be increased by increasing the intensity of radiation source. When the radiation source emits light in range of wavelength that pollutant can absorb radiation efficiently, the phenomenon is termed as direct photolysis. Among synthetic lights, the UVA lights have been the most generally utilized (Brillas et al. 2008; Flox et al. 2006; Borràs et al. 2013; El-Ghenymy et al. 2013; Moreira et al. 2015).

The PEF process may also be facilitated by using Fe^{3+} carboxylate complex; *Vilar* et al. recently degraded antibiotic trimethoprim in a lab-scale reactor equipped with BDD and carbon-PTFE cathode. The study revealed that the presence of complex promotes the generation of Fe^{3+} to Fe^{2+} at high pH and also decreased the formation of unwanted species like chloride, chlorate or sulphate in PEF process (Moreira et al. 2015). The iron for the EF or PEF may also be provided by non-conventional reagent; an antibiotic tetracycline has been removed in EF process utilizing chalcopyrite as a source of iron, and the study showed 90% of mineralization of recalcitrant antibiotic tetracycline (Barhoumi et al. 2017).

Due to the high electrical cost of PEF method, it limits the application of degradation of organic pollutants. However, SPEF process can be utilized to overcome the limitation of PEF method by utilizing solar light instead of UV light. A self-sustainable solar-assisted photo electro-Fenton (SPEF) system applied to degrade antibiotic trimethoprim (TMP) and SPEF process is then compared with other processes like anodic oxidation (RuO_2/Ti), AO-H_2O_2 and EF; it was demonstrated that SPEF mineralized the TMP antibiotic up to 8% in 6 h of treatment (Zhang et al. 2016). Furthermore SPEF was able to degrade sulphamethoxazole (SMX) in pilot plant; a study conducted elsewhere showed up to complete removal and 90% TOC was eliminated in SPEF process (Murillo-Sierra et al. 2018).

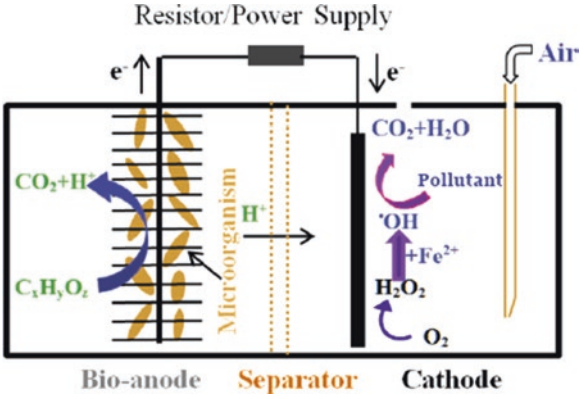

Fig. 17.8 Schematic diagram of Bio-Electro-Fenton process

17.6.3 Bio-Electro-Fenton

There are different bio-electrochemical systems such as microbial fuel cell, microbial electrolysis cell and microbial reverse-electro-dialysis cells which are used to treat the wastewater. The biodegradable waste could be easily degraded in these systems at anodic chamber; however bio-refractory materials like antibiotic could not be degraded by bacteria. Initially Zhu and Ni used microbial fuel cell electro-Fenton system for the degradation of refractory compound in cathodic chamber, and named the process Bio-electro-Fenton (Zhu and Ni 2009).

The electrons are released due to oxidation of organic matter (like acetate ion) through microorganisms in an anodic chamber (Eq. 17.42), and then moved to cathodic chamber by electrical connection, where H_2O_2 is produced by oxygen reduction reaction (Eq. 17.43). Fe^{2+} is produced there using some iron source, which consequently generated $^{\bullet}OH$ to treat the contaminant (Eqs. 17.43 and 17.44) as described in Fig. 17.8.

$$CH_3COO^- + 2H_2O \rightarrow 2CO_2 + 7H^+ + 8e^- \tag{17.42}$$

$$O_2 + 2H^+ + 2e^- \rightarrow H_2O_2 \tag{17.43}$$

$$Fe^{3+} + 2e^- \rightarrow Fe^{2+} \tag{17.44}$$

Some researchers coupled EF and biological system (Olvera-Vargas et al. 2016a; Ganzenko et al. 2018; Olvera-Vargas et al. 2017) and applied for the degradation of pharmaceuticals such as metoprolol, furosemide, ranitidine and real pharmaceutical wastewater. EF may be coupled with biological system either pre-treatment or post treatment.

The Bio-electro-Fenton was broadly classified as microbial fuel cell electro-Fenton (MFC), microbial electrolysis cell (MEC) electro-Fenton and microbial reverse-electro-dialysis cell (MREC) Electro-Fenton system and these systems are self-sustainable in terms of electricity. The *anode* and cathode materials are important in any electrochemical process; similarly in Bio-Electro-Fenton (BFE) mostly a cost-effective anode is used like activated carbon, graphite, carbon felt, carbon mesh, and carbon cloth (Li et al. 2018). The previous studies showed that anode must have strong compatibility with microorganism, good conductivity, chemical stability and high active surface area. Similarly, the *cathode material* in BEF process plays a vital role because the production of H_2O_2 through oxygen oxidation reaction takes place at cathode. The graphite (Zhang et al. 2015), carbon felt (Zhu and Ni 2009) and gas-diffusion electrodes (Rozendal et al. 2009) are widely used as cathode in BEF process. Recently some other carbon materials like graphene or reduced graphene oxide and some iron containing electrode like Fe@Fe_2O_3/carbon felt (Xu et al. 2011), Fe@Fe_2O_3/graphite (Yong et al. 2017), Fe@Fe_2O_3/NCF (Xu et al. 2013), CNT/FeOOH (Feng et al. 2010), Fe_2O_3/active carbon felt (Ling et al. 2016) and Carbon felt/FeOOH (Wang et al. 2014) have been applied ac cathode material for BEF. Mansour et al. (2014) studied sulphamethazine degradation by

combined EF-biological system of 0.2 mM. Batch biological treatment was performed under continuous stirring in the presence of activated sludge at 25 °C. While the EF process was performed in 1 L of undivided cell holding carbon felt cathode and a tube-shaped platinum anode. The solution was continuously stirred and oxygen was supped at 450 mL min^{-1}. EF process was initiated in the presence of 0.5 mM Fe^{2+}, 0.05 M sodium sulphate, pH 3 and 500 mA at 18 °C. After 6 h of EF process alone, the TOC removal was 6.5% with an optimum condition of 0.1 mM pollutant 0.1 mM Fe^{2+} and 200 mA, while 0% TOC removal was observed only for the biological system. After 0.5 h of operation, the TOC removal for the combined EF-biological system was 61.4% while the TOC removal was 78.8% and 93.9%, respectively, after 1 and 4 h of operation. Biodegradability was also assessed in the combination degradation process and was <0.4 after 0.5 h of operation and 0.5 after 1 h of operation.

Mansour et al. (2015) treated Tunisian pharmaceutical wastewater with 200 ppm of sulphamethazine. Fresh activated sludge was introduced in a batch reactor for the biological degradation investigation. The EF process was performed in 1 L undivided cell holding carbon felt cathode and a tube-shaped platinum anode. The solution was continuously stirred and oxygen was supplied at 450 mL min^{-1}. EF oxidative treatment was initiated in the presence of 0.5 mM Fe^{2+}, 0.05 M sodium sulphate, pH 3 and 500 mA at 18 °C. The TOC removal percentage for the EF system alone is 7.5%, while the TOC removal percentage for the combined EF-biological system is 81.4%, while BOD5/COD is 0.35 after 100 min.

Olvera-Vargas et al. (2016b) studied the 0.1 mM Ranitidine synthetic solution by combining the EF-biological system. Biological degradation was performed in batch reactor at 30 °C for 7 days, while 300 mL of undivided cell holding carbon felt cathode and BDD anode was used for EF oxidation. The solution was continuously stirred and dense air was supplied perpetually. The process was initiated in the presence of 0.1 mM Fe^{2+}, 0.05 M sodium sulphate, pH 3 and 500 mA applied current. 59% TOC removal was observed for the 1 h of EF operation, while, for the combine system, 94% TOC removal was observed for 1 h of operation. BOD_5/COD for the EF-biological system is 0.37 for 1 h of operation.

Furthermore, the *microorganisms* are important for BEF process, because they catalysed the electron from organic waste to anode (Li et al. 2017). A variety of domestic waste, municipal wastewater, brewery wastewater, and sewage sludge contain these electro-genic microorganisms which are able to transfer electron from waste to anode. However some exoelectrogenic microorganisms have been separated such as *Geobacter species* and *Shewanella species* which have high potential to generate power density as compared to mixed culture (Wang and Ren 2013). Ferrag-Siagh et al. (2013) reported the oxidation and degradation of tetracycline by coupling EF and biological system. Biological process was carried out from 21 to 25 days. Activated sludge was used as a biological substrate in 500 mL of biological reactor at 25 °C. While, for EF process, a Batch reactor containing carbon felt was used as a cathode and tube-shaped platinum was used as anode. The capacity of the reactor was I L and oxygen was supplied perpetually. The oxidative EF process was initiated in the presence of 0.1 mM Fe^{2+}, 0.05 M sodium sulphate and

300 mA. Efficiency of both processes was evaluated individually and then efficiency of combined EF and biological system was evaluated. It was concluded that EF process alone decreases COD: after 2 h = 66%, after 4 h = 86% and after 6 h = 93%. While, the TOC: after 2 h = 46%, after 4 h = 72%. The TOC removal efficiency of only biological system after 25 days was 10%. While, the TOC removal efficiency of combine system was: after 2 h = 69%, after 4 h = 86%.

17.6.3.1 Sono-Electrochemical Process

Ultrasound offer an alternative method for the treatment of contaminants and received a considerable attention in recent years (Serna-Galvis et al. 2019; Dietrich et al. 2017; Yang et al. 2016). The ultrasound is a sound wave with frequencies above 18 kHz, and therefore cannot be heard by human ear. There are two distinct categories of ultrasound: (a) ultrasound of high frequency or diagnostic ultrasound, with frequency between 2 and 10 MHz and is used mainly for medical applications, such as prenatal ultrasound, and (b) the ultrasound of low frequency or power ultrasound, which has frequency between 20 kHz and 2 MHz and is responsible for the phenomenon of cavitation.

The propagation of sound waves in a liquid medium causes cavitation. This is defined as the phenomenon that involves the formation, growth and violent collapse (implosion) of cavities or bubbles of steam and gases at high sound pressure. A sound wave consists of cycles of compression (positive pressure) and expansion (negative pressure) (Serna-Galvis et al. 2019). The formation and growth of bubbles occur in the expansion stage, and during the compression, occurs contraction and implosion of the bubbles. The collapse of bubbles generates highly reactive radical species and heat. On such drastic conditions, oxidizing species are generated by homolytic cleavage of molecules of gas or solvent. HO$^{\bullet}$ radicals and some other reactive oxidizing species are produced from sonolysis of water (Eqs. 17.45–17.49). In these equations "))))" represents the ultrasound waves.

$$H_2O+))) \rightarrow OH^{\cdot} + H^{\cdot} \tag{17.45}$$

$$O_2+))) \rightarrow 2O^{\cdot} \tag{17.46}$$

$$H_2O + 2O^{\cdot} \rightarrow 2HO^{\cdot} \tag{17.47}$$

$$O_2 + H^{\cdot} \rightarrow O^{\cdot} + OH^{\cdot} \tag{17.48}$$

$$2\,OH^{\cdot} \rightarrow H_2O_2 \tag{17.49}$$

The main advantages of electrochemical processes and sonochemical on other AOPs are: (a) the oxidizing species are generated in situ, and therefore it is unnecessary to add them to the solution being treated, (b) both processes do not require a rigorous control of pH of the solution and (c) at the end of the process, the treated

solution can be easily separated from the reaction system and is not necessary to introduce any additional steps.

Regarding the electrochemical process, another advantage is that the electrode can be reused several times. These characteristics show that the combination of electrochemistry with sonochemistry can result in a promising and interesting process for the destruction of organic contaminants in water which is known as sono-electrochemistry.

The hydroxyl radicals produced by electrochemical and sonochemical processes can oxidize organic contaminants. In addition to the generation of ROS, synergistic effects could be expected to couple the sonication and oxidation via electrochemical in one reactor (Ren et al. 2013). In addition, the physical effects of ultrasound such as streaming, microjets and acoustic cavitation (AC) shockwaves contribute to increased mass transfer, reduced electrode passivation and a reduction in the accumulation of gas bubbles in the electrodes (Pollet 2012; Neppolian et al. 2012). In sono-electrochemical process different kinds of electrode have been studied; Ren et al. (2014) degraded Triclosan using Niobium coated diamond electrode under high frequency ultrasound (850 kHz); the electrode gives the efficient result with affirmative synergistic effect which degraded 92% of pollutant in 15 min following pseudo-first-order kinetics. Similarly Tran et al. oxidized carbamazepine using Ti/PbO_2 with sonochemical process. The author observed that increasing applied current density enhances the gradation of synergy; however, it also increases the imposed ultrasonic power. Highest ultrasonic power was reported for highest synergy degree value, i.e. 33% at lowest current intensity of 1 A and 40 W of ultrasonic power remove 99.5% pollutant by combined effect of sono-electrochemical process (Tran et al., 2017). On the contrary, Pt and graphite electrochemical system couple with ultrasonic process, which effectively degrade SMX at nominal current density of 20 mA cm^{-2} in both sodium sulphate and sodium chloride medium. It was interesting to note that combined US-EC system also prevents the generation of complex chlorinated by-product that is normally detected with sodium chloride (Huang et al. 2017). The sono-electrochemical process is also applicable in different matrix; Ren et al. (2019) reported the combined effect of US-EC for the Chlorpyrifos effluent using different matrix. The combined US-EC successfully removed 93.3% chlorpyrifos under 20 V and 200 W US power. The couple process was observed to follow pseudo-first order kinetics.

17.6.4 Photo-Electrochemical Process or Photo-Electrocatalytic Process

The efficacy of an electrochemical process can be magnified by coupling with UV radiation and the process is known as photo-electrochemical or photo-electrocatalytic technique. In simple photocatalytic process various semiconductors such as TiO_2, ZnO, and WO_3 can be used for the photocalalytic process in suspension form and

the process is independent of mass transfer. However the suspension semiconductor needs filtration at final stage, which is a difficult step at industrial scale. The nanocrystalline from anatase of TiO_2 is a semiconductor material widely used in photocatalysis for light-initiated destruction of organic contaminants in wastewaters (Peralta-Hernández et al. 2006). It has exceptionally appealing characteristic for this application, for example, minimal effort, low danger and a wide band gape of 3.2 eV, which results in high strength and avoid photo-corrosion (Peralta-Hernández et al. 2006). As the photocatalytic material is irradiated with UV light having greater energy than bandgap of material, the electron in valance band (VB) is activated and promoted into the conduction band (CB), generating charged electron (e^-) and hole (h^+) (Eq. 17.50). The (h^+) has good oxidation ability and degraded the organic pollutants directly or it may react with H_2O and produce hydroxyl radical which indirectly oxidized the pollutant (Liu et al. 2019; Banerjee et al. 2015) (Eqs. 17.51 and 17.52).

$$TiO_2 + h\nu \rightarrow e^-_{CB} + h^+_{VB} \tag{17.50}$$

$$h^+_{VB} + H_2O_{(ads)} \rightarrow HO^{\cdot}_{(ads)} + H^+_{(ads)} \tag{17.51}$$

$$h^+_{VB} + RX_{(ads)} \rightarrow RX^{+\cdot}_{(ads)} \tag{17.52}$$

Similarly, the (e^-) reacts with O_2 and produces reactive oxygen species and some other species like superoxide $O^{2-\bullet}$, $HO_2^{\bullet}$, and H_2O_2 also produced $^{\bullet}OH$ by interaction of $h\upsilon$ and H_2O_2 (Pillai et al. 2017; Xie and Li 2006) (Eqs. 17.52–17.57).

$$O_{2(ads)} + e^-_{CB} \rightarrow O^{-}_{2(ads)} \tag{17.53}$$

$$O^{-}_{2(ads)} + H^+ \leftrightarrow HO^{\cdot}_{2(ads)} \tag{17.54}$$

$$2\,HO^{\cdot}_{2(ads)} \rightarrow H_2O_{2(ads)} + O_2 \tag{17.55}$$

$$H_2O_2 + h\nu \rightarrow 2\,HO^{\cdot} \tag{17.56}$$

$$H_2O_2 + O^{-}_{2(ads)} \rightarrow O_2 + HO^{\cdot} \tag{17.57}$$

However these electron (e^-) and hole (h^+) re-join each other by releasing heat and consequently decrease the efficiency of degradation process (Pillai et al. 2017; Bai et al. 2019; Peralta-Hernández et al. 2006; Robert and Malato 2002) (Eqs. 17.58 and 17.59).

$$e^-_{CB} + h^+_{VB} \rightarrow TiO_2 + \text{Heat} \tag{17.58}$$

$$e^-_{CB} + HO^{\cdot} \rightarrow HO^- \tag{17.59}$$

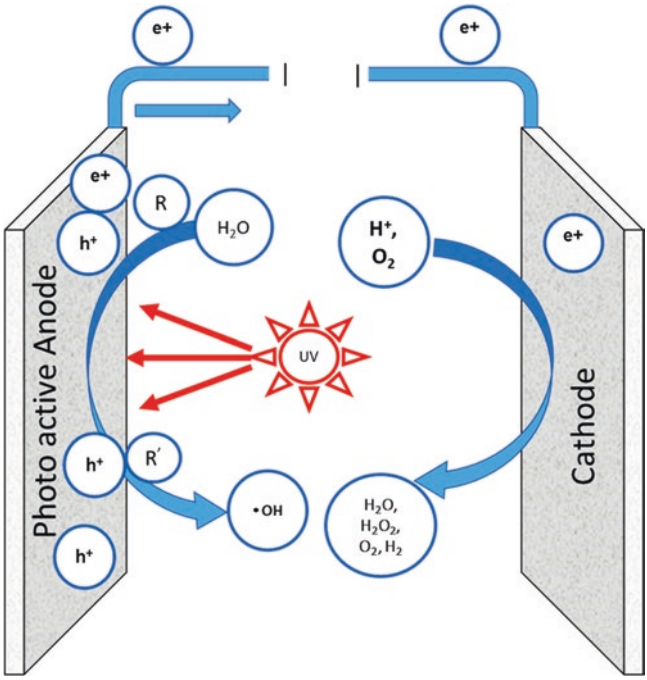

Fig. 17.9 Photo-electro-catalytic process for the pollutant (R) degradation

The photo-electrochemical process present different synergetic way to enhance degradation process like it avoid the recombination of these electron (e^-) and hole (h^+) by applying bias potential through external circuit. So the hole should be available for the sole degradation purpose and enhance the degradation of pollutant (Fig. 17.9). Similarly, the potential between the OER potential and redox potential enhances the direct electro-oxidation pollutant. Similarly, the indirect photocatalytic generated species minimize the electrode fouling. Likewise, the electrochemically generated O_2 takes the electrons and may produce H_2O_2 (Fig. 17.9).

Light-inducted electrochemical process can efficiently treat wastewater. The light-supported method applied either a constant i (current density) or a constant bias anodic potential (E_{anode}) to a TiO_2-based thin film anode such DSA® subjected to UV illumination. In this situation, external electrical circuit constantly release photo-induced electron from the anode which result in the prevention of reaction (Eq. 17.56) (Bessegato et al. 2014) and promote the generation of high sum of holes and heterogeneous $^{\bullet}OH$ from reaction (Eq. 17.51). Thus, there is generation of high photo-induced species, and it also increases the organic destruction relative to the photocatalysis (Bai et al. 2019; Bessegato et al. 2016; Xie and Li 2006; Zanoni et al. 2003; Peralta-Hernández et al. 2006). Commonly, most widely used photo-induced anode is Ti meshes or plates coated with TiO_2 (Carneiro et al. 2005; Zainal et al. 2005; Carneiro et al. 2004), or $Ti/Ru_{0.3}Ti_{0.7}O_2$ (Socha et al. 2006; Socha et al. 2005; Socha et al. 2007; Pelegrini et al. 1999; Catanho et al. 2006; Hussain et al. 2017).

Different parameters can influence the efficiency of photo-electrochemical degradation, such as pH, pollutant concentration and the most important is supporting electrolyte. Different studies described more efficient removal of pollutant using NaCl medium due to the electroformation of oxidizing species at the anode (Su et al. 2016; Zanoni et al. 2004; Yuan et al. 2011; Hussain et al. 2017). The reaction (Eqs. 17.60–17.65) described the mechanism for generating chlorine radicals during photo-electrochemical process.

$$HO^{\cdot} + Cl^{-} \rightarrow ClHO^{\cdot -} \tag{17.60}$$

$$ClHO^{\cdot -} + H^{+} \rightarrow Cl^{\cdot} + H_2O \tag{17.61}$$

$$Cl^{\cdot} + Cl^{-} \rightarrow Cl_2^{\cdot -} \tag{17.62}$$

$$Cl_2^{\cdot -} + H_2O \rightarrow H^{+}\, Cl^{-} \tag{17.63}$$

$$Cl_2^{\cdot -} + Cl_2^{\cdot -} \rightarrow Cl_2 + Cl^{-} \tag{17.64}$$

$$HOCl \leftrightarrow H^{+} + OCl^{-} \tag{17.65}$$

These radicals oxidize and mineralize the organic substances, which is more efficient than pure electrochemical process. In addition, photoactive substances can be cleaved into the solution, increasing the speed and efficiency of the oxidation reaction, and also break the bonds between C-Cl in chlorinated organic compounds and generate highly oxidizing species, improving the efficacy of the process (Eq. 17.66–17.68).

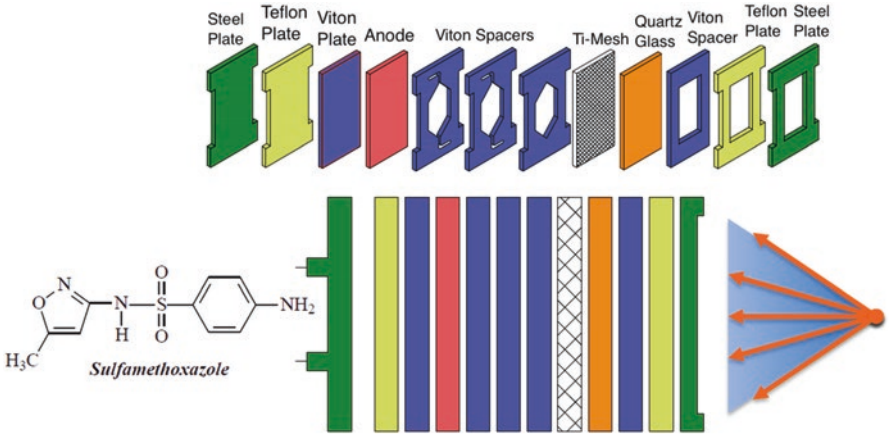

Fig. 17.10 Photo-electrochemical flow cell for the degradation of SMX antibiotic (Hussain et al. 2017)

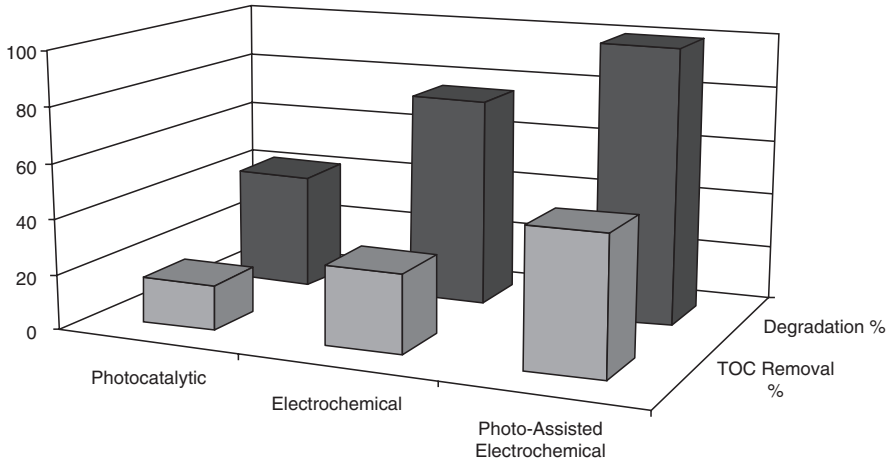

Fig. 17.11 Comparative TOC and degradation values of SMX in photo-catalytic, electrochemical and photo-assisted electrochemical process, duration 10 min

$$\mathrm{Cl-OH} + h\vartheta \rightarrow \mathrm{Cl^{\cdot}} + \mathrm{OH^{\cdot}} \tag{17.66}$$

$$\mathrm{Cl-O} + h\vartheta \rightarrow \mathrm{Cl^{\cdot}} + \mathrm{O^{-\cdot}} \tag{17.67}$$

$$\mathrm{Cl-Cl} + h\vartheta \rightarrow 2\,\mathrm{Cl} \tag{17.68}$$

The photo-assisted electrochemical process is generally more efficient than electrochemical and can efficiently oxidize the organic contaminants. Many studies have demonstrated that DSA® electrodes can be successfully used in photo-electrochemical oxidation processes (Pelegrini and Bertazzoli 2002; Alves et al. 2010). In a recent study Hussain et al. conducted the photochemical, electrochemical and photo-assisted electrochemical degradation of antibiotic SMX in a UV-illuminated electrochemical flow cell as presented in Fig. 17.10

The author compare the degradation and TOC removal of SMX in these three processes, and conclude that the photo-assisted electrochemical was more efficient to eliminate 100% of SMX within 10 min, while only 76.6% and 42.3% of the SMX could be removed by electrochemical and photocatalytic processes, respectively, as shown in Fig. 17.11. Similarly, TOC abatement of 16.5%, 28.6% and 49.6% of TOC was eliminated from SMX after 120 min of photocatalytic, electrochemical and photo-assisted electrochemical degradation, respectively. It is clear that combining UV irradiation with the electrochemical process generates a significant synergic effect to the degradation and mineralization of SMX. Furthermore, the energy consumption parameter for electrochemical and photoelectrochemcial process was compared; the electrical energy per order (E_{EO}) increased from 0.67 to 1.06 kW hm^{-3} $order^{-1}$ as the current density increased from 10 to 60 mA cm^{-2} and dropped from 8.82 to 0.57 kW hm^{-3} $order^{-1}$ which was less than sole electrochemical process.

Likewise another investigation also reported the SMX removal in TiO_2/Ti Photo anode (Su et al. 2016). The author observed that when increasing the anodic potential in range of 0–0.5 V, decreasing the pH from 10.1 to 2.7 and increasing the molar concentration of sodium chloride in range of 1–100 mM, the degradation rate of pollutant increases. After 70 min of operation and 0.5 V, almost 100% organic compound was removed.

Different pharmaceuticals and antibiotic have been studied; the degradation of ibuprofen and naproxen by electrochemical process assisted with radiation source (Zhao et al. 2009) using BDD modified with Bi_2MoO_6 film onto a BDD in cylindrical quartz assisted with light emission source of 150 W Xe lamp (wavelength > 420 nm). Bi_2MoO_6 has the capacity to absorb the visible light at 460 nm. The photo-assisted electrochemical process degraded both ibuprofen and naproxen in range of visible light radiation. However the degradation rate of these pollutants was greater in combined process than the sole process of photo catalysis and electrochemical process. The ibuprofen and naproxen were also productively mineralized in the combine process. Furthermore Cui et al. (2016) conducted a photo-electrochemical study for the degradation of acetylsalicylic in aqueous solution using Pt/TiO_2 NTs photo electrode, then electrochemical process coupled with photo catalytic process by using Xe lamp, power of 6.7 mW cm^{-2}. Almost 98.3% organic compounds was removed in 4 h of photo electrochemical process. Similarly, Li et al. (2016a) investigated same pollutant acetylsalicylic in aqueous solution by photo-electrochemical process using Pd/C-N-S-TiO_2 as anode, while electrochemical process was assisted by photo using 350 W Xe lamp. After 2.5 h of operation, nearly 90% organic compound was removed by combined UV+ visible radiation source assisting electrochemical process, while only 50% organic compound was removed by only visible light assisting electrochemical process. Kondalkar et al. (2014) studied cafetaxime as a model pollutant by photoelectrocatalytic process. TiO_2 was used as photo anode while UV light source, power of 5 mW cm^{-2}, was used to assist the electrochemical process. In 50 mg L^{-1} initial concentration of pollutant, 1.5 V and pH in range of 3–10 conditions, almost 60–95% organic compound can be removed. Li et al. (2016b) studied ciprofloxacin by anodic oxidation coupled with light emission source. TiO_2 NTs was used as photo anode, while UV light source was used. Changing the initial concentration of pollutant in range of 0.1–10 mg L^{-1}, almost 90–100% organic compound can be removed in 2 h of operation. Similarly, Liu et al. (2016) studied ofloxacin by combined photo electrochemical process. UV lamp with power 15 W was used as light emission source, while TiO2 NTs was used as working photo anode. In 0.05 mM of initial concentration of pollutant, applied current of 8 mA, and pH = 2 conditions, almost 50% organic compound was removed after 2 h of operation by the combined process. Tantis et al. (2015) studied omeprazole using UVA (1.5 mW cm^{-2}) and P25/FTO as a working photo anode. The author observed that omeprazole degraded at rate of 1.4 mg L^{-1} h^{-1}. $ZnFe_2O_4$ have been used as photo anode for the abetment of salicylic acid; the investigation reveals about 49% removal and almost 20 COD decrease was reported (Kumbhar et al. 2015).

17.7 Suggestion and Future Research Challenges

A key challenge in the successful implementation of electrochemical treatment of industrial and environmental application is to reduce the energy consumption and cost. Moreover, enhancing the long-term stability, electrolytic efficiency and reducing the cost of electrode materials and advancement in material science and nanotechnology can be vital. Additionally in some cases where NaCl is used as electrolyte the formation of organochlorine by-products is also one of the main challenges of electrochemical process. Laboratory scale experiment should be performed with real water samples and measurement of AOX (Adsorbable organic halogen), toxicity, chlorate and perchlorate is required when oxidation occurs. It may also concern the presence of low concentration of pollutants that may limit the electrochemical reactivity through mass transfer (such as contaminants concentration below that dissolved salts and organic compounds). Therefore, research should be focused towards authentic condition of wastewater. In addition, it is also necessary for complete evaluation of electrochemical system on the basis of appropriate figure of values that provide complete information on reactor configuration, operating parameters, and electrolytic composition. Electrochemical systems are especially suitable for the harmful organic waste treatment. For a case, aqueous film-foaming solution containing high concentration of fluorinated surfactants can be treated by electrodes such as BBD and Ti/SnO_2.

Electrochemical system has many beneficial aspects, such as no need of chemical storage and handling, operation control simplicity, reactor design compactness and the adaptability to varying organic load in wastewater. Referring to these essential benefits, the application of electrochemical process may be addressed in the fragmented treatment of water and wastewater, and also in dispersed point of entry and point of use water treatment.

17.8 Conclusion and Perspectives

Electrochemistry-based treatment technologies have a tremendous power to degrade antibiotic in aqueous solution. Some of these technologies like Electro-Fenton, Bio-electro-Fenton and photo-electrocatalytic obtain good focus in the last decades. Many of these processes efficiently degrade the antibiotics; however some process do not completely mineralize the antibiotics, because of slow degradation rates. Similarly the performance of Fenton process has been improved by coupling with photochemical or sonochemical process or by coupling with biological process, and by using solar radiation the energy of treatment process could be decreased.

Recently the self-sustainable Bio-electro-Fenton process has been developed which degrade the organic contaminants and also produce electricity. Some of them like reverse electro dialysis system (RED) generate electricity from salinity gradient from fresh and saline water, and on the other side it degrades the organic pollutant by providing electron at anodic chamber. Lately some new photocatalytic semicon-

ducting materials have been developed for photoelectrochemical process which degrade the antibiotic wastewater efficiently. However the electrochemical process still has some problem like scale up, electricity demand, electrode cost, and application towards real complex medium, so more work will be needed for full implementation on global scale. Finally it can be concluded that electrochemical technologies offer a promising way to treat wastewater containing antibiotics.

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Chapter 18
Recent Advances in Treatment Technologies for Antibiotics and Antimicrobial Resistance Genes

Hassan Waseem, Sana Jameel, Jafar Ali, Asif Jamal, and Muhammad Ishtiaq Ali

Abstract Various water and waste treatment technologies are gaining attention for having great potential to curb antimicrobial resistance (AMR) dissemination in the environment. Most of the treatment technologies treat AMR by being able to degrade antimicrobial resistance genes (ARGs), lyse antimicrobial resistant bacteria (ARBs) and/ or oxidize the antibiotics whose presence in the environmental matrices contribute in the development and spread of AMR. A number of treatment technologies like aerobic and anaerobic digestion, membrane bioreactors, composting, nanoparticles and some disinfection-based mechanisms have already been evaluated, at industrial scale, for treating the antibiotics and associated ARBs and ARGs. These technologies, at present, are in use from the perspective of waste management and/or alternative energy but their place in formulating a broad and comprehensive strategy for controlling the spread of AMR in clinical and environmental systems has

H. Waseem
Environmental Microbiology Laboratory, Department of Microbiology, Quaid-i-Azam University, Islamabad, Pakistan

Department of Biotechnology, University of Sialkot, Sialkot, Punjab, Pakistan

Department of Civil and Environmental Engineering, Michigan State University, East Lansing, MI, USA
e-mail: hassan.waseem@uskt.edu.pk

S. Jameel
Department of Biotechnology, University of Sialkot, Sialkot, Punjab, Pakistan

J. Ali
Key Laboratory of Environmental Nanotechnology and Health Effects, Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences, Beijing, China
e-mail: Jafarali_st@rcees.ac.cn

A. Jamal · M. I. Ali (✉)
Environmental Microbiology Laboratory, Department of Microbiology, Quaid-i-Azam University, Islamabad, Pakistan
e-mail: ishimrl@qau.edu.pk

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been realized. This chapter summarizes some of the important treatment technologies, illustrating their key features and efficacy in treating AMR. Studies that provide alternative views about the contributions of various treatment technologies in selecting resistant bacteria and genes are also highlighted in this chapter.

Keywords Recent advances · Treatment technologies · Antibiotics · Antimicrobial resistance genes

18.1 Introduction

Antibiotic resistance has started becoming a public health and safety concern worldwide (Roca et al. 2015). The worldwide escalation of antibiotic consumption and subsequent resistance is also a serious concern for the environment. The development and subsequent increase in the dissemination of AMR is already endangering the therapeutic efficacy of antibiotics and subsequently increasing the rates of treatment failures. All of this may, ultimately, lead to prolonged and persistent infections causing increased morbidity and mortality (Li and Webster 2018). The need for combating AMR through improved versions of current methods and development of new innovative technologies has already been realised. Scientific community is exploring different methodologies to decipher the intricate trends of ARGs persistence and dissemination in order to formulate strategies for curbing the dissemination of AMR (Li et al. 2015, Munir et al. 2017, Waseem et al. 2019). Implications are still present in developing and utilizing new techniques for AMR analysis (Williams et al. 2017). Different water and waste management treatment technologies have been evaluated by the researchers in order to gauge their impact on removing resistant microbes and associated ARGs particularly from the environmental matrices. Some of the selected treatment technologies with a great potential to be utilized on large scale for treatment of AMR have been described below.

18.2 Anaerobic Digestion

Anaerobic digestion is a complex series of microbial processes in which biological sludge (and other organic material) break down and biogas is produced in the absence of oxygen. Although the major advantages of AD are reduction in emission of greenhouse gases and renewable energy production (Zaks et al. 2011), it can also be used as a treatment technology for reducing ARGs in various environmental matrices (Ma et al. 2011). Diverse views are present on the removal efficiencies of AD (Waseem et al. 2017b). Some studies have suggested thermophilic AD to be efficient in removing ARGs (Sun et al. 2016; Xu et al. 2018), and others have

reported mesophilic anaerobic digestion can reduce potential pathogens along with ARGs but a chemical oxidation process is required for enhancing the reduction efficiency (Ihara et al. 2013). Similarly, some physicochemical pre-treatments before AD are also reported to enhance the ARGs removal efficiency of the anaerobic digestion process (Tong et al. 2016). There are also reports of selected removal of ARGs, during AD, on the basis of resistance mechanisms (Sui et al. 2016). Apart from directly mitigating the dissemination of ARGs from the sludge into the environment, AD can also indirectly control the accumulation of ARGs by direct degradation of antibiotics. Enhanced and faster degradation of antibiotics can be achieved during thermophilic digestion as compared to mesophilic anaerobic digestion (Massé et al. 2014).

The underlying processes which can influence the removal of ARGs during anaerobic sludge digestion are not yet completely understood. There is a dire need to keep in mind that the primary purpose of anaerobic digestion is methane production and not pathogens and/ or resistance mitigation. Addition of graphene oxide (GO) in the concentration of 500 mg/L has reportedly enhanced the reduction of resistant genes from 33.7% in control to 40.2% in the treatment group during the digestion of swine manure, but the addition of GO has also reduced the biogas-producing capability of the anaerobic digester by 17.1% (Zhang et al. 2017). The process optimization is generally performed to keep anaerobic digestion process stable and biogas production optimum. Anaerobic digestion can also face process instability due to lack of optimization in operational parameters, which in turn can also influence antimicrobial resistance genes survival in the digesters. Sudden changes in substrate loading and/or addition of exogenous compounds are believed to be one of the main causes of the process instability (Ferguson et al. 2016). Studies have highlighted that the substrate shock—sudden addition of large amount of carbon source—is often responsible for the process imbalance, causing organic acids build-up which can ultimately lead towards inhibition of biogas production (Chen et al. 2012). Effects of various operational parameters on antimicrobial resistance remain to be elucidative. It is perhaps due to these reasons that there is still a reluctance in the broader adoption of this technology.

An opposite point of view regarding the role of anaerobic digestion also exists among the scientific community. People are seeing the anaerobic digesters as a major source of antibiotic resistance dissemination into the environment, as studies have reported that the sludge contribution in releasing ARGs into the environment is more than the effluent wastewater (Calero-Cáceres et al. 2014). The presence of antibiotics and/or residual compounds can also select various antibiotic resistance genes in the digester. For example, risk of ARGs dispersal was increased when arsanilic acid is present in relatively higher concentrations (650 mg/kg) during anaerobic digestion (Sun et al. 2017). More emphasis is needed on the treatment processes of sludge for mitigating the spread of resistant bacteria and ARGs; therefore, further in-depth investigations into the use of anaerobic digesters for curbing antimicrobial resistance is required. Changes in design of anaerobic digesters to include physicochemical pre-treatments for enhancing cell lysis and extracellular DNA degradation may also be warranted.

18.3 Aerobic Digestion

In aerobic digestion microorganisms break down complex organic molecules in the presence of oxygen. In wastewater treatment plants (WWTPs), the process of aerobic digestion is also referred as activated sludge treatment. The major purpose of aerobic digestion is reduction of volume of sewage sludge during the process of sewage treatment. Being one of the integral parts of wastewater treatment systems, the efficacy of aerobic digestion as ARGs removal technology has been critically evaluated. Like anaerobic digestion, the ARGs removal efficiencies during activated sludge treatment are also variable (Waseem et al. 2018a). A study investigating the reduction of ARGs in municipal wastewater solids had concluded that the process of aerobic digestion can substantially decrease the abundance of ARGs but the rates of ARGs reduction are on the individual ARG under investigation and reactor design (batch vs continuous flow) (Burch et al. 2013). Other operational conditions and influent qualities are important drivers behind inconsistent removal efficiencies of aerobic digestion. For example, a study by Neyestani and colleagues has reported that presence of high antibiotics concentration, longer solid retention times (SRTs) and thermophilic conditions could increase the AMR in activated sludge systems (Neyestani et al. 2017b). Another study evaluating the effects of thermophilic aerobic digestion on 23 ARGs, 4 metal-resistant genes MRGs, *int1* and 16S rRNA gene has concluded that increased temperature decreased in ARGs due to reduction in overall resistome and bacterial diversity in sewage sludge (Thermophilic aerobic digestion). Another study has also confirmed that the longer SRTs during aerobic digestion can result in a 40% increase in the prevalence of ARBs in the presence of extracellular constituents. The authors of the study have suggested that either the antibiotic-resistant bacteria were positively selected and/or the results came out as false positive (Neyestani et al. 2017a).

Residual sub-inhibitory concentrations can play a vital role in the selection of ARGs in the environment. There are many reported instances where activated sludge digestion has not been able to clear the antibiotics completely from the biological sludge (Khan et al. 2017). A group of researchers, for example, has investigated the concentrations of nine selected antibiotics utilizing liquid chromatography tandem mass spectrometry in treated and reclaimed wastewater. Conventional activated sludge process has significantly reduced the antibiotic concentrations (>50%), during the experiments, but the complete elimination of the antibiotics was not attained (Kulkarni et al. 2017). Similarly an activated sludge floc can cause the sorption of the antibiotics and can bring bacteria and antibiotics in the vicinity of each other which may cause positive selection of resistant bacteria (Louvet et al. 2017).

Aerobic digestion could serve as a critical step in designing of wastewater treatment technologies for mitigation of ARGs into the environment; therefore, effective assessment about the influence of activated sludge process on AMR is essentially needed. Further research in investigating the role of aerobic digestion for reducing ARGs, ARBs and antibiotics will be useful for environmental engineers in comparing the advantages and limitations of this technology with other treatment technologies.

18.4 Membrane Bioreactors

Membrane bioreactors (MBRs) have also emerged as a promising and sustainable technology for the treatment of AMR in municipal and industrial wastewater (Fig. 18.1) (Aslam et al. 2017). It is important to understand the fundamental processes responsible for proliferation of ARGs in MBR systems so that efficient removal mechanism can be developed. In MBRs, foulant layer plays a very important role in the removal of ARGs whereas transmembrane pressure contributes in the removal of ARBs. Various aerobic and anaerobic MBRs have been employed in different studies and their contaminant removal efficiencies have also been evaluated. Role and contribution of membrane fouling, in reducing ARGs from WWTP effluents, is widely discussed in the literature. An anaerobic MBR, for example, has been employed highlighting the impact of membrane fouling on ARBs and ARGs removal; membrane fouling was found to be positively correlated with the removal of ARGs (Cheng and Hong 2017). A different anaerobic MBR treatment was able to remove selected ARGs and *int*1 in the range of 3.3–3.6 log units. In this case anaerobic MBR treatment had also decreased the biomass significantly as depicted by the reduction in the concentration of the housekeeping gene (Kappell et al. 2018). Another study, evaluating the repercussions of antibiotics presence on membrane fouling, has measured the ARGs removal capacity of anoxic/aerobic membrane bioreactor (A/O-MBR) (Zhu et al. 2018). Membrane fouling acted as an additional barrier that effectually stopped the flow of ARGs across the membrane.

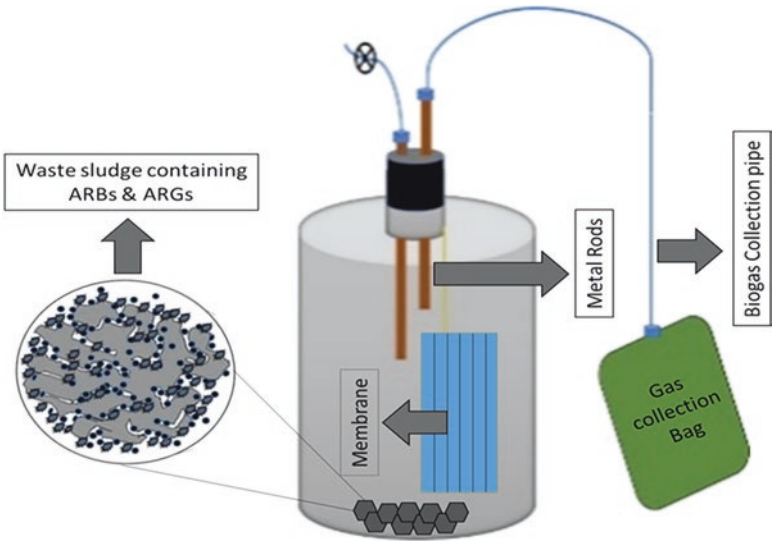

Fig. 18.1 Schematic diagram of a working membrane anaerobic digester for the treatment of ARGs and ARBs

Different treatment technologies, sometimes, are synergistically applied for the enhancement of AMR removal. Karaolia et al. (2017) have investigated the performance of a small-scale MBR combined together with a solar fenton oxidation system. The integrated system was able to remove >99% of bacteria, and the antibiotic removal efficiency was also improved. However, the integrated process was unable to remove some selected ARGs. In fact, few ARGs proliferated after 180 min of treatment. One probable reason for this increase of ARGs is the release of intracellular ARGs to the outside environment, after the treatment, as a result of bacterial cell lysis.

MBRs, particularly anaerobic ones, can play an important role in future plans for efficient AMR reduction from wastewater because they don't require any intensive energy demanding oxygen transfer and can also synthesize biogas during wastewater treatment. Filtration performance of MBRs can be substantially increased or decreased by variations in membrane fouling. Membrane fouling, on the other hand, is also viewed as a major drawback halting the general adoption of application of MBRs for treating AMR because it can reduce the lifespan of the membranes resulting in a considerable increase in operation costs.

18.5 Disinfection-Based Treatment Technologies

Disinfection is the process of inactivation and/or killing of microorganisms (>99%) from the inanimate objects. Various physical and chemical disinfection processes have been employed for tackling AMR in different environmental matrices (Di Cesare et al. 2016; Shi et al. 2013). Environmental cleaning and disinfection can play a role in curbing the spread of various resistant bacterial species like vancomycin-resistant *enterococci* (VRE) and methicillin-resistant *Staphylococcus aureus* (MRSA) (Carling and Huang 2013). Routine cleaning and disinfection of inanimate objects in patient rooms was associated with a significant reduction in MRSA, VRE, and *Clostridium difficile* infections in a hospital in Canada (Alfa et al. 2015). Surface disinfectants like hydrogen peroxide kills the pathogen by oxidative cellular destruction. The process is instantaneous, and occurs on multiple non-specific target sites making it challenging for the bacterial pathogen to develop resistance against surface disinfectants.

18.5.1 Chlorination

Chlorination is the most popular disinfection process for treating aquatic environments due to its effectiveness and availability. The number of systems employing free chlorine as a disinfectant is an order of magnitude greater than alternate disinfection technologies (AWWA 2017). Yuan and colleagues have recently studied the fates of nine ARBs and eight selected ARGs providing bacterial resistance to erythromycin and tetracycline during wastewater chlorination. A chlorine dose ranging from 15 to 300 milligrams-minutes per litre (mg-min/L) was able to remove 60%

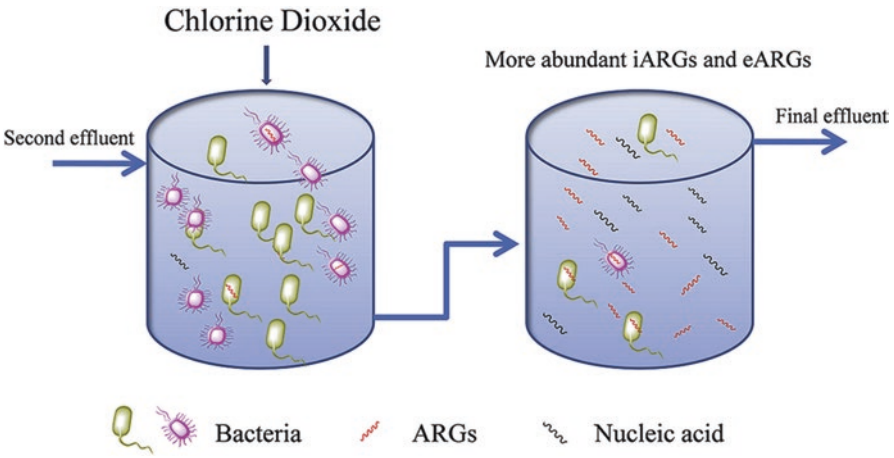

Fig. 18.2 Chlorine dioxide treatment more efficiently treating ARBs than ARGs (Liu et al. 2018) Reprinted from Water Research,136, Liu et al., Chlorine disinfection increases both intracellular and extracellular antibiotic resistance genes in a full-scale wastewater treatment plant, 131–136, Copyright (2018), with permission from Elsevier

and 20% of the ARGs, respectively. All resistant bacteria were inactivated with the chlorine dose of only 15 mg-min/L except sulphadiazine- and erythromycin-resistant bacteria which required an additional dosage of 60 mg-min/L (Yuan et al. 2015). Another group of scientists evaluated the chlorination impact on the removal of 282 ARGs from secondary effluent of a WWTP. A total of 4 mg of chlorine per litre with a contact time of 30 min was capable of reducing the ARGs abundance by 2.4–3.4 folds (Lin et al. 2016). A contact time of 10 min with a relatively higher chlorine concentration of 75 mg/L was also able to inactivate 90% of ARBs and 78.8% of pB10 plasmid in a different study (Pak et al. 2016). However a constant and long-term application of chlorine can also increase the environmental spread of AMR. Liu et al. (2018) have monitored the effects of chlorine disinfection, for a year, on intracellular and extracellular ARGs and found that ARGs were increased by 3.8-folds and 7.8-folds, respectively, thus enhancing the risk of AMR in the environment (Fig. 18.2). Apart from chlorination, potential of other relatively mature disinfectant treatment technologies like ozone and UV treatment for AMR elimination have also been investigated by researchers.

18.5.2 Ozone

Ozone is a strong disinfectant and oxidant which can be a promising choice for inactivating resistant microorganisms. Contact time is considered very critical for the ozone treatment. Addition of various catalysts like persulphate and monopursulphate can also significantly reduce the contact time for the treatment of ARGs

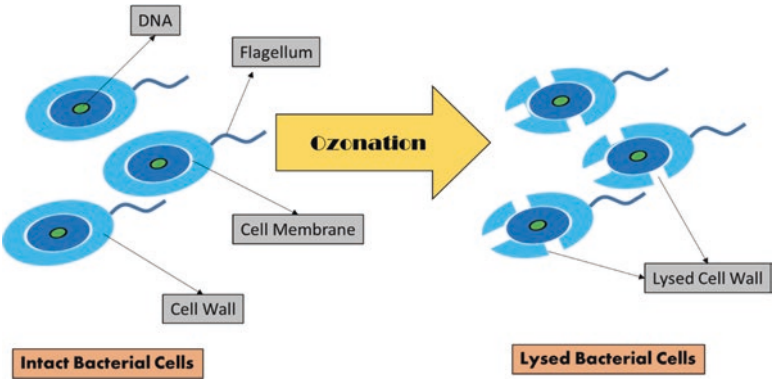

Fig. 18.3 Ozone's antibacterial action killing the ARBs by cell wall lysis

(Oh et al. 2014). Diverse trends in ARGs abundance in response to ozone treatment could be due to selective shifts among different bacterial groups within a community. Such diverse trends of ozone on ARGs were highlighted in wastewater where a concentration of 0.9 ± 0.1 g ozone per 1 g of dissolved organic content (DOC) was employed for treating wastewater. Although the ozone treatment was able to reduce *ermB* by two orders of the magnitude but at the same time two other resistant genes (*vanA, blaVIM*) increased within the survived bacterial population (Alexander et al. 2016). Ozone disinfection has better influence on resistant bacteria as compared to ARGs as ozone treatment of only 15 min was able to completely remove erythromycin and ertapenem *E. coli* in urban wastewater effluents (Michael-Kordatou et al. 2017). Ozone has the potential to degrade the bacterial cell wall (Fig. 18.3). It can also be employed for the degradation of various antibiotics and hence can also play an indirect role for the reduction of AMR in wastewaters (Ben et al. 2012; Lange et al. 2006).

18.5.3 UV

The impact of UV disinfection on various ARGs, ARBs and antibiotics have also been investigated in different studies. Increase in UV doses have been linked with the exponential decrease in ARGs in the effluent of a WWTP (Zheng et al. 2017). In most of the studies, strong oxidants like H_2O_2 have also been employed along with UV to enhance the treatment capability of UV. Degradation kinetics and transformation products of trimethoprim and sulphamethoxazole with UV, UV/H_2O_2 and UV peroxydisulphate treatments were explored in synthetic and hydrolysed urine (Zhang et al. 2016). Ferro and colleagues have reported the successful implementation of a UV/H_2O_2 disinfection treatment process on coliforms isolated from wastewater. Although the process had significantly inactivated the coliforms including the resistant *E. coli*, resistant genes including *blaTEM*, *qnrS* and *tetW* survived even

4 h of treatment process (Ferro et al. 2016). UV disinfection treatment can also negatively impact the human health by increasing the risk which it can pose during the use of reclaimed wastewater. A UV dose of 20 mJ/cm^2, for example, has initially inactivated tetracycline-resistant (3.0 log) and other heterotrophic bacteria (>4.0 log) but bacterial population reactivated during a dark repair period. Abundance of tetracycline-resistant bacteria was increased after reactivation due to more rigorous inactivation of non-resistant heterotrophic bacteria (Huang et al. 2016). There are also concerns that transduction by bacteriophages can be induced with the help of UV treatment, which in turn can enhance the transferability potential of ARGs among bacterial populations (Torres-Barceló, 2018).

Overall, disinfection treatment processes can decrease bacterial susceptibility and develop cross-resistance to therapeutically important antibiotics (Webber et al. 2006). Studies have shown sublethal concentrations of disinfectants can trigger resistance mechanism in bacteria (Buffet-Bataillon et al. 2012). Similarly, many different studies have also reported the role of disinfection by products in induction of AMR (Li et al. 2016, Lü et al. 2015). Evidence is available in literature to support both the viewpoints about the pros and cons of disinfectants for curbing the dissemination of AMR. Further research is required to decipher the intricate relationship between various disinfection mechanisms and AMR to conclude the usefulness of disinfection as an effective and sustainable AMR treatment technology.

18.6 Nanoparticles to Control Antibiotic Resistance

Metal nanoparticles (NPs) have emerged as a new tool to cope the antibiotic resistance due to their excellent antibacterial activities against deadly bacterial infections (Rai et al. 2009). Nanoparticle-based strategies are more effective in controlling ARBs and ARGs than conventional approaches. Silver, titanium, copper, zinc and iron are the metals extensively used in nanoparticle-based antimicrobial studies. Among all the nanomaterials, silver has been extensively used in various medicinal compounds such as bhasmas and Ayurveda used to treat numerous bacterial infections since time immemorial (Sharma et al. 2009). Antibacterial activities of silver nanoparticles (AgNPs) are due to their extremely minute size rendering them high surface area to volume ratio, which increase the contact area with microbes. Moreover, AgNPs enhance the chemical and biological activities and has the potential to target numerous bacterial structures (Fig. 18.4). Generally, metal nanoparticles disturb the physiology of cell membrane such as permeability and respiration (Xiu et al. 2012). Furthermore, AgNPs can bind with sulphur-containing amino acids and phosphorous-containing nucleotides, thus destroying the major molecular machinery of bacteria (Zheng et al. 2018).

AgNPs have been regarded as new weapon against the multidrug-resistant (MDR) bacteria. The orthodox chemical synthesis of AgNPs is rapidly becoming obsolete due to the hazardous nature, low yield and cost ineffectiveness of the process (Duan et al. 2015; Raza et al. 2016). So, people are exploring the facile, eco-

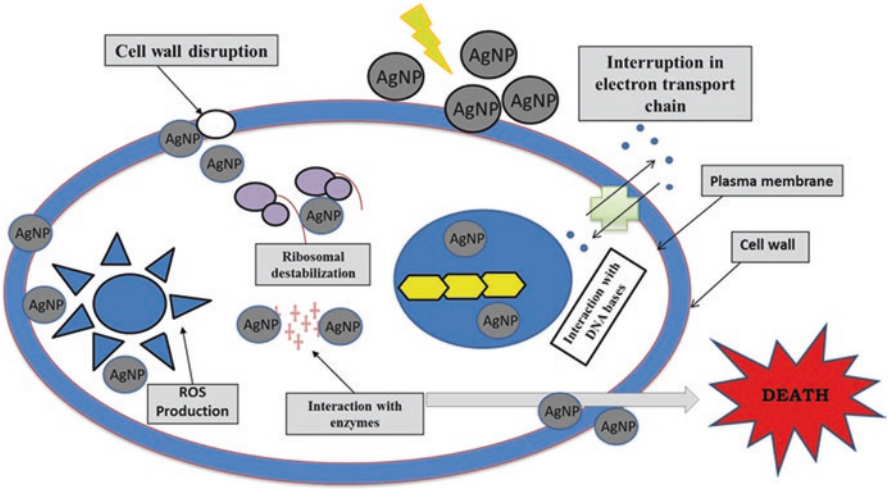

Fig. 18.4 Schematic representations of different antimicrobial mechanisms employed by silver nanoparticles against bacteria (AgNPs)

friendly and cost-effective alternative options (Shah et al. 2019). Influenced by the bio-reduction of silver ion (Ag^+) using microorganisms, green synthesis of AgNPs has become a timely topic (Ali et al. 2017, 2018). Although biogenic production of AgNPs has been evaluated using fungi, plants and enzymes, bacterial synthesis is considered as easy and facile fabrication (Ali et al. 2016). Silver compounds or AgNPs have been extensively used in antibacterial assessment against the both gram-positive and gram-negative pathogenic bacteria (Rana et al. 2018).

Even though metal nanoparticles have been effectively used against the antibiotic-resistant bacteria, they also pose some toxicity to mammalian cells (Park et al. 2007; Waseem et al. 2018b). Therefore, modifications and new combinations must be evaluated to minimize the toxicity of NPs. Recently, different amalgams of NPs with antibiotics, polymers, essential oils and antimicrobial peptides have been developed to reduce their toxicity (Hemaiswarya et al. 2008). The combined efficacy of AgNPs and antibiotics lessened the toxicity of both agents towards mammalian cells possibly because of lowered dosage requirements owing to synergistic antimicrobial effect. Likewise, selective/target specificity are major concerns in the use of AgNPs in targeted drug delivery and antimicrobial therapy against multidrug resistant (MDR) bacterial infections (Wang et al. 2018). A recent study combined the antibacterial potential of AgNPs with branched polyethylenimine (bPEI), a selective bacterial toxic agent, thus providing a cationic Ag-nanocluster as a potent antibacterial agent for MDR (Huma et al. 2018).

Despite the fact that metal NPs are the ray of hope in the war against AMR, there are some concerns that metal contamination may serve as a selective agent in the spread of antibiotic resistance and ARGs. Bacterial cells can develop metal resistance/tolerance by limiting the entry of metal into the cell envelop via synthesizing the various extracellular polymeric substances (EPS). Another important strategy is

to release the siderophores in the medium, which can protect the bacteria from metal toxicity. Bio-sorption and biofilm production are also essential ways opted by bacteria to protect the metal toxicity (Giovanella et al. 2017). A recent study described that pervasive metal pressure in the environment may facilitate the proliferation of antibiotic resistance by co-selection of metal resistance genes (MRGs) and ARGs. The co-selection process can be attributed to various underlying mechanisms depending upon the type and level of contaminations as documented in literature (Baker-Austin et al. 2006). This particular interaction may result from co-resistance (different resistance determinants present on same genetic element) and cross-resistance (only one genetic element responsible for both metal and antibiotic resistance) (Li et al. 2017). Moreover, indirect and shared regulatory responses to antibiotic and metals such as biofilm development also represent a co-selection mechanism in bacteria. Environmental factors associated with the dissemination of metal NPs and antibiotics must also be considered. An important factor in emergence of combined resistance is experimentally induced co-selection using microcosms amended with an antibiotics and variety of metals, or which resulted in an increased frequency of multiple resistance phenotypes (Wang et al. 2017a). Although a direct assessment of the critical role of metals in co-selection is often hindered by the manifestation of some other anthropogenic contaminants, these studies do link contaminant exposure with elevated antibiotic resistance. Current advances in microbial physiology, genomics, and biochemistry could provide the basis for the accurate determination of important steps involved in complex metal–antibiotic resistance interactions, particularly, the relative contributions of co-resistance determinants to the fitness of bacteria in different environmental and clinical settings (Pal et al. 2017). Despite of abovementioned studies on the existence of selection mechanism of metals and antibiotics, there are still some uncertainties which must be explored to fully understand this scientific mysteries about the co-selection mechanism and influence of horizontal gene transfer in the presence of metals (Seiler and Berendonk 2012). Therefore, metal contamination represents a long-standing, widespread and recalcitrant selection pressure with both environmental and clinical importance that potentially contributes to the maintenance and spread of antibiotic resistance factors.

18.7 Constructed Wetlands

Constructed wetlands (CWs) are artificially engineered wetlands that have been employed for either the treatment of wastewater or the reclamation of an effected land (Sheoran and Sheoran 2006, Wang et al. 2017b). Poor performance of individual septic systems and higher cost associated with centralized sewer systems have paved the use of constructed wetlands to treat various environmental contaminants including ARGs, ARBs and antibiotics. Abundance and distribution dynamics of ARGs before and after treatment of municipal wastewater with constructed wetland have been evaluated by researchers. A study, for example, has reported that

certain targeted resistance encoding genes *(tetA, tetB, tetM, ermB, sul1, ampC and qnrS)* were detectable in the mesocosm environment and the abundance of these genes was reduced after the remediation of wastewater in the constructed wetlands (Nõlvak et al. 2013). In addition to ARGs, mesocosm-scale constructed wetlands have also been used for the removal of antibiotics in domestic wastewater. Six CWs (mesocosm-scale) with three flow types and two different species of plants were set up to observe the removal of 8 antibiotics, 12 genes in different matrices through CWs. The removal efficiencies ranged from 75.8% to 98.6% and 63.5% to 84.00% for antibiotics and ARGs, respectively, where the presence of plants played an important role for removal of pollutants and other substrates (Chen et al. 2016). Another study reported the volcanic CWs with vertical flow cemetery in the removal of ARGs including three *tet* genes and 16S rRNA from swine wastewater which is reduced by 50% and affected by the nature of wetland medium (Liu et al. 2013).

Water remediation is often considered as a time-requiring process which in certain cases can take months and years. Concentrations of ARGs were also monitored over a period of 10 years in an integrated surface flow constructed wetland (ICW) where domestic sewage was the primary source of ARGs contribution, and 77.8% and 59.5% removal rates were determined in the winter and summer seasons, respectively. Significant correlations were observed between *intI*1 and many ARGs, signifying the role of mobile genetic elements dissemination of ARGs in an ICW (Fang et al. 2017). Constructed wetlands have also been employed to check their potential in the removal of antibiotic resistance bacteria from the saline aquaculture effluents. A study suggested that the removal percentages of both the total and resistant bacteria were relatively fewer and unstable during the first week of experiment in the CWs microcosms, stabilizing after the third week of the treatment to attain the maximum removal values of around 96% (Bôto et al. 2016). In another comparative study, percentages of antibiotic resistance bacteria were found to be similar in constructed wetlands and conventional wastewater treatment, and the abundance of bacteria was much low in CW as in activated sludge of WWTP which means much lower ARB spread in the environment. It was also supported by faecal indicator bacteria resistance to antibiotics in experimental constructed wetlands (Sidrach-Cardona and Bécares 2013).

In one particular study, the abundance and removal of sulphonamides and tetracycline were also examined other than ARGs using wetland plants *(Phragmites autralis)* under microcosm adsorption, biodegradation and sunlight photodegradation mechanisms. The study suggested that the physiochemical properties of plants play an important role in the removal of antibiotics. The average removal efficiencies of other selected antibiotics in this study were found to be 85% (SMA), 81.86% (SH), 49.43% (SHZ), 29.47% (CTC) and 22.26% (EFX) (Choi et al. 2016). Some CWs studies have also reported inconsistent removal of ARGs. A constructed wetland treatment study has reported inefficient removal of ARGs whereas successful removal of nutrients, suspended solids, pharmaceuticals and micro-pollutants was reported by the same wetland (Anderson et al. 2013). Another study encountered the continuous supply of antibiotics in constructed wetland to determine the antibiotic removal efficiency by CW and evaluate resistance development and expression

profiling. It suggested that short-term treatment of CW with antibiotics has no observed impacts on antibiotic resistance genes (Berglund et al. 2014).

18.8 Composting

Composting can also be an effective approach for mitigating the risk of AMR propagation via environmental routes. It can serve as a powerful treatment technique for curtailing ARGs, ARBs and residues of antibiotics. Aerobic composting has been reported to significantly reduce the ARGs and MGEs in cattle manure. In a 120-day-long microcosm incubation experiment, the abundance and diversity of resistome in manure-treated soils were significantly higher than those in compost-treated soils (Gou et al. 2018). Recently, researchers have found that removal of AMR can be significantly enhanced by increasing the temperature during composting in a 3-week-long experiment. Hyper-thermophilic composting is also better able to reduce the half-life of ARGs and MGEs as compared to conventional composting (0.8–1.3 vs. 1.9–4 days) (Liao et al. 2018). The major advantage of compositing is that it can be easily transformed into bigger industrial scale technology. Addition of biochar can also influence the removal capacity of ARGs during composting. Impacts of rice straw biochar and mushroom biochar on the behaviour of ARGs in a lab scale chicken manure compost were investigated by Cui and colleagues. Mushroom biochar had positively influenced the ARGs removal while addition of rice straw biochar had decreased the ARGs removal capacity of compost treatment (Cui et al. 2016) (Fig. 18.5).

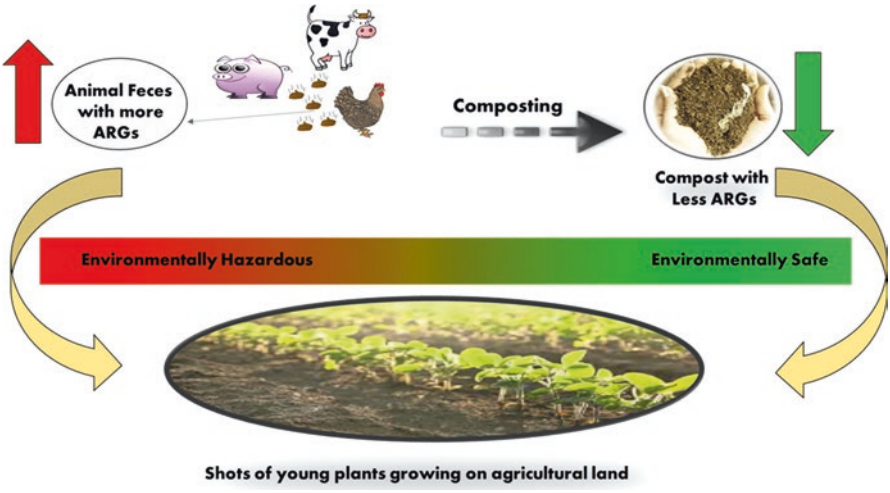

Fig. 18.5 Pictorial depiction of composting reducing ARGs in animal manure

Some researchers advocate against composting because they believe that ARGs are disseminated into the environment from animal faeces and even the treated manure can contribute significantly in the dispersion of ARGs into the environment. Reducing or eliminating compost, however, is not a viable option because traditional composting actually helps the agricultural sector to have less dependence on chemical fertilizers. This in turn could produce healthier and more environmentally friendly food, but we have to make it safe in the context of dissemination of ARGs and ABRs in the agricultural settings.

18.9 Conclusion

As the pace of new antibiotic development is not keeping up with the development and dissemination of AMR, the need for the development and validation of treatment technologies is realized greatly. Many of the reported treatment technologies are yet not yet able to achieve complete elimination of ARGs, ARBs and antibiotic residues. Thus, only treatment technologies alone cannot fully tackle the problem of AMR spread in the environment, so they should, instead, be a part of a broader and comprehensive AMR control strategy. Bioinformatics approaches for identifying the AMR risk should be explored (Waseem et al. 2017a, b). Other integrated approaches like rational prescription of the existing antibiotics, AMR stewardship programmes, controlled discharge of antibiotic resistance waste, on-site analysis of AMR and strict infection control along with the already developed potential treatment technologies are required for designing and implementing an efficacious and sustainable strategy to win the war against the ever-increasing threat of AMR.

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Chapter 19
Treatment Technologies for Removal of Antibiotics, Antibiotic Resistance Bacteria and Antibiotic-Resistant Genes

Paweł Krzemiński and Magdalena Popowska

Abstract This chapter describes current knowledge on the selected eco-friendly strategies for the treatment of main sources (manure and wastewater) of antibiotics, antibiotic resistance genes (ARGs) and antibiotic resistance bacteria (ARB), including bacteria pathogenic for humans and animals, also those mentioned on the WHO list of antibiotic-resistant priority pathogens. In the first part, known and used methods for manure treatment, like thermophilic composting and digestion, are described. In the second part, established methods of wastewater treatment (anaerobic-aerobic bioreactors, constructed wetlands, coagulation, membrane filtration and disinfection processes) as well as those tested only in a laboratory or small scale requiring further investigation (nanomaterials and biochar). The chapter concludes by highlighting the importance to develop effective treatment methods, management strategies and prevention activities, to eliminate or reduce the risk of the release of antibiotics, ARGs and ARB to the environment from manure and wastewater.

Keywords Antimicrobial resistance · Antibiotic resistance bacteria · Antibiotic resistance genes · Manure treatment methods · Wastewater treatment strategies

P. Krzemiński
Section of Environmental Chemistry and Technology, Norwegian Institute for Water Research (NIVA), Oslo, Norway
e-mail: pawel.krzeminski@niva.no

M. Popowska (✉)
Institute of Microbiology, Faculty of Biology, University of Warsaw, Warsaw, Poland
e-mail: magdapop@biol.uw.edu.pl

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19.1 Introduction

The knowledge about entry routes of antibiotics and antimicrobial resistance in the environment and the associated risks to humans and animals demonstrates an important, difficult yet urgent problem to be solved (Chaps. 1, 6 and 14). Many of the currently applied treatment methods applied for treatment of wastewaters and manure have been proven to have limited effectiveness in the elimination of antibiotics, ARB and ARGs. Therefore, it is extremely important to develop effective treatment strategies, especially for manure and municipal sewage, to eliminate or at least reduce the risk of the release of antibiotics, ARG and ARB to the environment. It is in these environments, referred to as hot spots, that the highest concentrations of antibiotics belonging to all known groups are detected, as well as bacteria from the list of antibiotic-resistant priority pathogens published by the WHO.

The following sections present current knowledge on selected treatment strategies applied for the treatment of manure and wastewater with regard to their removal of ARB and ARGs. Some of these treatment methods are already established and used in full-scale applications like anaerobic-aerobic bioreactors, constructed wetlands, coagulation, membrane filtration or disinfection processes (for wastewater), and thermophilic composting or anaerobic digestion (for manure). Others are either emerging or have only been tested in a laboratory or on a small scale such as use of nanomaterials and biochar.

19.2 Treatment Strategies

19.2.1 Manure Treatment Strategies

Animal manures are usually a mixture of faeces, urine, discarded bedding, and waste feed but with variable water content. Therefore, some manure treatment technologies can be more suitable than others to handle manure depending on if they are in solid, semi-solid, slurry, or liquid forms. The treatment technologies of manure are applied for different reasons: emissions reduction (including bioaerosols, NH3, odours); to reduce nutrients prior to soil, like nitrogen (N) and phosphorus (P); in order to liquefy and unify the form of manure and volume reduction and energy recovery; to get rid of pathogenic bacteria and ultimately antibiotics and other drugs or chemical compounds that may be a contamination of manure. Thermophilic composting and anaerobic digestion, which can facilitate the degradation of antibiotics and the reduction of ARG in animal manures (Pruden et al. 2013; Szogi et al. 2015; Youngquist et al. 2016), are discussed.

19.2.1.1 Thermophilic Composting of Manure

There are a lot of interest in composting manures for several reasons: to reduce bulk, concentrate nutrients, reduce odour, kill pathogens and weed seeds, and have a stabilized product for transport to the fields providing a source of slow release nutrients (Westerman and Bicudo 2005). There are several methods for composting manures: passive composting, aerated composting, windrow composting. in-vessel composting and vermicomposting. Those interested, I refer to the relevant, professional literature devoted to this. In addition, composting manure has also been shown to degrade antibiotics effectively, thus limiting the transfer of antibiotics to cultivated soil after its application (Kim et al. 2012; Selvam et al. 2012a, b). Literature data indicated that composting eliminates on average 50–70% of some antibiotics. In Sharma et al. (2009) was investigated how the composting process during 18 weeks will affect the reduction of total *E. coli*, *E. coli* resistant to ampicillin and tetracycline; and selection tetracycline (tet) and erythromycin resistance methylase (erm) genes. The compost windrows were prepared using manure collected from cattle (*Bos Taurus L.*) fed tylosin, chlortetracycline-sulphamethazine, and from control cattle (no antimicrobials). It was observed that just after two weeks (where the temperature was still relatively low—55 °C) composting reduced high initial levels of total *E.coli* and *E. coli* resistant to ampicillin and tetracycline. The tet and erm genes significantly decreased after 18 weeks of composting process (Sharma et al. 2009). In the other study was determined the effect of adding chlortetracycline, tylosin, and monensin to horse manure on distribution ARG. The manures, with and without antibiotics, were subjected to high-intensity management (sample 1) by composting or low-intensity management (sample 2). The samples were monitored for antibiotic concentrations and levels of tetracycline ARG [tet(W) and tet(O)] using quantitative real-time polymerase chain reaction. All three antibiotics dissipated more rapidly in sample 1 of manure, with twice shorter half-lives compared to sample 2 of manure (Storteboom et al. 2007). It was also shown that composting of pig manure resulted in decrease of cultivated aerobic heterotrophic erythromycin-resistant bacteria and tetracycline-resistant bacteria by more than 4 and 7 logs, respectively. Among six classes each of erm and tet genes quantified by class-specific real-time PCR assays, the abundance of erm(A), erm(C), erm(F), erm(T), erm(X), tet(G), tet(M), tet(O), tet(T), and tet(W) declined marginally during the first 17 days, but then within 31 days of the composting treatment dramatically decreased (Wang et al. 2012). Analysis of these results indicates that, antibiotic degradation to mainly occur only during the thermophilic phase over the first 2 weeks, and efficiency of degradation process depends on both duration and temperature. All treatments used during composting as watering, aeration, and turning significantly accelerate antibiotic degradation (Storteboom et al. 2007). The assessment of composting effectiveness is very difficult due to the lack of large-scale research, and not only on a laboratory scale. Only one work shows the reduction of the amount of antibiotic resistance genes (ARGs) in three types of animal (bovine, chicken, and pig) manure after industrial composting (Qian et al. 2018). The authors of this work explain this result suggesting that different animal species

had significant effects on the diversity, abundance, and persistence of ARGs, and hence these differences. Other study on composting showed that the manure composting process is more effective with addition of mushroom biochar compared to addition of rice straw biochar (Cui et al. 2016). In this work, tetracycline (tetA, tetB, tetL, tetM, tetW, tetQ, tetO and tetX), sulfonamide (sul1 and sul2), chloramphenicol (fexA, floR, cmlA, cfr and fexB) resistance genes, and integrase gene (intI1) were studied in lab-scale chicken manure composting test. The average removal rate of ARGs was 0.86 log units and was dependent on the biochar used and the presence of heavy metals. In the other research work, Guo et al. (2017) have analysed the effects of superabsorbent polymers (15 mg/kg) on the abundances of antibiotic resistance genes, mobile genetic elements, and the bacterial community during swine manure composting. After 35 days of composting, the abundances of ARGs and MGEs decreased to a different extent, and were more efficient in removing tetW, dfrA7, ermX, aac(6¢)-ib-cr and MGEs which exceeded 90% (Guo et al. 2017). Until now, research has also shown that there are ARBs and ARGs that even become persistent after composting (McKinney et al. 2010; Sharma et al. 2009), and ARGs can persist even in the absence of selection pressure (Johnsen et al. 2011).

19.2.1.2 Digestion of Manure

A new field of application for animal manure is in biogas plants. This method is not widely used but has a large development potential due to the possibility of production environmentally friendly energy (Nkoa 2014). As demonstrated in the studies of Mohring et al. (2009) such antibiotics as sulphadiazine, sulphamerazine, sulphamethoxazole, sulphadimethoxine, and trimethoprim were nearly completely eliminated during a 5-week fermentation process while sulphathiazole, sulphamethazine, and sulphamethoxypyridazine showed persistence. Noteworthy, sulphonamides and tetracyclines are frequently used veterinary pharmaceuticals in animal husbandry. Yet, despite the risk of their reintroduction in the environment, fermentation residues are often used as fertilizers on agricultural fields. Thus, the effective fermentation process may be an efficient way to reduce the load of selected veterinary antibiotics preventing their way into the environment. However, comparative studies indicate that the aerobic process removed some of the antibiotics (e.g. sulphamethoxazole and oxytetracycline) more effectively than anaerobic incubation of dairy lagoon water or composting process (Pei et al. 2007; Wang et al. 2012).

19.2.2 Wastewater Treatment Strategies

A proper treatment of wastewater is essential before its discharge into natural water reservoirs (e.g. rivers, lake) or before the water is reused. Choosing and applying the right treatment strategies allows to prevent the spread of ARB and ARGs into the environment. An analysis of numerous publications indicates that the high amount

of antibiotics, ARB and ARGs are released into the wastewater that may promote their dissemination into natural environments (Rizzo et al. 2013). Emerging microbial pathogens and increasing antibiotic resistance among emerging microbial pathogens is a global public health issue which has been recognized internationally (EU 2011; WHO 2015; UN 2016; EU 2017).

In a wastewater treatment plant (WWTP), many pathogenic bacteria resistant to antibiotics have been identified, including multidrug resistant (MDR) listed on the antibiotic-resistant "priority pathogens list" (Table 1.2, Chap. 1). The treatment methods applied to prevent the spread of ARB and ARGs into the environment should be able to destroy DNA and inactivate pathogens and the other ARB in the sewage. The selected methods like biological treatment reactors, constructed wetlands, membrane filtration, coagulation, biochar or nanomaterials, with a potential to limit a spread of AMR, are further discussed in this section.

19.2.2.1 Biological Treatment Reactors

Anaerobic–aerobic sequence (AAS) bioreactors combine anaerobic pre-treatment with aerobic post-treatment of anaerobic effluent. Like other anaerobic and aerobic bioreactors, AAS are low energy and environmentally friendly strategies and have a potential to remove ARB and ARGs. Fate of ARG in anaerobic, aerobic and AAS bioreactors was studied with metagenomic approaches (Christgen et al. 2015). For 6 months, five reactor configurations were monitored for treatment performance, energy use, and ARG abundance and diversity. The obtained results showed that AAS was more efficient compared with aerobic and anaerobic units. ARGs reductions were achieved in over 85% in AAS compared to 83% in aerobic and 62% in anaerobic conditions. In the other WWTP with the anaerobic/anoxic/aerobic membrane bioreactor (MBR) process the variation of ARGs [tet(G), tet(W), tet(X), sul(1), and intI(1)] in the influent and effluent of each treatment unit has been evaluated (Du et al. 2014). The results of these studies allow to conclude that anaerobic and anoxic conditions are more suitable to remove ARGs compared to aerobic conditions. It seems that in anaerobic conditions, the propagation of resistance genes is inhibited because microorganisms have lower bioactivity in these conditions. In another study, occurrence of tetracycline-resistant and sulphonamide-resistant bacteria, as well as three genes [sul(1), tet(W), and tet(O)], was monitored in the effluent of five WWTPs (Munir et al. 2011). The ARGs and ARB removal level was highest in MBR (from 2.6-log to 7.1-log) compared with activated sludge, oxidative ditch and rotatory biological contactors (from 2.4-log to 4.6-log). For comparison, in conventional WWTPs, the number of bacteria in the effluent decreases compared to the influent but there is still a significant number of ARBs whose resistance profile changes. An example may be the work on the level analysis of resistance to beta-lactams of *Aeromonas* spp. (Piotrowska et al. 2017). Five of the β-lactamases families (blaTEM, blaOXA, blaFOX, blaV EB, and cphA) were identified in all three isolation sites (influent, activated sludge and effluent). Most of the tested strains had a MDR phenotype (68%) and 62% of the isolates from all three points

of the WWTP carry plasmids and some of them coding blaFOX-4-like and blaGES genes. These results strongly suggest that WWTPs are hotspots of ARB and ARGs dissemination. Additionally, *Aeromonas* spp. are referred to as important vectors of ARGs in the environment (Berendonk et al. 2015; Piotrowska and Popowska 2014). The metagenomic study was also proven co-occurrence of ARGs and human bacterial pathogens in municipal sewage sludge digesters and poor performance in their treatment (Ju et al. 2016). In result of 323 ARGs and 83 human bacterial pathogens studied, it was found that most ARGs and a minor proportion of HBPs (mainly *Collinsella aerofaciens*, *Streptococcus salivarius* and *Gordonia bronchialis*) could not be removed by anaerobic digestion. It was also shown that ARGs of multidrug and macrolide-lincosamide-streptogramin tended to co-occur more with human bacterial pathogens. The metagenomic sequencing approach was applied also by Guo et al. (2017) to determine the occurrence, abundance and diversity of ARGs and MGEs in a full-scale WWTP treating domestic wastewater. The activated and digested sludge were a source of ARGs and different MGEs including plasmids, transposons, integrons (intI1) and insertion sequences (e.g. ISSsp4, ISMsa21 and ISMba16) responsible for horizontal transfer of resistance genes. The findings also corroborate the hypothesis that WWTPs are hotspots of ARGs and MGEs. It evidently points to biological risk of post-digestion sludge in disseminating antibiotic resistance and pathogenicity.

These studies strongly indicate that the use of only biological treatment in WWTP is not sufficient to limit the flow of ARB to the environment. However, when biological treatment is applied together in combination with membrane-based technologies (e.g. MBR), better ARG removal efficiency can be achieved.

19.2.2.2 The Man-Made Wetlands

Constructed Wetlands (CWs) are small semi-aquatic ecosystems that use natural processes, and which can be used as alternative approaches for treatment of municipal, industrial and agricultural wastewaters. In man-made systems, these wetlands are artificially created and are typically long, narrow trenches or channels. CWs have been used as a green technology to treat various wastewaters and offer a low-energy, and less-operational-requirements alternative to conventional treatment systems but are land-intensive as large areas are required (Wu et al. 2014, 2015a, b).

CWs are simple, cost efficient, and provide reduction of biochemical oxygen demand (BOD), suspended solids, nitrogen, metals, pathogens and some of the contaminants of emerging concern such as ARGs (Krzeminski et al. 2019). The following characteristics are important for ARB and ARGs removal efficiency: plant species, flow configuration and flow types including surface, horizontal subsurface and vertical subsurface flow. In this treatment solution, processes such as biodegradation, sedimentation, chemical precipitation and adsorption, and microbial interactions with BOD, solids, and nitrogen as well as plant uptake are responsible for decreasing the loadings of pollutants like nutrients, antibiotics, and ARGs (Chen et al. 2016; Wang et al. 2017). However, studies conducted during the winter and

summer in the wetland construction steadily operated over 10 years have shown removal of total targeted ARGs (78% and 60% in the winter and summer, respectively, but was observed that the concentrations of ARGs (sul1, sul3, tetA, tetC, tetE, and qnrS) were increased throughout the treatment process. In this work strong positive correlations between concentrations of intI1 and ARGs were also shown. This suggests that long-used wetlands can be reservoirs of specific ARGs and that mobile genetic elements affect the dissemination of ARGs in this system (Fang et al. 2017). Chan and other researchers in 2016 studied on the elimination of ARB and ARGs in differently constructed wetland and found that removal efficiencies of total antibiotics ranged from 78 to 99%, while those of total ARGs fluctuated between 64% and 84%. It was also shown that the presence of plants was beneficial to the removal of pollutants, and the subsurface flow constructed wetland had higher pollutant removal than the surface flow constructed wetlands.

19.2.2.3 Membrane Filtration

Membrane filtration processes include microfiltration (MF), ultrafiltration (UF), nanofiltration (NF) and reverse osmosis (RO). During membrane filtration a portion of water known as permeate passes through the membrane, while the constituents larger than the membrane pores are rejected by the membrane generating a concentrated stream containing the separated salts and other pollutants. Membranes are classified according to their pore sizes and molecular weight cut-off (MWCO).

The removal of ARB is expected to be comparable to removal of bacteria not containing antibiotic resistance. This has been verified by investigating the efficiency to remove antibiotic-resistant *E. coli* from WWTP effluents and demonstrating, as expected, complete removal of viable *E. coli* below the limit of quantification (10 CFU/mL) of the plating method (Schwermer et al. 2018). MF and UF are both capable of total removal of protozoa and effective removal of bacteria with up to 4 log removals for UF (Hai et al. 2014; Pecson et al. 2017). Due to even smaller pore size, NF and RO are considered to be absolute barriers for bacteria as long as the membrane is intact (Gerba et al. 2018).

Regarding the effectiveness against ARGs, only limited data exists in the literature on the performance and effects of the membrane filtration processes (Gwenzi et al. 2018). Most of the studies focused on MBRs (Munir et al. 2011; Rizzo et al. 2013; Yang et al. 2013; Du et al. 2014; Wang et al. 2015; Zhang et al. 2015; Sun et al. 2016; Threedeach et al. 2016; Le et al. 2018; Zhu et al. 2018b), which combine biological treatment with membrane separation. Only few others have investigated MF or UF (Arkhangelsky et al. 2008, 2011; Riquelme Breazeal et al. 2013; Krzeminski et al. 2018; Slipko et al. 2018).

Riquelme Breazeal et al. (2013) demonstrated in a lab-scale UF system a reduction of *vanA* and bla_{TEM} ARGs from WWTP effluent by 0.9, 3.5 and 4.2 log for membranes with MWCO of 100, 10 and 1 kDa, respectively. The removal of plasmid-associated ARGs was attributed to membrane retention and the removal of DNA was improved by colloids present in the water. The impact of colloids was

stronger at lower membrane pore size. Riquelme Breazeal et al. (2013) observed incomplete removal of plasmid, including also 1 kDa membrane, and pointed out that the effective size of DNA is smaller than predicted by molecular weight because DNA is a long, thin and flexible molecule.

In another lab-scale study with a dead-end system, authors focused on penetration of plasmid DNA through UF membranes (Arkhangelsky et al. 2008, 2011). The authors demonstrated that despite electrostatic repulsion and significant size difference between membrane pore sizes and plasmid, a circular double-stranded DNA was able to pass through the UF membrane with 20 kDa MWCO. They have speculated that at pressure exceeding 2–3 bars, under which UF, NF and RO membranes are typically operated, the DNA plasmid may be stretched allowing to penetrate through membrane pores. Although penetration mechanism is not yet fully verified, the stretching out of plasmid due to hydrodynamic pressure into long and flexible strands increases the plasmid penetration capability through the pores; the mechanism is in line with the findings of others (Thompson and Travers 2004; Marko et al. 2011). Transportation levels are supposed to be linearly correlated to transmembrane pressure (TMP).

Recently, more attention has been given to high-pressure membrane filtration such as NF and RO (Krzeminski et al. 2018; Slipko et al. 2018). Between 5.0–8.1 log reduction value (LRV) for NF and 5.3–9.5 LRV for RO were reported for ARGs removal from swine WWTP effluent (Lan et al. 2019). However, the LRVs were not calculated for NF or RO alone but for the whole treatment train of the WWTP. Krzeminski et al. (2018) demonstrated removal from ultrapure water spiked with cell-free DNA containing antibiotic resistance kanamycin and ampicillin genes by nine UF, NF, and RO membranes in a bench-scale system. The plasmid rejection varied between 2 and 7 log removal value (LRV, >99.2%) and was correlated with MWCO of the membranes. A LRV of 2 was observed for a UF membrane with MWCO of 100 kDa, between 3 and 4 LRV for a tight UF (50–1 kDa) and between 5 and 7 LRV for NF and RO membranes with MWCO below 0.4 kDa. Additionally, the membrane concentrate was effectively treated by UV-LED irradiation providing damage and inactivation of ARGs (Krzeminski et al. 2020). Slipko et al. (2018) studied nine MF, UF, NF and RO membranes in the lab-scale system feed with spiked ultrapure water or WWTP effluent. The authors reported up to 99.9% removal of free DNA by NF membrane. Due to a large pore size, the 0.3 μm MF membrane was able to remove only up to 20% of cell-free DNA. In MF and UF, the removal was attributed to size exclusion mechanism, whereas in NF and RO electrostatic repulsion also plays an important role (Slipko et al. 2019).

To conclude, membranes may be effective in reducing the risk of ARB and ARGs release and spreading of antibiotic resistance in the environment commonly providing 99.9% removal, but further investigations to verify if complete removal is achievable are needed. The separation mechanisms and factors impacting removal (i.e., feed composition, membrane properties, operating conditions) as well as concentrate treatment methods need to be addressed. Furthermore, the possible contribution of membrane filtration to conditions that induce the SOS response in bacteria

potentially leading to an increased mutation rate in bacteria should be evaluated (Karkman et al. 2018).

19.2.2.4 Coagulation

Coagulation/flocculation has been extensively used in wastewater treatment and drinking water production for removal of particles, natural organic matter, but also pathogens, heavy metals and phosphorous (Alexander et al. 2012). Through addition of a coagulant agent, electrical charges of small particles are neutralized during coagulation causing particles agglomeration. Flocculation promotes particle collision and growth of flocs, resulting in the formation of larger particles for easier separation from water during sedimentation or filtration (Bratby 2016). Coagulation can remove ARGs through electric-double-layer compression, charge neutralization, adsorption, and/or entrapment (Li et al. 2017; Yuan et al. 2019).

Although there are studies on antibiotics removal by coagulation (Choi et al. 2008; Alexander et al. 2012), there exist very few studies that have focused on ARG removal from wastewater by coagulation. Li et al. (2017) evaluated the potential of the jar-test coagulation for removal of two sulphonamide resistance genes (sulI and sulII), three tetracycline resistance genes (tetO, tetW and tetQ), and the class 1 integron (intI1 gene) from treated wastewater. Coagulation with $FeCl_3$ and polyferric chloride (PFC) resulted in removal of various ARGs between 0.5-log and 3.1-log reductions. Removal of ARGs was dependent on the coagulant type and dose, and was significantly correlated with the removal of dissolved NH_3-N and DOC suggesting that the co-removal of DOC, NH3-N, and ARGs could play a role.

Lee et al. (2017) studied changes in 12 ARGs which confer resistance to tetracycline (tetX, tetM, tetA), sulphonamide (sul1, sul2), macrolide (ermB, ermC), quinolone (qnrD, qnrS) and β–lactam (blaTEM, blaSHV, blaCTX) in two full-scale WWTPs treating municipal and industrial wastewater. Four of the ARGs (ermC, qnrS, blaSHV, and blaCTX) were not detected in the samples. Regarding coagulation process, contrasting results were observed between two WWTPs using polyaluminium chloride (PAC) as a coagulant. At one, tet, sul and bla ARGs were reduced by 48%, 75%, and 44%, respectively. At second WWTP, tet decreased by 76% whereas sul and bla increased by 36% and 152%, respectively. The difference in the reduction of ARGs was attributed to larger usage of coagulant at the plant with higher effectiveness.

Yuan et al. (2019) studied fate of five ARGs (sulI, sulII, tetO, tetQ, tetW) and class 1 integrase (intI1) in a full-scale WWTP treating municipal and industrial wastewater. Polyferric chloride (30–45 mg/L) was used as a coagulant. Coagulation was essential for the removal of the ARGs providing between 0.48-log and 1.86-log in terms of the absolute abundance. Among the five investigated genes, the lowest removal was observed for sulII.

The fate and removal of ARB and ARGs was also studied during drinking water treatment (Guo et al. 2014; Bai et al. 2015; Xu et al. 2016). With regard to ARBs, the antibiotic resistance rates of bacteria did not increase during coagulation and

sedimentation (Bai et al. 2015). With regard to ARGs, Guo et al. (2014) studied removal of ten sulphonamide (sulI, sulII) and tetracycline (tetC, tetG, tetX, tetA, tetB, tetO, tetM, tetW) resistance genes as well as 16S-rRNA genes in seven DWTPs. The relative abundance of ARGs was not changed significantly by coagulation process during drinking water production. The removal of different ARGs at two DWTPs varied, based on absolute abundance, between 0.2 and 0.7 LRV for coagulation/flocculation process and between 0.3 and 1.0 LRV for coagulation/flocculation followed by sedimentation (Xu et al. 2016).

Concluding, coagulations seem to be effective technology for ARG reduction in WWTPs, but studies should be devoted to optimizing coagulation process for enhanced ARG removal. The effectiveness of coagulation for ARG reduction in DWTPs is somewhat smaller and varies between the studies, and thus requires more investigation.

19.2.2.5 Biochar

Biochar is a porous carbon-rich product, which can be produced by pyrolysis of high organic content materials (biomass) such as sludge, algae, and waste from different sources and sectors, e.g. industrial, livestock, agricultural, household and garden. Biochar has been typically applied as a soil amendment to improve soil quality or for carbon sequestration. But, due to strong adsorption capacity, biochar can be also used for organic, inorganic and microbial contaminants removal from water and/or control in soils (Beesley et al. 2011; Gwenzi et al. 2017). Biochar can stabilize heavy metals in the contaminated soils, leading to a significant reduction in crop uptake of heavy metals (by reducing their bioavailability, but also phytotoxicity). It may regulate the concentration of organic pollutants in contaminated soils and consequently may affect other processes such as bioavailability, degradation, leaching, and volatilization of contaminants (Zhang et al. 2013).

However, until now, biochar research focused on AMR was predominantly concentrated on the applications related to soil, manure and solid waste, whereas studies on wastewater are scarce. Sun et al. (2018) assessed the effect of biochar on ARGs when applied to the organic solid produced during anaerobic digestion of wastewater. The authors studied resistance genes of tetracycline (tetA, tetB/P, tetC, tetE, tetG, tetM, tetO, tetQ, tetT, tetW, and tetX), sulphonamide (sul1, sul2 and dfrA7), fluoroquinolone (aac(6′)-Ib-cr, qnrA, parC, qnrC, and qnrS), macrolide (ermB, ermF, ermQ, and ermX), as well as mobile genetic elements (intI1, intI2, ISCR1, and Tn916/1545) in lab-scale anaerobic digestion experiments with cattle manure. The relative abundance was decreased for 5–7 out of 13 ARGs but the results were inconsistent. In another study, the type of wastewater used for irrigation of soil was of importance for the biochar effect. When piggery wastewater was used, after initial decrease, the relative abundance increased again suggesting that the effect of biochar on tet and sul genes in soil was time-dependent (Cui et al. 2018).

Application of biochar to a contaminated soil decreased the uptake of sulphonamides, ARB enrichment and abundance of sulI and sulII genes in lettuce tissues

during lettuce pot experiment (Ye et al. 2016). The ARGs levels, based on absolute abundance, were at least 1-log lower for lettuce tissues (roots and leaves) and between 0.2 and 0.8 log lower in the soil with 0.5% (w/w) biochar amendment. Furthermore, biochar was also found effective in controlling soil antibiotics, ARB, and ARGs disseminate to the edible part of potato (Jiao et al. 2018). In a study with ARGs of tetracycline (tetC, tetG, tetW, and tetX), sulfonamide (sul1 and sul2), and macrolide (ermF and ermX), ARGs levels were decreased in soil and lettuce tissues after biochar application in the lettuce pot experiments (Duan et al. 2017). The relative abundances of ARGs were reduced by 44%, 43%, and 52% in soil, roots, and lettuce leaves, respectively. Bacterial community influenced the variations in ARGs and intI1. However, due to high abundance of intI1 in soil and lettuce tissue, the spread of ARGs via horizontal gene transfer cannot be excluded. According to another pot experiment, the absolute abundance of ARGs decreased in non-planted soil after biochar application. Yet, biochar alone was reported to be insufficient to decrease ARGs level in planted soil and crops (Chen et al. 2018). Authors suggested maintaining or increasing diversity of bacterial community in soil as possibly more effective measure in mitigating ARG spread and accumulation. In addition, biochar weakened the effect caused by struvite application on intI1 ARG cassettes in soil, indicating biochar capability to mitigate the spread of resistance determinants from soils to vegetables (An et al. 2018).

Biochar was also reported to help adsorb heavy metals, reduce their availability, and subsequently contribute to reduce the selective pressure on ARB (Ezzariai et al. 2018). Therefore, biochar seems to be an effective amendment for reducing the abundances of antibiotics, ARB, and ARGs in soils. But large-scale trials are required to verify lab-scale findings before implementation. Furthermore, as reversible adsorption can occur, the desorption of contaminants adsorbed onto biochar should be carefully investigated (Safaei Khorram et al. 2016). Nevertheless, the clear attractiveness of biochar is in the possibility of using low-cost waste materials to minimize the bioavailability of ARB and ARGs for plant uptake and reduce the transfer of ARB and ARGs from the soil to plant (Piña et al. 2018). Consequently, by reducing uptake of ARB and ARGs by irrigated crops, biochar may help in restricting entry into the food chain.

19.2.2.6 Nanomaterials

Due to physical, chemical, and biological properties, nanomaterials are increasingly being used, or considered to be used, not only in medical but also in different water treatment applications. Nanotechnology and water treatment have attracted considerable attention of the research community. Among other topics, one of the frequently researched aspects is nanoparticles (NPs) with antibacterial properties. Until now, a vast number of inorganic, organic and hybrid NPs have been frequently proposed: silver (Ag), gold (Au), iron oxide (Fe_3O_4), titanium oxide (TiO_2), copper oxide (CuO), magnesium oxide (MgO), zinc oxide (ZnO), nitric oxide (NO) releasing NPs, chitosan, fullerenes, carbon nanotubes (CNTs), graphene oxide (GO),

reduced graphene (rGO), Polyethylenimine (PEI), quaternary ammonium compounds and nanoemulsion (Li et al. 2008; Hajipour et al. 2012; Moritz and Geszke-Moritz 2013; Yousefi et al. 2017). In addition, composites of NPs have been proposed to utilize a synergistic effect of different NPs. For example, superparamagnetic iron oxide NPs with conjugation of iron, zinc, and silver was effective inhibitor of antibiotic-resistant biofilms formation (Taylor et al. 2012).

Addition of silver nanoparticles (Ag NPs) to anaerobic digester treating a mixture of WWTP primary sludge and wasted activated sludge has not decreased the absolute or relative copy numbers of ARGs of tetracycline (tetO, tetW), sulphonamide (sulI, sulII) and intI1 (Miller et al. 2013). The anaerobic digestion itself reduces (1–2 log) but does not provide complete removal of ARGs. Thermophilic anaerobic digestions have been more effective towards reduction of tetracycline genes and digester operating conditions influence bacterial community composition and prevalence of ARGs.

Graphene, a two-dimensional carbon nanomaterial, has been intensively studied in the last years and GO has been considered to be an effective adsorbent (Liu 2012) and carrier for genes (Feng et al. 2011a, b). However, GO alone had a limited effect on ARB inactivation and on levels of tetracycline (tetA) and kanamycin (aphA) resistance genes (Guo and Zhang 2017). Only under high GO concentrations (>10 mg/L) relative abundance decreased suggesting that resistant plasmid damage was possible. In addition, GO promoted the conjugative transfer of ARGs which was GO concentration dependant.

On the other hand, GO nanosheets showed high efficiency in removal of four ARGs (tetA, sul2, ermB and ampC) in a cyclic (c-DNA) and double-stranded (ds-DNA) form, when applied for removal of ARGs spiked to a river water (Yu et al. 2017). For the cyclic DNA, the LRV were 1.2, 1.6, 1.7, and 2.0 for c-tetA, c-sul2, c-ermB, and c-ampC, respectively. For the double-stranded DNA, the removal rates were generally higher and the LRV were 2.1, 0.7, 2.5 and 2.3 for ds-tetA, ds-sul2, ds-ermB and ds-ampC, respectively. After 5 regeneration cycles of GO nanosheets, the removal ability of c-ARGs and ds-ARGs decreased less than 40%.

Combination of gold, graphene oxide and cobalt oxide hollow sphere (Au/GO-Co3O4) acted as inhibitor for tetracycline-resistant genes (tetA) limiting its replication/damaging its bioactivities (Yu et al. 2018). Au/GO-Co3O4 showed excellent binding effect towards tetA. The short (210 bp) DNA fragments were easier removed than the long (bacteria content after cracking) DNA fragments. The removal depended on composite concentration and was in range of 1.5–4.6 log for short DNA fragments, and in range of 1–3.6 log for long DNA fragments. The authors concluded that damage of tet-ARGs in water was due to combined effects of the composites properties, released ions from Au/GO-Co3O4, and coated gold NPs.

Yousefi et al. (2017) reviewed application of GO nanoparticles and its nanohybrids as antimicrobial agent. The authors concluded high antibacterial efficiency of GO due to its capability to damage cell membranes. Nevertheless, to increase the antibacterial effects of graphene oxide, functionalization of graphene surface was pointed out for future research. Another promising field are NPs in combination

with other processes such as membranes (Liu et al. 2016; Ying et al. 2017; Zhu et al. 2018a) or photocatalysis (Hwangbo et al. 2019). However, although nanomaterials might be effective against antimicrobial resistance, there is a risk associated with the use of nanomaterials in water treatment (Sharma et al. 2016). Some nanomaterials, such as silver, can have toxic effects on the environment, while others may facilitate the development of antibiotic resistance. The dissemination and propagation of ARGs in aquatic environments may be enhanced, for example, by ZnO (Wang et al. 2018), nanoalumina (Al_2O_3) but also other NPs (Qiu et al. 2012). Therefore, knowledge on the impact of the NPs on potential development of the resistance and the mechanisms of ARGs transfer is needed.

Nanostructured materials (e.g. metallic, organic, carbon nanotubes) possess antimicrobial activity and may circumvent existing drug resistance mechanisms in bacteria. Additionally, due to the specific mechanism of action of nanoparticles, no resistance was observed among bacteria. In addition to their antimicrobial potential, nanoparticles may inhibit biofilm formation and other processes in bacterial cell. The studies showed that nanoparticles can inhibit the activity of bacterial efflux pumps, formation of biofilms, and interference of quorum sensing, which confirms the possibility of their use in the strategies to combat MDR bacteria (AlMatar et al. 2017; Baptista et al. 2018; Barancheshme and Munir 2018; Bassegoda et al. 2018; Katva et al. 2018; Siddiqi et al. 2018; Zaidi et al. 2017). Aruguete et al. (2013) prepared a combination of nanomaterials with antibiotics and proved they are toxic to MDR *Pseudomonas aeruginosa* strain. In many other studies, the activity of various nanoparticles on ARB in an aqueous solution was shown, e.g. methicillin or vancomycin or multidrug-resistant *Staphylococcus. aureus*; antibiotic-resistance *E. coli*, *Salmonella* spp., *Enterococcus faecalis*, *S. epidermidis*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Mycobacterium tuberculosis*, *Acinetobacter baumanii* and MDR *Streptococcus pneumoniae* (Adegboyega et al. 2014; Luo et al. 2013; Fayaz et al. 2011; Singh et al. 2014; Taylor et al. 2012; Tran et al. 2010; Qiu et al. 2012). On the other hand, as pointed out earlier, some of the nanomaterials have shown toxic effects on the proper microflora of water or soil and more importantly on plants, animals, and humans, and can also encourage the development of antibiotic resistance in the environment (Aruguete et al. 2013; Miller et al. 2013). Therefore, before widespread use of nanoparticles or nanomaterials, more information is needed concerning the mechanisms of their antimicrobial activity and their potential for influencing the development of resistance and their toxic effect. In addition, efforts should be made to develop less toxic forms of nanomaterials and innovate with the combined use of plant-based antimicrobials and nanoparticles.

19.3 Summary and Perspectives

Due to the ubiquitous antibiotic resistance in the environment, strategies for manure, wastewater, water and soil treatment that could aid in mitigating risks of dissemination and development of antimicrobial resistance in the environment are necessary.

Despite the treatment methods mentioned in this chapter and the mechanisms governing these methods are generally well-known, there are still gaps in knowledge. For example, the dominant removal mechanism governing the removal of ARGs are not yet fully explored. In particularly, the diversification of methodologies used for screening and quantification of ARGs makes the comparison of effectiveness of different treatment solutions difficult. Thus, there is an urgent need for standardization and uniformization of AMR surveillance. In addition, many of the treatment methods have been investigated in a lab- or pilot-scale, using synthetic wastewater, and/or over short operation time. Therefore, there is limited full-scale evidence on removal of ARB and ARGs and effectiveness against AMR. Moreover, altough different advanced treatment methods are being developed and proposed for ARB and ARGs removal single treatment method is likely not going to be sufficient (Rizzo et al. 2020). Due to the costs, the optimal method should be not only effective but also economical. To confirm this, it is necessary to conduct large-scale testing under real environmental conditions. Especially in the case of WWTP, further investigation of advanced treatment systems should be carried out, to discover a suitable and cost-effective method to remove ARGs and ARB from WWTP effluents.

The further development of innovative treatment approaches should be parallel to the management strategies aiming at reduced use of antimicrobials and prevention activities related to improved sanitation and access to clean water.

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Chapter 20
Bio-management of Antibiotics

Saadia Andleeb, Sumbal Sardar, and Mahnoor Majid

Abstract Antibiotic resistance is a major threat to the survival of mankind, and it is being consistently rising every year. The use of unregulated antimicrobials in the aquaculture, livestock, and agriculture industries has played a major role in its rampant dissemination. It has become crucial to devise effective strategies to manage and promote the optimized use of antibiotics so as to save them from becoming completely useless. This chapter focuses on the various antimicrobial strategic plans being implemented across the globe for the bio-management of antibiotics and discusses few case studies that have shown promising results in controlling the adverse outcomes of antibiotic resistance.

Keywords Antibiotics · Antibiotic resistance · Global action plans · Antimicrobial management · Quality improvement plans

20.1 Bio-management of Antibiotics

Antibiotics are the most integral therapeutics which have been compromised lately due to the emergence of antibiotic resistance. Consequently, the most important pathogens are acquiring the resistance on daily basis due to the polluted environments, which in return hold the responsibility of disseminating resistant genes in associated surroundings (Walsh 2003). This chapter will mainly focus on the management of antibiotics and how they can be saved from further aggravating the rampant spread of the resistant genes (ARGs).

S. Andleeb (✉) · S. Sardar · M. Majid
Atta-ur-Rahman School of Applied Biosciences, National University of Sciences and Technology, Islamabad, Pakistan

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20.2 Optimization of the Use of Antibiotics

The application of antibiotics in the manure and livestock has played a significant role in the rise of antibiotic resistance which have rendered many antibiotics useless. Therefore, it is time to handle this case seriously as according to a recent study, almost 70% of the antibiotics that are prescribed for various disease are given to livestock as well (Criticism for WHO guidance on using antimicrobials in food-producing animals, Anon 2017). Due to the unregulated use of antibiotics in the livestock and agricultural sector, ARGs are consistently evolving and disseminating among the humans and animals (Wu et al. 2010). The first and most direct control that can be employed to manage the resistance is to minimize the use of those antibiotics which are used for the treatment of critical clinical diseases. Secondly, the use of growth promoters in the animals also assist in the spread of ARGs which should be managed on urgent basis. Denmark is one of the very few countries that was able to reverse the effects of antibiotics where in 1990, the growth promoters were banned for their use in animal feed. Later on, due to the decrease in the application of subtherapeutic antibiotics, there was a prominent decrement in the antibiotic resistance which set an example that the antibiotic resistance can be reversed by controlling the feed among the animal population (Aarestrup et al. 2001).

20.3 Bio-management Strategies

Reduction of antibiotics and antibiotic resistance entry in the environment can be considered by opting different bio-management options such as:

20.3.1 Confining Agriculture Sources

Large proportion of antibiotics are used in agricultural sources. WHO recommends that the use of antibiotics should be restricted in healthy animals in order to reduce the dissemination of antibiotic resistance because overuse of antibiotics contributes to the spreading of resistance among animals and humans (World Health Organization 2017). Analysis from one of the studies shows that limiting antibiotic use in food-producing animals highly contributes to the reduction in antibiotic resistance (Tang et al. 2017). Antibiotics used in livestock for nontherapeutic purposes for long period of time is observed as main route for the spread of antibiotic resistance (Manyi et al. 2018). Strategies must be followed to reduce nonmedical and therapeutic use of antibiotics by improving nutrition, hygienic conditions, and waste management which can result in improved immunity and lesser number of infections (Manyi et al. 2018).

20.3.2 Management of Aquaculture

Different antibiotics are commonly used in aquaculture for economic purposes, such as quinolones, tetracycline, and sulfonamide (Smith 2008). One of the studies estimates that about 150 pounds of antibiotics are used per acre in the United States for salmon harvesting. As a result of these antibiotics, resistant bacteria present in the aquaculture directly interacts with the environment (Serrano 2005). And if these antimicrobial agents occur for long period of time, they can provide excellent conditions for the development of antimicrobial resistance genes (AMR) and bacteria by horizontal gene transfer. Studies suggest that large number of mobile elements including integrons, plasmids, and transposons present in the aquatic bacteria can easily pass to new environments and results in the development of different combinations of antibiotic resistance genes (ARGs) and mobile elements (Santos and Ramos 2018). Thus, strict measurements and regulatory laws should be followed to optimize the use of antibiotics in aquacultures which shall limit antibiotic resistance level.

20.3.3 Waste Water Treatment

Waste water acts as a main reservoir of antibiotic resistance genes (ARGs) and antibiotic-resistant bacteria. It is because of ideal conditions provided by the waste water pool such as presence of large number of nutrients, stable temperature, and bearable pH (Manaia et al. 2018). These waste water treatment plants act as a junction between different environments and thus provide a path for the transfer of mobile elements such as ARGs (Schmieder and Edwards 2012).

Waste water reuse is important for water sustainability, but care should be taken for better evaluation of water after treatment, to avoid antibiotic resistance and other unintentional consequences. Different strategies to be followed to combat antimicrobial resistance coming from wastewater such as use of aerobic and anaerobic water treatments plants are considered good for removing antibiotics and resistant bacteria (Barancheshme and Munir 2018). Secondly, nowadays nanotechnology applications are widely used for waste water treatments because nanomaterials have the potential to trap antibiotics and resistant bacteria (Aruguete et al. 2013).

20.4 Implementation of Strategies

Implementation needs to be strengthened. Routine programs are required to make availability of all the data related to antibiotics and antibiotic resistance. Monitoring system should also be introduced that provide baseline for different strategies such as Global Action Plan (GAP) on AMR and its implementation. This system should

also make sure the availability of results of the effects of GAP on antimicrobial resistance and health.

20.4.1 Antimicrobial Management Programs

Reducing antimicrobial resistance relies on institutional programs. To ensure the reduction of antimicrobial resistance, antimicrobial resistance programs have two major objectives. One is to ensure the use of appropriate and adequate antimicrobial agents and second is to make it possible that antibiotics are not wasted. Some antibiotic management programs are based on education while others follow "front-end approach," and a "back-end approach," or a combination of both of the practices (Paterson 2006).

20.4.2 Antimicrobial Stewardship Program

Education is the primitive tool that serves as a mean to reduce antimicrobial resistance. The purpose is to develop programs for educating the physicians and clinicians about the proper use of antibiotics, antibiotic resistance, and consequences of resistance.

Antimicrobial stewardship program is a set of coordinated programs that supports the appropriate uses of antibiotics, enhances patient consequences, lowers antimicrobial resistance, and reduces the number of infections caused by multidrug-resistant pathogens (Fishman 2006).

Summary of Core Elements of Hospital Antibiotic Stewardship Programs (Pollack and Srinivasan 2014).

- Leadership Commitment: Dedicating necessary human, financial, and information technology resources.
- Accountability: To improve outcomes of program, appoint one leader. It is effective to appoint physician as a leader as it is confirmed from the experience of successful programs.
- Drug Expertise: In order to improve antibiotic use, appoint one pharmacist leader.
- Action: Implementing at least one recommended action, such as systemic evaluation of ongoing treatment need after a set period of initial treatment (i.e., "antibiotic time out" after 48 h).
- Tracking: Continuously monitoring of antibiotics use, prescribing, and resistance development patterns.
- Reporting: Regular reporting information on the use of antibiotic and resistance development to doctors, nurses, and relevant staff.
- Education: To educate and aware clinicians about the appropriate prescribing of antibiotics and resistance development.

20.4.2.1 Use of Front-End Approach for Reduction of Antibiotic Resistance

This approach is also known as pre-prescription approach. In this approach, certain antimicrobial use requires permission of authorities for using it because it is considered restricted. If a clinician wants to order the particular antimicrobials, they must need approval from the designated authority. Antimicrobials can be approved for specific duration for which cultural data shall be obtained (Doron and Davidson 2011; Fishman 2006). Front-end approach targets those antimicrobials which are overused or misused. It ensures the use of antimicrobial based on formulatory data provided by the hospital.

20.4.2.2 Use of Back-End Approach for Reduction of Antimicrobial Resistance

This approach is also known as post-prescription approach. Antibiotics having broad spectrum are used. Post-prescription reviews and then discontinues the antibiotic therapy on second or third day if not working well and decision is supported by susceptibility testing and patient response (Fishman 2006). The clinician has the recommendation by the antimicrobial stewards, according to which they can persist, change, or quit the therapy after microbiological testing. This approach is based on de-escalation, which means the modification of initial antimicrobial plan based on the available cultural data. De-escalation is an important aspect of appropriate use of antibiotics and it includes changing of broad-spectrum antibiotics to narrow spectrum, poly-therapy to one single therapy, and discontinuation of therapy if the drug is no more needed (Doron and Davidson 2011) (Fig. 20.1).

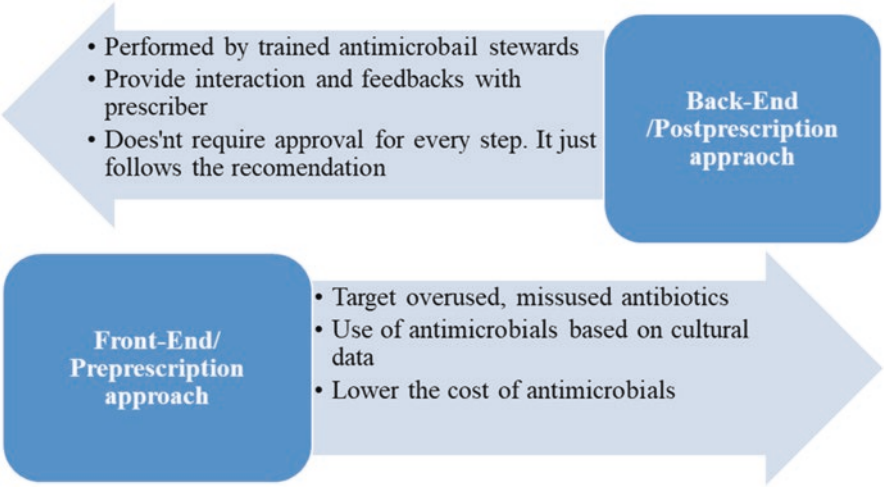

Fig. 20.1 Post-prescription and pre-prescription approaches (Doron and Davidson 2011)

20.5 Response of WHO on the Emergence of Antibiotic Resistance

In order to reduce the misuse and overuse of antibiotics, and to eliminate the antimicrobial resistance, WHO gave global action plan on May 2015. This plan has five important points.

- To provide education about antimicrobial resistance and to develop awareness.
- To enhance scrutiny and research about antimicrobials and resistance.
- To lower the number of infections.
- Optimization of the use of antimicrobial.
- To assure tenable investment for retaliation of antimicrobial resistance.

World antibiotic week is also celebrated every November with the theme "antibiotics, handle with care." Many other initiatives were taken by the WHO to monitor antimicrobial use and antimicrobial resistance (Who.int 2019) (Fig. 20.2).

20.6 Global Antimicrobial Surveillance System

GLASS was launched in 2015 and the basic aim of this program is to extend the research on global surveillance and thus heighten the data about antimicrobial resistance (AMR). The objectives of GLASS are (World Health Organization 2018):

- To investigate the extended burden of AMR by specific indicators.
- To interpret and publish worldwide data on AMR.

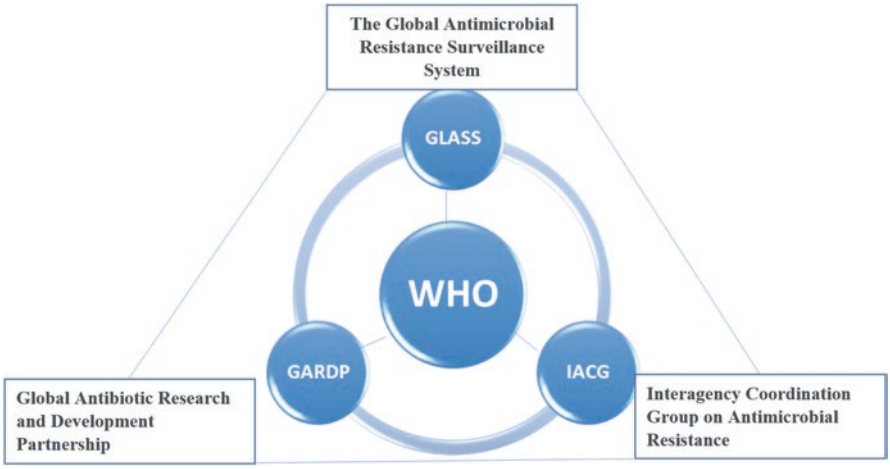

Fig. 20.2 Interlinked programs for appropriate use of antimicrobials and reducing antimicrobial resistance (Who.int 2019)

- To identify new resistance and how it spreads internationally.
- Implementation of control programs.
- To evaluate the impact of new interventions.

20.7 Global Antibiotic Research and Development Partnership

GARDP is a joint program of WHO and Drugs for Neglected Disease *initiative* (DND*i)* established in 2016 and it is a not-for-profit organization. The aim of GARDP is to develop and improve new antibiotics treatment to assure sustainable access. It encourages research and development on new antibiotics in response to antimicrobial resistance (GARDP 2019).

20.8 Interagency Coordination Group on Antimicrobial Resistance (IACG)

IACG was established in 2016 by the Secretary General of United Nations and the aim of this organization is to ensure the appropriate global action plan against the antimicrobial resistance which is a serious threat to health and to establish proper coordination between different international organizations (World Health Organization 2019).

20.9 Example Case Study: A Quality Improvement Program in Tertiary Neonatal Unit in the UK

The subject program was initiated in the year 2016 with the sole objective of minimizing the use of antibiotics without affecting the safety and stability of the neonatal patients in the UK. The program encompassed the arrangement of awareness webinars and meetings, at national and international scale where the participants were educated about the impact of antibiotics and how their overuse can lead to severe consequences. Moreover, posters and training sessions were carried out for general public, healthcare staff, and workers as they play an integral role in the management of the patients. This resulted in a positive response, and a major decline in overuse of antibiotics was observed afterwards. This program can serve as a standard example of plan, do, study, and act (PDSA) which should be implemented across the globe, especially in the developing countries. The need to educate the masses about the grave side effects of antibiotics should be made the major objective of national health programs (Makri et al. 2018).

20.10 Introduction of Computer-Assisted Programs for the Management of Antibiotics

With the recent bloom in the field of artificial intelligence and deep learning, the management of antibiotics and their use have been shifted towards the computer-assistance programs where specialized models are designed to help the physicians in determining the condition of patients and assist them in selecting the right category of antibiotics. Such a model program has been devised by various groups of scientists which aim to shift the intriguing cases on artificial intelligence. In healthcare facilities, such programs are built to gather the patients' information such as their temperature, diagnosis, radiographs, and multiple tests, which are further screened via a decision support logic of the program. This algorithm helps the clinicians in determining the appropriate and personalized set of medicine for individual patient and indicates the need of a new therapy. Moreover, computerized antibiograms are employed to make decisions based on the antibiotic susceptibility profiling. The attractive aspect of such programs is the patient-specific prediction which the algorithm generates after checking the patients and their specific conditions. Such programs have been incorporated in various hospitals particularly in the USA and the UK where automated programs have revolutionized the diagnostics and therapeutics (Evans et al. 1998).

20.11 Biological Control of Antibiotic-Resistant Microorganisms

Apart from implementing the above mentioned management strategies for the effective reduction of antibiotics, it is imperative to search for alternative ways to somehow control the emerging antibiotic-resistant organisms as well. The employment of bacteriophages is highly considered in this regard as they can confer benefits to the host while destroying the pathogenic microbes. Moreover, extracts from plants and animal sources have also proven to be useful in ameliorating the side effects of antibiotic resistance. Natural sources are a gateway for the effective management of antibiotics which need to be exploited more.

20.12 Conclusion

With the emergence of antibiotic resistance across the globe, it has become crucial to look for alternative strategies to manage the existing antibiotics and save them from becoming resistant. Therefore, various antimicrobial management programs based on holistic approaches need to be initiated so as to raise awareness and education among the population for gaining productive outcomes.

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Index

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