Thesis for doctoral degree (Ph.D.)
2021

Integrated assessment of environmental and human health risks of antibiotic residues and resistance for environmental and health policy

Nada Hanna

Karolinska Institutet

From the Department of Global Public Health
Karolinska Institutet, Stockholm, Sweden

Integrated assessment of environmental and human health risks of antibiotic residues and resistance for environmental and health policy

Nada Hanna

Karolinska Institutet

Stockholm, 2021

Cover illustration by Jean Hanna

Published by Karolinska Institutet.
Printed by Universitetsservice US-AB, 2021

ISBN 978-91-8016-167-1

Integrated assessment of environmental and human health risks of antibiotic residues and resistance for environmental and health policy

THESIS FOR DOCTORAL DEGREE (Ph.D.)

By

Nada Hanna

Public defence
Wednesday 19th May 2021 at 10:00
Inghesalen, Widerströmska Huset, Karolinska Institutet, Solna, Sweden

Principal Supervisor:
Professor Cecilia Stålsby Lundborg
Karolinska Institutet
Department of Global Public Health
Health Systems and Policy

Co-supervisor:
Professor Ashok J. Tamhankar
R.D. Gardi Medical college
Department of Environmental Medicine
Karolinska Institutet
Department of Global Public Health
Health Systems and Policy

Opponent:
Professor Alistair B. A. Boxall
University of York
Department of Environment and Geography

Examination Board:
Associate professor Åsa Sjöling
Karolinska Institutet
Department of Microbiology, Tumor and Cell Biology

Professor Björn Wettermark
University of Uppsala
Department of Pharmacy, Social Pharmacy

Doctor Andreas Sandgren
University of Uppsala
Department of Medical Sciences, ReAct

To my family

Environmental factors are key determinants of human health.

- Hippocrates "father of medicine", on Airs, Waters and Places, Circa 400 B.C.

POPULAR SCIENCE SUMMARY OF THE THESIS

We are in the midst of the silent pandemic of antibiotic resistance that continues to grow around the world. If no urgent actions are taken, antibiotic resistance will have a devastating impact. The reduced efficiency of therapeutic antibiotic use can lead to increased hospitalisations for patient infections caused by antibiotic-resistant bacteria, and to treatment failure and mortality. Deaths from antimicrobial-resistant infections will increase from the current 700,000 to 10 million annually by 2050 and cost up to USD 100 trillion. In addition, concerns are growing about the impact of antibiotic resistance on the environment. Antibiotics enter the environment through different sources such as pharmaceutical plants, hospitals, and municipals. Accordingly, antibiotic residues have been detected in the environment such as drinking water, vegetables, river water, ground water, and wastewater treatment plants. Antibiotic residues are likely to lead to the development of antibiotic resistant bacteria in the environment. Further, there is the potential risk of antibiotic residues via drinking water or food consumption disrupting our gut flora, inducing the emergence of resistance, and also posing toxic effects.

Our understanding of the occurrence of antibiotic resistance in the environment, and its potential risk to human health, is limited. This must be further explored to protect human health and the environment. The studies included in this thesis therefore examined the environmental contamination of antibiotic residues and resistance, assessed their risk to the environment and human health in the Western Pacific Region (WPR) and South-East Asia Region (SEAR) of the World Health Organization, particularly China and India, and developed methods for the prioritisation of antibiotics for environmental and health policy.

Antibiotic residues and antibiotic resistance were present in various environmental compartments of the WPR and SEAR, in particular China and India. Antibiotics were observed to pose resistance risk in the wastewater of Shandong province in China. Antibiotic residues and antibiotic resistant bacteria in the water of the Kshipra river of India were associated with different seasons, sites, and water quality parameters over a 3-year period. The concentration levels of some antibiotics were estimated to pose an environmental risk for the development of antibiotic resistance in the environment of the WPR, the SEAR, China, and India. Antibiotics appear to pose a resistance risk to human health from environmental exposure via drinking water. A list of priority antibiotics based on their environmental and human health risks of resistance, toxicity, and ecotoxicity in all types of aquatic environmental compartments was developed. Ciprofloxacin posed the greatest risk. The highest risks of antibiotic residues were observed in wastewater and wastewater treatment plants' influents and effluents. Wastewater and wastewater treatment plants act as a major source and primary pathway for environmental contamination by antibiotic residues and antibiotic resistance in these regions.

The knowledge generated in this thesis can help decision-makers to undertake well-directed actions towards monitoring and mitigating antibiotic residues and antibiotic resistance. Antibiotic residues can be targeted for remediation, wherever there appears to be a high risk of development of resistance within the environments of the WPR, the SEAR, China, and India. The outcome from the proposed approach can be used to develop targeted policies which

would prevent and minimise the environmental and human health risks of antibiotic residues, and to help focus research efforts.

As a whole, the emergence of antibiotic residues and antibiotic resistance in the environment caused by human actions and inactions show how their defined and measurable risks can influence our health and the environment. This has to stop, with us tackling antibiotic resistance with more political commitment and coordinated collaborated efforts.

ABSTRACT

Background: Antibiotic resistance is a global health crisis and a serious threat to progress in achieving the Sustainable Development Goals. This requires a One Health response that recognises the link between human and animal health and the environment. While within One Health, human and animal health feature prominently in terms of research and implementation, the efforts to tackle antibiotic resistance in the environment lag far behind in attention, evidence base, and political commitment. The knowledge of the emergence of antibiotic residues and antibiotic resistance in the environment, and the magnitude of the human health risk posed by it, remain limited. Various anthropogenic sources such as the pharmaceutical industry, hospital, and municipal wastewater release antibiotic residues into the environment. To define the risks to the environment and human health and to inform policy, there is a particular need to assess antibiotic concentrations in the environment at which resistance might develop and pose a health threat.

Aim: To assess the potential environmental and human health risks of exposure to antibiotic residues and antibiotic resistance in various environmental compartments of the Western Pacific Region (WPR) and South-East Asia Region (SEAR) of the World Health Organization, particularly China and India, and to develop methods for the prioritisation of antibiotics to deal with aspects of their resistance and the toxicity risks on human health and various aquatic environmental compartments.

Methods: In Study I, quantitative methods to determine antibiotic concentrations in various environmental compartments in rural Shandong province in China were used, and risk assessment methods to characterise environmental and health risks of antibiotic residues were performed. In study II, quantitative methods to investigate antibiotic residue levels and water quality in the river water samples from the Kshipra river in India during different seasons, and sampling sites over a 3-year period were applied, and microbiological and molecular methods to test antibiotic resistant *Escherichia coli* (*E. coli)* and antibiotic resistance genes were used. In study III, I conducted a systematic review of the literature (WPR (n=218), SEAR (n=22), China (n=168), and India (n=15)) published between 2006 and 2019, to investigate the occurrence and concentration of the reported antibiotic residues in various aquatic environmental compartments of the WPR and SEAR. I also used risk assessment methods including Probabilistic Environmental Hazard Assessments, to assess antibiotic exposures in various aquatic environmental compartments for concentrations of antibiotic residues that are above Predicted No Effect Concentrations for resistance development. In Study IV, I developed an integrated environment–human risk approach for a quantitative environmental (resistance and ecotoxicity) and human health (resistance and toxicity) risk assessment of antibiotic residues and a prioritisation system thereof. I propose a risk-based approach; the approach combines data on the exposure, toxicity, resistance, and chemical structure of antibiotics with Probabilistic Environmental Hazard Assessments and a Threshold of Toxicological Concern. The utility of the approach and the system was demonstrated using data from China as an example. The proposed approach can be used for other settings.

Findings: Antibiotic residues were present in various environmental compartments of the WPR, the SEAR, China, and India (Study I, Study II, and Study III). The concentration levels of enrofloxacin, levofloxacin, and ciprofloxacin in wastewater were estimated to pose environmental risks for the development of antibiotic resistant bacteria in the environment of Shandong province, in China (Study I). Antibiotic resistant *E. coli* were present in the water and sediment of the Kshipra river in India and showed significant seasonal and spatial variations over a 3-year period, and had varying associations with measured water quality parameters (Study II). In the WPR, 92 antibiotics were detected, and in the SEAR, 45 antibiotics were detected. Values of predicted threshold concentrations corresponding to different centiles for environmental exposure distributions of the maximum measured environmental concentrations of antibiotic residues, and the likelihood of exceedances of antibiotic Predicted No Effect Concentrations for resistance development of the WPR, the SEAR, China, and India were indicated (Study III). The highest environmental risks of antibiotic residues were observed in wastewater, and wastewater treatment plants' influents and effluents (up to 100%) (Study III). Antibiotic residues appear to pose an appreciable human health risk from environmental exposure via drinking water of the WPR and China, and the highest risk was observed for ciprofloxacin (62.5%) (Study III). A list of priority antibiotics from different classes for China was developed by ranking antibiotics in descending order, based on their a) overall risk, b) resistance risk on environment, c) ecotoxicity risk, d) overall environmental risk, e) resistance risk on human health, f) toxicity risk on human health, and g) overall human health risk. Ciprofloxacin posed the greatest risk (Study IV).

Conclusions: A novel assessment of the health risk due to the antibiotic residues in the aquatic environment of the WPR, the SEAR, China, and India were presented in this thesis. Antibiotic residues and antibiotic resistance were ubiquitous. There is evidence that residual concentrations of some antibiotics exceeded the thresholds for the development of resistance in various proportions of exposure in various aquatic environments of the WPR, the SEAR, China, and India, and posed an ecotoxicity effect. Wastewater and wastewater treatment plants serve as a hot spot for the development of antibiotic resistance in these regions. Antibiotic residues appear to pose an appreciable resistance and toxicity risk to human health from environmental exposure via drinking water. The emergence of antibiotic residues and resistance in drinking water further emphasises the need to place these threats to humans in the perspectives of environmental and health policy. These findings can help decision-makers to derive special risk reduction measures and focus mitigations towards priority antibiotics and high-risk sites, to decide the desired level of protection based on the proportions of exposure impacted, to implement eco-pharmacovigilance, and to help focus research efforts. This has the potential to assist decision-makers in efficiently allocating resources, which is especially vital for resource-poor settings e.g., in the WPR and SEAR.

Key words: antibiotic residues, antibiotic resistance, environment, human health, integrated quantitative risk assessment, prioritisation system, health policy, eco-pharmacovigilance.

LIST OF SCIENTIFIC PAPERS

I. **Hanna N**, Sun P, Sun Q, Li X, Yang X, Ji X, Zou H, Ottoson J, Nilsson LE, Berglund B, Dyar O, Tamhankar AJ, Stålsby Lundborg C.
Presence of antibiotic residues in various environmental compartments of Shandong province in eastern China: Its potential for resistance development and ecological and human risk.
Environment International. 2018 May; 114:131–42.

II. **Hanna N**, Purohit M, Diwan V, Chandran SP, Riggi E, Parashar V, Tamhankar AJ, Stålsby Lundborg C.
Monitoring of Water Quality, Antibiotic Residues, and Antibiotic-Resistant *Escherichia coli* in the Kshipra River in India over a 3-Year Period.
International Journal of Environmental Research and Public Health. 2020 Jan; 17(21):7706.

III. **Hanna N**, Tamhankar AJ, Stålsby Lundborg C.
Assessment of antibiotic exposure concentrations for the development of antibiotic resistance in aquatic environments of the Western Pacific and South-East Asia Regions of the WHO, based on a systematic review and probabilistic environmental hazard assessment.
Submitted.

IV. **Hanna N**, Tamhankar AJ, Stålsby Lundborg C.
The development of an integrated environment–human risk approach for the prioritisation of antibiotics for eco-pharmacovigilance and health policy decisions.
Submitted.

These Studies are referred to in the text by their Roman numerals [I-IV].

OTHER SCIENTIFIC CONTRIBUTION

I. Diwan V, **Hanna N**, Purohit M, Chandran S, Riggi E, Parashar V, Tamhankar AJ, Stålsby Lundborg C.
Seasonal Variations in Water-Quality, Antibiotic Residues, Resistant Bacteria and Antibiotic Resistance Genes of *Escherichia coli* Isolates from Water and Sediments of the Kshipra River in Central India.
International journal of environmental research and public health. 2018; 15 (6).

CONTENTS

LIST OF ABBREVIATIONS

ADI	Accepted Daily Intake
EC	Effective Concentration
EEDs	Environmental Exposure Distributions
EDI	Estimated Daily Intake
EPA	Environmental Protection Agency
EUCAST	European Committee for Antimicrobial Susceptibility Testing
FAO	Food Agriculture Organisation
HPLC-MS/MS	High performance liquid chromatography-tandem mass spectrometry
K_{ow}	Octanol/water partitioning coefficient
LOEC	Lowest Observed Effect Concentration
LMICs	Low- and Middle-Income Countries
mMECs	Maximum Measured Environmental Concentrations
MICs	Minimum Inhibitory Concentrations
MSCs	Minimum Selective Concentrations
NOEC	No Observed Effect Concentration
OIE	World Organisation for Animal Health
PEHA	Probabilistic Environmental Hazard Assessments
PNECs	Predicted No Effect Concentrations for the development of resistance
$PNEC_E$	Predicted No Effect Concentrations for ecotoxicity
PRISMA	Preferred reporting items for systematic reviews and meta-analyses
RQ	Risk Quotient
STPs	Sewage Treatment Plants
SEAR	South East Asia Region
TTC	Threshold of Toxicological Concern
WHO	World Health Organization
WPR	Western Pacific Region
WWTPs	Wastewater Treatment Plants

OPERATIONAL DEFINITION

Antimicrobial	Substance of natural, semi-synthetic, or synthetic origin that in *in vivo* concentrations kills or inhibits the growth of microorganisms, such as bacteria, fungi, viruses, and protozoans. Antimicrobials with activity against bacteria are called antibacterial agents or antibiotics.

1 BACKGROUND

1.1 ANTIBIOTIC RESISTANCE IS A GLOBAL CRISIS

Antibiotic resistance is a global health crisis of the 21st century and a serious threat to progress in achieving the Sustainable Development Goals. Antibiotics are important tools for preventing and treating bacterial infectious diseases in humans, animals, aquaculture, and agriculture. However, the emergence of resistance to these medicines threatens a century of progress in human health. Common infections have become resistant to several antibiotics that have been used in treating them, and lifesaving medical procedures and treatments are risky to perform. At the same time, there is a lack of scientific innovation resulting in part from market failure, with too few new antibiotics, diagnostic tools, vaccines, and alternatives to antibiotics in the research and development pipeline (1, 2).

Unless the world acts urgently, antibiotic resistance will have a devastating impact. The reduced efficiency of therapeutic antibiotic use can lead to increased hospitalisations for patient infections caused by antibiotic-resistant bacteria, and to treatment failure and mortality. Antimicrobial-resistant infections have already caused at least 700,000 global deaths annually, and this figure could increase to 10 million by 2050 if no action is taken. The economic consequences of uncontrolled antimicrobial resistance would also be disastrous. It has been estimated that it would cost up to USD 100 trillion globally by 2050, and millions of people could be forced into extreme poverty, mainly in low- and middle-income countries (LMICs). As antibiotic resistant pathogens spread, food security—including global trade in livestock, food, and feed—would also be increasingly jeopardised. In addition, concerns are growing about the impact of antibiotic resistance on animal health, environment, water and sanitation, social development, business and trade, and travel and tourism. Antibiotic resistance does not recognise geographical or ecological borders, impacting many countries worldwide (3, 4).

1.2 A ONE HEALTH RESPONSE TO ADDRESSING ANTIBIOTIC RESISTANCE

Addressing the complex global challenge of antibiotic resistance on multiple fronts requires an integrated and holistic One Health response that considers the connections between human and animal health and the environment (5, 6). One Health is the collaborative effort of multiple disciplines—working locally, nationally and globally—to attain optimal health of people, animals, and the environment. Antibiotic resistance has been described as the "quintessential One Health issue" as it exists in all three sectors. The World Health Organization (WHO) Global Action Plan on Antimicrobial Resistance (7), declaration from the 2016 high-level meeting on antimicrobial resistance at the United Nations General Assembly (8) and the Food and Agriculture Organisation of the United Nations (FAO)/the World Organisation for animal health (OIE)/the WHO (9) —all of which emphasise the importance of a One Health approach to tackling antibiotic resistance.

1.3 THE CURRENT GLOBAL RESPONSE TO ANTIBIOTIC RESISTANCE IN THE ENVIRONMENT IS INADEQUATE

While the impact of antibiotic resistance on human health has received considerable attention, political commitment and stakeholder involvement in these areas are still inadequate nationally and globally. Antibiotic resistance in animals requires more attention and political commitment, while efforts in tackling antibiotic resistance in the environment lag far behind in attention, evidence base, advocacy, and political commitment. Our limited understanding of the relation between the resistance in humans, animals, and the environment might be responsible for the lack of any significant environmental focus in existing policies and Antibiotic Resistance Action Plans. This might hinder the ability of the environmental decision-makers and regulators from delivering environmental and human health protection from antibiotic residues and antibiotic resistance. Without consideration of all the drivers, pathways, and impact of antibiotic residues and antibiotic resistance in the environment, Antimicrobial Resistance Action Plans are insufficient and at risk of not achieving the desired goals of tackling antibiotic resistance and ensuring and improving the efficacy of the existing and future antibiotics (10–12).

1.4 THE DRIVERS OF ANTIBIOTIC RESISTANCE

Although the development of antibiotic resistance is a natural phenomenon for bacteria, the emergence of antibiotic resistance in human pathogens is a recent event in evolutionary terms, which has occurred after the introduction of such medicines as antibacterial agents (13). The use of antibiotics accelerated the emergence of antibiotic-resistant bacteria, reducing their therapeutic potential against human and animal pathogens (14). As such, the current problem of antibiotic resistance should be considered as an example of anthropogenic-driven evolution.

Antibiotics have been extensively used for therapeutic and non-therapeutic purposes in humans, animals, aquaculture, and agriculture. Veterinary antibiotics have been used in the prevention and treatment of infectious diseases in animals, but they are gradually added as prophylactics and growth promoters, which currently far exceeds their use as animal therapeutics (15). Although bans on the use of antibiotics as growth promoters have been introduced in many countries such as China, how these policies are implemented, monitored, and enforced remain to be seen. Global antibiotic use in humans is projected to increase by 200% by 2030 (16). This increase is expected to be faster in LMICs as their economies grow and access to health services improves. The estimated global antimicrobial consumption in food animal production is projected to rise by 67% by 2030. For LMICs such as China and India, the increase in antimicrobial consumption will be 99%. The use of antibiotics to routinely prevent disease and promote growth in healthy animals without appropriate significance and in the absence of good agriculture and practices to prevent infectious diseases in animals are further contributing to the development and spread of antibiotic resistance (17).

The drivers of antibiotics use in human and veterinary medicine, crop protection, and aquaculture – especially in many LMICs – include a lack of awareness and knowledge; poor prescription practices and a lack of patient adherence to treatment; oversight including over-the-counter sales; social and cultural factors; weak regulation and economic incentives; inadequate access to safe water, sanitation and hygiene in health care facilities, households, and farms settings; poor

prevention of infection and disease; a lack of equitable access to affordable and quality-assured antibiotics, diagnostics, and vaccines; weak food safety and feed production; the large and growing burden of animal diseases; the increasing livestock production; the insufficient investment in veterinary services and animal health; and inadequate sewage/wastewater infrastructures all contribute to the emergence and transmission of antibiotic resistance in humans, animals, plants, food, and the environment (18–20).

1.5 HOW DO ANTIBIOTICS ENTER THE ENVIRONMENT?

Antibiotics can enter the environment through various anthropogenic sources and pathways such as municipal, hospital, and pharmaceutical manufacturing effluents, livestock and plant production, and fish and seafood farming. Substantial amounts (up to 90%) of antibiotics administered to humans and animals are excreted into waste streams, through urine and feces, in their biologically active forms or as active metabolites (21). However, sewage treatment plants/wastewater treatment plants (STPs/WWTPs) and other waste management systems are only partially effective in their removal that are subsequently released in treated effluent (22, 23). Antibiotics can be introduced into soil through manure and sludge land application to crops or landfill, and irrigation with reclaimed water (24). They may accumulate in vegetables through uptake from manure-amended cropland. Other sources may include agriculture runoff from fields containing animal manure and aquaculture ponds (25), and the discharge of landfill leachates of antibiotic disposal (26). Accordingly, antibiotic residues have been detected in various compartments of the environment including effluents from hospitals, municipal and pharmaceutical manufacturing, STPs/WWTPs, receiving environmental compartments (e.g., river, lake, sea, ground, and drinking water), aquaculture, soil, manure, and plant in several parts of the world (27–31).

1.6 POTENTIAL ENVIRONMENTAL AND HUMAN HEALTH RISKS OF ANTIBIOTIC RESIDUES

Antibiotic residues are considered as contaminants of emerging concern because of their continuous introduction into the environment and their potential adverse effects on the environment and human health. In particular, antibiotic residues may promote the emergence of antibiotic resistant bacteria and antibiotic resistance genes (32, 33) (Figure 1).

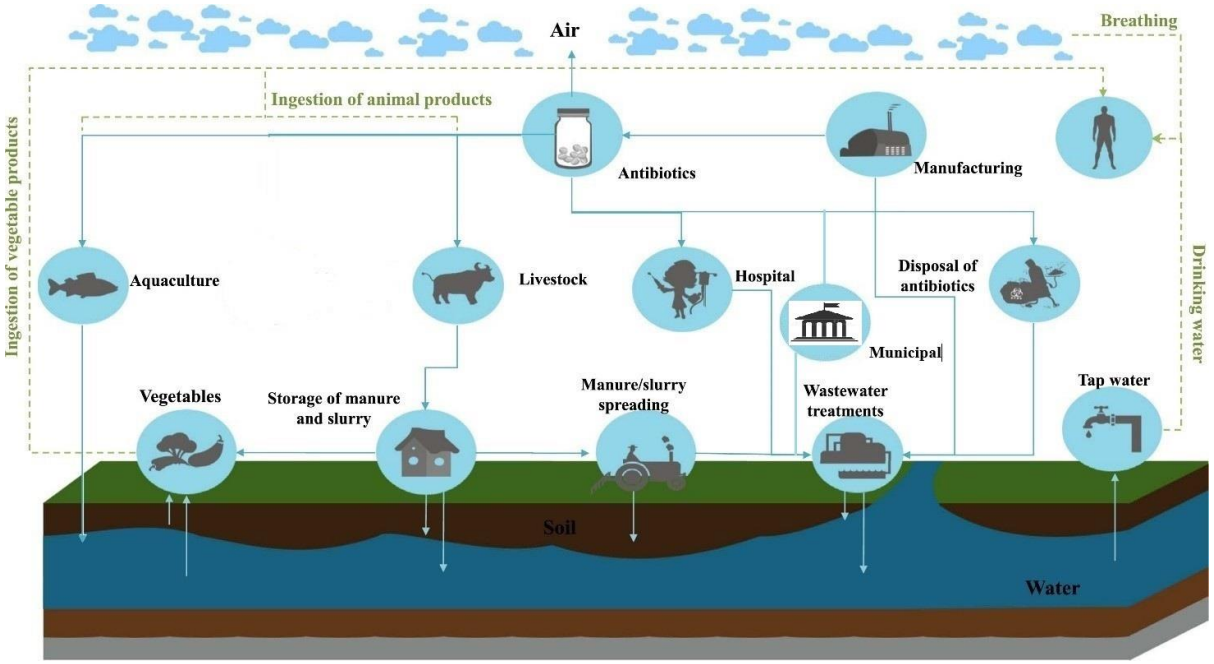


Figure 1. Environmental and human exposure to antibiotic residues and antibiotic resistance in the environment (34)

1.6.1 Potential environmental risk of antibiotic residues

Antibiotic residues in the environment create a selection pressure on the environmental bacteria and thus generate environmental reservoirs of antibiotic resistant bacteria and antibiotic resistance genes. Antibiotic residues may lead to acquired changes in susceptible bacteria, allowing the bacteria to survive and further proliferate as antibiotic resistant bacteria that carry antibiotic resistant genes. Mobile genetic elements such as plasmids, transposons, and integrons would further enhance the promotion and dissemination of genetic recombination of antibiotic resistance genes by conjugation, transformation, or transduction collectively referred to as horizontal gene transfer (35). The environmental release of antibiotics in effluents and sludge via STPs/WWTPs from anthropogenic sources, combined with direct contact between natural bacterial communities and discharged antibiotic resistant bacteria and their resistance genes, is a driver for the emergence of resistant strains in various aquatic environmental compartments including hospital wastewater (36, 37), municipal wastewater (38), industrial effluent (39), STPs/WWTPs (40), and the receiving environment (e.g., river, lake, sea, ground, and drinking water) (41–43), aquaculture (44, 45), soil, and vegetables (46, 47). In addition, antibiotic residues can have ecotoxicity effects on non-target organisms such as fish, daphnia, algae and other aquatic organisms (48).

Bacterial responses to antibiotics depend on their concentrations; diverse biological responses occur in bacteria at different concentrations. Antibiotic residues provide a further opportunity for the selection and persistence of antibiotic resistant bacteria in the environment, and this might occur at much lower levels than previously assumed. Exposures to very low levels of antibiotic concentrations below the Minimum Inhibitory Concentrations (MICs, refers to the lowest concentration of an antibiotic that inhibits the visible growth of a target bacteria during the incubation period) are sufficient for selecting resistant bacteria. Furthermore, each new

compound added for certain a combination of antibiotics lowered the Minimum Selective Concentrations (MSCs, refers to the lowest concentration of an antibiotic that selects for resistance) of the others (49, 50). It should be pointed out that these experiments were performed *in vitro* for single bacterial species and there is a need to further investigate how well these MSCs values correlate to their proposed effects in the environment, as the MSCs are likely to differ in more complex contexts with multiple species under exposure from multiple antibiotics.

To determine the potential of antibiotic concentrations in promoting the development of antibiotic resistant bacteria, thresholds of Predicted No-Effect Concentrations (PNECs) for the development of resistance in the environment (51) were assessed. These PNECs were based on applying extrapolation techniques to available MICs for clinically relevant bacteria to promote the development and selection of antibiotic resistance included in the MIC-database of the European Committee for Antimicrobial Susceptibility Testing (EUCAST) (52). The estimation of the upper boundary MSCs from the MICs is analogous to the Lowest Observed Effect Concentration (LOEC) values used in the ecotoxicity risk assessment of chemicals in the environment.

1.6.2 Potential human health risk of antibiotic residues

1.6.2.1 Potential human health risk of exposure to antibiotic residues

The potential human health risk of ingested antibiotic residues through drinking water or food consumption is disrupting the intestinal microbiome compositions and function and inducing the emergence of an antibiotic resistant intestinal microbiome. Antibiotic exposure is strongly associated with alterations in the intestinal microbiome due to the broad-spectrum effect on the host bacterial community rather than on the target bacteria. Once an intestinal microbiome homeostasis disturbs, it can lead to various diseases such as an increased susceptibility to infections such as diarrhoea, compromised immune homeostasis and tolerance such as inflammatory and autoimmune diseases and asthma, and deregulated metabolism such as obesity and diabetes. The human intestinal microbiome is an important reservoir and transporter of antibiotic resistance genes, and these genes may be transferred in the gut from one bacterium to another, including to human pathogens. The dissemination of resistant bacteria can also occur among individuals and thus further increase the prevalence of antibiotic resistance in a population and across continents. The effects of antibiotics may be cumulative in humans. It can therefore be expected that the effects of environmental exposure to antibiotic residues on the human microbiome may cumulate across generations (53–58, 58–61). Furthermore, given the especial prenatal and early-life susceptibility to antibiotics during critical windows of early development, unpredicted adverse consequences may result (62–65). Also, human exposure to antibiotic residues might pose toxicity effects, such as allergic reactions, thyroid toxicity, fatal aplastic anaemia, carcinogenicity, and mutagenicity (66–71).

The potential risks of antibiotics to human health are mainly assessed by determining the short-term effects of the treatments involving high oral doses that usually comprise a single type of antibiotic targeting specific pathogens. In contrast, exposure to environmental antibiotic residues exhibits a different mode of exposure: long-term exposure to a mixture of antibiotics with a high proportion of broad-spectrum antibiotics at a low-dose exposure level. When chemicals from the

surrounding environment are combined, a combined adverse effect can potentially occur, even if each individual component is harmless or present at low levels, and adverse effects are not expected. Chronic exposure to mixtures of antibiotics can potentially be associated with increased human health risks (72, 73). The main challenges are how to determine the degree to which humans are co-exposed to chemicals, what interactions can occur among these chemicals, and what specific human health effects are associated with these chemical mixtures. Possible human health risks due to the unintended intake of antibiotics through various exposure routes, such as inhalation and dermal contact, have not been fully explored (74, 75). The understanding of the absorption of antibiotics whose intake is through these exposure routes and the corresponding metabolic response of the intestinal microbiome is limited. This lack of understanding is due to the technical challenge of analysing the metabolites of the re-ingested antibiotics at the environmental level in the highly heterogeneous gastrointestinal system.

Assessments of human health risk of antibiotic residues were performed to establish the safety of antibiotic residues in human food and drinking water. In order to assess the effect of antibiotic residues in food and drinking water on the human intestinal microbiome, a harmonised approach to determine the threshold dose that might adversely disturb the microbiome and induce the emergence of antibiotic resistant intestinal bacteria was established (76). International regulatory bodies have developed guideline VICH GL36 (R) for determining the threshold of microbiological Accepted Daily Intake (ADI, refers to a level of daily intake of a compound that should not result in an adverse human health effect from direct exposure of an individual in a population) for antibiotics. This guideline takes into consideration relevant data including MICs data against human intestinal bacteria. To date, thresholds of microbiological ADI for antibiotics have not yet been determined due to various challenges. These challenges include how to quantify resistance and define thresholds' levels of change in the gastrointestinal tract with the complexity of the intestinal microbiome composition? Data of MICs clinical breakpoints are unavailable for some antibiotics. It is worth noting that even where this has been done, breakpoints have generally only been determined from clinical isolates, i.e. infections, rather than including isolates from healthy people. Thus the human health risk of antibiotic residues remain poorly understood.

1.6.2.2 Potential human health risk of antibiotic resistance development

Antibiotic residues could accelerate the emergence of antibiotic resistant bacteria and antibiotic resistance genes in the environment, and the risks associated with the environmental antibiotic resistance refer to the transmission of environmental antibiotic resistant bacteria and antibiotic resistance genes to humans through various exposure routes such as drinking water, food consumption, inhalation, and dermal contact (77–79). Pathogenic and the human commensal antibiotic resistant bacteria have the ability to colonise and proliferate in the human body, and pathogenic antibiotic resistant bacteria have the ability to cause infectious disease. Most commensal antibiotic resistant bacteria are not pathogens, but they could cumulatively reside in the human body and may harbour crucial virulence genes and therefore cause a disease, or they could transfer the genes conferring crucial virulence to other commensal human microbiomes. A potential transfer of antibiotic resistant bacteria and resistance genes between environment, animals, and humans, e.g., through horizontal gene transfer, can occur, posing health threats to

humans and animals (80–82). This has led to the recognition of the role of the environment in the emergence and dissemination of antibiotic resistance from a One Health Perspective (83).

Microbial risk assessment is used to evaluate the exposure level and the subsequent risk to human health from microbiological hazards. In the context of antibiotic resistant bacteria, environmental quantitative microbial risk assessment is in its infancy but is needed to address antibiotic resistant bacteria. Human health risk assessments dealing with the behaviour of pathogenic and commensal antibiotic resistant bacteria in various environmental compartments, the transfer of antibiotic resistant bacteria from the environment to humans, the transmission of antibiotic resistant bacteria via various environmental routes, and formal dose−response models with respect to a pathogenic antibiotic resistant bacteria are sparse. To date, no assessment of microbial thresholds for significant antibiotic resistant bacteria development has been conducted. The lack of thresholds data has prevented microbial risk assessment analysis to be developed.

1.7 ECO-PHARMACOVIGILANCE

The assessment of the environmental and human health impacts of antibiotic residues is crucial for developing targeted policies and risk mitigation measures. Therefore, it is essential to emphasise the eco-pharmacovigilance system for assessing the potential risk of antibiotic residues on the environment. Eco-pharmacovigilance has been proposed as a kind of pharmacovigilance for the environment (84, 85). Eco-pharmacovigilance involves activities associated with the detection, evaluation, understanding, and prevention of adverse effects of pharmaceuticals in the environment. This can be assessed by implementing the environmental risk assessment for the existing and new pharmaceuticals. The concerns about the environmental health paved the way in developing regulatory guidelines for assessing the risk of pharmaceuticals in the environment. However, mechanism exists in regulatory bodies to include environmental risk assessment for all new marketing authorisation applications, provided risks are identified before approval. Pharmaceuticals that have already been authorised, are not subject to a retrospective environmental assessment. Thus, the significance of trace levels of these pharmaceuticals in the environment and human health is often poorly understood. Importantly, most of the current guidelines do not recognise that the issue of antibiotic resistance development may be the most important risk associated with the occurrence of antibiotic residues in the environment (86). An addendum emphasising the need to assess the risks posed by antibiotics in promoting antibiotic resistance emergence should be included in these guidelines. In the case of antibiotics, information on potential environmental impacts must be taken into account in the pharmacovigilance system. However, compliance with risk mitigation measures, therefore, has only a voluntary character, and their implementation is not systematically verified nor followed up on. As such, this asserts the need to implement eco-pharmacovigilance systematically. In recognition of the One Health perspective, the impact of pharmaceuticals in the environment on One Health might need to be included in the definition of eco-pharmacovigilance (84, 87, 88). In doing so, eco-pharmacovigilance would be defined as “the science and activities associated with the detection, evaluation, understanding and prevention of adverse effects of pharmaceuticals in the environment, and its potential One Health impacts”.

2 THESIS RATIONALE

Antibiotic residues are recognised as contaminants of emerging concern due to their environmental and human health effects, especially their potential risk in promoting antibiotic resistance. Various anthropogenic sources such as the pharmaceutical industry, hospitals, and municipal wastewater release antibiotic residues into the environment. However, the understanding of the environment as a source and dissemination route for antibiotic residues and antibiotic resistance, and the magnitude of the human health risks posed by it, currently has been emphasised as a critical research need, as antibiotic resistance can also be co-released into the environment with antibiotic residues (33, 89). Antibiotic residues in various environmental compartments have been measured in several countries but how exactly they pose a health or resistance built up threat, has not been demonstrated. To define the risks to the environment and human health and to inform policy, there is a particular need to assess the antibiotic concentrations in all types of the environmental compartments at which resistance might develop and pose a health threat. These risks are more threatening in LMICs, including those populated in the Western Pacific Region (WPR) and the South-East Asia Region (SEAR) of the WHO, particularly China and India, which are large densely populated nations and are among the world's largest producers and consumers of antibiotics in human and veterinary medicines (17, 18, 90). In addition, the knowledge and the relative quantification about the adverse effects of antibiotic residues on the human health and non-targeted organisms, such as the chronic toxicity and resistance, remain scarce (91). Since multiple numbers of antibiotics are in use concurrently in variable quantities, the challenge is to identify which of these antibiotics should be prioritised for further assessment and environmental management of antibiotic residues. Therefore, a quantitative risk assessment approach to understanding the environmental and human health risks of antibiotic residues in an integrated manner, and adequate evaluation prioritisation strategies are of great environmental importance and need (92).

3 AIM AND OBJECTIVES

3.1 AIM

The overall aim was to assess the potential environmental and human health risks of exposure to antibiotic residues and antibiotic resistance in various environmental compartments of the WPR, the SEAR, particularly China and India, and to develop methods for the prioritisation of antibiotics to deal with aspects of their resistance and toxicity risks on human health and various aquatic environmental compartments.

3.2 OBJECTIVES

The specific objectives were:

- To investigate the occurrence of antibiotic residues in different types of environmental samples including water samples in rural eastern China, and to characterise the environmental risk for the development of resistance to antibiotic residues in the environment as well as the potential human health risk of exposure to antibiotic residues via drinking water and vegetables. **(Study I)**

- To investigate the occurrence of antibiotic residues and antibiotic-resistant *Escherichia coli* (*E. coli*) in the water and sediment of the Kshipra river in India at seven selected sites during different seasons over a 3-year period, and to investigate the association between antibiotic residues and antibiotic-resistant *E. coli* in water and sediment and measured water quality parameters of the river. **(Study II)**

- To investigate the occurrence and concentration of reported antibiotic residues in various aquatic environmental compartments of the WPR and SEAR, to assess antibiotic exposures in various aquatic environmental compartments for concentrations of antibiotics that are likely to select for resistance, and to identify hot spots and subsequent hazards of antibiotic residues for antibiotic resistance emergence in the aquatic environments of the WPR, the SEAR, China, and India. **(Study III)**

- To develop an integrated environment–human risk approach for assessing and quantifying both the environmental (resistance and ecotoxicity) and human health (resistance and toxicity) risks of antibiotics in various proportions of exposures, and developing a system for the prioritisation of antibiotics in various aquatic environmental compartments for eco-pharmacovigilance and policy decisions. **(Study IV)**

4 METHODS

4.1 OVERVIEW OF STUDIES

An overview of the studies included in the thesis is presented in Table 1.

Table 1. Overview of studies and their methods

Study	Study design	Settings	Sampling and data collection	Study period	Data analysis
I	Quantitative methods Risk assessment methods	12 villages in Shandong province in eastern China	Various environmental compartments Samples (n = 214) HPLC–MS/MS was used to determine the concentration of antibiotic residues	2015	Risk assessment
II	Quantitative methods Microbiological methods Molecular methods	Kshipra river in India	Water and sediment samples Different seasons, seven sampling sites over a 3-year period HPLC–MS/MS was used to determine the concentration of antibiotic residues	2014, 2015, and 2016	ANOVA Post-hoc analysis Tukey correction Pearson's rank correlation test
III	Systematic review Risk assessment methods (Probabilistic Environmental Hazard Assessments (PEHA))	Western Pacific Region (WPR) of the WHO South East Asia Region (SEAR) of the WHO China India	All reported measured antibiotic residues All types of aquatic environmental compartments Included studies (218 from WPR, 22 from SEAR, 168 from China, 15 from India)	2006-2019	Risk assessment Linear regression
IV	Methodology development based on risk assessment methods	China	All reported measured antibiotic residues All type of aquatic environmental compartments	2006-2019	Risk assessment Linear regression

4.2 STUDY SETTINGS

4.2.1 The WPR, the SEAR, China, and India

Study I, Study II, Study III, and Study IV were conducted in the WPR, the SEAR, China, and India. The Asia Pacific Region comprises the WPR and the SEAR and include 48 countries and areas with 53% of the world's total population (93, 94). China belonging to the WPR, with 1.43 billion people in 2019, and India belonging to the SEAR, with 1.37 billion, have long been the two most populous countries of the world, comprising 19 and 18 percent, respectively, of the

global total in 2019 (95). China and India are among the world's largest producers and consumers of antibiotics in human and veterinary medicines and are also major contributors to global pharmaceutical production (17, 18, 90). Antibiotic residues and antibiotic resistance are widely detected in the various environmental compartments of the WPR and the SEAR (31, 96–98). Antibiotic resistance and exposure patterns of antibiotic residues in the environment in these regions are influenced by the following aspects: a) population growth and expanding urban centers (99), b) the rapid growth of antibiotic use in livestock production reflects the increase in the demand for meat products following the increase in income per capita, c) the shifting of global pharmaceutical manufacturing to Asia (100), and d) the inefficient removal of antibiotics by STPs and WWTPs. Further, in these regions ~ 80–90% of wastewater is released untreated into various water sources (101).

4.2.2 Study areas

Study I was conducted in 12 villages in Shandong province in eastern China. The province has a population of 96 million in 17 cities and 140 counties, of which around half are rural (Figure 2).

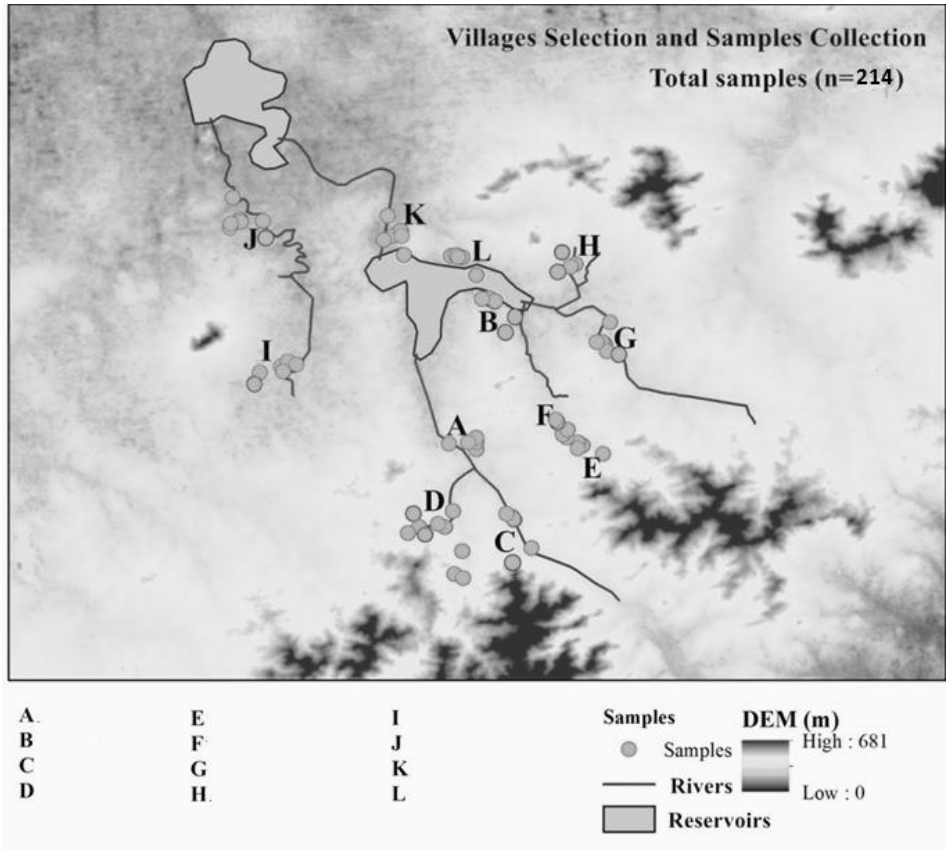


Figure 2. The distribution of the sampling sites for environmental samples in 12 villages (A–L) of Shandong province of China

Study II was conducted within the reaches of the Kshipra river that flows through the city of Ujjain in India. The Kshipra River is 195-km long, of which 93 km flows through Ujjain District. It originates in the Kokri Bardi Hills (747-m high). After crossing a 70-km pathway, it enters Ujjain District (Figure 3).

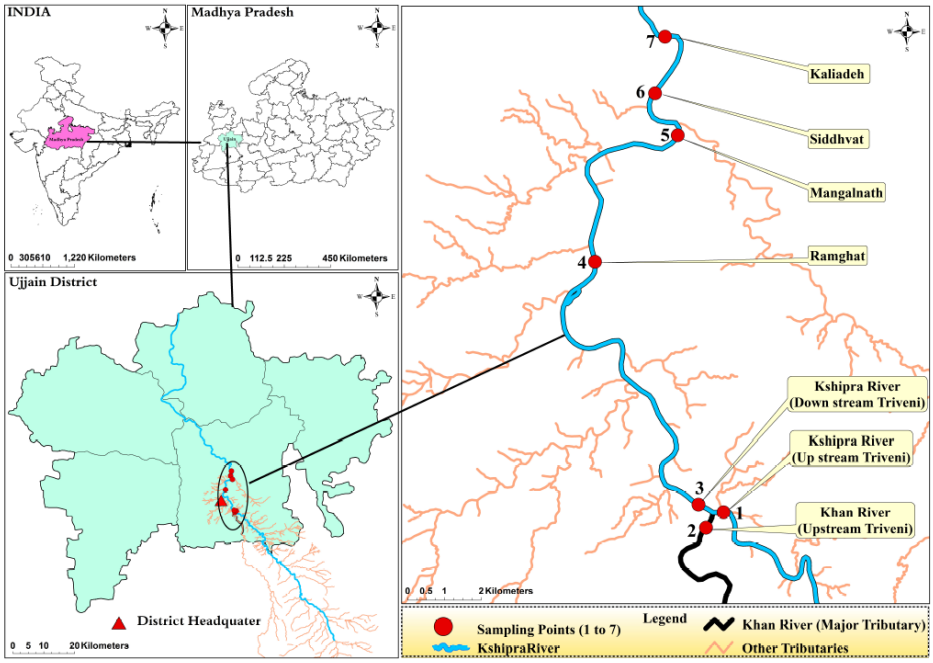


Figure 3. Geographical location of the study site. The map shows (clockwise) India, Madhya Pradesh, the sampling points on the Kshipra river, and Ujjain district.

Study III was conducted in the WPR and SEAR, the WPR, including American Samoa (USA), Australia, Brunei Darussalam, Cambodia, China, Cook Islands, Fiji, French Polynesia (France), Guam (USA), Hong Kong SAR (China), Japan, Kiribati, Lao People's Democratic Republic, Macao SAR (China), Malaysia, Marshall Islands, Federated States of Micronesia, Mongolia, Nauru, New Caledonia (France), New Zealand Niue, Northern Mariana Islands, Commonwealth of the (USA), Palau, Papua New Guinea, Philippines, Pitcairn Islands (UK), Republic of Korea (South Korea), Samoa, Singapore, Solomon Islands, Tokelau, Tonga, Tuvalu, Taiwan, Vanuatu, Viet Nam, Wallis and Futuna (France), and the SEAR, including Bangladesh, Bhutan, Democratic People's Republic of Korea, India, Indonesia, Maldives, Myanmar, Nepal, Sri Lanka, Thailand, and Timor-Leste.

Study IV was methodology development, to demonstrate the utility of the proposed approach, it was applied to the situation of China, China being just an example.

4.3 STUDY DESIGN, DATA MANAGEMENT, AND ANALYSES

Study I

Quantitative methods for determining antibiotic concentrations in various environmental compartments of Shandong province in China were used, and risk assessment methods to characterise its environmental and health risks were performed.

Environmental samples (n = 214) were collected from the 12 villages, over six consecutive days in July 2015. The samples consisted of different types of water (well, surface, tap, and wastewater), sediments, pig manure, soil, and parts of the vegetables that are edible for humans.

In each village, at least two households with animal breeding were included in the study. In each household, one sample each was taken from one human and one animal drinking water container. If for humans and animals different drinking water sources were used, e.g., well water for animals and tap water for humans, then both would be sampled. If humans and animals used the same drinking water, only one drinking water sample was taken. In addition, wastewater, manure, and outlet sediments were also sampled from the same household. If the village was near the river, water and sediment from the river were sampled. For vegetables, different types of edible parts such as cucumbers, tomatoes, lettuces, and kidney beans etc. were sampled.

To test the level of antibiotic residues in the environmental samples, target antibiotics were selected based on their usage in humans and animals in China, particularly in the target area, as well as their behaviours in the environment. Norfloxacin, levofloxacin, ciprofloxacin, enrofloxacin, doxycycline, sulfapyridine, sulfamethoxazole, metronidazole, florfenicol, and chloramphenicol were analysed by using the high performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS) technique.

The concentrations of antibiotic residues are reported as median, mean, and range for each target antibiotic for each type of sample.

The environmental risk for the development of resistance to antibiotics was estimated by means of risk quotient (RQ) values. $PNEC_S$ (ng/L) for the development of resistance (51) were employed for these calculations. Based on the MICs for 111 antibiotics from the database of the EUCAST (52), these $PNEC_S$ were assessed with an assessment factor of 10, corresponding to the median MICs / MSCs ratio for clinically relevant bacteria to promote the development of antibiotic resistance.

The RQ values are expressed as the ratio of the Measured Environmental Concentrations (MECs) to the PNECs for development of resistance, for any particular antibiotic compound as shown in Eq. 1.

$$RQ = MECs/PNECs \quad (1)$$

The risk was classified into three levels, i.e. low risk with the RQ values ranging between 0.01 and 0.1, moderate risk with the RQ values ranging between 0.1 and 1, and high risk with the RQ values > 1 (102).

To assess the potential risk to human health through exposure to antibiotic residues in drinking water, the approach of ADI was used in this study to assess the potential risk to human health through the exposure to antibiotic residues in drinking water. It was combined with the standard assumptions to derive Predicted No Effect Concentrations for drinking water ($PNEC_{DW}$). $PNEC_{DW}$ was estimated for both adults and children using a general exposure equation which was consistent with those used by the U.S. Environmental Protection Agency (U.S. EPA) for developing concentration limits to protect against threshold-type effects, such as the Ambient

Water Quality Criteria (AWQC), for the protection of human health, or maximum allowed contaminant levels. $PNEC_{DW}$ was calculated using Eq. 2.

$$PNEC_{DW} = (1000 * ADI * BW * AT)/(IngR_{DW} * EF * ED) \quad (2)$$

$PNEC_{DW}$ represents what the predicted no effect concentrations for drinking water is (ng/L); 1000 is a conversion factor (ng/μg); ADI is the accepted daily intake (μg/kg/day); BW is the child or adult body weight (kg/person); AT is the average time of exposure (days); $IngR_{DW}$ is the child or adult drinking water ingestion rate (L/person/day); EF is the exposure frequency (days/year); ED is the exposure duration (years). The equation was applied using human exposure parameters recommended by the U.S. EPA guidance for deriving AWQC and WHO Guidelines for Drinking water Quality (103,104).

I calculated the ratios of MECs/$PNEC_{DW}$ for each compound to estimate the potential health risk, a ratio of MECs/$PNEC_{DW}$ of > 1 was identified as a potential risk to human health.

I calculated the potential risk by vegetable consumption according to the ADI in μg/kg/day of an antibiotic present in the vegetables. ADI is established to provide a guide for the maximum quantity of a single chemical or drug that can be taken daily in food without an appreciable health risk to the consumers (105).

The Estimated Daily Intake (EDI) values were calculated for adult and child, using Eq. 3.

$$EDI = \left(C_{food} * IR_{veg} * \beta_{g/cup} * \beta_{ww/dw}\right)/BW \quad (3)$$

C_{food} represents the mean concentration of an antibiotic in vegetables (ng/g dry weight (dw)). IR_{veg} represents 3.45 cup equivalents of vegetables per day for an adult in China (276 g/d), and 2.86 cup equivalents of vegetables per day for a child in China (228.8 g/d) (i.e. 1 cup = 80 g of raw vegetable) (106, 107). $\beta_{g/cup}$ represents the mass of a cup of fresh tissue diced to ≤ 0.5 cm^3 (i.e. g/cup equivalent), which was determined in the laboratory. $\beta_{ww/dw}$ represents the mean wet-to-dry conversion factor used by the U.S. EPA for plant tissue in the development of soil screening values (i.e. 0.085) (108). BW is the child or adult body weight (kg/person).

I calculated the ratios of EDI/ADI for each antibiotic to estimate the potential health risk, a ratio of EDI/ADI of > 0.1 was identified as a potential risk to human health.

Study II

Quantitative methods to investigate the antibiotic residues' levels and water quality in the river water and sediment samples from the Kshipra river during different seasons, seven sampling sites over a 3-year period were used, and microbiological and molecular methods to test antibiotic resistant *E. coli* and antibiotic resistance genes were applied.

Water and sediment samples from the Kshipra river were collected from seven selected sites, and water samples were collected in duplicate. Sampling was conducted once during each of the four seasons for three consecutive years: 2014, 2015, and 2016. The criteria for the sampling sites included both point and non-point sources of pollution, and the locations were chosen at places which have industries and agriculture activities, and at the confluence of the

Khan and Kshipra rivers. The Khan river brings pollutants from pharmaceutical industries nearby. Further, there were mass-bathing spots or spiritually important places where the pollution load was expected to be high due to the bathing of people.

The methods are described in brief here; a detailed description of the methods is available in the published protocol (109). Water and sediment water quality parameters were measured. Further, river water and sediment samples were analysed for the presence of antibiotics: ceftriaxone, ofloxacin, norfloxacin, ciprofloxacin, sulfamethoxazole, metronidazole, and total residual antibiotics as β-lactam. These antibiotics were selected based on antibiotic residues previously found in the same geographical area (110), environmental stability, and known and suspected environmental impacts of the antibiotic and the degree of antibiotic metabolism (111). Antibiotic residues were detected by using the LC-MS/MS technique.

Susceptibility tests for eight different classes of antibiotics inclusive of ampicillin, cefotaxime, ceftazidime, cefepime, nalidixic acid, ciprofloxacin, nitrofurantoin, gentamicin, amikacin, tetracycline, tigecycline, imipenem, meropenem, co-trimoxazole, and sulfamethiazole were conducted using the Kirby Bauer disc-diffusion method (112). Clinical and Laboratory Standard Institute (CLSI) guidelines were used to measure and interpret the zone diameter of bacterial growth inhibition (113). Antibiotic resistance genes such as ESBL-coding, plasmid-mediated quinolone resistance, carabapenemase resistance, and sulfonamide resistance genes were analysed from the river water and sediment samples by polymerase chain reaction (PCR).

An analysis of variance (ANOVA) was conducted to determine seasonal variation in antibiotic residues and resistance among the seven sites over a 3-year period. A post-hoc analysis was applied to test the difference in antibiotic residues and resistance between seasons within each year. The Tukey correction was used to adjust p-values for multiple pairwise comparison. Correlations between antibiotics and water quality parameters were analysed with Pearson's rank correlation test.

Study III

I conducted a systematic review of the literature published between 2006 and 2019 to investigate the occurrence and concentrations of the reported antibiotic residues in various aquatic environmental compartments of the WPR and SEAR. I also used risk assessment methods including Probabilistic Environmental Hazard Assessments (PEHA) to assess antibiotic exposures in various aquatic environmental compartments for concentrations of antibiotics that are likely to select for resistance.

The Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines were followed (114).

Eligibility criteria were specified a priori as original research articles of any quantitative design related to the subject matter. Studies were included if they a) were original, b) reported antibiotics or antibacterials employed for systemic use in humans or animals, c) reported any type, group, or class of antibiotic, d) collected any type of water samples from the environment, such as: river water, lake water, drinking water, ground water, sea water, or other water compartments (surface water, canal, pond, stream, surrounding aquaculture, reservoir drainage, estuary, nearshore, and

offshore), wastewater (municipal, hospital, and pharmaceutical manufacturing), and STPs'/WWTPs' influents and effluents, e) measured antibiotic residue concentrations in one or several of the aquatic environments, f) for studies conducted in the WPR and SEAR, g) published from 2006 to 2019, and h) there were no language restrictions and grey literature (conference abstracts, dissertations) was searched. Studies were not included if they only reported antibiotic residues in non-water samples (soil, sediment, manure, and vegetables).

I conducted the literature search using various scientific literature databases such as Medline (OVID), Web of Science, Embase, Global Health (OVID), GreenFile, Dissertations and Theses (ProQuest), and WHO IRIS. I performed the searches in October 2018 and updated it in December 2019. The first screening stage (relevance screening) consisted of an evaluation of titles and abstracts of all records retrieved, to retain only those relevant to the review question. The full texts of eligible papers were assessed according to the pre-defined inclusion and exclusion criteria in the second screening stage.

Considering the threats to internal validity, I assessed each included study for risk of bias in three domains: selection bias, information bias, and confounding. For each domain, each study was evaluated as having either a low, high, or unclear risk of bias. For each study, I assessed overall risk of bias by combining risk of bias from each domain. In this study, I adapted the format for assessing risk of bias and critical appraisal criteria to assess the studies from the Cochrane Collaboration Risk of Bias Tool and other tools from previous studies (115, 116). They were adjusted to our research question of environmental interest to create our own tool. Studies were included irrespective of how it was judged in terms of quality and risk of bias.

I extracted data on antibiotic concentrations, for reported measured antibiotic residues from the papers and clustered them by each aquatic environmental compartmental media/system to include the following items: a) author, b) year, c) journal, d) location, e) country, f) compartment, g) chemical, h) concentration ng/L (min, max, mean, and median), i) removal efficiency for STPs/WWTPs, j) detection limit, k) instrumentation, l) sample size/sampling notes, m) notes, and n) full citation.

After the data were extracted and compiled from the primary literature, they were utilised to perform PEHA. In which the likelihood of the environmental occurrence of each antibiotic exceeding the thresholds for the development of antibiotic resistance, in various proportions of exposure, in various aquatic environmental compartments of the WPR, the SEAR, China, and India were estimated. The PEHA approach is currently being assessed for incorporation into assessment procedures in a number of regulatory jurisdictions and literatures. PEHA is used to estimate the exposures of populations and communities to potentially hazardous materials and their responses to these exposures, and to predict the magnitude and probability of the effects, where the probability is the characterisation, quantitatively, of the uncertain variables (117–120).

I formulated PEHA models using Environmental Exposure Distributions (EEDs) of the maximum Measured Environmental Concentrations (mMECs) of antibiotics for each water compartment. As maximum, mean, and median values were not consistently reported in the studies/literature and to conservatively maximise the estimates of exposure, mMECs of antibiotics were used for hazard assessments of aquatic environmental compartments.

Reported mMECs of antibiotic were ranked in ascending order, and the percent rank assigned using a Weibull formula:

$$j = (i * 100)/(n + 1)$$

Where, j is the percent rank, i is the rank assigned to the mMECs of an antibiotic, n is the number of data points. A linear regression was then fitted to the percent rank versus mMECs plot (probability transformed and log normal scales, respectively; SigmaPlot 12.0). For each regression related to each aquatic environmental compartment, the slope, intercept, and r^2 were determined. The resulting slope and intercept from the linear regression were used to estimate the probabilities of observing mMECs at given concentrations with the NORMDIST function in Microsoft Excel, using the equation:

$$centile\ value\ = NORMDIST\ ((m * log_{10}(x)) + b)$$

Where NORMSDIST returns the standard normal cumulative distribution function, x is the threshold value, m is the slope of the regression, and b is the intercept.

I calculated the predicted threshold concentrations of the corresponding different centiles (1, 5, 25, 50, 75, 95, and 99) for the measured environmental concentrations distribution of the maximum reported antibiotic residues in various aquatic environments, and I determined the percent exceedances of antibiotic thresholds for the development of resistance. The thresholds of PNECs (ng/L) for the development of resistance (51) were employed for these calculations.

PEHA does not generate a single point estimate, but rather produces a likelihood and range that a particular exposure and effect will occur. Accordingly, this type of assessment allows the risk assessors or managers (e.g., decision making and/or regulatory agency, policy maker, or industries) to conduct the assessment independent of most value judgments, to predict the likelihood that a certain level of protection would be attained. For instance, the assessor may require that the environmental concentration associated with the level of protection be exceeded only 5% of the time (alternatively, 95% of all exposures (water concentrations) would be expected to be equal to or less than the required environmental concentration). Using these centile levels depending on the resources available to the risk managers and the capacity and applicability, they then take appropriate actions. Then they do not work in a void but have some understanding of what resistance reduction their actions are likely to have. PEHA also allows the analysis of variability (refers to the heterogeneity and diversity) and uncertainty (refers to imperfect knowledge or a lack of a precise knowledge) to be incorporated into exposure and/or hazard assessments.

Study IV

I developed an integrated environment–human risk approach for the quantitative environmental and human health risk assessment of antibiotics and a prioritisation system thereof.

Conceptually, the specificities of the approach I propose are: a) a risk-based approach suitable for the initial assessment and prioritisation of antibiotics that are of interest for management purposes and further research; b) the approach combines data on exposure, data on toxicity,

data on resistance, and the chemical structures of antibiotics with PEHA and the Threshold of Toxicological Concern (TTC) approach; c) the approach enables the researcher to assess and quantify the environmental risks (resistance and ecotoxicity) and the human health risks (resistance and toxicity) regarding the chronic exposure of maximum concentrations of antibiotic residues in various aquatic environmental compartments in various proportions of exposure and d) the approach enables the prioritisation of antibiotics in various aquatic environmental compartments. Based on the data availability, this approach could be adjusted for any environmental compartments. Previous studies that compared a set of previously proposed ranking methods / prioritisation schemes for environmental risk assessment reported that risk-based approaches are preferred over hazard-based methods since environmental risk reflects both exposure and impact or inherent hazards of the pharmaceuticals (24). For risk-based decisions, when the risk and data gap of antibiotics are identified, follow-up work is required to identify targeted policies in accordance with the level of risk, to minimise it or to refine the environmental risk assessment.

The following sections present descriptions of the development of the data collection and assessment schemes and the antibiotic prioritisation system.

Data collection and assessment

I conducted a systematic review of the literature published between 2006 and 2019 to investigate antibiotic residues in all aquatic environmental compartments of China. After data on environmental concentrations of antibiotic residues were extracted and compiled from primary literature, they were utilised to assess the environmental risk and human health risk of antibiotics by performing PEHA (our unpublished data from Study III, to be published separately).

Environmental risk

I assessed environmental risk for resistance risk and ecotoxicity risk of antibiotic residues in surface water (river water, lake water, sea water, other water compartments, waste water, WWTPs' influents and WWTPs' effluents) and ground water in China.

Resistance risk on environment

Resistance risk was estimated by combining actual data on the exposure of the mMECs of antibiotic residues for each water compartment including surface water (river water, lake water, sea water, other water compartments, waste water, WWTPs' influents and WWTPs' effluents) and ground water in China, the thresholds of $PNEC_S$ for the development of resistance, and PEHA.

Ecotoxicity risk

The likelihood of the environmental occurrence of each antibiotic exceeding the threshold for ecotoxicity risk, in various proportions of exposure, in surface water (river water, lake water, sea water, other water compartments, waste water, WWTPs' influents and WWTPs' effluents) and ground water in China were estimated by combining actual data on the exposure of the mMECs

of antibiotic residues for each aquatic compartment, the thresholds of the $PNEC_E$ which represent the ecotoxicity, and PEHA.

The Environmental Risk Assessment aims to establish the safe concentrations for the protection of ecosystem structure and function, wildlife populations, and includes the calculation of $PNEC_E$ for aquatic organisms, namely $PNEC_{Egroundwater}$ ($PNEC_{Egw}$), $PNEC_{Esurfacewater}$ ($PNEC_{Esw}$) and $PNEC_{Emicroorganism}$ (86). The $PNEC_{Egw}$ is based on a chronic test with Daphnia magna (121) and $PNEC_{Esw}$ is calculated from the toxicity for three aquatic species – green algae, invertebrate and fish. $PNEC_{Egw}$ and $PNEC_{Esw}$ were calculated by taking the lowest No Observed Effect Concentration (NOEC, the test concentration at which there is no statistically significant effect in the response being tested, such as on growth rate or reproduction), or 50% Effective Concentration (EC_{50}, refers to the concentration of a toxic compound inducing the response to 50% of the maximal possible effect from an organism) if NOEC was not available for aquatic species, and applying an assessment factor (AF) according to the European technical guidance (102). The AF was applied to account for inter-species and intra-species variations; the extrapolation of short-term toxicity towards long-term toxicity; and the laboratory data to field impact extrapolation, as described in the regulatory guidance (86). The $PNEC_{Egw}$ and $PNEC_{Esw}$ were calculated using Eq. (1). In chronic tests (long-term), lethal and sub-lethal effects are measured. The latter includes evaluations of growth reduction, reproductive impairment, lack of mobility and inhibition of some regulatory functions such as development, fertility, changes in behaviour and maintenance of homeostasis (122). The NOEC or EC_{50} of antibiotics was retrieved from a systematic review, which reported the lowest 'reliable' NOEC and EC_{50} of taxa commonly used in environmental risk assessment for antibiotics (123).

$$PNEC = NOEC\ or\ EC_{50}/AF \quad (1)$$

The bioaccumulation of an antibiotic is represented using its octanol–water partition coefficient (LogKow) value. This indicator was used to represent the chemical's bioaccumulation attribute as it has been correlated with the bioconcentration factor for different ionic and non-ionic compounds as well. A LogKow value of more than 4.5 indicates a potential for the bioaccumulation of a chemical (86). Information on the bioaccumulation of antibiotics was retrieved from a review study of Physicochemical properties of antibiotics (124) and the PubChemDatabase, 2019.

Human health risk

I assessed the human health risk regarding resistance risk and toxicity risk of antibiotic residues in both drinking water and ground water (in the literature, antibiotic residues' information was available separately for drinking water and ground water).

Resistance risk on human health

The likelihood of the environmental occurrence of each antibiotic exceeding the threshold for the development of antibiotic resistance was assessed by combining actual data on the exposure of the mMECs of antibiotic residues in drinking water and ground water in China, thresholds of $PNEC_S$ for the development of resistance, and PEHA.

Due to the lack of human health thresholds for the development of resistance data, the assessment of the human health risk of antibiotic residues via drinking water and ground water was based on the threshold of $PNEC_S$ for the development of resistance. The assumption was made that if the likelihood of exceedances of antibiotic $PNEC_S$ for the development of resistance are observed for antibiotic residues in drinking water and ground water, these antibiotics have the potential risk for the development of antibiotic resistance in human intestinal microbiome.

Toxicity risk on human health

The likelihood of the environmental occurrence of each antibiotic exceeding the threshold for toxicity risk was assessed by combining actual data on the exposure of the mMECs of antibiotic residues in drinking water and ground water in China, Threshold of Toxicological Concern (TTC) approach, and PEHA.

The TTC approach is a screening tool that has been developed in order to assess substances of unknown toxicity present at low levels in the food and drinking water, and to prioritise chemicals. TTC values are intended to represent exposure thresholds below which there is no appreciable risk to human health over a lifetime of daily exposure for chemicals of that class, based upon structural characteristics of the chemical in question and existing toxicity data for other substances in an identified database. A large database of reference substances was compiled from which a distribution of NOELs could be derived. Three classes were identified based on the toxicity in ascending order: Cramer class I, II, and III (125). TTC utilises different systemic endpoints, such as developmental and reproductive toxicity, immunotoxicity and neurotoxicity.

A Cramer classification of each of the antibiotics was performed using ToxTree version software (https://apps.ideaconsult.net/data/ui/toxtree). The TTC approach relies on conservative daily exposure thresholds (NOEL) for each Cramer class, derived from a database of sub-chronic, chronic and reproductive / developmental oral toxicity data on more than 600 chemicals (126, 127). Each TTC value represents the 5th percentile NOEL of all compounds in the dataset for that particular class (i.e., 95% of the compounds in the class were less toxic). Short-term testing is generally not applicable for human pharmaceuticals since continuous exposure of the aquatic environment via drinking water is assumed. TTC values were obtained by dividing the respective TTC values for the appropriate Cramer class (1800, 540 and 90 ug/day for classes I, II and III, respectively) by 60 (adult body weight in kilograms), to express the values per unit of body weight. Accordingly, Cramer class I chemicals have human exposure threshold values of 30 ug/kg body weight per day; class II, 9 ug/kg body weight per day; and class III, 1.5 ug/kg body weight per day. A TTC value of 1.5 ug/kg body weight per day would provide an adequate margin of safety for both non-cancer and cancer endpoints (128).

The TTC values are total oral exposures per person per day. To derive drinking water target values, these therefore need to be translated to drinking water concentrations. According to the WHO guidelines for drinking water quality 2011, there is variation in both the body weight of consumers and volume of daily water consumed. Therefore, assumptions were applied in order to determine a guideline value. The default assumption for body weight is 60 kg, whereas the default assumption for adult consumption is 2 litres of water per day. In order to account for the variations in exposure from different sources of default values, generally 10 % is used. Wherever

possible, country- or site-specific information should be used in assessments of this type. If no specific data are available, an approximate risk estimate can be based on default values (129).

The standard WHO drinking water consumption rate of 2 L/day for adults (60 kg) is used. Using the TTC value of 1.5 ug/kg body weight per day, the following target value can be derived:

(1.5 ug/kg body weight per day $*$ 10%) / 2 L $\approx$ 0.1 ug/L $\approx$ 100 ng/L.

According to EMA 2015 (130), as part of the environmental risk assessment, the concentrations in groundwater of antibiotic residues need to be compared against the value of 100 ng/L. Concentrations above 100 ng/L have been considered unacceptable for all substances, regardless of their intrinsic hazardous properties. In this situation, applicants could refine the concentrations of groundwater and drinking water with additional data (e.g., modelling, more studies e.g., on degradation in manure, mitigation measures). For concentrations below 100 ng/L, no risk was anticipated and no further regulatory action was required.

Antibiotic prioritisation system

The proposed antibiotic prioritisation system consists of criteria, attributes, sub-attributes and sub-sub-attributes.

Criteria, attributes, sub-attributes and sub-sub-attributes

The ranking of antibiotics in this study was based on the overall scores of two criteria: a) environmental risk and b) human health risk of antibiotic residues.

The first criterion "environmental risk" was represented using two attributes: a) resistance risk: was represented as the likelihood of the environmental occurrence of each antibiotic exceeding the thresholds for the development of antibiotic resistance, which was represented using eight sub-attributes (river water, lake water, ground water, sea water, other water compartments, waste water, WWTPs' influents and effluents), and b) ecotoxicity risk: was represented using two sub-attributes: b1) ecotoxicity, which was represented as the likelihood of the environmental occurrence of each antibiotic exceeding the thresholds for ecotoxicity, which was represented using eight sub-sub-attributes (river water, lake water, ground water, sea water, other water compartments, waste water, WWTPs' influent and effluent), and b2) bioaccumulation, which was represented using its octanol–water partition coefficient (LogKow) values.

The second criterion, "human health risk", was represented using two attributes: a) resistance risk: was represented as the likelihood of the environmental occurrence of each antibiotic exceeding the thresholds for the development of antibiotic resistance, and was represented using two sub-attributes (ground water and drinking water), and b) toxicity risk: was represented as the likelihood of the environmental occurrence of each antibiotic exceeding the thresholds for human toxicity, which was represented using two sub-attributes (ground water and drinking water).

Utility functions

All of the criteria, attributes, sub-attributes and sub-sub-attributes have numerical values. Expressions of utility functions were chosen to reflect the fact that an antibiotic with a high

potential of resistance risk, a high potential of toxicity or a high logKnow (value indicates a potential for bioaccumulation) should have a high overall rank score and prioritisation in water. However, other variations of utility functions are possible. For example, the overall ecotoxicity risk attribute can be expressed separately using individual ecotoxicity risk attributes for aquatic species. Further, in the absence of any data, the utility function of an attribute was assigned 0.

Multiple criteria, attributes, sub-attributes and sub-sub-attributes have different importances in influencing the overall ranking of antibiotics in water and were expressed as importance weights. For each category (i.e., criteria, attributes, sub-attributes or sub-sub-attributes), the total sum of importance weights remains 1. The calculated importance weights of the components of a representative utility function are presented in Table 2. An illustration of the steps for calculating rank scores and data gaps is presented in Appendix 1 using ciprofloxacin as a representative antibiotic.

Table 2. Calculated importance weights of components of a representative utility function of criterion, attribute, sub-attribute, and sub-sub-attribute

Category	Component	Importance weights *
Criterion	**Environmental risk**	1/2
Attribute	Resistance risk	1/2
Sub-attribute	River water	1/8
	Lake water	1/8
	Ground water	1/8
	Sea water	1/8
	Other water compartments	1/8
	Wastewater	1/8
	WWTP influent	1/8
	WWTP effluent	1/8
Attribute	Ecotoxicity risk	1/2
Sub-attribute	Ecotoxicity	1/2
	River water	1/8

Sub-sub-attribute	Lake water	1/8
	Ground water	1/8
	Sea water	1/8
	Other water compartments	1/8
	Wastewater	1/8
	WWTP influent	1/8
	WWTP effluent	1/8
Sub-attribute	Bioaccumulation	1/2
Criterion	**Human health risk**	1/2
Attribute	Resistance risk	1/2
Sub-attribute	Drinking water	1/2
	Ground water	1/2
Attribute	Toxicity risk	1/2
Sub-attribute	Drinking water	1/2
	Ground water	1/2

*In this study, all criteria are assumed to be equally important; all attributes for a given criterion are assumed to be equally important; all sub-attributes for the given attribute are assumed to be equally important; all sub-sub-attributes for the given sub-attributes are assumed to be equally important.

Overall rank score ($R_{overall}$)

I calculated the overall score of a given ith antibiotic using Eq. (2), where $R_{i,overall}$ represents the overall rank scores of the antibiotic, $R_{j,i}$ represents the score of the corresponding criterion, $W_{j,i}$ represents the importance weight of the jth criterion and N represents the total number of criteria. Similarly, as the two criteria were also considered equally important to avoid any judgment bias, the value of $W_{j,i}$ was set as 1/2.

$$R_{i,overall} = \sum_{j=1}^{N} R_{j,i} * W_{j,i} \quad (2)$$

An expression for calculating an overall rank score of a given ith antibiotic can be written as $R_{i,overall} = R_{e,i} * W_{e,i} + R_{h,i} * W_{h,i}$ where $W_{e,i}$ and $W_{h,i}$ represent the importance weights of the

environmental risk (e) and the human health risk (h) criteria, respectively (each assumed to be equal to ½ in this study). Here, $R_{e,i}$ and $R_{h,i}$ represent the rank scores of the environmental risk and the human health risk criteria, respectively.

The rank scores of multiple criteria ($R_{j,i}$) were calculated using Eq. (3), where $R_{k,j,i,}$ represents an attribute-based rank score, $W_{k,j,i}$ is the importance weight of each attribute for a kth attribute of a jth criterion, and N represents the total number of attributes. If the criterion has two attributes, both of them are considered equally important. Therefore, the importance weight $W_{k,j,i}$ was assigned ½ .

$$R_{j,i} = \sum_{K=1}^{N} R_{k,j,i} * W_{k,j,i} \quad (3)$$

An expression for calculating a rank score for the environmental risk criterion ($R_{e,i}$) (attributes: resistance risk (e1) and ecotoxicity risk (e2)); can be written as $R_{e,i} = R_{e1,e,i} * W_{e1,e,i} + R_{e2,e,i} * W_{e2,e,i}$ where $W_{e1,e,i}$ and $W_{e2,e,i}$ represent the importance weights of the resistance risk and the ecotoxicity risk attributes, respectively. Here, $R_{e1,e,i}$ and $R_{e2,e,i}$ represent rank scores of the resistance risk and the ecotoxicity risk attributes, respectively. A similar expression was developed for calculating the rank scores of the attributes, sub-attributes and sub-sub-attributes.

An expression for calculating a rank score for the human health criterion ($R_{h,i}$) (attributes: resistance risk (h1) and toxicity risk (h2)); can be written as $R_{h,i} = R_{h1,h,i} * W_{h1,h,i} + R_{h2,h,i} * W_{h2,h,i}$ where $W_{h1,h,i}$ and $W_{h2,h,i}$ represent the importance weights of the resistance risk and the toxicity risk attributes, respectively (assumed to be equal to ½ for each attribute). Here, $R_{h1,h,i}$ and $R_{h2,h,i}$ represent the rank scores of the resistance risk and the toxicity risk attributes, respectively. Similar expressions were developed for calculating the rank scores of the attributes and sub-attributes.

Database uncertainty

A database uncertainty of an antibiotic is represented by calculating an overall data gap rank score. For calculating this score, values of utility functions are assigned ½ for attributes, sub-attributes and sub-sub-attributes with missing data for a given antibiotic and assigned 0 for attributes, sub-attributes and sub-sub-attributes with available antibiotic data. In these calculations, the values of the importance weights of the criteria, attributes, sub-attributes and sub-sub-attributes are kept constant, obtaining values from Table 2. The overall data gap was quantitatively calculated using Eq. (4), where $R_{j,i,datagap}$ represent data gap scores of multiple criteria. A data gap score of a criterion is calculated using Eq. (5) and depends on $W_{k,j,i}$ and the data gap scores of multiple attributes ($R_{k,j,i,datagap}$). A similar expression was developed for calculating the data gap scores of the attributes, sub-attributes and sub-sub-attributes.

$$R_{i,datagap} = \sum_{j=1}^{N} R_{j,i,datagap} * W_{j,i} \quad (4)$$

$$R_{j,i,datagap} = \sum_{k=1}^{N} R_{k,j,i,datagap} * W_{k,j,i} \quad (5)$$

4.4 ETHICAL CONSIDERATIONS

Ethical approval was obtained for Study I from the first Affiliated Hospital, College of Medicine, Zhejiang University, China, reference number 2015#185. Ethical approval was obtained for Study II from the Ethics Committee of the R.D. Gardi Medical College, Ujjain, MP, India (No: 2013/07/17-311). In Study III, a systematic review and risk assessment methods were performed. Since all data were extracted from the published literature, an ethical approval was not required. Study IV was methodology development based on risk assessment methods and did not require ethical approval.

5 FINDINGS

5.1 Antibiotic residues were detected in various environmental compartments of rural Shandong province, China. The concentration levels of enrofloxacin, levofloxacin, and ciprofloxacin in wastewater were estimated to pose environmental risks for the development and selection of antibiotic resistance in bacteria. The concentration of antibiotic residues in drinking water and vegetables were estimated to pose no appreciable direct risk to human health through consumption (Study I)

Water samples and non-water samples taken from villages in Shandong province in eastern China were found to contain metronidazole, sulfapyridine, norfloxacin, levofloxacin, ciprofloxacin, enrofloxacin, doxycycline, sulfamethoxazole, florfenicol, and chloramphenicol. In water samples, the mean concentrations detected in river water varied from 0.101 ng/L (metronidazole) to 4.359 ng/L (norfloxacin). In wastewater, the range was from 0.0309 ng/L (chloramphenicol) to 646.715 ng/L (levofloxacin). The highest concentration in drinking water was for ciprofloxacin (6.205 ng/L). For non-water samples, the highest mean concentrations in river sediment, outlet sediment, pig manure, soil, and vegetables were found for enrofloxacin (2.205 μg/kg), ciprofloxacin (80.749 μg/kg), norfloxacin (32.782 μg/kg), ciprofloxacin (2.805 μg/kg), and ciprofloxacin (44.584 μg/kg), respectively.

The RQ values for the estimated environmental risk of antibiotic resistance selection were > 1 for enrofloxacin (1.821) and levofloxacin (2.587). The RQ value for ciprofloxacin was 0.127, and all other RQ values were < 0.1 (Table 3). MECs/$PNEC_{DW}$ ratios were < 1 from exposure to antibiotics through drinking water for adults and children. EDI/ADI ratios were < 0.1 from exposure to antibiotics through the consumption of vegetables.

Table 3. Environmental risk of antibiotic residues in water samples

Antibiotics	PNECs µg/L	River water RQ	Waste water RQ	Drinking water RQ
Sulfapyridine	10	0.0000135	0.0000569	0.0000052
Sulfamethoxazole	16	0.0000813	0.000087	0.00013
Ciprofloxacin	0.064	0.0135	0.127	0.096
Enrofloxacin	0.064	0.043	1.821	0.0067
Levofloxacin	0.25	0.00213	2.587	-
Norfloxacin	0.5	0.0087	0.0035	0.00048
Chloramphenicol	8	-	0.0000037	-
Florfenicol	2	0.00046	0.0020	0.00089
Doxycycline	2	0.000163	0.0065	0.000015
Metronidazole	0.125	0.00081	0.00139	-

Abbreviations: PNECs: predicted no effect concentrations for the development of antibiotic resistance, RQ: risk quotient.

5.2 Antibiotic residues and resistant *E. coli* were present in the water and sediment of the Kshipra river in India and showed significant seasonal and spatial variations over a 3-year period, and had varying associations with measured water quality parameters (Study II)

Antibiotics including norfloxacin, ofloxacin, metronidazole, and sulfamethoxazole were detected in the water of the Kshipra river in various seasons from the seven sampling sites over a 3-year period. The mean concentrations detected in the river water varied from 0.27 µg /L (metronodazole) to 4.66 µg /L (sulfamethoxazole). Significant ($p < 0.05$) seasonal and spatial variations in the occurrence of sulfamethoxazole and ofloxacin were found over a 3-year period (Figure 4).

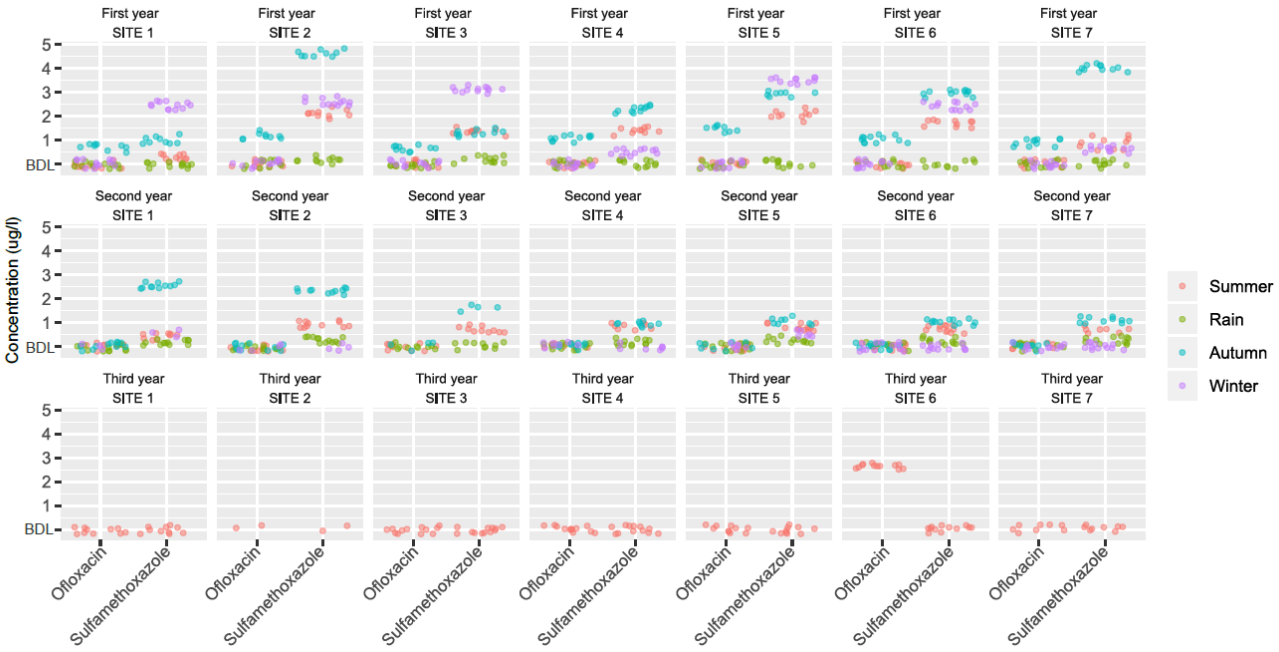


Figure 4. Concentrations of antibiotic residues measured in waters of the Kshipra river in India in various seasons and at various sites over a 3-year period.
Note: Significant ($p < 0.05$) seasonal and spatial variations in the occurrence of sulfamethoxazole and ofloxacin were found over a 3-year period.

Antibiotic resistant *E. coli* were present in water and sediment during various seasons and at various sampling sites over the 3-year period. In water, significant ($p < 0.05$) seasonal and spatial variations in the resistance of *E. coli* to ampicillin, cefepime, amikacin, tetracycline, meropenem, nalidixic acid, co-trimoxazole, and sulfamethizole were found (Figure 5). There were significant seasonal and spatial variations in the occurrence of multidrug resistant (MDR) *E. coli*. In *E. coli* from sediment samples, significant ($p < 0.05$) seasonal and spatial variations in the resistance were found for ampicillin, cefotaxime, ceftazidime, meropeneme, and nitrofurantoin. There were significant seasonal and spatial variations in the occurrence of extended spectrum β-lactamase (ESBL) and MDR *E.coli*. Antibiotic resistance genes detected in resistant *E. coli* isolated from water and sediment samples of the river. Most *E. coli* isolates from both river water and sediment belonged to phylogenetic groups A and B1.

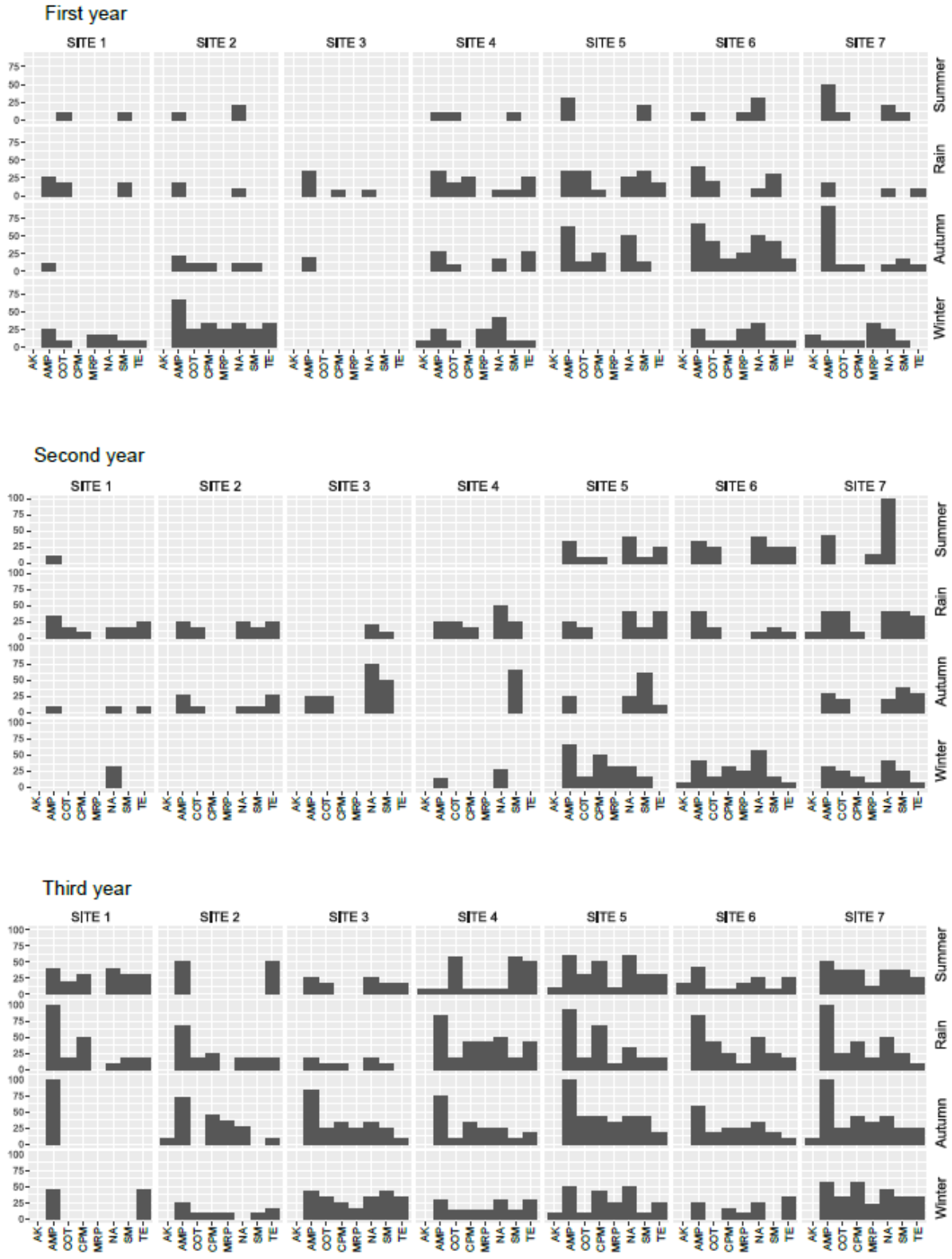


Figure 5. Antibiotic resistance patterns in *E. coli* isolated from water samples of the Kshipra river in India in various seasons and at various sites over a 3-year period.
Note: Significant ($p < 0.05$) seasonal and spatial variations in the resistance of *E. coli* to amikacin, ampicillin, co-trimoxazole, cefepime, meropenem, nalidixic acid, sulfamethizole, and tetracycline were found over a 3-year period.
Abbreviations: AK: Amikacin, AMP: Ampicillin, COT: Co-trimoxazole, CPM: Cefepime, MRP: Meropenem, NA: Nalidixic Acid, SM: Sulfamethizole, TE: Tetracycline.

Sulfamethoxazole was significantly correlated with water quality parameters ($p < 0.05$). The resistance of *E. coli* to antibiotics (e.g., sulfamethiazole, norfloxacin, ciprofloxacine, cefotaxime, co-trimoxazole, ceftazidime, meropenem, ampicillin, amikacin, metronidazole, tetracycline, and tigecycline) had varying associations with measured water and sediment quality parameters.

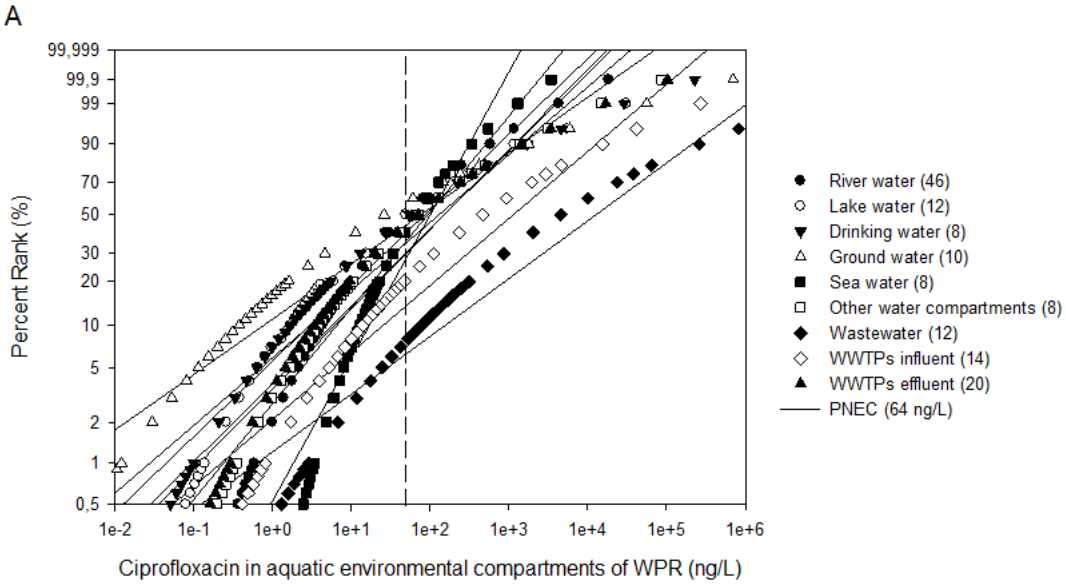
5.3 In various aquatic compartments of the environment of the WPR, the SEAR, China, and India, a) antibiotic residues were ubiquitous, b) residual concentrations of some antibiotics exceeded PNECs for the development of resistance in various proportions of exposure, c) the highest likelihood of exceedances of antibiotic PNECs (up to 100%) were observed in wastewater, and WWTPs' influents and effluents, d) the likelihood of exceedances of antibiotic PNECs were also observed in receiving aquatic environments, and e) the highest risk for the development of resistance in drinking water of the WPR and China was observed for ciprofloxacin (62.5%) (Study III)

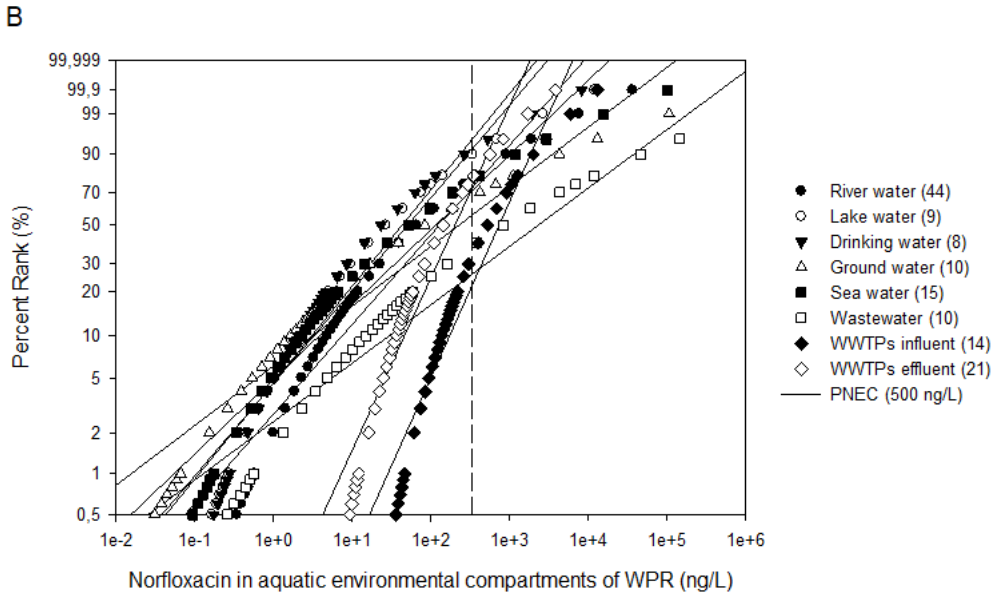
Studies measuring antibiotic residue concentrations in the aquatic environment have been reported. The systematic review included 218 studies out of 9346 screened from the WPR, 22 studies out of 4148 screened from the SEAR, and have largely been from China (n=168) and India (n=15), between 2006 and 2019. In the WPR, 92 antibiotics were detected, and in the SEAR, 45 antibiotics were detected. The most updated and precise analytical procedure, LC-MS/MS was used in most published studies. The overall risk of bias in the included studies were low.

Antibiotic residues were detected in diverse aquatic environmental compartments of the WPR and SEAR. Antibiotics from various classes such as fluoroquinolones (e.g., ciprofloxacin, norfloxacin, and levofloxacin), macrolides (e.g., erythromycin, azithromycin, clarithromycin, and roxithromycin), tetracyclines (e.g., tetracycline, oxytetracycline, chlortetracycline, and doxycycline), β-lactams (e.g., penicillins), lincosamides (e.g., lincomycin), sulfonamides (e.g., sulfamethoxazole and sulfamethazine), amphenicols (e.g., chloramphenicol and florfenicol), glycopeptides (e.g., vancomycin), and aminoglycosides (e.g., gentamicin) were reported to be found in wastewater (municipal, hospital, and pharmaceutical manufacturing/industry), STPs'/WWTPs' influents and effluents, and receiving aquatic environments of the WPR and SEAR. The most frequently detected antibiotics include fluoroquinolones, macrolides, tetracyclines, and sulfonamides in hospital, municipal, and industrial wastewater, and STPs/WWTPs of the WPR and SEAR, and azithromycin, ciprofloxacin, ofloxacin, norfloxacin, enrofloxacin, sulfamethoxazole, sulfamethazine, sulfadiazine, tetracycline, chlortetracycline, oxytetracycline, clarithromycin, roxithromycin, erythromycin, and trimethoprim in the receiving aquatic environments of the WPR and SEAR.

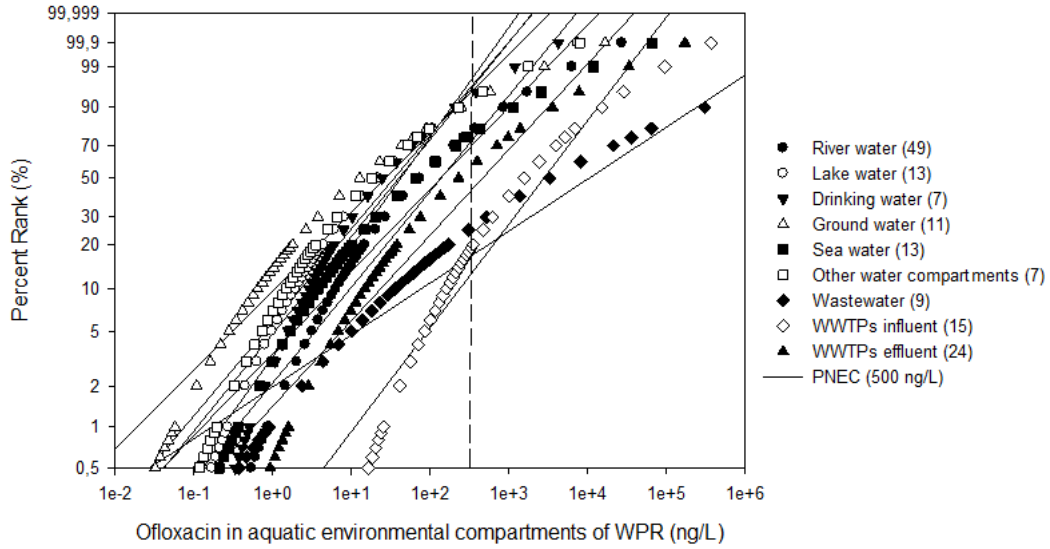
The results from the PEHA indicated values of predicted threshold concentrations corresponding to different centiles (1, 5, 25, 50, 75, 95, and 99) for the EEDs of the mMECs of antibiotics. They also indicated the likelihood of exceedances of antibiotic PNECs, for fluoroquinolones, macrolides, tetracyclines, β-lactams, lincosamides, sulfonamides, amphenicols, and trimethoprim for the WPR and China. The analysis for the SEAR indicated the likelihood of exceedances of antibiotic PNECs for fluoroquinolones, macrolides, sulfonamides, and

trimethoprim for aquatic environments of the SEAR, and for fluoroquinolones for aquatic environments of India. The results are presented in Appendix 2, Appendix 3, Appendix 4, and Appendix 5, and some antibiotics are presented as an example in Figure 6 and Figure 7.

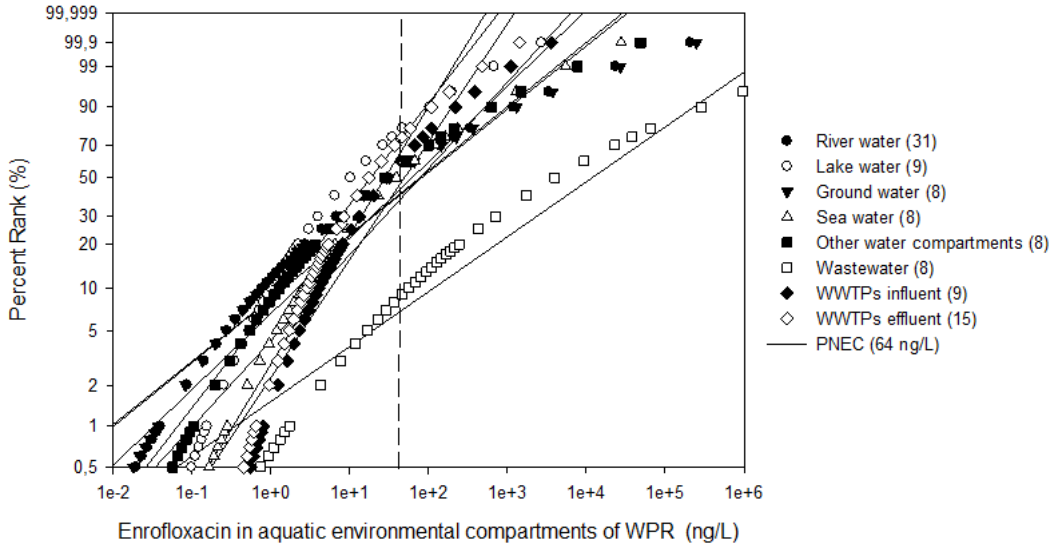




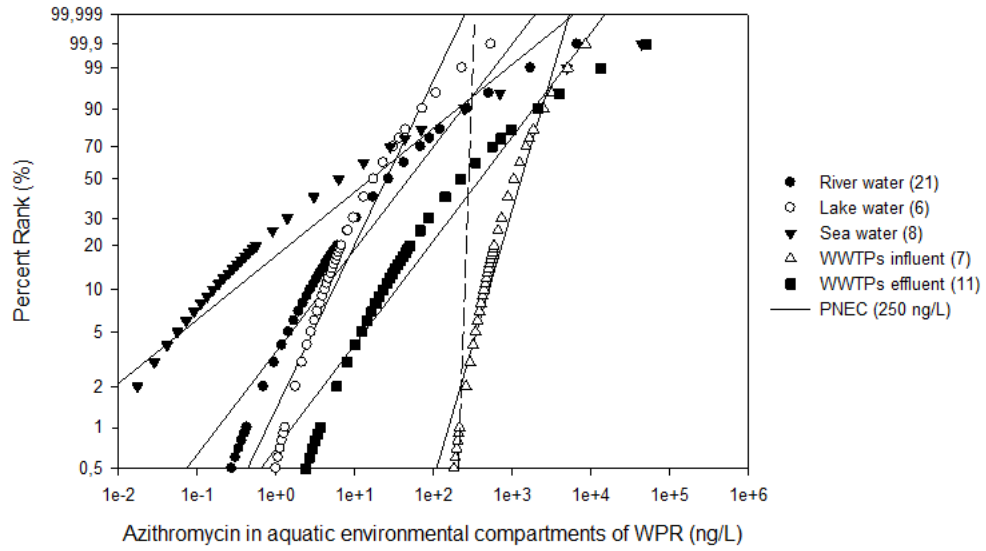
C


99,999
99,9
99
90
70
50
30
20
10
5
2
1
0,5
Percent Rank (%)
1e-2
1e-1
1e+0
1e+1
1e+2
1e+3
1e+4
1e+5
1e+6
Ofloxacin in aquatic environmental compartments of WPR (ng/L)
River water (49)
Lake water (13)
Drinking water (7)
Ground water (11)
Sea water (13)
Other water compartments (7)
Wastewater (9)
WWTPs influent (15)
WWTPs effluent (24)
PNEC (500 ng/L)

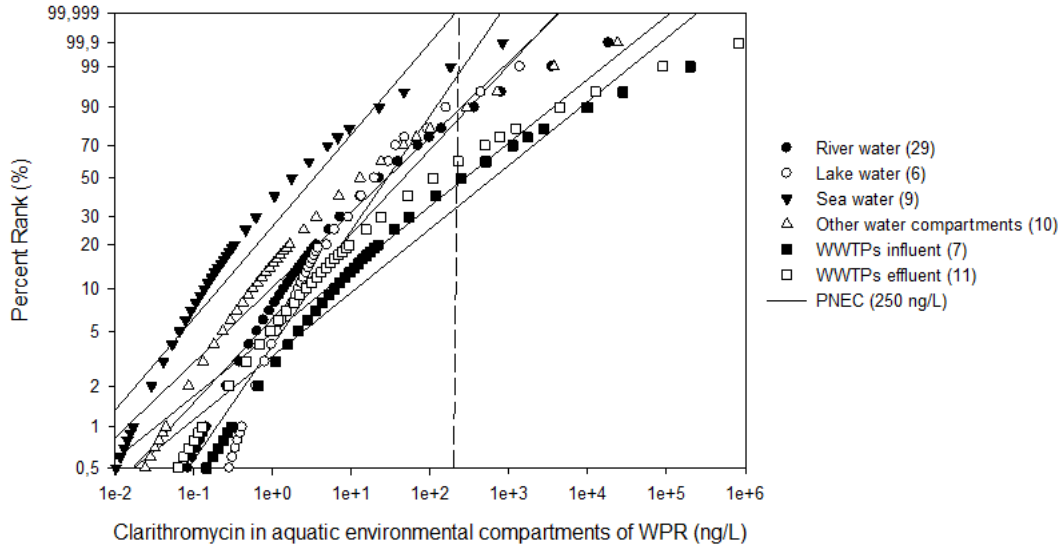
D


99,999
99,9
99
90
70
50
30
20
10
5
2
1
0,5
Percent Rank (%)
1e-2
1e-1
1e+0
1e+1
1e+2
1e+3
1e+4
1e+5
1e+6
Enrofloxacin in aquatic environmental compartments of WPR (ng/L)
River water (31)
Lake water (9)
Ground water (8)
Sea water (8)
Other water compartments (8)
Wastewater (8)
WWTPs influent (9)
WWTPs effluent (15)
PNEC (64 ng/L)

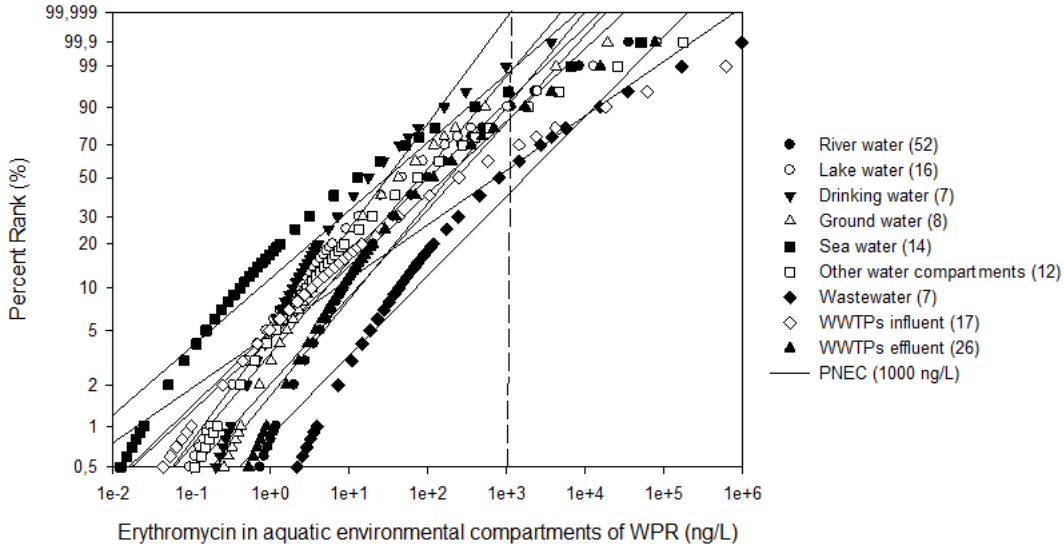
E


99,999
99,9
99
90
70
50
30
20
10
5
2
1
0,5
Percent Rank (%)
1e-2
1e-1
1e+0
1e+1
1e+2
1e+3
1e+4
1e+5
1e+6
Azithromycin in aquatic environmental compartments of WPR (ng/L)
River water (21)
Lake water (6)
Sea water (8)
WWTPs influent (7)
WWTPs effluent (11)
PNEC (250 ng/L)

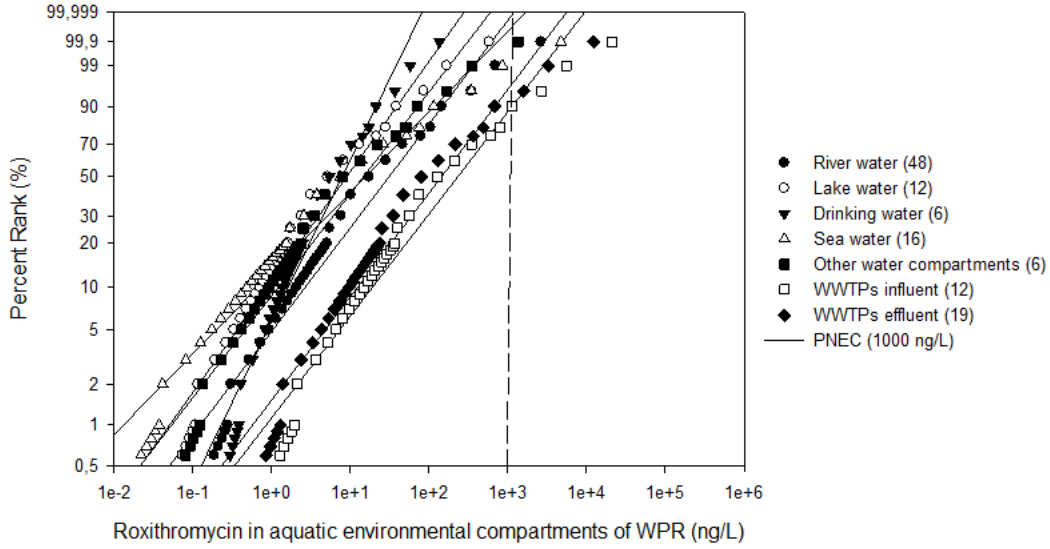
F


99,999
99,9
99
90
70
50
30
20
10
5
2
1
0,5
Percent Rank (%)
1e-2
1e-1
1e+0
1e+1
1e+2
1e+3
1e+4
1e+5
1e+6
Clarithromycin in aquatic environmental compartments of WPR (ng/L)
River water (29)
Lake water (6)
Sea water (9)
Other water compartments (10)
WWTPs influent (7)
WWTPs effluent (11)
PNEC (250 ng/L)

G


99,999
99,9
99
90
70
50
30
20
10
5
2
1
0,5
Percent Rank (%)
1e-2
1e-1
1e+0
1e+1
1e+2
1e+3
1e+4
1e+5
1e+6
Erythromycin in aquatic environmental compartments of WPR (ng/L)
River water (52)
Lake water (16)
Drinking water (7)
Ground water (8)
Sea water (14)
Other water compartments (12)
Wastewater (7)
WWTPs influent (17)
WWTPs effluent (26)
PNEC (1000 ng/L)

H


99,999
99,9
99
90
70
50
30
20
10
5
2
1
0,5
Percent Rank (%)
1e-2
1e-1
1e+0
1e+1
1e+2
1e+3
1e+4
1e+5
1e+6
Roxithromycin in aquatic environmental compartments of WPR (ng/L)
River water (48)
Lake water (12)
Drinking water (6)
Sea water (16)
Other water compartments (6)
WWTPs influent (12)
WWTPs effluent (19)
PNEC (1000 ng/L)

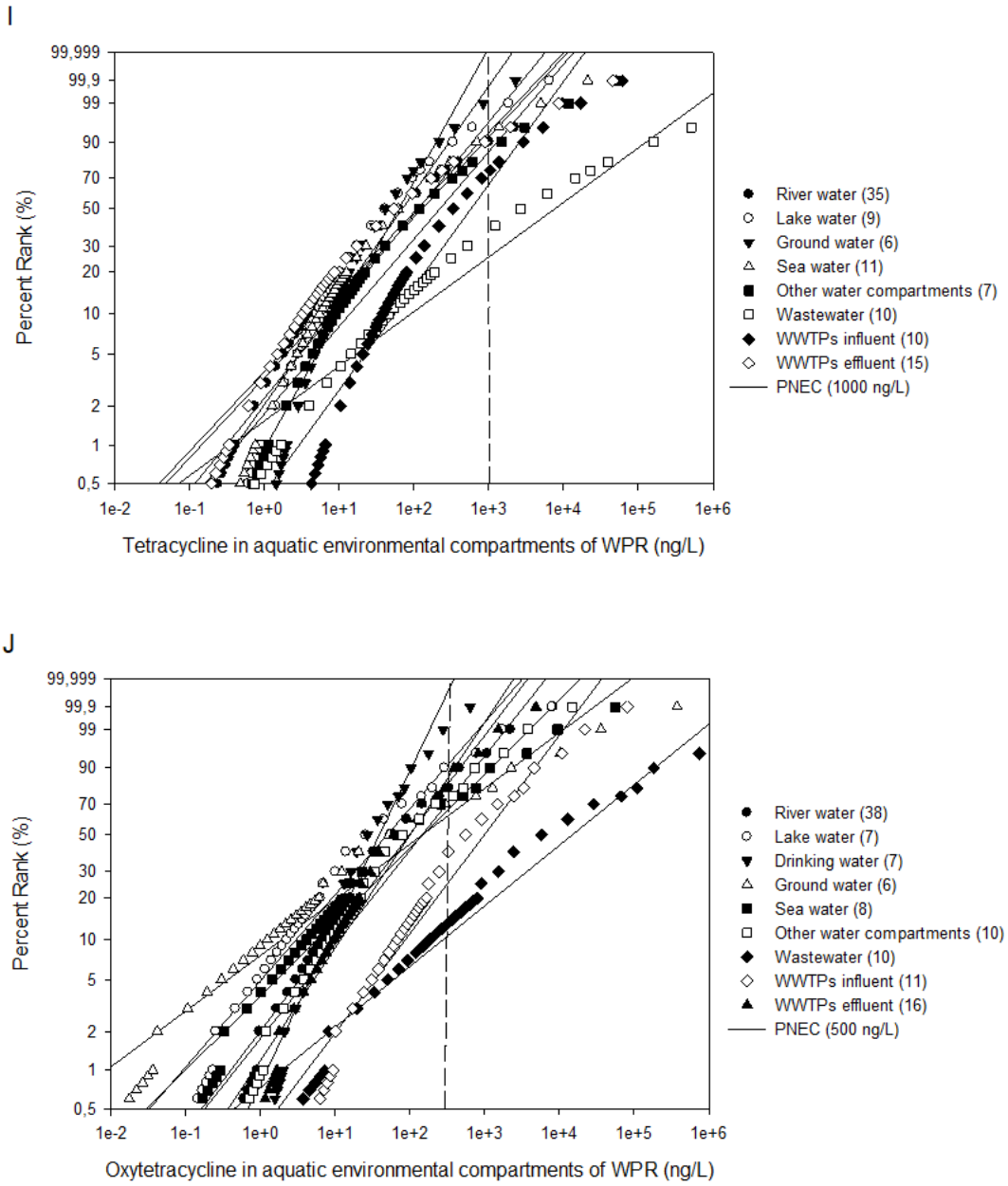
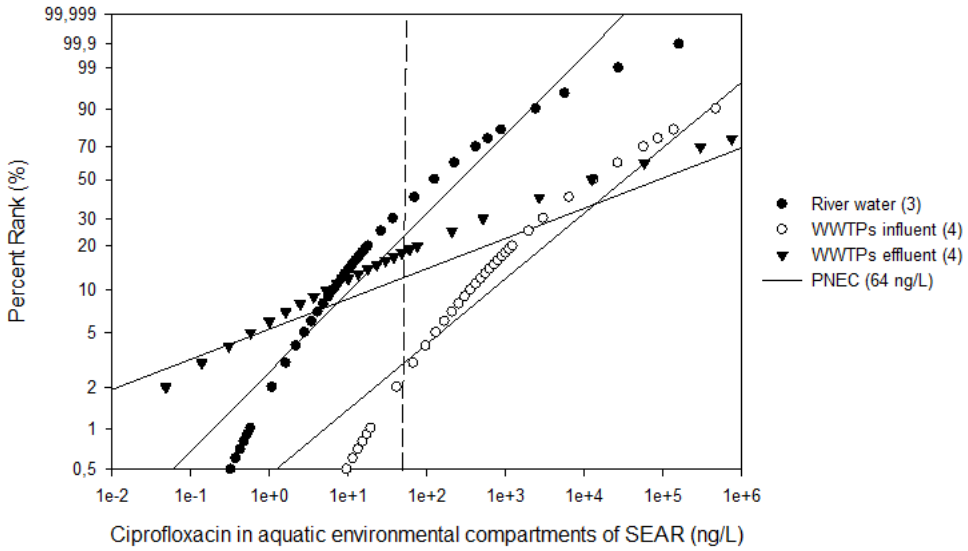
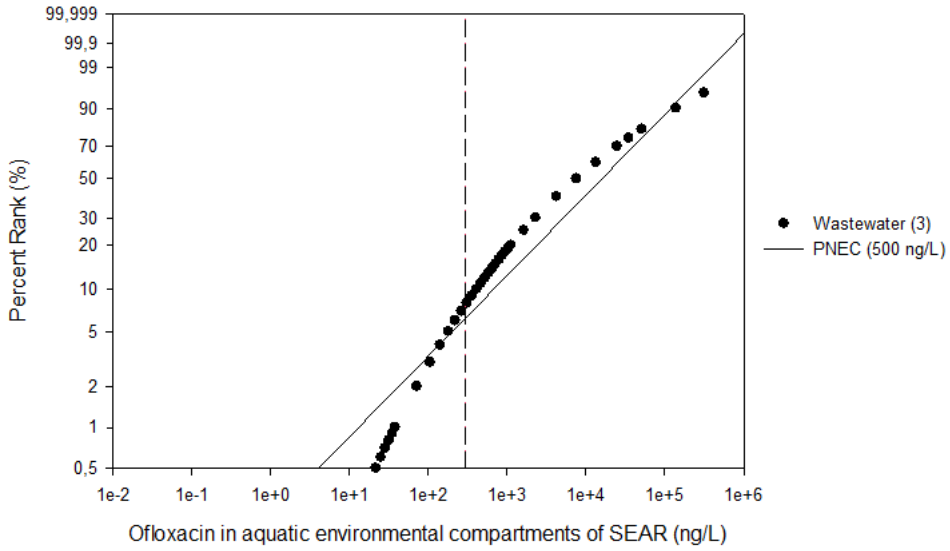


Figure 6. Environmental exposure distributions of measured environmental concentrations (MECs) in various aquatic environmental compartments of the Western Pacific Region (WPR) of the WHO, for: (A) ciprofloxacin; (B) norfloxacin; (C) ofloxacin; (D) enrofloxacin; (E) azithromycin; (F) clarithromycin; (G) erythromycin; (H) roxithromycin; (I) tetracycline; and (J) oxytetracycline. Vertical lines correspond to antibiotic Predicted No Effect Concentration (PNEC; ng/ L) for the development of antibiotic resistance. Numbers in parenthesis indicate number of data points.

A


99,999
99,9
99
90
70
50
30
20
10
5
2
1
0,5
Percent Rank (%)
1e-2
1e-1
1e+0
1e+1
1e+2
1e+3
1e+4
1e+5
1e+6
Ciprofloxacin in aquatic environmental compartments of SEAR (ng/L)
River water (3)
WWTPs influent (4)
WWTPs effluent (4)
PNEC (64 ng/L)

B


99,999
99,9
99
90
70
50
30
20
10
5
2
1
0,5
Percent Rank (%)
1e-2
1e-1
1e+0
1e+1
1e+2
1e+3
1e+4
1e+5
1e+6
Ofloxacin in aquatic environmental compartments of SEAR (ng/L)
Wastewater (3)
PNEC (500 ng/L)

C

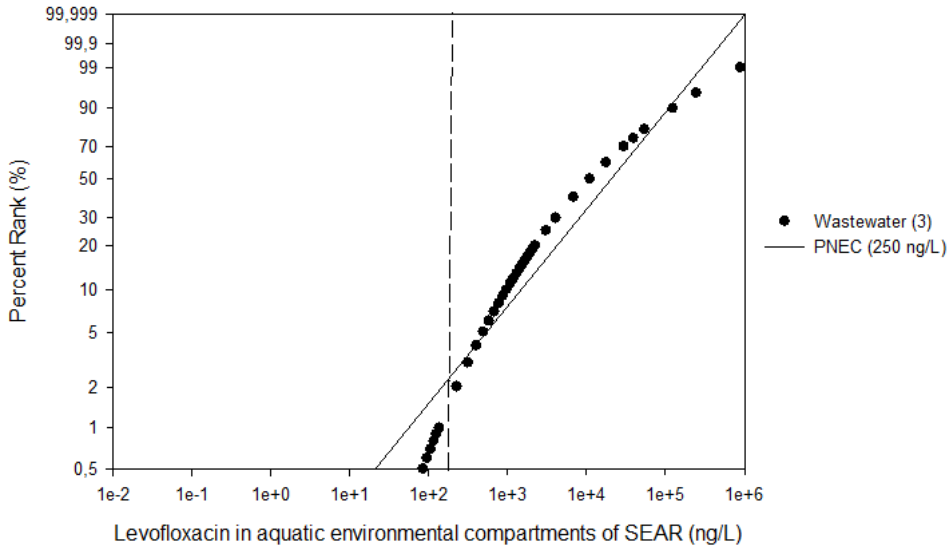

99,999
99,9
99
90
70
50
30
20
10
5
2
1
0,5
Percent Rank (%)
1e-2
1e-1
1e+0
1e+1
1e+2
1e+3
1e+4
1e+5
1e+6
Levofloxacin in aquatic environmental compartments of SEAR (ng/L)
Wastewater (3)
PNEC (250 ng/L)

Figure 7. Environmental exposure distributions of measured environmental concentrations (MECs in various aquatic environmental compartments of the South East Asia Region (SEAR) of the WHO, for: (A) ciprofloxacin; (B) ofloxacin; (C) levofloxacin. Vertical lines correspond to antibiotic Predicted No Effect Concentration (PNEC; ng/ L) for the development of antibiotic resistance. Numbers in parenthesis indicate number of data points.

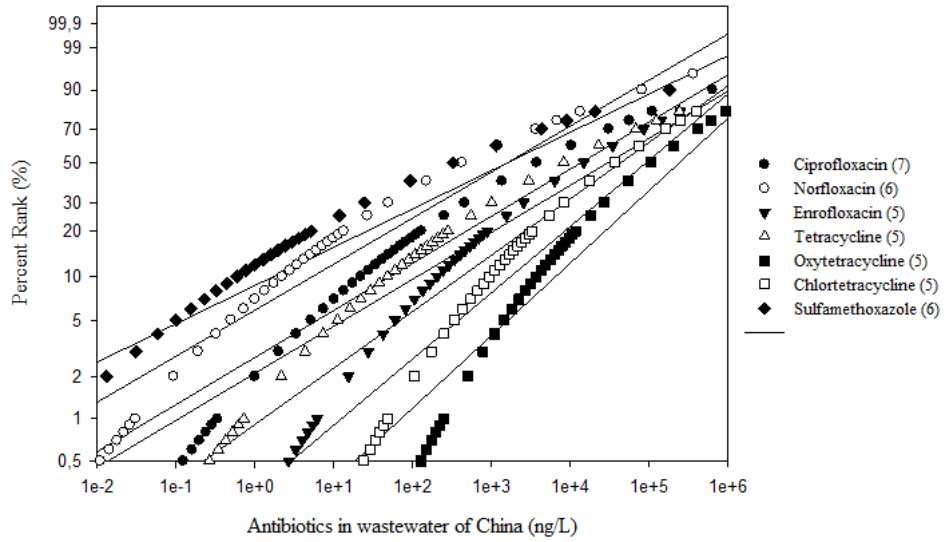
If the 5th centiles were considered, the highest levels of predicted threshold concentrations of most antibiotics and the highest likelihood of exceedances of antibiotic PNECs (up to 100%) were observed in wastewater and WWTPs' influents of the WPR, the SEAR, China, and India.

In receiving aquatic environments, the likelihood of exceedances of antibiotic PNECs for the development of resistance was only observed for ciprofloxacin in the river water of the SEAR. The likelihoods of exceedances of antibiotic PNECs for the development of resistance were regularly observed for fluoroquinolones, macrolides, and tetracyclines in most receiving aquatic environmental compartments of the WPR and China. Contamination of drinking water by ciprofloxacin, norfloxacin, ofloxacin, erythromycin, roxithromycin, tylosin, oxytetracycline, doxycycline, penicillin, lincomycin, sulfamethoxazole, sulfadiazine, sulfamethazine, sulfamonomethoxin, sulfapyridine, and trimethoprim was found in the WPR and China. If the 95th centiles were considered, the highest levels and likelihood of exceeding antibiotic PNECs (62.5%) were observed for ciprofloxacin in the drinking water of the WPR and China (no drinking water results were found for the SEAR and India).

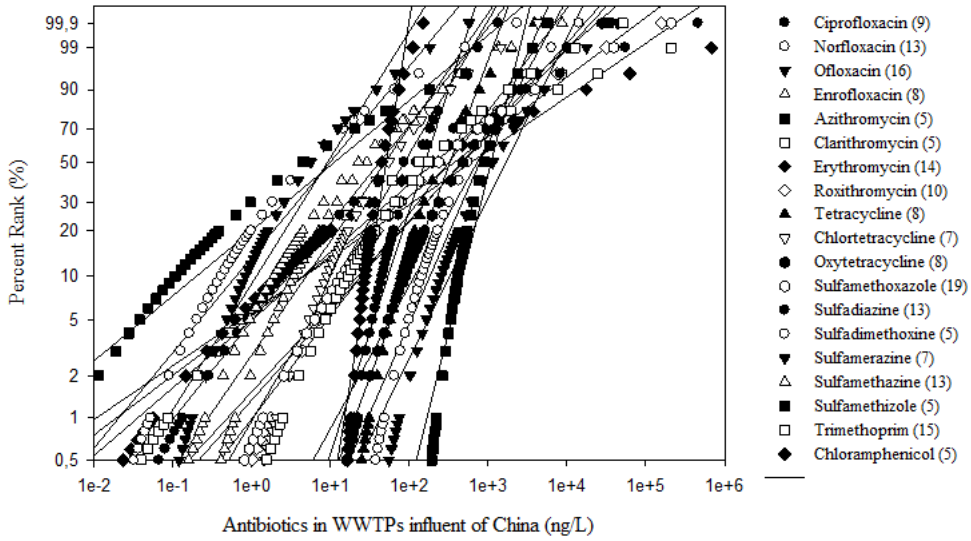
5.4 A list of priority antibiotics in various aquatic environmental compartments of China was developed by ranking antibiotics in descending order, based on their a) overall risk, b) resistance risk on environment, c) ecotoxicity risk, d) overall environmental risk, e) resistance risk on human health, f) toxicity risk on human health, and g) overall human health risk. Ciprofloxacin posed the greatest risk (Study IV)

Studies measuring the antibiotic residue concentrations in the aquatic environment have been reported from China in 168 publications, between 2006 and 2019. Data for 41 antibiotics were utilised to perform the proposed approach (our unpublished data, to be published separately). Results from PEHA indicated values of predicted threshold concentrations corresponding to various centiles (1, 5, 25, 50, 75, 95, and 99) for measured environmental concentration distributions of the maximum reported antibiotic concentrations (ng/L) in the aquatic compartments of China, and the likelihood of exceedances of antibiotics of PNECs for the development of antibiotic resistance, $PNEC_E$ for ecotoxicity, and TTC for toxicity to human health, for 41 antibiotics from various classes (Figure 8 and Appendix 4).

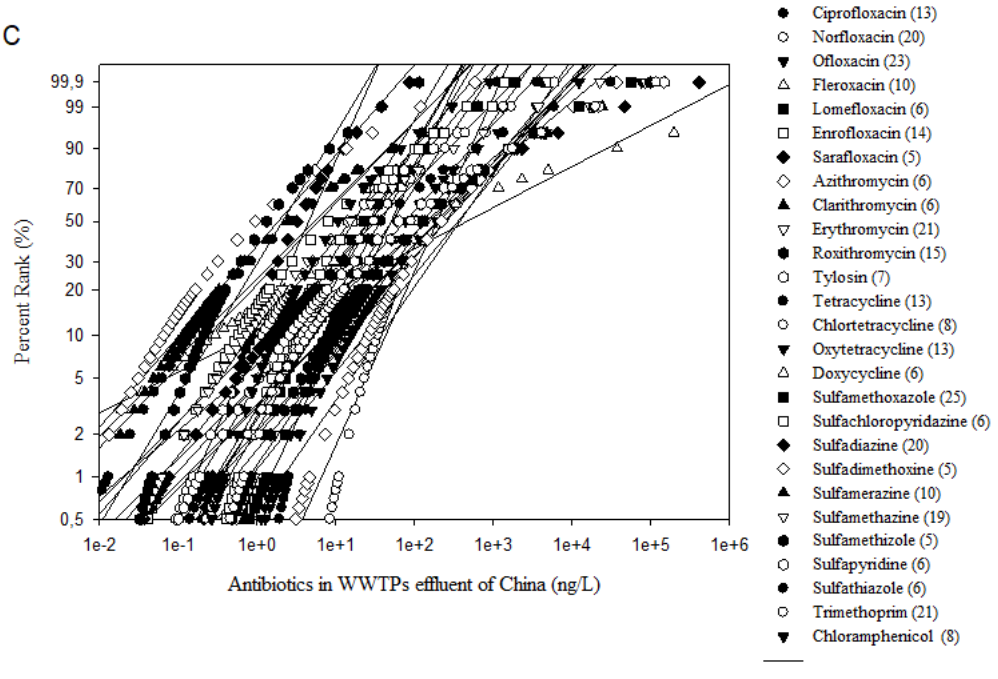
A


Percent Rank (%)
99,9
99
90
70
50
30
20
10
5
2
1
0,5
1e-2
1e-1
1e+0
1e+1
1e+2
1e+3
1e+4
1e+5
1e+6
Antibiotics in wastewater of China (ng/L)
Ciprofloxacin (7)
Norfloxacin (6)
Enrofloxacin (5)
Tetracycline (5)
Oxytetracycline (5)
Chlortetracycline (5)
Sulfamethoxazole (6)

B


Percent Rank (%)
99,9
99
90
70
50
30
20
10
5
2
1
0,5
1e-2
1e-1
1e+0
1e+1
1e+2
1e+3
1e+4
1e+5
1e+6
Antibiotics in WWTPs influent of China (ng/L)
Ciprofloxacin (9)
Norfloxacin (13)
Ofloxacin (16)
Enrofloxacin (8)
Azithromycin (5)
Clarithromycin (5)
Erythromycin (14)
Roxithromycin (10)
Tetracycline (8)
Chlortetracycline (7)
Oxytetracycline (8)
Sulfamethoxazole (19)
Sulfadiazine (13)
Sulfadimethoxine (5)
Sulfamerazine (7)
Sulfamethazine (13)
Sulfamethizole (5)
Trimethoprim (15)
Chloramphenicol (5)

C


Percent Rank (%)
99,9
99
90
70
50
30
20
10
5
2
1
0,5
1e-2
1e-1
1e+0
1e+1
1e+2
1e+3
1e+4
1e+5
1e+6
Antibiotics in WWTPs effluent of China (ng/L)
Ciprofloxacin (13)
Norfloxacin (20)
Ofloxacin (23)
Fleroxacin (10)
Lomefloxacin (6)
Enrofloxacin (14)
Sarafloxacin (5)
Azithromycin (6)
Clarithromycin (6)
Erythromycin (21)
Roxithromycin (15)
Tylosin (7)
Tetracycline (13)
Chlortetracycline (8)
Oxytetracycline (13)
Doxycycline (6)
Sulfamethoxazole (25)
Sulfachloropyridazine (6)
Sulfadiazine (20)
Sulfadimethoxine (5)
Sulfamerazine (10)
Sulfamethazine (19)
Sulfamethizole (5)
Sulfapyridine (6)
Sulfathiazole (6)
Trimethoprim (21)
Chloramphenicol (8)

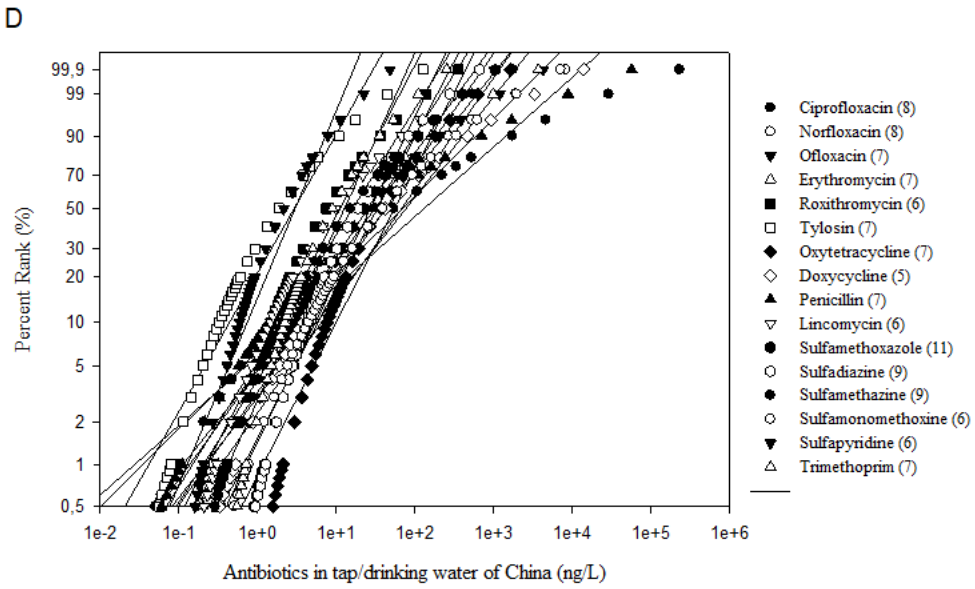


Figure 8. Environmental exposure distributions of measured environmental concentrations (MECs) of antibiotics in: (A) wastewater; (B) WWTPs influents; (C) WWTPs effluents; and (D) tap/drinking water of China. Numbers in parenthesis indicate the number of data points.

Overall and criterion ranking

Risk antibiotic residues in various aquatic environmental compartments of China based on the overall, criterion and attribute utility scores are presented in Figure 9. A list of priority antibiotics in various aquatic environmental compartments of China was developed by ranking antibiotics in descending order, based on overall, criterion, and attribute utility scores of their a) overall risk, b) resistance risk on environment, c) ecotoxicity risk, d) overall environmental risk, e) resistance risk on human health, f) toxicity risk on human health, and g) overall human health risk. Ciprofloxacin posed the greatest risk (Figure 10 and Appendix 6).

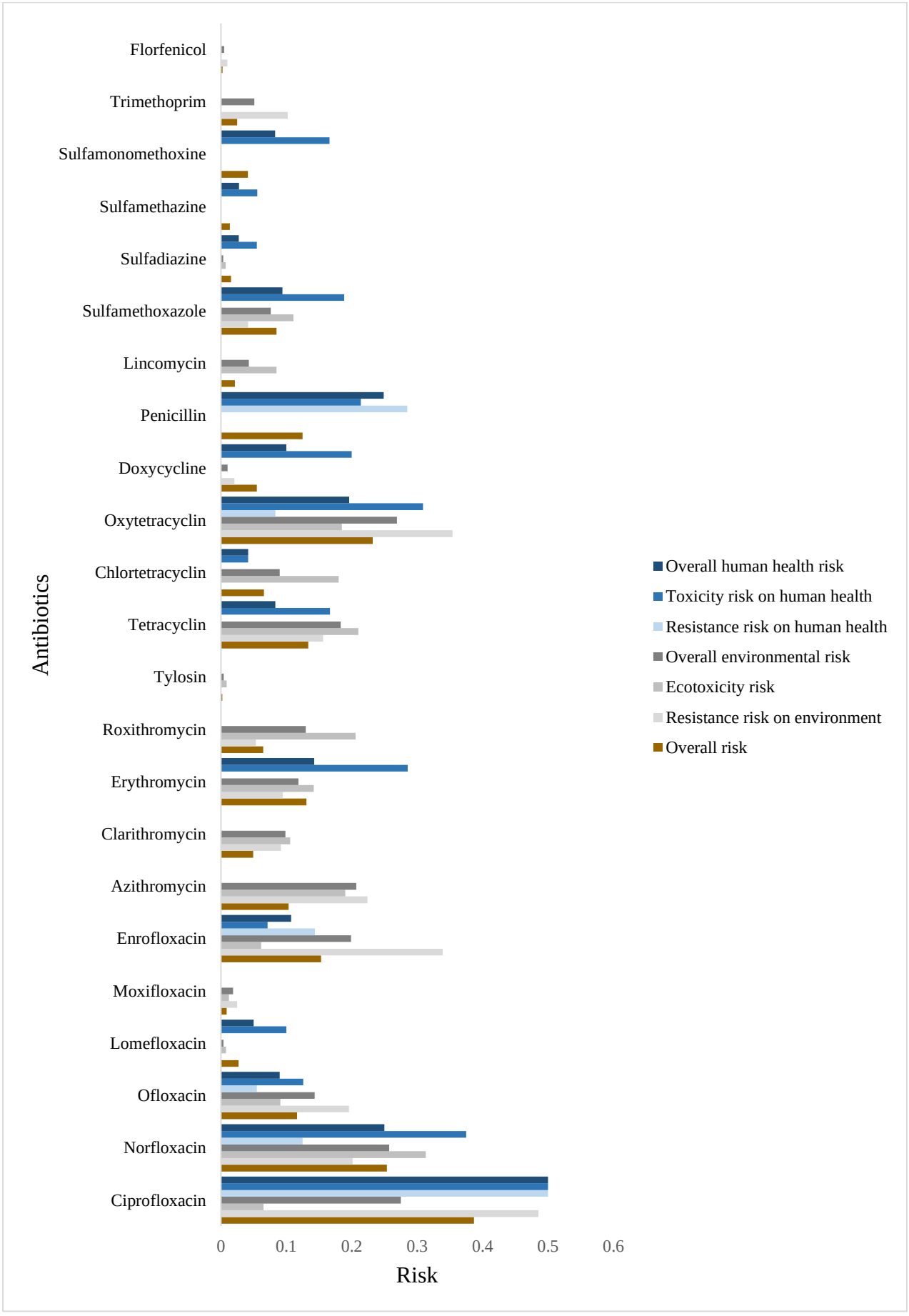


Figure 9. Potential risks of antibiotic residues in various aquatic environmental compartments of China, based on overall, criterion, and attribute risk utility scores (calculations made using data from publications from China between 2006-2019)

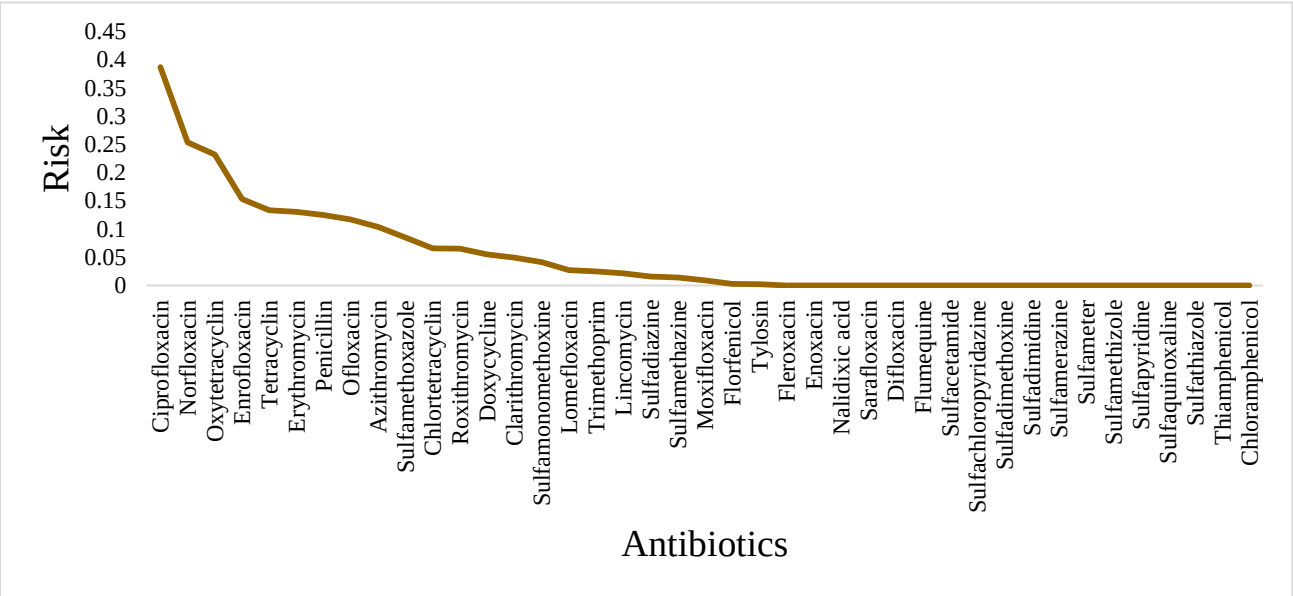


Figure 10. Ranking of risk from various antibiotics in aquatic environmental compartments of China, based on descending order of overall risk scores (calculations made using data from publications from China between 2006-2019)

Data gap-based ranking

Percentage data gaps of antibiotic residues in various aquatic environmental compartments of China based on overall, criterion and attribute data gap scores are presented in Figure 11. A list of data gaps of antibiotics in various aquatic environmental compartments of China was developed by ranking antibiotics based on descending orders of overall, criterion, and attribute data gap scores of their a) overall risk, b) resistance risk on environment, c) ecotoxicity risk, d) overall environmental risk, e) resistance risk on human health, f) toxicity risk on human health, and g) overall human health risk, and are presented in Figure 12 and Appendix 7.

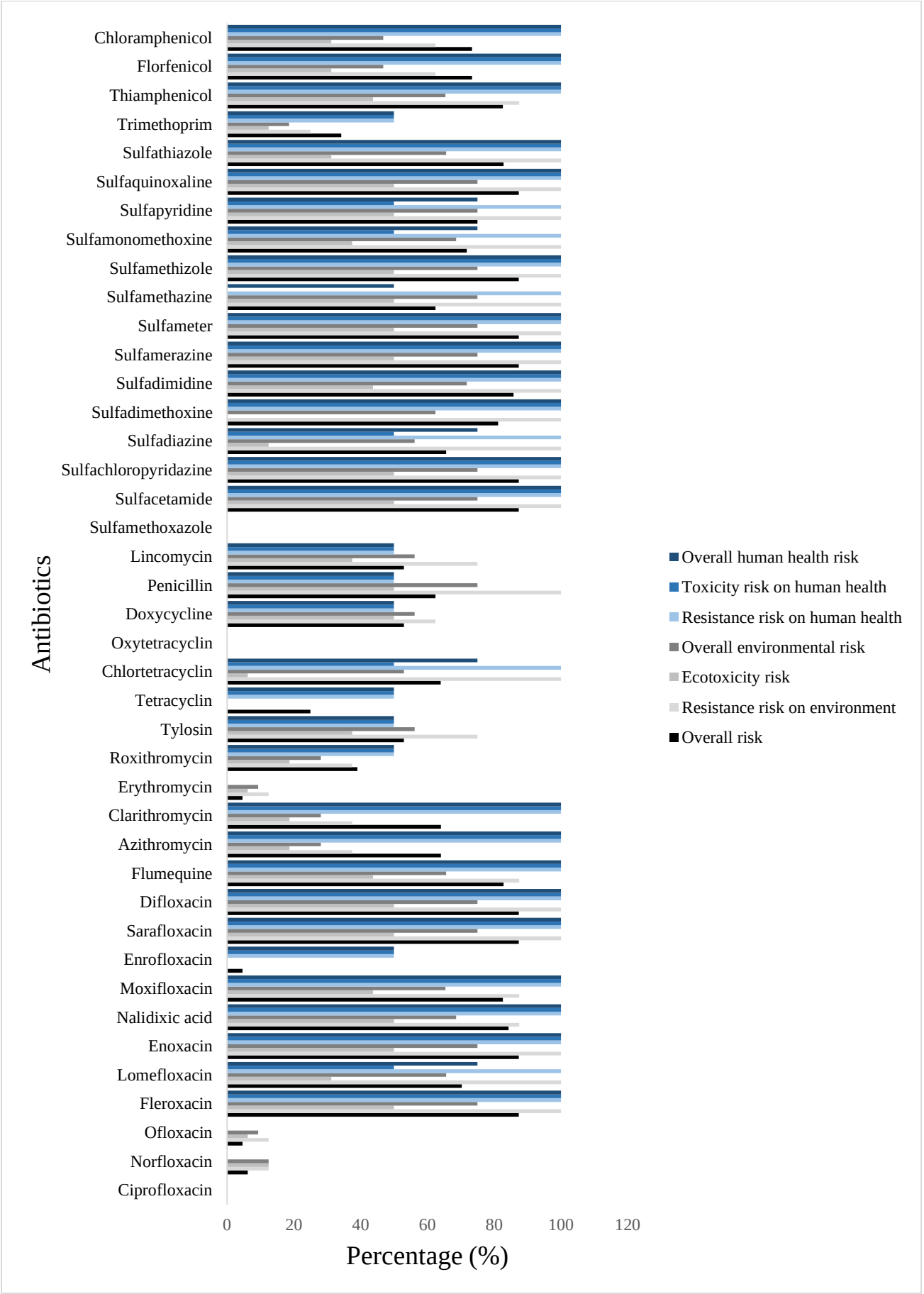


Figure 11. Percentage data gaps for antibiotics in various aquatic environmental compartments of China, based on overall, criterion, and attribute data gap scores (calculations made using data from publications from China between 2006-2019)

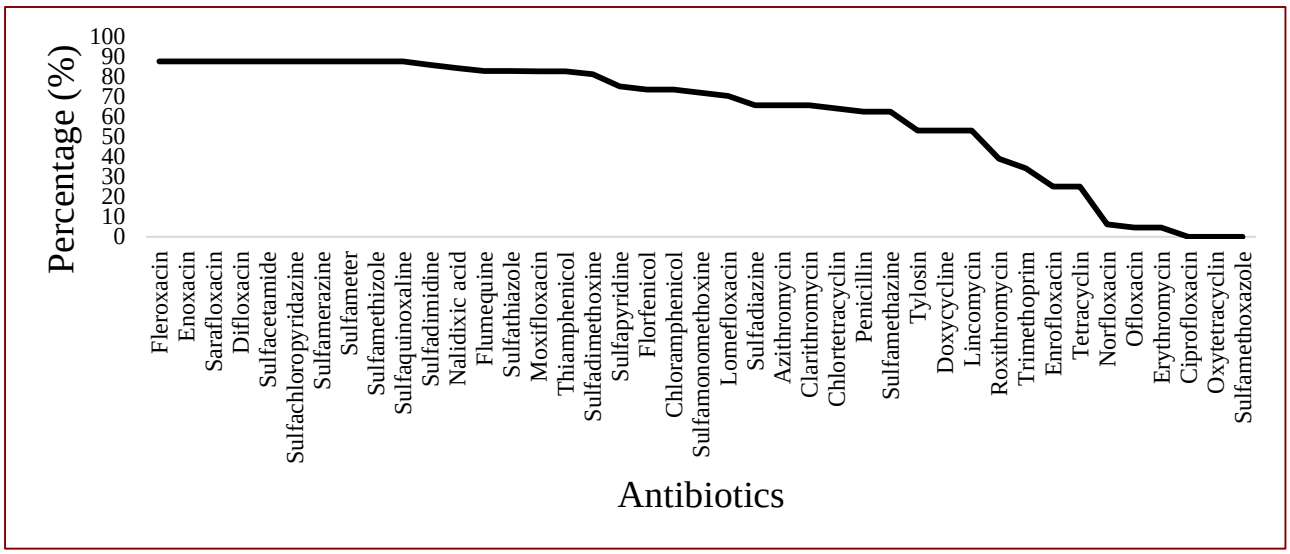


Figure 12. Ranking of data gap of antibiotics in various aquatic compartments of China, based on descending order of overall data gap scores (calculations made using data from publications from China between 2006-2019)

6 DISCUSSION

This thesis sheds light on the environmental and human health risks of antibiotic residues and antibiotic resistance in various environmental compartments of the WPR and SEAR, particularly China and India. I also further developed methods for a quantitative environmental and human health risk assessment of antibiotics and a prioritisation system. Antibiotic residues were present in various environmental compartments of the WPR, the SEAR, China, and India (Study I, Study II, and Study III). Antibiotic residues and antibiotic resistant *E. coli* were present in the water and sediment of the Kshipra river in India and showed significant seasonal and spatial variations over a 3-year period, and had varying associations with measured water quality parameters. Most *E. coli* isolates from both river water and sediment belonged to phylogenetic groups A and B1 (Study II). The most frequently detected antibiotics include fluoroquinolones, macrolides, tetracyclines, and sulfonamides in hospital, municipal, and industrial wastewater, and STPs/WWTPs of the WPR and the SEAR, and azithromycin, ciprofloxacin, ofloxacin, norfloxacin, enrofloxacin, sulfamethoxazole, sulfamethazine, sulfadiazine, tetracycline, chlortetracycline, oxytetracycline, clarithromycin, roxithromycin, erythromycin, and trimethoprim in the receiving aquatic environments of the WPR and SEAR. The values of predicted threshold concentrations corresponding to different centiles for the EEDs of the mMECs of antibiotics, and the likelihood of exceedances of antibiotic PNECs, for fluoroquinolones, macrolides, tetracyclines, β-lactams, lincosamides, sulfonamides, amphenicols, and trimethoprim for the WPR and China were indicated. The analysis for the SEAR indicated the likelihood of exceedances of antibiotic PNECs for fluoroquinolones, macrolides, sulfonamides, and trimethoprim for aquatic environments of the SEAR, and for fluoroquinolones for the aquatic environments of India (Study III). The highest environmental risks of antibiotic residues were observed in wastewater, STPs'/WWTPs' influents and effluents (Study I and Study III). Antibiotic residues appear to pose an appreciable human health risk (resistance and toxicity) from environmental exposure via drinking water of the WPR and China, and the highest risk was observed for ciprofloxacin. An ecotoxicity risk of antibiotic residues in the environment of China was also observed (Study III and Study IV). A list of priority antibiotics for China was developed by ranking antibiotics in descending order, based on their a) overall risk, b) resistance risk on environment, c) ecotoxicity risk, d) overall environmental risk, e) resistance risk on human health, f) toxicity risk on human health, and g) overall human health risk. Ciprofloxacin posed the greatest risk (Study IV).

6.1 THE OCCURRENCE OF ANTIBIOTIC RESIDUES IN THE ENVIRONMENT OF THE WPR, THE SEAR, CHINA, AND INDIA.

Antibiotic residues were ubiquitous in various environmental compartments of the WPR, the SEAR, China, and India. An enormous diversity in antibiotic residue levels and patterns was observed among various aquatic environmental compartments of the WPR, the SEAR, China, and India (Study I, Study III, and Study III). Such occurrence and variability are influenced by anthropogenic input such as antibiotic use; hospital, industrial, municipal, aquaculture, and

agricultural activities; inefficient wastewater treatment managements, and the physicochemical properties of the antibiotics that are reviewed below.

6.1.1 Antibiotic use

The antibiotic use in human and veterinary medicines is widely regarded as a major driving force in the presence of antibiotic residues in the environment (7, 131). A WHO report on the surveillance of antibiotic consumption in humans in 2015 showed notable variation in the total amount and type of antibiotics consumed between the countries within the regions. In most of the countries of the WPR, the most frequently used antibiotic classes were β-lactams, quinolones, macrolides, and tetracyclines. In the SEAR, the analysis of the different classes of antibiotics found a high level of consumption of quinolones in some of the countries (132). The consumption patterns of antibiotics in the WPR and the SEAR are reflected in their presence in various compartments of the environment, similar classes of antibiotics have been found in the aquatic environment of these regions (Study III).

6.1.2 Hospital, industrial, municipal, aquaculture, and agricultural activities

The occurrence of antibiotic residues in the environment can be attributed to hospital, industrial, municipal, aquaculture, and agricultural activities. High levels of sulfamethoxazole, norfloxacin, and ofloxacin were detected at site 2 (Khan) of the Kshipra river. Site 2 (Khan) is dominated by agricultural activities and is located on the banks of the river Khan, a sub-tributary of the Kshipra river. The Khan river brings pollutants from pharmaceutical industries nearby. The Khan is the major contributor of the contamination and degradation of the water quality of the Kshipra river (133) (Study II). Findings from Study III showed that high levels of antibiotic residues were typically associated with aquatic compartments routinely receiving urban wastewater discharge; high levels of antibiotics were found in the Haihe River, China, especially the sulfonamides, tetracyclines, and macrolides (134). High concentrations of antibiotics, including fluoroquinolones, in surface, ground, and drinking water were reported from Hyderabad, India, and the study suggested that the area in the vicinity of pharmaceutical plants was highly prone to antibiotic contamination, especially when WWTPs were inefficient (31). Detection of antibiotic residues in drinking water impacted by wastewater discharge has also been reported in the WPR and SEAR (29, 135, 136). For example, fluoroquinolones, including ciprofloxacin, were reported with considerably high concentration in the drinking waters of China (137, 138).

6.1.3 Inefficient wastewater treatment managements

Wastewater and STPs/WWTPs act as a major source and primary pathway for environmental contamination of antibiotic residues in the WPR, the SEAR, China, and India (Study III). The concentrations of antibiotic residues in STPs'/WWTPs' influents and effluents, and eventually in the receiving aquatic environment show significant spatial and temporal variations. For instance, in the STPs/WWTPs, antibiotics may mainly undergo biodegradation or sorption onto the activated sludge and transformation and precipitation, depending on the technology used in the STPs/WWTPs. The removal efficiencies were highly variable for different antibiotics and

different STPs/WWTPs. The conventional treatment plants often did not completely remove the antibiotics, consequently antibiotics could still be detected in the treated wastewater (effluents) (139–144). Whereas advanced wastewater treatment processes can generally achieve higher removal rates for antibiotics compared with conventional treatment (145). Treatment plants are, however, a major challenge in these regions, because of the intensive investment and associated high cost needed. According to a 2017 United Nations report, the most common constraint in Asia is the lack of the human and financial resources needed to enforce environmental regulations and develop water services and infrastructure (146).

6.1.4 Physicochemical properties of antibiotics

The persistence of antibiotic residues in the aquatic environment is determined by their physicochemical properties such as their degradation and their sorption to organic particles (147, 148). Various factors affect these processes, such as the half-life; water quality (149); seasons (150,151); and characteristics of the soil (152). A variety of degradation processes may occur in the environment like hydrolysis, photo-degradation by sunlight, and biodegradation by bacteria under aerobic and anaerobic conditions (147). Some of the antibiotics are easily degraded, such as β-lactams, while others are more stable, such as fluoroquinolones and tetracyclines, allowing them to persist longer in the environment, to spread further, and accumulate in higher concentrations (153–155). Further, the mobility and transport of antibiotic residues in the environment depend on their sorption behaviour. Sulfamethoxazole is characterised by a high water solubility and is found to be the most persistent sulfonamide in water, with a low biodegradability and a high transport propensity (156) (Study I, Study II, and Study III).

6.2 POTENTIAL ENVIRONMENTAL RISK OF ANTIBIOTIC RESISTANCE DEVELOPMENT

The findings from Study III demonstrate that residual concentrations of some antibiotics exceeded the thresholds for the development of resistance (PNECs) in various aquatic environments of the WPR, the SEAR, China, and India. The highest environmental risks of antibiotics were observed in wastewater, and STPs'/WWTPs' influents and effluents. In Study I, high and moderate environmental risks of some antibiotics were observed in wastewater of China. Wastewater and STPs/WWTPs served as major reservoirs of antibiotic residues, antibiotic resistance, and hot spots for the selection and spread of antibiotic resistance (157). Further, wastewater and STPs/WWTPs act as a dominant emission pathway for the release of antibiotic resistant bacteria and resistance genes to the receiving aquatic environments such as drinking water.

Findings from Study II showed that river pollutants deteriorate and alter water quality, affecting the occurrence and the level of antibiotic residues in the river water. Water quality might contribute to the persistence of antibiotic residues in aquatic environments. Antibiotics that persist in the environment may therefore pose a risk with respect to antibiotic resistance development (158). Furthermore, it might cause a shift in the bacterial community composition

(159, 160). In addition, the bacterial community influences the shaping, abundance, and diversity of antibiotic resistance (161). Rivers are important reservoirs of antibiotic resistance genes where the exchange and transfer of genes can take place among pathogenic and commensal *E. coli* strains. Therefore, the resistant pathogenic *E. coli* load will increase, and if this results in infection, the disease will be more difficult to treat. The majority of the *E. coli* isolates from the Kshipra river water and sediment belong to commensal phylogenetic groups A and B1. There is an association between phylogenetic groups and the host species; human commensal strains belong mostly to groups A and B1, and strain isolates from animals fall mostly in group B1. The presence of phylogenetic groups A and B1 can be thus considered an indicator of anthropogenic activities (162). The environmental dissemination of antibiotic resistant bacteria and resistance genes has been of the emerging contaminants.

6.3 POTENTIAL HUMAN HEALTH RISK OF ANTIBIOTIC RESIDUES

Findings from Study I and Study III showed that the contamination of drinking water by antibiotic residues was found in the WPR and China. Antibiotic residues appear to pose resistance and toxicity risk to human health from environmental exposure via drinking water of the WPR and China, and the highest risk was observed for ciprofloxacin (Study III and Study IV). The human exposure to antibiotic residues or directly to antibiotic resistant bacteria via contaminated drinking water could increase the potential human or population health risks of infections with antibiotic resistant bacteria. Dissemination of resistant bacteria can also occur between individuals, which can further increase the prevalence of antibiotic resistance in a population. Drinking water has been a significant vehicle for the spread of antibiotic resistant bacteria and resistance genes in different countries (163). Antibiotic resistance may cause outbreaks of bacterial infections (164–166). Furthermore, the population density is one of the important factors in increasing the prevalence of antibiotic resistance (167) and this warrants special attention in regions such as the WPR and SEAR, which have high population densities, poor health services and sanitation, and unsafe drinking water. The human health risk of exposure to antibiotic resistance through drinking water is not only a risk to human individuals, but to the human population. The emergence of antibiotic residues and resistance in drinking water further emphasises the need to place these threats to humans in perspectives of environmental and health policy.

The disruption of the human commensal gut microbiome by exposure to antibiotics can affect human health in the long-term and can promote long-term disease. In addition, the potential combined adverse effects resulting from chronic exposure to antibiotic mixtures that can be formed in the aquatic environments and during the treatment processes used in the WWTPs remain as significant knowledge gaps. Further, the potential human health risk via different exposure/transmission routes have not been assessed yet. This is of special concern in the case of the countries in the WPR and SEAR, which use aquatic systems such as rivers and lakes for bathing and washing and as source waters for drinking water supplies. Antibiotic contaminants and antibiotic resistance in drinking water should always be considered as a priority for management and control, to ensure negligible risks to public health.

6.4 PRIORITISATION OF ANTIBIOTICS FOR ECO-PHARMACOVIGILANCE AND HEALTH POLICY DECISIONS

I developed an integrated environment–human risk approach for the quantitative environmental and human health risk assessment of antibiotic residues and a risk based prioritisation system thereof (Study IV). This approach addressed two main issues in an integrated manner: first, the potential environmental risk of antibiotic residues including the resistance risk and the ecotoxicity risk; and second, the potential human health risk of antibiotic residues encompassing the resistance risk and toxicity risk. In addition, it considered the complexity of environmental exposure pathways associated with antibiotic residue risks and the large uncertainty in the input data for these pathways, in order to prioritise risks and guide decision-making. The outcome from this research can be used to implement eco-pharmacovigilance and to develop targeted policies which would prevent and minimise the environmental and human health risks of antibiotic residues. The utility of the approach and the system was demonstrated using data from China as an example. The proposed approach can be used for any other country/region/setting. A list of priority antibiotics from different classes such as fluoroquinolones, macrolides, tetracyclines, β-lactams, lincosamides, sulfonamides, amphenicols, and trimethoprim for China was developed. Ciprofloxacin posed the greatest risk. The use of this list of priority antibiotics will allow for a country/region/setting to a) optimise the use of antibiotics and their prescribing practices, b) effectively mitigate and monitor strategies, c) minimise the discharge of antibiotic residues, and d) help focus research efforts.

As a whole, the knowledge generated in this thesis can help decision-makers to make better decisions and to undertake well-directed actions towards monitoring and mitigating antibiotic residues and antibiotic resistance, and to implement eco-pharmacovigilance (Study I, Study II, Study III, and Study IV). Antibiotic residues can be targeted to wherever there appears to be a high risk of the development of resistance within the aquatic environments of the WPR, the SEAR, China, and India. The findings also allow the risk manager and risk assessor to decide the desired level of protection based on the proportions of exposure impacted. The proposed approach can be used for any other country/region/setting to derive special risk reduction measures and to focus mitigations towards priority antibiotics and high-risk sites, and focus research efforts, provided the likelihood of exceedances of each antibiotic PNECs, $PNEC_E$, and TTC, for every aquatic environmental compartment and its threshold concentration, both overall and criterion risk utility scores and both overall and criterion uncertainty scores, and data unavailability for exposures data for every compartment and environmental risk data (NOEC, EC_{50}, PNECs, $PNEC_E$, and LogKow) were specified. As such, this has the potential to assist policymakers in efficiently allocating resources, which is especially vital for resource-poor settings e.g., in the WPR and SEAR (Study III and Study IV).

6.5 METHODOLOGICAL CONSIDERATIONS

The studies in this thesis used various methods: quantitative methods (Study I and Study II), microbiological and molecular methods (Study II), systematic review (Study III) and risk assessment methods (Study I, Study III, and Study IV).

6.5.1 Strengths

The occurrence of antibiotic residues in the environment of rural Shandong province, China was investigated. Samples from various environmental compartments of a reasonably large number of villages (12 villages) were evaluated (Study I). In order to monitor antibiotic residues and antibiotic resistant *E. coli* in the Kshipra river in India long-term, water samples from the river were collected in duplicate from seven selected sites during four seasons for three consecutive years. Sediment samples were also collected from the same seven sites. In addition, different water and sediment quality parameters were examined (Study II). For both Study I and Study II, samples were stored, protected, and transferred for analysis and analysed using appropriate standard methods. The most updated and precise analytical procedure, HPLC–MS/MS, was used to determine the concentration of antibiotic residues. The analytical methods are crucial for generating high precision data on antibiotic residue levels in the aquatic environment and for the consequent determination of the environmental and human health risk associated with exposure to them (168–170). Furthermore, antibiotic susceptibility testing was conducted following CLSI guidelines (113).

Study III used a systematic review of the literature published between 2006 and 2019 to investigate all reported antibiotic residue concentrations from various aquatic environmental compartments of the WPR and SEAR. The overall risk of bias in the included studies were low. Literature indicated that LC-MS/MS was used in most studies. A large number of antibiotics were evaluated, 92 antibiotics in the WPR, and 45 antibiotics in the SEAR. PEHA does not generate a single point estimate, but rather produces a likelihood and range that a particular exposure and effect will occur. Accordingly, this type of assessment allows the risk assessors or decision-maker to conduct the assessment independent of most value judgments, to predict the likelihood that a certain level of protection would be attained. PEHA also permits the characterisation of uncertainty and variability, which can help inform decisions.

In Study IV, the proposed approach enables the researcher to assess and quantify the environmental risks (resistance and ecotoxicity) and the human health risks (resistance and toxicity) regarding the chronic exposure of maximum concentrations of antibiotics in the various aquatic environmental compartments in various proportions of exposure. It also permits the analysis of uncertainty.

6.5.2 Limitations

Relatively few antibiotics and samples were analysed due to financial constraints (Study I and Study II). For the assessment of the risk, the MICs are derived from *in vitro* experiments and are not necessarily representative of the concentrations at which effects are expected in more complex systems in the environment. Studies on MSCs in complex microbial communities are scarce and further experimental validation are necessary to evaluate how well the PNECs estimate the potential for antibiotic resistance development and selection. Furthermore, some parameters derived from the U.S. population were applied, as Chinese data was not available (Study I), this can affect the estimate of the risk.

Study III was subject to some degree of uncertainty resulting from non-availability of a very exhaustive data from many of the countries in the region; the lack of data on the environmental occurrence of antibiotic residues for some antibiotics and compartments (studies analysed few antibiotic compounds); the lack of data on PNECs for the development of resistance for some important antibiotics such as chlortetracycline and most sulfonamides (although the EUCAST database have MIC data on most clinically relevant species, most environmental bacterial species cannot be cultivated and thus have unknown MICs); and the extrapolation of *in vitro* bacterial susceptibility data to the field conditions with complex microbial communities under different antibiotic exposure, and variability or heterogeneity e.g., sites, seasons, rural and urban area, upstream and downstream conditions, water quality, different analysed antibiotic compounds, characteristics of the environmental compartment, regulations, and sampling and analytical methods. Uncertainty and variability have the potential to result in overestimates or underestimates of the risk. Further, the maximum measured concentrations were used, which is a conservative assumption and that would overestimate the risk. In addition, there may be a publication bias among the included studies. Studies with a full text which could not be retrieved by any resource available were excluded, introducing a degree of selection bias to the review. This might challenge the definitive conclusions that can be drawn. However, the use of a relatively large number of included studies (218 from the WPR and 22 from the SEAR) in the analysis might mitigate this problem.

In Study IV, data on the environmental occurrence of antibiotic residues for some antibiotics and compartments, NOEC, EC_{50}, PNECs, and $PNEC_E$ were not available thus, the prioritisation of these antibiotics was limited. Assumptions were intentionally selected to be conservative and that would overestimate rather than underestimate risk for a chronic lifetime exposure via drinking water ingestion: For this case study, the maximum measured concentrations were used, which is a conservative assumption, and the TTC is a conservative approach.

6.5.3 Generalisability and transferability

For the type of environmental research in general, the findings are very specific to the context. For instance, the occurrence of antibiotic residues and resistance in the environment is very related to the context because it is based on various factors such as the drivers, environmental condition, seasons, sites, and policies. Although the findings are context-specific, the methods used could be transferred and adapted in other contexts (Study I, Study II, and Study III). However, the WPR and SEAR are the largest and most populous of the WHO regions and include 48 countries with 53% of the world's total population and also include countries across the spectrum of all income groups like High, Medium, and Low. The findings from Study III therefore provide many insights and are likely to be relevant to other settings also. The utility of the proposed approach and the system was demonstrated using data from China as an example. The results and discussion are focused on China, however, the approach can be used for any other country/region/setting and in that case, the context, results, and various eco-pharmacovigilance and health policy implications will vary according to that context (Study IV).

7 CONCLUSIONS

- Antibiotic residues were present in various environmental compartments of Shandong province of China. Risk estimates indicated a potential for the measured levels of enrofloxacin, levofloxacin, and ciprofloxacin in wastewater to pose an environmental risk for resistance selection. The investigated antibiotics did not appear to pose an appreciable direct human health risk from environmental exposure through drinking water or vegetables consumption. However, they might still pose a risk for resistance development.

- Antibiotic residues and antibiotic resistant *E. coli* were present in the water and sediment of the river in India and showed significant seasonal and spatial variations over a 3-year period, and had varying associations with measured water quality parameters.

- A novel assessment of the health risk due to the antibiotic residues in the aquatic environment of the WPR, the SEAR, China, and India were presented. There is evidence that antibiotic residues were ubiquitous, and residual concentrations of some antibiotics exceeded the thresholds for the development of resistance in various proportions of exposure in various aquatic environments. Wastewater and WWTPs serve as a hot spot for the development of antibiotic resistance in these regions. Antibiotic residues appear to pose an appreciable human health risk from environmental exposure via drinking water. This can aid in developing effective monitoring and mitigation measures to combat antibiotic resistance, and to protect environmental and human health.

- I developed an integrated environment–human risk approach, for the quantitative environmental (resistance and ecotoxicity) and human health (resistance and toxicity) risks assessment of antibiotic residues in the aquatic environment and a prioritisation system thereof. The utility of the approach and the system was demonstrated using data from China as an example and a list of priority antibiotics from different classes was developed. Ciprofloxacin posed the greatest risk. The proposed approach could be customised for other settings. Thus, the outcome from this research can be used to implement eco-pharmacovigilance, to develop collective actions and targeted policies which would prevent and minimise the environmental and human health risks of antibiotic residues, and to help focus research efforts.

8 RECOMMENDATIONS FOR RESEARCH, PRACTICE, AND POLICY

The emergence of antibiotic residues and antibiotic resistance in the environment caused by human actions and inactions show how their defined and measurable risks can influence our health and the environment. This requires collective actions and a sustained integrated One Health response with shared vision and goals. The findings in this thesis provide several suggestions of multipronged strategies to reduce their impact on human health and the environment in the WPR and SEAR and also to some extent for any other setting in general. These include:

8.1 STRENGTHENING SURVEILLANCE AND MONITORING SYSTEMS

Strengthening surveillance and systematic evidence generation regarding antibiotic residues, antibiotic resistant bacteria, and resistance genes in wastewater, drinking water, and food to pursue policymakers to implement stringent regulations.

The implementation of One Health surveillance systems, such as the analysis of integrated surveillance data to ensure that data is used as a guide for responses at the country and regional levels, and supporting global-level surveillance through initiatives such as the WHO Global Antimicrobial Resistance Surveillance System (GLASS) and surveillance work undertaken by OIE and FAO.

8.2 IMPROVING WASTEWATER TREATMENT MANAGEMENT SYSTEMS

The development of cost-effective, efficient, and sustainable wastewater treatment systems such as off-grid and decentralised water treatment to reduce antibiotic residues and the antibiotic resistance burden in water sources.

The development of an additional or a specialised drinking water treatment management system to reduce the very low concentrations of antibiotic residues in drinking water.

8.3 IMPLEMENTATION OF ECO-PHARMACOVIGILANCE SYSTEMATICALLY

The implementation of eco-pharmacovigilance systematically by countries in their health systems and to consider it in their selection of essential medicines list, their recommendations for prescription practices of antibiotics, their drinking water and food management systems, and also regulations for their pharmaceutical industries, will allow to optimise the use of antibiotics, to effectively prevent and reduce the environmental and human health risks of antibiotic residues and antibiotic resistance, to increase the compliance with risk mitigation measures and the verification and follow up with their implementation, and to increase the transparency and availability of environmental data for antibiotics.

8.4 ENFORCEMENT OF REGULATIONS

Regulations of pharmaceutical industries and hospitals concerning the discharge of antibiotics into the environment, of hazardous industrial wastes, and of antibiotic residues and antibiotic resistance in drinking water and food. The implementation of incentives for various stakeholders for implementing the strategies for reducing the antibiotic resistance burden.

The development and implementation of a circular economy model for industries, hospitals, municipals, and farmers to reduce the antibiotic residues and antibiotic resistance in effluents.

8.5 FURTHER INVESTIGATIONS

Research could further address what specific human health impacts are associated with exposure to antibiotic residues, their mixtures, interaction through various exposure routes, including drinking water, food consumption, inhalation, and dermal contact, to understand the significant relationship between antibiotic residues exposure and the human microbiome.

Research should determine the thresholds of microbiological ADIs of antibiotics for the disruption of the human intestinal microbiome and the selection of resistance, the microbial thresholds of antibiotics for significant human antibiotic resistant bacteria development, the PNECs thresholds of some antibiotics for resistance development in the environment, and the $PNEC_E$ of some antibiotics for ecotoxicity in order to perform environmental and human health risk assessment.

The development of easily degradable antibiotics (171), compounds that trigger the antibiotic degradation (172–174), systems to target an antibiotic to the infection point (175), and adsorbents (some already in Phase II of development) that remove antibiotics from the human gut or in the water would all help reduce selective pressure on the human microbiome (176) or the environmental bacteria (177). Biorestoration of antibiotic-susceptible bacteria to remove resistant bacteria and control antibiotic resistance (178–183). Some of these approaches are still in their infancy, and further research is needed to see what their contribution might be.

8.6 ACCELERATING THE DEVELOPMENT AND IMPLEMENTATION OF THE NATIONAL AND GLOBAL ONE HEALTH ANTIMICROBIAL RESISTANCE ACTION PLAN

Building and sustaining effective and tailored national responses through increased political commitment and more integrated collaborated multi- or transdisciplinary efforts including human and veterinary medicine, agriculture, finance, environment, industry, scientists, farmers, and consumers across the One Health spectrum.

Integrated multi-level actions in optimising antibiotic use in human and veterinary medicine, crop protection, and aquaculture. Strengthening key national systems for vaccination; infection prevention and hygiene in health care and farming settings; and integrated laboratory systems for human health, animal health, and the environment. Prioritising interventions and actions that are specific to the national context, infrastructure, and capacity. Strengthening implementation and operational research and research coordination and collaboration.

9 ACKNOWLEDGEMENTS

I'm deeply convinced that our lives are opportunities to create who we are and claim the fullness of our beings, but, most of all, to acknowledge that all we are and have is given to us as a gift of love, a gift to be celebrated with joy. Thank you to all people who have been sharing their own joy of living, their own inner peace, their own sense of well-being, their own sorrows, and their solitude with my life's journey. Thank you to all who have brought me to where I am now. Your presence made this journey delightful and for that I will always be grateful.

Cecilia Stålsby Lundborg, my main supervisor. I have had the privilege of working with you. Thank you for introducing me to the research field and for giving me the opportunities to pursue so many dreams. I'm very grateful to you for giving me the freedom to independently develop as a researcher, and for your valuable advice and guidance throughout this journey.

Dr Ashok J Tamhankar, It has been such a pleasure having you as my co-supervisor. I convey my deepest gratitude for all your constructive suggestions and comments. Thank you for being so supportive along my training and for giving me the confidence to develop my critical thinking.

Thank you all my colleagues, co-authors, and collaborators especially in China and India for all your valuable input.

I would also like to thank Nele for the mentorship and encouragement.

My sincere gratitude to all members of our research subgroup – Anna M, Erika, Gaetano, Guobin, Hager, Jaran, Joe, Keira, Lien, Manju, Megha, Oliver, Oyang, Philip, Shweta, Vishal, Weirong, and Yuan for being supportive and inspiring colleagues and friends, and for sharing all your experiences and passion in improving the use of medicines. Viji, thank you for all your great support.

To my colleagues of the Health System and Policy research group and the Department of Global Public Health: Thank you to all my senior colleagues for your inspiring research and for making the right environment for us so we can thrive - Marie Hasselberg, Asli Kulane, Bo Burström, Birger Forsberg, Claudia Hanson, Emilie Agardh, Helle Mölsted Alvesson, Johan von Schreeb, Lucie Laflamme, Mariano Salazar, Stefan Peterson, Tobias Alfvén, and all others. Thank you to my colleagues and friends - Alicia, Anna K, Anders, Anneli, Carina, Constance, Dell, Elin, Emili, Fadhlun, Galit, Dorcus, Ida, Juliet, Jhon, Karima, Kristi, Lisa, Max, Martina, Moa, Otto, Primus, Regine, Ritva, Sara, Siddarth, Susanne, Tjede, Veronika, and many others for your encouragement and all the times we have shared. Thank you Olivia, Linda, and Sharon for our little PhD support group.

A special thanks to all my administrative colleagues - Amanda, Anastasia, Anita, Amina, Bo, Gun-Britt, Emma, Josefine, Lena, Linus, Malin, Maria, Per, Pia, Sakis, Sofia, and Therese for all your great support and kindness.

Thank you to all my friends – Jinan, Nisryn R, and Nassrin W for being by my side! Antoine, Andrea, Alessio, Aya, Carolina, Christina, Fenia, Gina, Jacqueline, Jizel, Joanna, Jessica, Lia, Lina, Leila, Maha, Maria, Marco, Pia, Rima, Sara, Vanessa, and all others thank you for the good times we spent together, but most of all, for your friendship.

Dalia, Jina, Lena, and Sanaa, my dear friends, I am so honoured to have you in my life. I will forever cherish the wonderful friendship we created a very long time ago. Thank you for so many dreams and memories we have shared.

I would also like to thank all my extended family throughout the world - uncles, aunts, cousins, and many more others for your encouragement. Especially, my grandma Janet, thank you for your prayers and beautiful soul.

My brother Jean, thank you for creating the cover page of my thesis.

To my powerful and inspiring family, my parents and siblings - Dad, Mom, Roula, Jean, and Milad who have always believed in me. Thank you for your prayers and endless love and care. Despite the difficult time you have been passing through during the Syrian war, you have always been there for me. Your love, faith, and dedication make me realise the light that shines in the darkness, and claim responsibility for my challenges and move beyond them. I am ever so grateful and couldn't thank you enough.

10 REFERENCES

1. Laxminarayan R, Matsoso P, Pant S, Brower C, Røttingen J-A, Klugman K, et al. Access to effective antimicrobials: a worldwide challenge. The Lancet. 2016 Jan 9;387(10014):168–75.

2. Jasovský D, Littmann J, Zorzet A, Cars O. Antimicrobial resistance—a threat to the world's sustainable development. Ups J Med Sci. 2016 Aug;121(3):159–64.

3. O'NEILL J. TACKLING DRUG-RESISTANT INFECTIONS GLOBALLY: FINAL REPORT AND RECOMMENDATIONS. 2016; Available from: https://amr-review.org/sites/default/files/160518_Final%20paper_with%20cover.pdf

4. World Bank. DRUG-RESISTANT INFECTIONS A Threat to Our Economic Future. Washington (DC): 2016; Available from: http://pubdocs.worldbank.org/en/527731474225046104/AMR-Discussion-Draft-Sept18updated.pdf

5. Hernando-Amado S, Coque TM, Baquero F, Martínez JL. Defining and combating antibiotic resistance from One Health and Global Health perspectives. Nature Microbiology. 2019 Sep;4(9):1432–42.

6. Laxminarayan R, Duse A, Wattal C, Zaidi AKM, Wertheim HFL, Sumpradit N, et al. Antibiotic resistance—the need for global solutions. The Lancet Infectious Diseases. 2013 Dec 1;13(12):1057–98.

7. WHO. Global action plan on antimicrobial resistance. 2015; Available from: http://www.wpro.who.int/entity/drug_resistance/resources/global_action_plan_eng.pdf

8. OPGA/WHO/FAO/OIE. OPGA/WHO/FAO/OIE (2016) High-Level Meeting on Antimicrobial Resistance. 2016; Available from: https://www.un.org/pga/71/2016/09/21/press-release-hl-meeting-on-antimicrobial-resistance/

9. FAO/OIE/WHO. FAO/OIE/WHO (2017) The Tripartite's Commitment—Providing multi-sectoral, collaborative leadership in addressing health challenges. FAO/OIE/WHO. 2017; Available from: https://www.who.int/zoonoses/tripartite_oct2017.pdf

10. Iossa G, White PC. The natural environment: a critical missing link in national action plans on antimicrobial resistance. Bulletin of the World Health Organization. 2018 Dec 1;96(12):858–60.

11. Essack SY. Environment: the neglected component of the One Health triad. The Lancet Planetary Health. 2018 Jun 1;2(6):e238–9.

12. IACG. NO TIME TO WAIT: SECURING THE FUTURE FROM DRUG-RESISTANT INFECTIONS. 2019; Available from: https://www.who.int/docs/default-source/documents/no-time-to-wait-securing-the-future-from-drug-resistant-infections-en.pdf?sfvrsn=5b424d7_6

13. Martinez JL. The role of natural environments in the evolution of resistance traits in pathogenic bacteria. Proc Biol Sci. 2009 Jul 22;276(1667):2521–30.

14. Davies J, Davies D. Origins and Evolution of Antibiotic Resistance. Microbiol Mol Biol Rev. 2010 Sep;74(3):417–33.

15. Gelband H, Miller-Petrie M, Pant S. The State of the World's Antibiotics. Washington, DC: Centre for Disease Dynamics, Economics&Policy. 2015; Available from: https://cddep.org/publications/state_worlds_antibiotics_2015/

16. Klein EY, Boeckel TPV, Martinez EM, Pant S, Gandra S, Levin SA, et al. Global increase and geographic convergence in antibiotic consumption between 2000 and 2015. PNAS. 2018 Apr 10;115(15):E3463–70.

17. Van Boeckel TP, Brower C, Gilbert M, Grenfell BT, Levin SA, Robinson TP, et al. Global trends in antimicrobial use in food animals. Proc Natl Acad Sci USA. 2015 May 5;112(18):5649–54.

18. O'NEILL. ANTIMICROBIALS IN AGRICULTURE AND THE ENVIRONMENT: REDUCING UNNECESSARY USE AND WASTE. THE REVIEW ON ANTIMICROBIAL RESISTANCE. 2015; Available from: https://amr-review.org/sites/default/files/Antimicrobials%20in%20agriculture%20and%20the%20environment%20-%20Reducing%20unnecessary%20use%20and%20waste.pdf

19. Karkman A, Pärnänen K, Larsson DGJ. Fecal pollution can explain antibiotic resistance gene abundances in anthropogenically impacted environments. Nature Communications. 2019 Jan 8;10(1):80.

20. Holmes AH, Moore LSP, Sundsfjord A, Steinbakk M, Regmi S, Karkey A, et al. Understanding the mechanisms and drivers of antimicrobial resistance. Lancet. 2016 Jan 9;387(10014):176–87.

21. Heberer T. Occurrence, fate, and removal of pharmaceutical residues in the aquatic environment: a review of recent research data. Toxicol Lett. 2002 May 10;131(1–2):5–17.

22. Wang Q, Wang P, Yang Q. Occurrence and diversity of antibiotic resistance in untreated hospital wastewater. The Science of the total environment. 2018;621:990–9.

23. Zhang T, Li B. Occurrence, transformation, and fate of antibiotics in municipal wastewater treatment plants. Critical Reviews in Environmental Science and Technology. 2011;41(11):951–98.

24. Kang DH, Gupta S, Rosen C, Fritz V, Singh A, Chander Y, et al. Antibiotic Uptake by Vegetable Crops from Manure-Applied Soils. J Agric Food Chem. 2013 Oct 23;61(42):9992–10001.

25. Li Y-W, Wu X-L, Mo C-H, Tai Y-P, Huang X-P, Xiang L. Investigation of sulfonamide, tetracycline, and quinolone antibiotics in vegetable farmland soil in the Pearl River Delta area, southern China. Journal of agricultural and food chemistry. 2011;59(13):7268–76.

26. Glassmeyer ST, Hinchey EK, Boehme SE, Daughton CG, Ruhoy IS, Conerly O, et al. Disposal practices for unwanted residential medications in the United States. Environment International. 2009 Apr 1;35(3):566–72.

27. Shimizu A, Takada H, Koike T, Takeshita A, Saha M, Rinawati, et al. Ubiquitous occurrence of sulfonamides in tropical Asian waters. The Science of the total environment. 2013;452–453:108–15.

28. Done HY, Halden RU. Reconnaissance of 47 antibiotics and associated microbial risks in seafood sold in the United States. J Hazard Mater. 2015 Jan 23;282:10–7.

29. Hanna N, Sun P, Sun Q, Li X, Yang X, Ji X, et al. Presence of antibiotic residues in various environmental compartments of Shandong province in eastern China: Its potential for resistance development and ecological and human risk. Environ Int. 2018 May;114:131–42.

30. Rodriguez-Mozaz S, Chamorro S, Marti E, Huerta B, Gros M, Sanchez-Melsio A, et al. Occurrence of antibiotics and antibiotic resistance genes in hospital and urban wastewaters and their impact on the receiving river. Water research. 2015 Feb;69:234–42.

31. Fick J, Söderström H, Lindberg RH, Phan C, Tysklind M, Larsson DGJ. Contamination of surface, ground, and drinking water from pharmaceutical production. Environ Toxicol Chem. 2009 Dec;28(12):2522–7.

32. Harris SJ, Cormican M, Cummins E. Antimicrobial Residues and Antimicrobial-Resistant Bacteria: Impact on the Microbial Environment and Risk to Human Health—A Review. Human and Ecological Risk Assessment: An International Journal. 2012 Jul 1;18(4):767–809.

33. Xu J, Xu Y, Wang H, Guo C, Qiu H, He Y, et al. Occurrence of antibiotics and antibiotic resistance genes in a sewage treatment plant and its effluent-receiving river. Chemosphere. 2015 Jan;119:1379–85.

34. Ben Y, Fu C, Hu M, Liu L, Wong MH, Zheng C. Human health risk assessment of antibiotic resistance associated with antibiotic residues in the environment: A review. Environmental Research. 2019 Feb 1;169:483–93.

35. Martínez JL, Coque TM, Baquero F. What is a resistance gene? Ranking risk in resistomes. Nat Rev Microbiol. 2015;13(2):116–23.

36. Diwan V, Chandran SP, Tamhankar AJ, Stålsby Lundborg C, Macaden R. Identification of extended-spectrum β-lactamase and quinolone resistance genes in Escherichia coli isolated from hospital wastewater from central India. J Antimicrob Chemother. 2012 Apr;67(4):857–9.

37. Wang Q, Wang P, Yang Q. Occurrence and diversity of antibiotic resistance in untreated hospital wastewater. Sci Total Environ. 2018 Apr 15;621:990–9.

38. Chen H, Zhang M. Occurrence and removal of antibiotic resistance genes in municipal wastewater and rural domestic sewage treatment systems in eastern China. Environment International. 2013 May 1;55:9–14.

39. Marathe NP, Regina VR, Walujkar SA, Charan SS, Moore ERB, Larsson DGJ, et al. A Treatment Plant Receiving Waste Water from Multiple Bulk Drug Manufacturers Is a Reservoir for Highly Multi-Drug Resistant Integron-Bearing Bacteria. PLoS One. 2013 Oct 29 [cited 2019 Mar 30];8(10). Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3812170/

40. Harris S, Morris C, Morris D, Cormican M, Cummins E. Antimicrobial resistant Escherichia coli in the municipal wastewater system: effect of hospital effluent and environmental fate. Sci Total Environ. 2014 Jan 15;468–469:1078–85.

41. Dhawde R, Macaden R, Saranath D, Nilgiriwala K, Ghadge A, Birdi T. Antibiotic Resistance Characterization of Environmental E. coli Isolated from River Mula-Mutha, Pune District, India. Int J Environ Res Public Health. 2018 Jun [cited 2019 Mar 30];15(6). Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6025386/

42. Flach C-F, Johnning A, Nilsson I, Smalla K, Kristiansson E, Larsson DGJ. Isolation of novel IncA/C and IncN fluoroquinolone resistance plasmids from an antibiotic-polluted lake. J Antimicrob Chemother. 2015 Oct;70(10):2709–17.

43. Xi C, Zhang Y, Marrs CF, Ye W, Simon C, Foxman B, et al. Prevalence of Antibiotic Resistance in Drinking Water Treatment and Distribution Systems. Appl Environ Microbiol. 2009 Sep 1;75(17):5714–8.

44. Novais C, Campos J, Freitas AR, Barros M, Silveira E, Coque TM, et al. Water supply and feed as sources of antimicrobial-resistant Enterococcus spp. in aquacultures of rainbow trout (Oncorhyncus mykiss), Portugal. Sci Total Environ. 2018 Jun 1;625:1102–12.

45. Zhao Y, Zhang X-X, Zhao Z, Duan C, Chen H, Wang M, et al. Metagenomic analysis revealed the prevalence of antibiotic resistance genes in the gut and living environment of freshwater shrimp. J Hazard Mater. 2018 May 15;350:10–8.

46. Pan M, Chu LM. Occurrence of antibiotics and antibiotic resistance genes in soils from wastewater irrigation areas in the Pearl River Delta region, southern China. The Science of the total environment. 2018;624:145–52.

47. Wang F-H, Qiao M, Chen Z, Su J-Q, Zhu Y-G. Antibiotic resistance genes in manure-amended soil and vegetables at harvest. J Hazard Mater. 2015 Dec 15;299:215–21.

48. Isidori M, Lavorgna M, Nardelli A, Pascarella L, Parrella A. Toxic and genotoxic evaluation of six antibiotics on non-target organisms. Sci Total Environ. 2005 Jun 15;346(1–3):87–98.

49. Gullberg E, Albrecht LM, Karlsson C, Sandegren L, Andersson DI. Selection of a Multidrug Resistance Plasmid by Sublethal Levels of Antibiotics and Heavy Metals. mBio. 2014 Oct 31;5(5):e01918-14.

50. Gullberg E, Cao S, Berg OG, Ilbäck C, Sandegren L, Hughes D, et al. Selection of Resistant Bacteria at Very Low Antibiotic Concentrations. PLoS Pathog. 2011 Jul [cited 2017 May 19];7(7). Available from: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3141051/

51. Bengtsson-Palme J, Larsson DGJ. Concentrations of antibiotics predicted to select for resistant bacteria: Proposed limits for environmental regulation. Environ Int. 2016 Jan;86:140–9.

52. EUCAST. European Committee on Antimicrobial Susceptibility Testing, 2014. Data from the EUCAST MIC distribution website, accessed 11–26. 2014;

53. Cerniglia CE, Kotarski S. Evaluation of veterinary drug residues in food for their potential to affect human intestinal microflora. Regul Toxicol Pharmacol. 1999 Jun;29(3):238–61.

54. Ferrer M, Méndez-García C, Rojo D, Barbas C, Moya A. Antibiotic use and microbiome function. Biochem Pharmacol. 2017 15;134:114–26.

55. Francino MP. Early development of the gut microbiota and immune health. Pathogens. 2014 Sep 24;3(3):769–90.

56. Turnbaugh PJ, Ley RE, Mahowald MA, Magrini V, Mardis ER, Gordon JI. An obesity-associated gut microbiome with increased capacity for energy harvest. Nature. 2006 Dec 21;444(7122):1027–31.

57. Lawley TD, Clare S, Walker AW, Goulding D, Stabler RA, Croucher N, et al. Antibiotic treatment of clostridium difficile carrier mice triggers a supershedder state, spore-mediated transmission, and severe disease in immunocompromised hosts. Infect Immun. 2009 Sep;77(9):3661–9.

58. Young VB, Schmidt TM. Antibiotic-associated diarrhea accompanied by large-scale alterations in the composition of the fecal microbiota. J Clin Microbiol. 2004 Mar;42(3):1203–6.

59. Miller SA, Wu RKS, Oremus M. The association between antibiotic use in infancy and childhood overweight or obesity: a systematic review and meta-analysis. Obes Rev. 2018;19(11):1463–75.

60. Patrick DM, Sbihi H, Dai DLY, Mamun AA, Rasali D, Rose C, et al. Decreasing antibiotic use, the gut microbiota, and asthma incidence in children: evidence from population-based and prospective cohort studies. The Lancet Respiratory Medicine. 2020 Nov 1;8(11):1094–105.

61. Bengtsson-Palme J, Angelin M, Huss M, Kjellqvist S, Kristiansson E, Palmgren H, et al. The Human Gut Microbiome as a Transporter of Antibiotic Resistance Genes between Continents. Antimicrob Agents Chemother. 2015 Oct;59(10):6551–60.

62. Vries LE de, Vallès Y, Agersø Y, Vaishampayan PA, García-Montaner A, Kuehl JV, et al. The Gut as Reservoir of Antibiotic Resistance: Microbial Diversity of Tetracycline Resistance in Mother and Infant. PLOS ONE. 2011 Jun 28;6(6):e21644.

63. Fouhy F, Guinane CM, Hussey S, Wall R, Ryan CA, Dempsey EM, et al. High-throughput sequencing reveals the incomplete, short-term recovery of infant gut microbiota following parenteral antibiotic treatment with ampicillin and gentamicin. Antimicrob Agents Chemother. 2012 Nov;56(11):5811–20.

64. Karami N, Nowrouzian F, Adlerberth I, Wold AE. Tetracycline resistance in Escherichia coli and persistence in the infantile colonic microbiota. Antimicrob Agents Chemother. 2006 Jan;50(1):156–61.

65. Zimmermann P, Curtis N. Effect of intrapartum antibiotics on the intestinal microbiota of infants: a systematic review. Archives of Disease in Childhood - Fetal and Neonatal Edition. 2020 Mar 1;105(2):201–8.

66. Commission regulation (EU). Commission regulation EU/37/2010 on pharmacologically activesubstances and their classification regarding maximumresidue limits in foodstuffs of animal origin. Off. J. Eur. Union. 2010. Available from: https://ec.europa.eu/health/sites/health/files/files/eudralex/vol-5/reg_2010_37/reg_2010_37_en.pdf

67. Heuer OE, Kruse H, Grave K, Collignon P, Karunasagar I, Angulo FJ. Human health consequences of use of antimicrobial agents in aquaculture. Clin Infect Dis. 2009 Oct 15;49(8):1248–53.

68. Jones O a. H, Voulvoulis N, Lester JN. Potential ecological and human health risks associated with the presence of pharmaceutically active compounds in the aquatic environment. Crit Rev Toxicol. 2004 Aug;34(4):335–50.

69. Patterson R, DeSwarte RD, Greenberger PA, Grammer LC, Brown JE, Choy AC. Drug allergy and protocols for management of drug allergies. Allergy Proc. 1994 Oct;15(5):239–64.

70. Solensky R. Allergy to β-lactam antibiotics. J Allergy Clin Immunol. 2012 Dec;130(6):1442-1442.e5.

71. Yunis AA. Chloramphenicol toxicity: 25 years of research. Am J Med. 1989 Sep;87(3N):44N-48N.

72. WHO. Chemical mixtures in source water and drinking-water. 2017; Available from: https://www.who.int/water_sanitation_health/publications/chemical-mixtures-in-water/en/

73. Zhang Y, Keerthisinghe TP, Han Y, Liu M, Wanjaya ER, Fang M. 'Cocktail' of Xenobiotics at Human Relevant Levels Reshapes the Gut Bacterial Metabolome in a Species-Specific Manner. Environ Sci Technol. 2018 Oct 2;52(19):11402–10.

74. Xie JW, Jin L, Luo XS, Zhao Z, Li XD. Seasonal Disparities in Airborne Bacteria and Associated Antibiotic Resistance Genes in PM2.5 between Urban and Rural Sites. Environmental Science & Technology Letters. 2018 Feb;5(2):74–9.

75. Li J, Cao J, Zhu Y, Chen Q, Shen F, Wu Y, et al. Global Survey of Antibiotic Resistance Genes in Air. Environ Sci Technol. 2018 Oct 2;52(19):10975–84.

76. VICH GL36. Studies to evaluate the safety of residues of veterinary drugs in human food: General approach to establish a microbiological ADI. 2012; Available from: http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2012/07/WC500130367.pdf

77. Manaia CM. Assessing the Risk of Antibiotic Resistance Transmission from the Environment to Humans: Non-Direct Proportionality between Abundance and Risk. Trends in Microbiology. 2017 Mar 1;25(3):173–81.

78. Vaz-Moreira I, Nunes OC, Manaia CM. Bacterial diversity and antibiotic resistance in water habitats: searching the links with the human microbiome. FEMS Microbiol Rev. 2014 Jul;38(4):761–78.

79. Li N, Ho KWK, Ying G-G, Deng W-J. Veterinary antibiotics in food, drinking water, and the urine of preschool children in Hong Kong. Environment international. 2017;108:246–52.

80. Rhodes G, Huys G, Swings J, McGann P, Hiney M, Smith P, et al. Distribution of oxytetracycline resistance plasmids between aeromonads in hospital and aquaculture environments: implication of Tn1721 in dissemination of the tetracycline resistance determinant tet A. Appl Environ Microbiol. 2000 Sep;66(9):3883–90.

81. Mughini-Gras L, Dorado-García A, Duijkeren E van, Bunt G van den, Dierikx CM, Bonten MJM, et al. Attributable sources of community-acquired carriage of Escherichia coli containing β-lactam antibiotic resistance genes: a population-based modelling study. The Lancet Planetary Health. 2019 Aug 1;3(8):e357–69.

82. Knetsch CW, Connor TR, Mutreja A, van Dorp SM, Sanders IM, Browne HP, et al. Whole genome sequencing reveals potential spread of Clostridium difficile between humans and farm animals in the Netherlands, 2002 to 2011. Euro Surveill. 2014 Nov 13;19(45):20954.

83. Tamhankar AJ, Stålsby Lundborg C. Antimicrobials and Antimicrobial Resistance in the Environment and Its Remediation: A Global One Health Perspective. International Journal of Environmental Research and Public Health. 2019 Jan;16(23):4614.

84. Holm G, Snape JR, Murray-Smith R, Talbot J, Taylor D, Sörme P. Implementing ecopharmacovigilance in practice: challenges and potential opportunities. Drug Saf. 2013 Jul;36(7):533–46.

85. Velo G, Moretti U. Ecopharmacovigilance for better health. Drug Saf. 2010 Nov 1;33(11):963–8.

86. EMEA. Guideline on the environmental risk assessment of medicinal products for human use. European Medicines Agency (EMEA), Committee for medicinal products for human use (CHMP). 2006; Available from: https://www.ema.europa.eu/en/documents/scientific-guideline/guideline-environmental-risk-assessment-medicinal-products-human-use-first-version_en.pdf

87. WHO. World Health Organisation. The importance of pharmacovigilance: safety monitoring of medicinal products. Geneva: World Health Organisation; 2002. 2002; Available from: http://apps.who.int/medicinedocs/en/d/Js4893e/

88. EC. European Commission. Regulation (EU) No 1235/2010 of the European parliament and of the Council. Volume 1. Pharmaceutical legislation medicinal products for human use. 2010. Available from URL. 2010; Available from: https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX:32010L0084

89. Allen HK, Donato J, Wang HH, Cloud-Hansen KA, Davies J, Handelsman J. Call of the wild: antibiotic resistance genes in natural environments. Nat Rev Microbiol. 2010 Apr;8(4):251–9.

90. Van Boeckel TP, Gandra S, Ashok A, Caudron Q, Grenfell BT, Levin SA, et al. Global antibiotic consumption 2000 to 2010: an analysis of national pharmaceutical sales data. Lancet Infect Dis. 2014 Aug;14(8):742–50.

91. Brooks BW, Huggett DB, Boxall ABA. PHARMACEUTICALS AND PERSONAL CARE PRODUCTS: RESEARCH NEEDS FOR THE NEXT DECADE. Environmental Toxicology & Chemistry. 2009;28(12):2469–72.

92. Pruden A, Larsson DGJ, Amézquita A, Collignon P, Brandt KK, Graham DW, et al. Management Options for Reducing the Release of Antibiotics and Antibiotic Resistance Genes to the Environment. Environ Health Perspect. 2013 Aug;121(8):878–85.

93. WHO. World Health Statistics 2010, 2010Geneva, SwitzerlandDepartment of Health Statistics and Informatics, World Health Organization. 2010; Available from: https://books.google.se/books?hl=en&lr=&id=Z69vxfRfFIsC&oi=fnd&pg=PA1&ots=cIJN9hKBaE&sig=VXTrIMkfe6aYjwOgVqNhyCNmg8E&redir_esc=y#v=onepage&q=population&f=false

94. WHO. Population situation and policies in the South East Asia region. 2008; Available from: https://apps.who.int/iris/bitstream/handle/10665/205635/B2414.pdf?sequence=1&isAllowed=y

95. UN. World Population Prospects. 2019; Available from: https://population.un.org/wpp/Publications/Files/WPP2019_Highlights.pdf

96. Larsson DGJ, de Pedro C, Paxeus N. Effluent from drug manufactures contains extremely high levels of pharmaceuticals. J Hazard Mater. 2007 Sep 30;148(3):751–5.

97. Stålsby Lundborg C, Tamhankar AJ. Antibiotic residues in the environment of South East Asia. BMJ. 2017 Sep 5;358:j2440.

98. Zhang Q-Q, Ying G-G, Pan C-G, Liu Y-S, Zhao J-L. Comprehensive evaluation of antibiotics emission and fate in the river basins of China: source analysis, multimedia modeling, and linkage to bacterial resistance. Environ Sci Technol. 2015 Jun 2;49(11):6772–82.

99. Rehman MSU, Rashid N, Ashfaq M, Saif A, Ahmad N, Han J-I. Global risk of pharmaceutical contamination from highly populated developing countries. Chemosphere. 2015 Nov;138:1045–55.

100. Kookana RS, Williams M, Boxall ABA, Larsson DGJ, Gaw S, Choi K, et al. Potential ecological footprints of active pharmaceutical ingredients: an examination of risk factors in low-, middle- and high-income countries. Philosophical Transactions of the Royal Society B-Biological Sciences. 2014 Nov;369(1656).

101. UNESCAP. UNESCAP (United Nations Economic and Social Commission for Asia and the Pacific). 2010. Statistical Yearbook 2009. Bangkok, UNESCAP. 2010;

102. EC. Technical guidance document in support of Commission directive 93/67/EEC on risk assessment for new notified substances and Commission regulation (EC) no 1488/94 on risk assessment for existing substances. Ispra: Joint Research Center; 1996. 2003;

103. US EPA O. Fact Sheet: Methodology for Deriving Ambient Water Quality Criteria for the Protection of Human Health - Revised Methodology (2000). 2000 [cited 2017 May 18]. Available from: https://www.epa.gov/wqc/fact-sheet-methodology-deriving-ambient-water-quality-criteria-protection-human-health-revised

104. WHO. Guidelines for drinking water quality, 4th ed. Geneva, World Health Organization. 2001. WHO. 2011 [cited 2017 May 18]. Available from: http://www.who.int/water_sanitation_health/publications/2011/dwq_guidelines/en/

105. EC. EC. (2005). European Community. Notice to applicants and note for guidance: Establishment of maximum residue limits (MRLs) for residues of veterinary medicinal products in foodstuffs of animal origin.Volume 8. https://ec.europa.eu/health//sites/health/files/files/eudralex/vol-8/pdf/vol8_10-2005_en.pdf. 2005. Available from: https://ec.europa.eu/health//sites/health/files/files/eudralex/vol-8/pdf/vol8_10-2005_en.pdf

106. Agudo A. Agudo, A., 2004. Measuring Intake of Fruit and Vegetables. FAO/WHO, Japan. 2004; Available from: https://apps.who.int/iris/bitstream/handle/10665/43144/9241592826_eng.pdf

107. China National Bureau of Statistics. Measures for Fifth National Population Census. 2002; Available from: http://www.stats.gov.cn/english/LF/SR/200204/t20020423_27180.html

108. USEPA. Supplemental Guidance for Developing Soil Screening Levels for Superfund Sites, Background Discussion for Soil–Plant–Human Exposure Pathway. United States Environmental Protection Agency, Washington, DC, US. 1996; Available from: https://www.epa.gov/superfund/superfund-soil-screening-guidance

109. Diwan V, Purohit M, Chandran S, Parashar V, Shah H, Mahadik VK, et al. A Three-Year Follow-Up Study of Antibiotic and Metal Residues, Antibiotic Resistance and Resistance Genes, Focusing on Kshipra-A River Associated with Holy Religious Mass-Bathing in India: Protocol Paper. Int J Environ Res Public Health. 2017 29;14(6).

110. Diwan V, Tamhankar AJ, Khandal RK, Sen S, Aggarwal M, Marothi Y, et al. Antibiotics and antibiotic-resistant bacteria in waters associated with a hospital in Ujjain, India. BMC Public Health. 2010 Jul 13;10:414.

111. Huang C-H, Renew J, Smeby K, Pinkston K, Sedlak D. Assessment of Potential Antibiotic Contaminants in Water and Preliminary Occurrence Analysis. Journal of Contemporary Water Research and Education. 2011 Feb 23;120(1). Available from: http://opensiuc.lib.siu.edu/jcwre/vol120/iss1/4

112. Nataro J, Bopp C, Fields P, Kaper J, Strockbine N. Escherichia, Shigella, and Salmonella. In Manual of Clinical Microbiology. ASM Press. 2007; Available from: http://www.asmscience.org/content/book/10.1128/9781555816728.chap35

113. CLSI. Performance Standards for Antimicrobial Susceptibility Testing; Twenty- Fourth Informational Supplement. Clinical And Laboratory Standards Institute. 2014; Available from: http://www.facm.ucl.ac.be/intranet/CLSI/CLSI-2015-M100-S25-original.pdf

114. Moher D, Liberati A, Tetzlaff J, Altman DG, PRISMA Group. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. J Clin Epidemiol. 2009 Oct;62(10):1006–12.

115. Higgins J, Thomas J, Chandler J, Cumpston M, Li T, Welch V. Cochrane Handbook for Systematic Reviews of Interventions version 6.0. 2019 Jul;

116. Sanderson S, Tatt ID, Higgins JPT. Tools for assessing quality and susceptibility to bias in observational studies in epidemiology: a systematic review and annotated bibliography. Int J Epidemiol. 2007 Jun;36(3):666–76.

117. Saari GN, Scott WC, Brooks BW. Global scanning assessment of calcium channel blockers in the environment: Review and analysis of occurrence, ecotoxicology and hazards in aquatic systems. Chemosphere. 2017 Dec;189:466–78.

118. Corrales J, Kristofco LA, Steele WB, Yates BS, Breed CS, Williams ES, et al. Global Assessment of Bisphenol A in the Environment. Dose Response. 2015 Jul 29 [cited 2020 Feb 4];13(3). Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4674187/

119. USEPA. USEPA, 2000. Technical Progress Report of the Implementation Plan for Probabilistic Ecological Assessments: Aquatic Systems. 2000;

120. Solomon K, Giesy J, Jones P. Probabilistic risk assessment of agrochemicals in the environment. Crop Protection. 2000 Sep 12;19(8):649–55.

121. OECD. Guidance for the Initial Assessment of Aquatic Effects. 2002; Available from: http://www.oecd.org/chemicalsafety/risk-assessment/2483645.pdf

122. Crisp TM, Clegg ED, Cooper RL, Wood WP, Anderson DG, Baetcke KP, et al. Environmental endocrine disruption: an effects assessment and analysis. Environ Health Perspect. 1998 Feb;106(Suppl 1):11–56.

123. Le Page G, Gunnarsson L, Snape J, Tyler CR. Integrating human and environmental health in antibiotic risk assessment: A critical analysis of protection goals, species sensitivity and antimicrobial resistance. Environ Int. 2017;109:155–69.

124. Jafari Ozumchelouei E, Hamidian AH, Zhang Y, Yang M. Physicochemical properties of antibiotics: A review with an emphasis on detection in the aquatic environment. Water Environ Res. 2020 Feb;92(2):177–88.

125. Cramer GM, Ford RA, Hall RL. Estimation of toxic hazard--a decision tree approach. Food Cosmet Toxicol. 1978 Jun;16(3):255–76.

126. Munro IC, Ford RA, Kennepohl E, Sprenger JG. Thresholds of toxicological concern based on structure-activity relationships. Drug Metab Rev. 1996 May;28(1–2):209–17.

127. Munro IC, Ford RA, Kennepohl E, Sprenger JG. Correlation of structural class with no-observed-effect levels: a proposal for establishing a threshold of concern. Food Chem Toxicol. 1996 Sep;34(9):829–67.

128. Kroes R, Kleiner J, Renwick A. The Threshold of Toxicological Concern Concept in Risk Assessment. Toxicol Sci. 2005 Aug 1;86(2):226–30.

129. WHO. Guidelines for Drinking-water Quality. 2011; Available from: https://www.who.int/water_sanitation_health/publications/2011/dwq_guidelines/en/

130. EMA. Guideline on assessing the toxicological risk to human health and groundwater communities from veterinary pharmaceuticals in groundwater EMA/CVMP/ERA/103555/2015. 2015; Available from: https://www.ema.europa.eu/en/documents/scientific-guideline/draft-guideline-

assessing-toxicological-risk-human-health-groundwater-communities-veterinary_en.pdf

131. FAO. The FAO Action Plan on Antimicrobial Resistance 2016-2020 | Global Forum on Food Security and Nutrition (FSN Forum). 2016 [cited 2019 Mar 29]. Available from: http://www.fao.org/3/a-i5996e.pdf

132. WHO. WHO | WHO Report on Surveillance of Antibiotic Consumption. WHO. 2018 [cited 2019 Mar 20]. Available from: https://apps.who.int/iris/bitstream/handle/10665/277359/9789241514880-eng.pdf?ua=1

133. Abhineet N, Dohare ERD. Physico-Chemical Parameters for Testing of Present Water Quality of Khan River at Indore, India. International Research Journal of Environment Sciences. 2014; Available from: https://www.researchgate.net/publication/318761695_Physico-Chemical_Parameters_for_Testing_of_Present_Water_Quality_of_Khan_River_at_Indore_India

134. Luo Y, Xu L, Rysz M, Wang Y, Zhang H, Alvarez PJJ. Occurrence and transport of tetracycline, sulfonamide, quinolone, and macrolide antibiotics in the Haihe River Basin, China. Environ Sci Technol. 2011 Mar 1;45(5):1827–33.

135. Leung HW, Jin L, Wei S, Tsui MMP, Zhou B, Jiao L, et al. Pharmaceuticals in tap water: human health risk assessment and proposed monitoring framework in China. Environmental health perspectives. 2013;121(7):839–46.

136. Sharma BM, Becanova J, Scheringer M, Sharma A, Bharat GK, Whitehead PG, et al. Health and ecological risk assessment of emerging contaminants (pharmaceuticals, personal care products, and artificial sweeteners) in surface and groundwater (drinking water) in the Ganges River Basin, India. The Science of the total environment. 2019;646:1459–67.

137. Chen YH, Chen HJ, Zhang L, Jiang Y, Gin KYH, He YL. Occurrence, Distribution, and Risk Assessment of Antibiotics in a Subtropical River-Reservoir System. Water. 2018 Feb;10(2):104.

138. Meng T, Cheng W, Wan T, Wang M, Ren J, Li Y, et al. Occurrence of antibiotics in rural drinking water and related human health risk assessment. Environ Technol. 2019 Jul 23;23:1–11.

139. Lei K, Zhu Y, Chen W, Pan H-Y, Cao Y-X, Zhang X, et al. Spatial and seasonal variations of antibiotics in river waters in the Haihe River Catchment in China and ecotoxicological risk assessment. Environment international. 2019;130:104919.

140. Li WL, Zhang ZF, Ma WL, Liu LY, Song WW, Li YF. An evaluation on the intra-day dynamics, seasonal variations and removal of selected pharmaceuticals and personal care products from urban wastewater treatment plants. Science of the Total Environment. 2018 Nov;640:1139–47.

141. Sinthuchai D, Boontanon SK, Boontanon N, Polprasert C. Evaluation of removal efficiency of human antibiotics in wastewater treatment plants in Bangkok, Thailand. Water Sci Technol. 2016;73(1):182–91.

142. Tewari S, Jindal R, Kho YL, Eo S, Choi K. Major pharmaceutical residues in wastewater treatment plants and receiving waters in Bangkok, Thailand, and associated ecological risks. Chemosphere. 2013;91(5):697–704.

143. Ashfaq M, Li Y, Wang Y, Chen W, Wang H, Chen X, et al. Occurrence, fate, and mass balance of different classes of pharmaceuticals and personal care products in an anaerobic-anoxic-oxic wastewater treatment plant in Xiamen, China. Water research. 2017;123:655–67.

144. Chang X, Meyer MT, Liu X, Zhao Q, Chen H, Chen J, et al. Determination of antibiotics in sewage from hospitals, nursery and slaughter house, wastewater treatment plant and source water in Chongqing region of Three Gorge Reservoir in China. Environ Pollut. 2010 May;158(5):1444–50.

145. Sui Q, Huang J, Deng S, Yu G, Fan Q. Occurrence and removal of pharmaceuticals, caffeine and DEET in wastewater treatment plants of Beijing, China. Water research. 2010;44(2):417–26.

146. UN. Wastewater, the untapped resource. the united nations world water development report. 2017;

147. Poirier-Larabie S, Segura PA, Gagnon C. Degradation of the pharmaceuticals diclofenac and sulfamethoxazole and their transformation products under controlled environmental conditions. Sci Total Environ. 2016 01;557–558:257–67.

148. Cycoń M, Mrozik A, Piotrowska-Seget Z. Antibiotics in the Soil Environment-Degradation and Their Impact on Microbial Activity and Diversity. Front Microbiol. 2019;10:338.

149. Loftin KA, Adams CD, Meyer MT, Surampalli R. Effects of ionic strength, temperature, and pH on degradation of selected antibiotics. J Environ Qual. 2008 Apr;37(2):378–86.

150. Beausse J. Selected Drugs in Solid Matrices: A Review of Environmental Determination, Occurrence and Properties of Principal Substances. TrAC Trends in Analytical Chemistry. 2004 Nov 1;23:753–61.

151. Yao L, Wang Y, Tong L, Li Y, Deng Y, Guo W, et al. Seasonal variation of antibiotics concentration in the aquatic environment: a case study at Jianghan Plain, central China. The Science of the total environment. 2015;527–528:56–64.

152. Tolls J. Sorption of veterinary pharmaceuticals in soils: a review. Environ Sci Technol. 2001 Sep 1;35(17):3397–406.

153. Li B, Zhang T. Biodegradation and adsorption of antibiotics in the activated sludge process. Environ Sci Technol. 2010 May 1;44(9):3468–73.

154. Maia AS, Ribeiro AR, Amorim CL, Barreiro JC, Cass QB, Castro PML, et al. Degradation of fluoroquinolone antibiotics and identification of metabolites/transformation products by liquid chromatography-tandem mass spectrometry. J Chromatogr A. 2014 Mar 14;1333:87–98.

155. Kümmerer K. Antibiotics in the aquatic environment – A review – Part II. Chemosphere. 2009 Apr 1;75(4):435–41.

156. Patrolecco L, Rauseo J, Ademollo N, Grenni P, Cardoni M, Levantesi C, et al. Persistence of the antibiotic sulfamethoxazole in river water alone or in the co-presence of ciprofloxacin. Science of The Total Environment. 2018 Nov 1;640–641:1438–46.

157. Kim S, Aga DS. Potential Ecological and Human Health Impacts of Antibiotics and Antibiotic-Resistant Bacteria from Wastewater Treatment Plants. Journal of Toxicology and Environmental Health, Part B. 2007 Nov 23;10(8):559–73.

158. Zhang Y, Boyd SA, Teppen BJ, Tiedje JM, Li H. Role of tetracycline speciation in the bioavailability to Escherichia coli for uptake and expression of antibiotic resistance. Environ Sci Technol. 2014 May 6;48(9):4893–900.

159. Gupta RC, Gupta AK, Shrivastava RK. ASSESSMENT OF WATER QUALITY STATUS OF HOLY RIVER KSHIPRA USING WATER QUALITY INDEX. Vol 32, No 1-2, January-April, 2012. 2012;(1–2). Available from: http://www.iwrs.org.in/journal/jan2012/4jan.pdf

160. Prasad B, Antony R, Malviya J, Kaushal A, Tiwari S. Bacterial screening of river Kshipra water at Ujjain after Kumbh (Religious Fair). International Journal of Applied Research. 2017 Jun 1;3(6):310–2.

161. Zhou Z-C, Zheng J, Wei Y-Y, Chen T, Dahlgren RA, Shang X, et al. Antibiotic resistance genes in an urban river as impacted by bacterial community and physicochemical parameters. Environmental science and pollution research international. 2017;24(30):23753–62.

162. Escobar-Páramo P, Le Menac'h A, Le Gall T, Amorin C, Gouriou S, Picard B, et al. Identification of forces shaping the commensal Escherichia coli genetic structure by comparing animal and human isolates. Environmental Microbiology. 2006 Nov;8(11):1975–84.

163. Walsh TR, Weeks J, Livermore DM, Toleman MA. Dissemination of NDM-1 positive bacteria in the New Delhi environment and its implications for human health: An environmental point prevalence study. The Lancet Infectious Diseases. 2011;11(5):355–62.

164. Qamar FN, Yousafzai MT, Khalid M, Kazi AM, Lohana H, Karim S, et al. Outbreak investigation of ceftriaxone-resistant Salmonella enterica serotype Typhi and its risk factors among the general population in Hyderabad, Pakistan: a matched case-control study. The Lancet Infectious Diseases. 2018 Dec 1;18(12):1368–76.

165. Ma L, Li B, Zhang T. New insights into antibiotic resistome in drinking water and management perspectives: A metagenomic based study of small-sized microbes. Water Research. 2019 Apr 1;152:191–201.

166. Ma Q, Xu X, Luo M, Wang J, Yang C, Hu X, et al. A Waterborne Outbreak of Shigella sonnei with Resistance to Azithromycin and Third-Generation Cephalosporins in China in 2015. Antimicrobial agents and chemotherapy. 2017;61(6).

167. Bruinsma N, Hutchinson JM, van den Bogaard AE, Giamarellou H, Degener J, Stobberingh EE. Influence of population density on antibiotic resistance. J Antimicrob Chemother. 2003 Feb 1;51(2):385–90.

168. Qiao M, Ying G-G, Singer AC, Zhu Y-G. Review of antibiotic resistance in China and its environment. Environment international. 2018;110:160–72.

169. Tran NH, Chen H, Do TV, Reinhard M, Ngo HH, He Y, et al. Simultaneous analysis of multiple classes of antimicrobials in environmental water samples using SPE coupled with UHPLC-ESI-MS/MS and isotope dilution. Talanta. 2016 Oct 1;159:163–73.

170. Buchberger WW. Current approaches to trace analysis of pharmaceuticals and personal care products in the environment. J Chromatogr A. 2011 Jan 28;1218(4):603–18.

171. Chin W, Zhong G, Pu Q, Yang C, Lou W, De Sessions PF, et al. A macromolecular approach to eradicate multidrug resistant bacterial infections while mitigating drug resistance onset. Nature Communications. 2018 Mar 2;9(1):917.

172. Chen L, Yang S, Huang Y, Zhang B, Kang F, Ding D, et al. Degradation of antibiotics in multi-component systems with novel ternary AgBr/Ag3PO4@natural hematite heterojunction photocatalyst under simulated solar light. Journal of Hazardous Materials. 2019 Jun 5;371:566–75.

173. Kokai-Kun JF, Roberts T, Coughlin O, Sicard E, Rufiange M, Fedorak R, et al. The Oral β-Lactamase SYN-004 (Ribaxamase) Degrades Ceftriaxone Excreted into the Intestine in Phase 2a Clinical Studies. Antimicrob Agents Chemother. 2017 Mar;61(3).

174. Connelly S, Fanelli B, Hasan NA, Colwell RR, Kaleko M. Oral Metallo-Beta-Lactamase Protects the Gut Microbiome From Carbapenem-Mediated Damage and Reduces Propagation of Antibiotic Resistance in Pigs. Front Microbiol. 2019;10:101.

175. Lehar SM, Pillow T, Xu M, Staben L, Kajihara KK, Vandlen R, et al. Novel antibody-antibiotic conjugate eliminates intracellular S. aureus. Nature. 2015 Nov 19;527(7578):323–8.

176. de Gunzburg J, Ghozlane A, Ducher A, Le Chatelier E, Duval X, Ruppé E, et al. Protection of the Human Gut Microbiome From Antibiotics. J Infect Dis. 2018 Jan 30;217(4):628–36.

177. Chahm T, de Souza LF, Dos Santos NR, da Silva BA, Rodrigues CA. Use of chemically activated termite feces a low-cost adsorbent for the adsorption of norfloxacin from aqueous solution. Water Sci Technol. 2019 Jan;79(2):291–301.

178. Jebastin T, Narayanan S. In silico epitope identification of unique multidrug resistance proteins from Salmonella Typhi for vaccine development. Comput Biol Chem. 2019 Feb;78:74–80.

179. Withey S, Cartmell E, Avery LM, Stephenson T. Bacteriophages--potential for application in wastewater treatment processes. Sci Total Environ. 2005 Mar 1;339(1–3):1–18.

180. Vestergaard M, Nøhr-Meldgaard K, Bojer MS, Nielsen CK, Meyer RL, Slavetinsky C, et al. Inhibition of the ATP Synthase Eliminates the Intrinsic Resistance of Staphylococcus aureus towards Polymyxins. mBio. 2017 Nov 8 [cited 2021 Feb 18];8(5). Available from: https://mbio.asm.org/content/8/5/e01114-17

181. Millan B, Park H, Hotte N, Mathieu O, Burguiere P, Tompkins TA, et al. Fecal Microbial Transplants Reduce Antibiotic-resistant Genes in Patients With Recurrent Clostridium difficile Infection. Clin Infect Dis. 2016 Jun 15;62(12):1479–86.

182. Ronda C, Chen SP, Cabral V, Yaung SJ, Wang HH. Metagenomic engineering of the mammalian gut microbiome in situ. Nat Methods. 2019 Feb;16(2):167–70.

183. Kang YK, Kwon K, Ryu JS, Lee HN, Park C, Chung HJ. Nonviral Genome Editing Based on a Polymer-Derivatized CRISPR Nanocomplex for Targeting Bacterial Pathogens and Antibiotic Resistance. Bioconjug Chem. 2017 Apr 19;28(4):957–67.

Appendix 1. An illustration of steps for calculating rank scores using ciprofloxacin as a representative antibiotic in aquatic environment of China (Study IV)

Ciprofloxacin				
Parameters	Date value	Utility function value	Uncertainty utility function value	Justification
Environmental risk				
Resistance risk (Percentage Exceedance PNEC (%))				
River water	42.5%	=42.5/100=0.425	0	
Lake water	18.18%	=18.18/100=0.1818	0	
Ground water	37.5%	=37.5/100=0.375	0	
Sea water	62.5%	=62.5/100=0.625	0	
Other water compartments	33.33%	=33.33/100=0.3333	0	
Wastewater	85.71%	=85.71/100=0.8571	0	
WWTPs influent	77.77%	=77.77/100=0.7777	0	
WWTPs effluent	30.76%	=30.76/100=0.3076	0	
Overall utility score		=(1/8)×(0.425)+(1/8)×(0.1818)+ (1/8)×(0.375)+(1/8)×(0.625)+ (1/8)×(0.3333)+(1/8)×(0.8571)+ (1/8)×(0.7777)+(1/8)×(0.3076)= 0.4853		
Uncertainty score			=(1/8)×(0)+(1/8)×(0)+ (1/8)×(0)+(1/8)×(0)+ (1/8)×(0)+(1/8)×(0)+ (1/8)×(0)+(1/8)×(0)=0	
Ecotoxicity risk				
Ecotoxicity (Percentage Exceedance $PNEC_E$ (%))				
River water	10%	=10/100=0.1	0	

Lake water	9.09%	=9.09/100=0.0909	0	
Ground water	12.5%	=12.5/100=0.125	0	
Sea water	0%	=0/100=0	0	
Other water compartments	16.66%	=16.66/100=0.1666	0	
Wastewater	57.14%	=57.14/100=0.5714	0	
WWTPs influent	0%	=0/100=0	0	
WWTPs effluent	0%	=0/100=0	0	
Overall utility score		=(1/8)×(0.1)+(1/8)×(0.0909)+ (1/8)×(0.125)+(1/8)×(0)+ (1/8)×(0.1666)+(1/8)×(0.5714)+ (1/8)×(0)+(1/8)×(0)=0.131		
Uncertainty score			=(1/8)×(0)+(1/8)×(0)+ (1/8)×(0)+(1/8)×(0)+ (1/8)×(0)+(1/8)×(0)+ (1/8)×(0)+(1/8)×(0)=0	
Bioaccumulation	0.28	0	0	1 if LogKow>4.5 and 0 if LogKow<4.5
Overall utility score		=(½)× 0=0		
Uncertainty score			= (1/2)×0=0	
Ecotoxicity risk overall score		=(1/2)×(0.131)+(1/2)×(0) =0.065		
Ecotoxicity risk uncertainty score			=(1/2)×(0)+(1/2)×(0) =0	
Environmental risk overall score		=(1/2)×(0.4853)+ (1/2)×(0.065)=0.2751		
Environmental risk uncertainty score			=(1/2)×(0)+(1/2)×(0)=0	
Human health risk				
Resistance risk (Percentage Exceedance PNEC (%))				
Drinking water	62.5%	=62.5/100=0.625	0	
Ground water	37.5%	=37.5/100=0.375	0	
Overall utility score		=(½)× (0.625)+(½)× (0.375)=0.5		

Uncertainty score			=(1/2)×(0)+(1/2)×(0) =0	
Toxicity risk (Percentage Exceedance TTC (%))				
Drinking water	62.5%	=62.5/100=0.625	0	
Ground water	37.5%	=37.5/100=0.375	0	
Overall utility score		=(½)×(0.625)+(½)×(0.375)=0.5		
Uncertainty score			=(1/2)×(0)+(1/2)×(0) =0	
Human health risk overall score		=(1/2)×(0.5)+ (1/2)×(0.5)=0.5		
Human health risk uncertainty score			=(1/2)×(0)+(1/2)×(0)=0	
Total risk				
Overall utility score		= (1/2)×(0.275)+ (1/2)×(0.5)=0.387		
Overall uncertainty score			=(1/2)×(0)+(1/2)×(0)=0	

Abbreviations: PNEC: predicted not effect concentration for the development of antibiotic resistance, $PNEC_E$: predicted no effect concentration for ecotoxicity, WWTPs: wastewater treatment plants, K_{ow}: octanol/water partitioning coefficient, TTC: threshold of toxicological concern.
0≤Utility function value or risk index ≤1

Appendix 2. Values of equations for regression analysis and values corresponding to various centiles for measured environmental concentration distributions of the maximum reported antibiotic concentrations (ng/L) in aquatic compartments of the Western Pacific Region of the WHO, and percentage exceeding a predicted no effect concentration (PNEC) for the development of antibiotic resistance (Study III)

Western Pacific Region of the WHO														
Antibiotic	**Compartment**	**n**	**R^2**	**a**	**b**	**Centile values (predicted threshold concentrations) (ng/L)**							**PNEC (ng/L)**	**Percentage Exceedance PNEC**
						1%	**5%**	**25%**	**50%**	**75%**	**95%**	**99%**		
Ciprofloxacin	River water	46	0.929	1,205	-2.052	0.592	2.177	13.905	50.455	183.083	1169.306	4300.151	64	43.48 (20/46)
	Lake water	12	0.853	1.013	-1.461	0.140	0.658	5.976	27.685	128.258	1164.114	5479.541	64	25 (3/12)
	Drinking water	8	0.877	0.852	-1.475	0.100	0.632	8.701	53.854	333.331	4589.866	28951.823	64	62.5 (5/8)
	Ground water	10	0.85	0.698	-0.989	0.012	0.115	2.822	26.116	241.670	5935.117	56205.919	64	30 (3/10)
	Sea water	8	0.915	1.8	-3.279	3.383	8.088	27.987	66.323	157.175	543.844	1300.416	64	62.5 (5/8)
	Other water compartments	8	0.934	1.004	-1.866	0.348	1.661	15.373	72.205	339.132	3139.541	14984.605	64	37.5 (3/8)
	Wastewater	12	0.965	0.727	-2.658	2.859	24.751	534.99	4530.238	38361.216	829169.327	7178758.530	64	91.67 (11/12)
	WWTPs influent	14	0.808	0.843	-2.251	0.814	5.236	74.151	467.978	2953.465	41823.072	269049.014	64	85.72 (12/14)

	WWTPs effluent	20	0.791	0.977	-1.805	0.293	1.459	14.359	70.387	345.033	3396.786	16928.006	64	40 (8/20)
Norfloxacin	River water	44	0.914	1.128	-2.052	0.571	2.296	16.642	65.940	261.277	1893.880	7612.268	500	9.09 (4/44)
	Lake water	9	0.949	1.167	-1.666	0.272	1.043	7.073	26.767	101.289	687.170	2636.544	500	0 (0/9)
	Drinking water	8	0.985	1.213	-1.657	0.281	1.023	6.456	23.229	83.577	527.298	1922.566	500	0 (0/8)
	Ground water	10	0.890	0.751	-1.444	0.067	0.540	10.584	83.709	662.052	12971.420	104818.172	500	20 (2/10)
	Sea water	15	0.885	0.941	-1.614	0.175	0.927	9.964	51.903	270.381	2905.235	15395.888	500	13.34 (2/15)
	Wastewater	10	0.922	0.735	-2.146	0.568	4.807	100.47	831.243	6877.032	143755.339	1215701.743	500	50 (5/10)
	WWTPs influent	14	0.978	2.215	-6.022	46.609	94.654	259.55	523.282	1054.984	2892.900	5874.972	500	57.14 (8/14)
	WWTPs effluent	21	0.889	2.168	-4.676	12.128	25.011	70.100	143.492	293.723	823.233	1697.717	500	14.29 (3/21)
Ofloxacin	River water	49	0.982	1.205	-2.25	0.864	3.178	20.299	73.658	267.278	1707.040	6277.678	500	16.33 (8/49)
	Lake water	13	0.943	1.222	-1.634	0.271	0.980	6.098	21.735	77.465	482.155	1741.301	500	7.69 (1/13)
	Drinking water	7	0.774	1.382	-1.923	0.511	1.590	8.006	24.630	75.773	381.645	1187.904	500	0 (0/7)
	Ground water	11	0.91	0.993	-1.097	0.058	0.281	2.664	12.727	60.810	577.008	2802.078	500	9.09 (1/11)
	Sea water	13	0.844	1.033	-1.879	0.369	1.685	14.657	65.913	296.426	2577.991	11776.188	500	15.38 (2/13)

	Other water compartments	7	0.93	1.175	-1.488	0.193	0.735	4.924	18.466	69.250	463.723	1763.005	500	0 (0/7)
	Wastewater	9	0.95	0.653	-2.298	0.905	10.005	306.36	3304.779	35649.356	1091592.85	12069703.21	500	66.67 (6/9)
	WWTPs influent	15	0.834	1.303	-4.16	25.542	85.167	473.13	1558.233	5131.875	28509.610	95062.810	500	86.67 (13/15)
	WWTPs effluent	24	0.933	1.077	-2.544	1.593	6.837	54.431	230.206	973.608	7751.254	33276.901	500	37.5 (9/24)
Fleroxacin	River water	6	0.946	1.037	-1.380	0.122	0.555	4.790	21.417	95.760	825.900	3750.645	-	-
	WWTPs effluent	10	0.955	1.642	-2.06	0.688	1.790	6.979	17.971	46.274	180.429	469.186	-	-
Lomefloxacin	River water	9	0.810	1.137	-1.514	0.193	0.767	5.475	21.457	84.099	600.116	2385.694	-	-
	Ground water	5	0.787	1.328	-1.482	0.231	0.754	4.056	13.061	42.059	226.234	737.449	-	-
	WWTPs effluent	6	0.978	1.626	-2.226	0.868	2.277	8.999	23.389	60.788	240.214	630.553	-	-
Enoxacin	River water	7	0.858	1.529	-2.383	1.089	3.039	13.104	36.186	99.923	430.830	1202.315	-	-
Nalidixic acid	River water	6	0.906	3.569	-5.143	6.155	9.553	17.866	27.607	42.658	79.780	123.834	16000	0 (0/6)
Moxifloxacin	River water	5	0.819	1.398	-1.945	0.534	1.639	8.106	24.619	74.772	369.700	1135.867	125	20 (1/5)
Enrofloxacin	River water	31	0.797	0.806	-1.198	0.040	0.279	4.462	30.644	210.469	3365.997	23585.226	64	35.48 (11/31)
	Lake water	9	0.934	1.278	-1.294	0.156	0.531	3.053	10.292	34.697	199.330	680.492	64	22.22 (2/9)

	Ground water	8	0.656	0.794	-1.194	0.037	0.271	4.511	31.899	225.560	3761.685	27144.853	64	25 (2/8)
	Sea water	8	0.847	1.085	-1.729	0.281	1.196	9.373	39.224	164.134	1286.893	5465.725	64	25 (2/8)
	Other water compartments	8	0.922	0.955	-1.387	0.104	0.537	5.573	28.337	144.088	1495.257	7732.544	64	25 (2/8)
	Wastewater	8	0.911	0.691	-2.49	1.725	16.714	424.01	4013.038	37982.186	963538.814	9334965.078	64	100 (8/8)
	WWTPs influent	9	0.971	1.485	-2.193	0.813	2.339	10.533	29.976	85.304	384.073	1104.923	64	22.22 (2/9)
	WWTPs effluent	15	0.925	1.622	-2.025	0.652	1.715	6.802	17.720	46.163	183.041	481.621	64	13.33 (2/15)
Sarafloxacin	WWTPs effluent	5	0.833	2.170	-1.106	0.274	0.565	1.581	3.234	6.615	18.521	38.170	-	-
Difloxacin	River water	6	0.950	1.100	-1.520	0.185	0.770	5.870	24.089	98.853	753.598	3138.195	-	-
	Lake water	5	0.967	0.960	-1.013	0.043	0.220	2.252	11.356	57.253	586.945	3009.452	-	-
Flumequine	River water	7	0.796	1.745	-1.932	0.594	1.461	5.256	12.799	31.167	112.143	275.622	250	0 (0/7)
Azithromycin	River water	21	0.941	1.293	-1.853	0.430	1.449	8.156	27.108	90.105	507.251	1707.213	250	9.52 (2/21)
	Lake water	6	0.981	2.074	-2.576	1.319	2.812	8.257	17.460	36.920	108.424	231.056	250	0 (0/6)
	Sea water	8	0.899	0.804	-0.642	0.008	0.057	0.911	6.288	43.394	698.788	4920.112	250	12.5 (1/8)
	WWTPs influent	7	0.917	3.393	-10.239	214.809	341.12	659.02	1041.558	1646.160	3180.256	5050.275	250	100 (7/7)

	WWTPs effluent	11	0.872	1.309	-3.068	3.686	12.224	67.376	220.686	722.845	3984.250	13212.001	250	63.63 (7/11)
Clarithromycin	River water	29	0.977	1.061	-1.438	0.145	0.638	5.243	22.663	97.957	804.658	3531.218	250	17.24 (5/29)
	Lake water	6	0.923	1.536	-1.737	0.413	1.148	4.917	13.516	37.152	159.121	441.986	250	0 (0/6)
	Sea water	9	0.846	1.155	-0.283	0.017	0.066	0.458	1.758	6.745	46.681	181.625	250	0 (0/9)
	Other water compartments	10	0.949	0.945	-1.049	0.044	0.234	2.491	12.884	66.650	708.988	3730.755	250	10 (1/10)
	WWTPs influent	7	0.898	0.799	-1.91	0.301	2.147	35.182	245.747	1716.546	28127.326	200476.015	250	57.14 (4/7)
	WWTPs effluent	11	0.909	0.796	-1.619	0.129	0.928	15.366	108.123	760.797	12598.494	90462.207	250	45.45 (5/11)
Erythromycin	River water	52	0.961	1.21	-2.419	1.193	4.363	27.653	99.810	360.249	2283.267	8351.704	1000	13.46 (7/52)
	Lake water	16	0.986	0.956	-1.599	0.173	0.895	9.270	47.054	238.851	2472.591	12764.753	1000	12.5 (2/16(
	Drinking water	7	0.985	1.332	-1.659	0.315	1.025	5.484	17.599	56.477	302.253	981.755	1000	0 (0/7)
	Ground water	8	0.93	1.164	-1.892	0.423	1.631	11.117	42.211	160.283	1092.783	4207.365	1000	0 (0/8)
	Sea water	14	0.977	0.858	-0.947	0.025	0.154	2.078	12.698	77.598	1049.080	6532.671	1000	7.14 (1/14)
	Other water compartments	12	0.904	0.914	-1.705	0.209	1.164	13.411	73.354	401.218	4624.318	25743.351	1000	16.66 (2/12)

	Wastewater	7	0.915	1.005	-2.921	3.906	18.612	171.92	806.246	3780.957	34925.122	166433.852	1000	57.14 (4/7)
	WWTPs influent	17	0.600	0.686	-1.64	0.100	0.984	25.553	245.850	2365.330	61435.071	605129.343	1000	23.53 (4/17)
	WWTPs effluent	26	0.872	1.098	-2.273	0.894	3.733	28.565	117.524	483.523	3699.753	15446.903	1000	7.69 (2/26)
Roxithromycin	River water	48	0.956	1.182	-1.719	0.306	1.155	7.650	28.465	105.913	701.294	2645.210	1000	2.08 (1/48)
	Lake water	12	0.915	1.258	-1.155	0.117	0.408	2.410	8.282	28.463	168.128	585.285	1000	0 (0/12)
	Drinking water	6	0.941	1.844	-1.608	0.408	0.955	3.208	7.448	17.290	58.079	136.017	1000	0 (0/6)
	Sea water	16	0.977	0.92	-1.054	0.041	0.228	2.585	13.985	75.647	858.097	4723.796	1000	6.25 (1/16)
	Other water compartments	6	0.774	1.162	-1.311	0.134	0.516	3.530	13.435	51.131	349.755	1349.733	1000	0 (0/6)
	WWTPs influent	12	0.860	1.163	-2.705	2.116	8.158	55.709	211.778	805.078	5497.951	21192.415	1000	16.66 (2/12)
	WWTPs effluent	19	0.921	1.180	-2.500	1.403	5.305	35.241	131.415	490.056	3255.276	12306.238	1000	10.52 (2/19)
Tylosin	River water	7	0.930	1.286	-1.351	0.174	0.591	3.358	11.234	37.586	213.595	723.642	4000	0 (0/7)
	Drinking water	7	0.822	1.695	-0.467	0.080	0.202	0.754	1.886	4.715	17.617	44.462	4000	0 (0/7)
	WWTPs effluent	8	0.924	1.085	-1.066	0.069	0.293	2.295	9.605	40.192	315.125	1338.406	4000	0 (0/8)
Tetracycline	River water	35	0.953	1.063	-1.912	0.408	1.784	14.594	62.905	271.143	2218.464	9708.608	1000	11.43 (4/35)

	Lake water	9	0.910	1.407	-2.269	0.910	2.777	13.592	40.987	123.604	604.925	1845.275	1000	0 (0/9)
	Ground water	6	0.939	1.771	-2.859	1.999	4.848	17.119	41.147	98.900	349.231	847.073	1000	0 (0/6)
	Sea water	11	0.939	1.220	-2.183	0.763	2.761	17.238	61.566	219.890	1372.729	4968.059	1000	9.09 (1/11)
	Other water compartments	7	0.954	1.159	-2.393	1.142	4.421	30.392	116.068	443.271	3047.275	11800.859	1000	14.28 (1/7)
	Wastewater	10	0.948	0.724	-2.485	1.656	14.468	316.74	2705.954	23117.247	506078.301	4420873.757	1000	70 (7/10)
	WWTPs influent	10	0.803	1.363	-3.439	6.551	20.717	106.72	333.511	1042.240	5369.081	16978.360	1000	10 (1/10)
	WWTPs effluent	15	0.857	1.056	-1.831	0.340	1.501	12.450	54.188	235.843	1956.717	8647.338	1000	13.33 (2/15)
Chlortetracycline	River water	28	0.942	1.158	-1.932	0.457	1.770	12.188	46.601	178.178	1226.931	4756.963	-	-
	Lake water	8	0.961	1.138	-1.758	0.317	1.257	8.956	35.060	137.250	977.699	3882.021	-	-
	Ground water	6	0.657	0.547	-1.193	0.008	0.149	8.870	151.701	2594.526	154181.485	2715866.149	-	-
	Sea water	5	0.803	0.964	-1.101	0.054	0.273	2.770	13.871	69.470	705.336	3592.031	-	-
	Other water compartments	5	0.916	2.457	-6.551	52.413	99.267	246.46	463.724	872.524	2166.286	4102.836	-	-
	Wastewater	7	0.931	0,926	-4.058	74.137	403.64	4507.1	24115.12	129026.844	1440751.99	7844118.402	-	-

	WWTPs influent	11	0.815	0.726	-1.631	0.110	0.957	20.773	176.424	1498.324	32523.335	282418.010	-	-
	WWTPs effluent	11	0.943	0.989	-1.909	0.378	1.850	17.711	85.159	409.472	3920.855	19162.620	-	-
Oxytetracycline	River water	38	0.943	1.184	-2.313	0.974	3.667	24.203	89.856	333.594	2201.820	8286.443	500	15.79 (6/38)
	Lake water	7	0.846	1.035	-1.709	0.253	1.153	9.989	44.793	200.858	1739.555	7922.949	500	14.28 (1/7)
	Drinking water	7	0.887	1.877	-2.942	2.128	4.910	16.145	36.931	84.477	277.789	640.902	500	0 (0/7)
	Ground water	6	0.684	0.669	-1.403	0.042	0.435	12.273	125.072	1274.576	35961.270	375414.482	500	16.66 (1/6)
	Sea water	8	0.911	0.889	-1.890	0.323	1.887	23.296	133.655	766.815	9467.020	55309.344	500	25 (2/8)
	Other water compartments	10	0.881	1.132	-2.403	1.169	4.675	33,647	132.676	523.155	3765.671	15061.532	500	20 (2/10)
	Wastewater	10	0.945	0.728	-2.992	8.210	70.872	1525.4	12879.23	108739.422	2340477.29	20203327.50	500	90 (9/10)
	WWTPs influent	11	0.938	1.192	-3.525	10.129	37.784	246.24	906.181	3334.739	21733.293	81067.893	500	54.54 (6/11)
	WWTPs effluent	16	0.952	1.345	-2.665	1.779	5.668	29.519	92.948	292.677	1524.238	4857.045	500	25 (4/16)
Doxycycline	River water	17	0.967	1.824	-2.044	0.700	1.655	5.634	13.201	30.931	105.293	248.900	2000	0 (0/17)
	Lake water	5	0.677	1.245	-2.271	0.903	3.184	19.157	66.695	232.196	1397.217	4927.753	2000	0 (0/5)
	Drinking water	5	0.800	1.226	-1.987	0.529	1.901	11.764	41.756	148.208	916.981	3297.830	2000	0 (0/5)

	Wastewater	6	0.969	0.758	-2.255	0.805	6.382	121.65	943.917	7324.234	139612.926	1106609.914	2000	33.33 (2/6)
	WWTPs effluent	7	0.787	0.543	-1.093	0.005	0.096	5.898	103.013	1799.060	110176.180	1982173.076	2000	14.28 (1/7)
Amoxicillin	River water	5	0.954	0.979	-1.423	0.119	0.593	5.815	28.413	138.380	1360.381	6757.299	250	20 (1/5)
Penicillin	Drinking water	7	0.925	0.950	-1,421	0.111	0.581	6.107	31.318	160.612	1687.386	8801.906	64	57.14 (4/7)
Lincomycin	River water	14	0.969	1.289	-2.191	0.785	2.653	15.014	50.092	167.122	945.882	3195.485	2000	0 (0/14)
	Lake water	9	0.929	0.981	-1.324	0.095	0.471	4.593	22.369	108.942	1062.562	5260.751	2000	0 (0/9)
	Drinking water	6	0.962	1.540	-1.536	0.307	0.850	3.626	9.940	27.251	116.276	322.120	2000	0 (0/6)
	Other water compartments	9	0.982	0.965	-1.754	0.255	1.297	13.142	65.708	328.526	3327.577	16917.639	2000	11.11 (1/9)
	Wastewater	5	0.902	0.740	-2.948	6.919	57.678	1181.1	9633.493	78569.940	1609010.78	13412110.63	2000	40 (2/5)
	WWTPs influent	7	0.847	0.839	-2.132	0.587	3.807	54.601	347.633	2213.306	31740.472	206007.506	20000	14.28 (1/7)
	WWTPs effluent	8	0.785	0.812	-1.759	0.200	1.382	21.657	146.641	992.907	15557.434	107452.517	2000	12.5 (1/8)
Sulfamethoxazole	River water	73	0.924	1.495	-2.813	2.116	6.045	26.943	76.139	215.164	959.051	2739.624	16000	0 (0/73)
	Lake water	20	0.974	1.237	-2.471	1.309	4.654	28.334	99.443	349.010	2124.647	7554.604	16000	0 (0/20)
	Drinking water	11	0.900	1.846	-2.486	1.220	2.855	9.579	22.218	51.532	172.875	404.488	16000	0 (0/11)

	Ground water	9	0.938	0.942	-1.328	0.087	0.461	4.940	25.690	133.593	1431.844	7574.434	16000	0 (0/9)
	Sea water	17	0.939	1.361	-1.679	0.334	1.059	5.471	17.126	53.609	276.831	876.889	16000	0 (0/17)
	Other water compartments	18	0.981	1.041	-2.067	0.563	2.544	21.760	96.737	430.047	3678.422	16607.909	16000	0 (0/18)
	Wastewater	15	0.987	0.507	-1.424	0.017	0.367	30.083	643.693	13773.142	1129706.77	24953751.52	16000	26.66 (4/15)
	WWTPs influent	26	0.947	1.027	-2.603	1.859	8.570	75.476	342.426	1553.546	13682.859	63060.078	16000	3.84 (1/26)
	WWTPs effluent	35	0.923	1.168	-2.658	1.923	7.369	49.913	188.662	713.114	4830.032	18510.627	16000	2.85 (1/35)
Sulfacetamide	River water	6	0.994	1.333	-1.126	0.126	0.408	2.181	6.994	22.424	119.856	388.963	-	-
Sulfachloropyridazine	River water	12	0.930	1.680	-1.432	0.294	0.747	2.824	7.118	17.942	67.836	172.628	-	-
	WWTPs influent	5	0.968	1.064	-1.522	0.175	0.767	6.259	26.943	115.977	947.035	4138.742	-	-
	WWTPs effluent	7	0.972	1.058	-1.153	0.078	0.343	2.833	12.297	53.371	441.036	1943.608	-	-
Sulfadiazine	River water	52	0.977	1.364	-2.247	0.875	2.763	14.219	44.398	138.629	713.288	2253.691	-	-
	Lake water	15	0.923	1.112	-1.737	0.295	1.210	9.026	36.480	147.437	1099.601	4509.099	-	-
	Drinking water	9	0.908	1.992	-2.533	1.270	2.792	8.570	18.689	40.755	125.117	275.063	-	-
	Ground water	5	0.922	3.106	-2.920	1.553	2.574	5.284	8.712	14.364	29.491	48.877	-	-

	Sea water	15	0.940	1.100	-0.406	0.018	0.075	0.570	2.339	9.600	73.183	304.756	-	-
	Other water compartments	10	0.980	1.201	-1.834	0.389	1.437	9.235	33.656	122.650	788.190	2911.185	-	-
	WWTPs influent	13	0.974	0.829	-1.604	0.134	0.893	13.220	86.072	560.376	8298.568	55089.777	-	-
	WWTPs effluent	20	0.917	0.805	-1.430	0.077	0.541	8.680	59.758	411.408	6602.280	46373.583	-	-
Sulfadimethoxine	River water	14	0.913	1.674	-1.103	0.186	0.475	1.803	4.559	11.530	43.802	111.839	-	-
	Sea water	5	0.948	1.204	0.0914	0.010	0.036	0.231	0.840	3.050	19.509	71.824	-	-
	WWTPs influent	7	0.969	1.034	-1.119	0.068	0.310	2.691	12.084	54.265	470.948	2148.117	-	-
	WWTPs effluent	6	0.932	0.898	-0.261	0.005	0.029	0.346	1.953	11.009	132.535	760.736	-	-
Sulfadimidine	River water	12	0.972	1.248	-2.243	0.857	3.015	18.064	62.701	217.637	1303.969	4584.969	-	-
Sulfamerazine	River water	9	0.847	0.678	-0.599	0.003	0.029	0.774	7.647	75.562	2039.476	20638.227	-	-
	WWTPs influent	7	0.976	1.541	-1.162	0.176	0.486	2.072	5.676	15.551	66.290	183.523	-	-
	WWTPs effluent	10	0.880	0.966	-0.380	0.010	0.049	0.496	2.474	12.348	124.773	633.290	-	-
Sulfameter	River water	11	0.946	1.103	-1.112	0.079	0.329	2.493	10.190	41.655	315.801	1309.994	-	-
Sulfamethazine	River water	47	0.952	1.291	-2.205	0.805	2.716	15.329	51.048	169.994	959.557	3235.578	-	-

	Lake water	14	0.810	1.207	-0.936	0.070	0.259	1.647	5.963	21.592	137.480	504.495	-	-
	Drinking water	9	0.953	1.500	-1.773	0.428	1.217	5.399	15.205	42.821	189.919	540.628	-	-
	Ground water	6	0.916	1.350	-1.110	0.126	0.402	2.102	6.641	20.982	109.808	351.110	-	-
	Sea water	9	0.957	1.226	-0.952	0.076	0.272	1.684	5.977	21.216	131.266	472.084	-	-
	Other water compartments	12	0.946	1.034	-2.127	0.642	2.926	25.395	114.041	512.119	4444.547	20272.758	-	-
	Wastewater	9	0.934	0.762	-3.210	14.443	113.23	2125.40	16315.42	125243.610	2350709.62	18430978.44	-	-
	WWTPs influent	16	0.900	1.047	-1.693	0.248	1.112	9.393	41.400	182.479	1541.760	6901.106	-	-
	WWTPs effluent	23	0.973	0.985	-1.309	0.093	0.456	4.407	21.327	103.204	997.324	4905.785	-	-
Sulfamethizole	River water	5	0.980	0.730	-0.442	0.003	0.023	0.480	4.032	33.841	722.277	6198.083	-	-
	WWTPs influent	5	0.891	0.796	-0.512	0.005	0.038	0.625	4.398	30.943	512.407	3679.287	-	-
	WWTPs effluent	5	0.970	1.599	-0.199	0.047	0.125	0.504	1.332	3.518	14.228	37.960	-	-
Sulfamonomethoxine	River water	12	0.986	1.591	-2.622	1.534	4.113	16.752	44.465	118.021	480.694	1288.880	-	-
	Lake water	6	0.818	1.206	-1.800	0.366	1.345	8.575	31.084	112.672	718.504	2639.463	-	-
	Drinking water	6	0.895	1.368	-2.177	0.778	2.449	12.541	39.028	121.457	621.946	1958.488	-	-

Sulfapyridine	River water	26	0.988	1.305	-1.743	0.357	1.189	6.588	21.659	71.200	394.509	1313.029	-	-
	Lake water	8	0.962	1.184	-0.692	0.042	0.157	1.035	3.841	14.260	94.123	354.226	-	-
	Drinking water	6	0.951	2.290	-0.769	0.209	0.415	1.100	2.167	4.269	11.326	22.474	-	-
	WWTPs effluent	7	0.891	0.934	-1.803	0.275	1.477	16.153	85.194	449.323	4914.644	26372.016	-	-
Sulfaquinoxaline	River water	12	0.969	1.884	-2.014	0.683	1.570	5.140	11.722	26.731	87.512	201.277	-	-
Sulfathiazole	River water	21	0.906	1.318	-1.241	0.150	0.494	2.690	8.741	28.401	154.728	508.906	-	-
	Lake water	6	0.950	1.953	-1.838	0.562	1.256	3.942	8.732	19.341	60.720	135.607	-	-
	WWTPs influent	7	0.966	0.773	-1.181	0.033	0.251	4.521	33.714	251,406	4525.815	34460.325	-	-
	WWTPs effluent	10	0.922	0.928	-0.762	0.021	0.112	1.243	6.624	35.314	392.276	2127.948	-	-
Trimethoprim	River water	50	0.926	1.141	-2.071	0.597	2.363	16.747	65.324	254.808	1805.777	7144.010	500	22 (11/50)
	Lake water	10	0.953	1.536	-1.357	0.234	0.650	2.782	7.647	21.018	90.019	250.042	500	0 (0/10)
	Drinking water	7	0.844	2.127	-2.022	0.719	1.504	4.301	8.926	18.524	52.961	110.753	500	0 (0/7)
	Ground water	5	0.821	0.961	-0.722	0.021	0.110	1.121	5.640	28.390	290.340	1486.135	500	0 (0/5)
	Sea water	12	0.990	1.234	-1.655	0.288	1.019	6.149	21.451	74.832	451.595	1595.935	500	0 (0/12)

	Other water compartments	14	0.923	1.422	-2.480	1.282	3.866	18.608	55.465	165.328	795.685	2398.786	500	7.14 (1/14)
	Wastewater	10	0.861	0.705	-2.297	0.909	8.415	200.186	1811.991	16401.330	390195.036	3613601.409	500	60 (6/10)
	WWTPs influent	22	0.830	0.897	-2.095	0.552	3.176	38.337	216.552	1223.214	14766.941	84925.650	500	36.36 (8/22)
	WWTPs effluent	33	0.872	0.974	-1.973	0.434	2.172	21.536	106.088	522.591	5181.181	25948.656	500	21.21 (7/33)
Thiamphenicol	River water	7	0.949	0.944	-1.084	0.048	0.255	2.715	14.070	72.913	777.562	4098.799	1000	0 (0/7)
Florfenicol	River water	14	0.969	1.089	-2.176	0.728	3.074	23.922	99.578	414.505	3225.442	13626.596	2000	7.14 (1/14)
	Lake water	10	0.898	1.334	-2.942	2.894	9.384	50.094	160.471	514.058	2744.233	8897.874	2000	0 (0/10)
	Sea water	5	0.912	1.517	-1.399	0.245	0.689	3.003	8.360	23.272	101.507	285.584	2000	0 (0/5)
Chloramphenicol	River water	19	0.92	1.357	-1.66	0.323	1.026	5.324	16.722	52.521	272.528	866.200	8000	0(0/19)
	WWTPs influent	6	0.938	5.533	-9.376	18.798	24.962	37.382	49.495	65.533	98.139	130.320	8000	0(0/6)
	WWTPs effluent	9	0.986	1.552	-1.744	0.421	1.158	4.888	13.296	36.166	152.595	419.418	8000	0 (0/9)

Abbreviations: n: the total number of data points or studies, a: slope, b: intercept, PNEC: predicted not effect concentration for the development of antibiotic resistance, WWTPs: waste water treatment plants.

Appendix 3. Values of equations for regression analysis and values corresponding to various centiles for measured environmental concentration distributions of the maximum reported antibiotic concentrations (ng/L) in aquatic compartments of the South East Asia Region of the WHO, and percentage exceeding a predicted no effect concentration (PNEC) for the development of antibiotic resistance (Study III)

South East Asia of the WHO														
Antibiotic	**Compartment**	**n**	**R^2**	**a**	**b**	**Centile values (predicted threshold concentrations) (ng/L)**							**PNEC (ng/L)**	**Percentage Exceedance PNEC**
						1%	**5%**	**25%**	**50%**	**75%**	**95%**	**99%**		
Ciprofloxacin	River water	3	0.846	0.996	-2.091	0.580	2.805	26.436	125.718	597.855	5634.522	27232.545	64	33.33 (1/3)
	WWTPs influent	4	0.984	0.823	-3.387	19.443	130.86	1976.42	13044.61	86095.702	1300284.63	8751842.279	64	100 (4/4)
	WWTPs effluent	4	0.861	0.380	-1.555	0.009	0.580	207.555	12362.47	736339.825	263431338.99	16370930045.983	64	100 (4/4)
Ofloxacin	Wastewater	3	1.000	1.014	-3.933	38.412	180.53	1635.012	7563.072	34984.486	316841.340	1489110.402	500	100 (3/3)
Levofloxacin	Wastewater	3	0.989	1.219	-4.929	136.48	494.46	3091.465	11052.95	39517.754	247072.813	895127.807	250	100 (3/3)
Roxithromycin	Other water compartments	3	1.000	1.646	-2.159	0.791	2.053	7.978	20.496	52.655	204.629	530.883	1000	0 (0/3)
Sulfamethoxazole	River water	4	0.873	0.633	-0.992	0.008	0.093	3.174	36.910	429.225	14643.522	174685.045	16000	0 (0/4)

	Other water compartments	3	0.785	1.335	-3.387	6.230	20.183	107.608	344.412	1102.337	5877.312	19039.754	16000	0 (0/3)
	WWTPs influent	4	0.966	0.921	-2.660	2.303	12.654	143.159	772.974	4173.593	47218.064	259452.880	16000	0 (0/4)
	WWTPs effluent	3	0.777	1.221	-2.861	2.741	9.909	61.767	220.375	786.265	4901.148	17719.120	16000	0 (0/3)
Sulfamethazine	Other water compartments	3	0.832	7.063	-13.04	32.950	41.148	56.459	70.345	87.645	120.258	150.177	-	-
Trimethoprim	Other water compartments	3	0.975	2.571	-5.706	20.631	37.984	90.578	165.718	303.190	723.006	1331.103	500	0 (0/3)

Abbreviations: n: the total number of data points or studies, a: slope, b: intercept, PNEC: predicted not effect concentration for the development of antibiotic resistance, WWTPs: waste water treatment plants.

Appendix 4. Values of equations for regression analysis and values corresponding to various centiles for measured environmental concentration distributions of the maximum reported antibiotic concentrations (ng/L) in aquatic compartments of China, and percentage exceeding a predicted no effect concentration (PNEC) for the development of antibiotic resistance (Study III)

China														
Antibiotic	**Compartment**	**n**	**R^2**	**a**	**b**	**Centile values (predicted threshold concentrations) (ng/L)**							**PNEC (ng/L)**	**Percentage Exceedance PNEC**
						1%	**5%**	**25%**	**50%**	**75%**	**95%**	**99%**		
Ciprofloxacin	River water	40	0.944	1.148	-1.948	0.468	1.837	12.863	49.758	192.486	1347.918	5288.099	64	42.5 (17/40)
	Lake water	11	0.823	0.971	-1.346	0.098	0.492	4.915	24.333	120.458	1202.760	6053.780	64	18.18 (2/11)
	Drinking water	8	0.877	0.852	-1.475	0.100	0.632	8.701	53.854	333.331	4589.866	28951.823	64	62.5 (5/8)
	Ground water	8	0.781	0.730	-1.300	0.039	0.337	7.192	60.370	506.735	10815.487	92811.080	64	37.5 (3/8)
	Sea water	8	0.915	1.800	-3.279	3.383	8.088	27.987	66.323	157.175	543.844	1300.416	64	62.5 (5/8)
	Other water compartments	6	0.924	1.022	-1.795	0.302	1.403	12.485	57.064	260.814	2321.699	10780.279	64	33.33 (2/6)
	Wastewater	7	0.971	0.575	-2.054	0.336	5.147	250.696	3733.997	55615.976	2708874.69	41495050.99	64	85.71 (6/7)
	WWTPs influent	9	0.981	2.967	-6.204	20.273	34.405	82.084	123.311	208.129	441.961	750.024	64	77.77 (7/9)

	WWTPs effluent	13	0.976	2.045	-3.161	2.559	5.513	16.440	35.133	75.084	223.898	482.280	64	30.76 (4/13)
Norfloxacin	River water	41	0.915	1.097	-1.995	0.499	2.085	15.986	65.856	271.297	2079.730	8694.437	500	9.75 (4/41)
	Lake water	9	0.949	1.167	-1.666	0.272	1.043	7.073	26.767	101.289	687.170	2636.544	500	0 (0/9)
	Drinking water	8	0.985	1.213	-1.657	0.281	1.023	6.456	23.229	83.577	527.298	1922.566	500	0 (0/8)
	Ground water	8	0.815	0.795	-1.805	0.221	1.590	26.426	186.398	1314.786	21849.350	157276.557	500	25 (2/8)
	Sea water	12	0.914	0.879	-1.607	0.152	0.906	11.505	67.331	394.050	5006.015	29839.978	500	16.66 (2/12)
	Wastewater	6	0.760	0.562	-1.476	0.031	0.501	26.679	422.994	6706.474	357371.844	5831006.803	500	33.33 (2/6)
	WWTPs influent	13	0.980	2.197	-6.036	48.810	99.701	275.663	558.963	1133.412	3133.751	6401.144	500	61.53 (8/13)
	WWTPs effluent	20	0.888	2.130	-4.553	11.101	23.191	66.206	137.265	284.591	812.438	1697.230	500	15 (3/20)
Ofloxacin	River water	44	0.981	1.187	-2.207	0.793	2.976	19.547	72.328	267.633	1758.055	6594.231	500	15.90 (7/44)
	Lake water	12	0.930	1.361	-1.664	0.326	1.033	5.334	16.697	52.265	269.894	854.916	500	0 (0/12)
	Drinking water	7	0.774	1.382	-1.923	0.511	1.590	8.006	24.630	75.773	381.645	1187.904	500	0 (0/7)
	Ground water	9	0.901	0.983	-1.228	0.076	0.377	3.657	17.752	86.177	836.636	4128.733	500	11.11 (1/9)
	Sea water	11	0.808	1.034	-1.822	0.325	1.484	12.876	57.821	259.657	2253.495	10278.787	500	9.09 (1/11)

	Other water compartments	6	0.877	1.077	-1.449	0.153	0.658	5.238	22.152	93.685	745.863	3202.062	500	0 (0/6)
	WWTPs influent	16	0.942	1.956	-5.986	74.300	165.73	519.393	1149.019	2541.899	7966.259	17769.148	500	85.71 (12/14)
	WWTPs effluent	23	0.937	1.188	-2.707	2.091	7.835	51.389	189.941	702.056	4604.436	17251.418	500	34.78 (8/23)
Fleroxacin	River water	6	0.946	1.037	-1.38	0.122	0.555	4.790	21.417	95.760	825.900	3750.645	-	-
	WWTPs effluent	10	0.955	1.642	-2.06	0.688	1.790	6.979	17.971	46.274	180.429	469.186	-	-
Lomefloxacin	River water	8	0.789	1.145	-1.644	0.254	0.998	7.026	27.278	105.897	745.352	2934.626	-	-
	Ground water	5	0.787	1.328	-1.482	0.231	0.754	4.056	13.061	42.059	226.234	737.449	-	-
	WWTPs effluent	6	0.978	1.626	-2.226	0.868	2.277	8.999	23.389	60.788	240.214	630.553	-	-
Enoxacin	River water	7	0.858	1.529	-2.383	1.089	3.039	13.104	36.186	99.923	430.830	1202.315	-	-
Nalidixic acid	River water	5	0.949	3.322	-4.853	5.762	9.241	18.106	28.898	46.122	90.366	144.928	16000	0 (0/5)
Moxifloxacin	River water	5	0.819	1.398	-1.945	0.534	1.639	8.106	24.619	74.772	369.700	1135.867	125	20 (1/5)
Enrofloxacin	River water	30	0.786	0.800	-1.156	0.034	0.245	3.998	27.861	194.139	3170.057	22539.010	64	33.33 (10/30)
	Lake water	8	0.908	1.478	-1.272	0.193	0.559	2.537	7.255	20.748	94.084	272.023	64	12.5 (1/8)
	Ground water	7	0.615	1.522	-2.774	1.968	5.519	23.958	66.466	184.400	800.425	2244.309	64	28.57 (2/7)

	Sea water	8	0.847	1.085	-1.729	0.281	1.196	9.373	39.224	164.134	1286.893	5465.725	64	25 (2/8)
	Other water compartments	5	0.989	0.850	-0.985	0.026	0.167	2.319	14.415	89.607	1241.496	7865.084	64	20 (1/5)
	Wastewater	5	0.952	0.689	-2.871	6.173	60.201	1541.58	14686.17	139909.98	3582731.50	34939812.64	64	100 (5/5)
	WWTPs influent	8	0.971	1.393	-2.035	0.618	1.906	9.477	28.899	88.120	438.203	1351.771	64	37.5 (3/8)
	WWTPs effluent	14	0.942	1.614	-1.963	0.595	1.574	6.285	16.453	43.066	171.929	454.557	64	14.28 (2/14)
Sarafloxacin	WWTPs effluent	5	0.833	2.170	-1.106	0.274	0.565	1.581	3.234	6.615	18.521	38.170	-	-
Difloxacin	River water	6	0.950	1.100	-1.520	0.185	0.770	5.870	24.089	98.853	753.598	3138.195	-	-
	Lake water	5	0.967	0.960	-1.013	0.043	0.220	2.252	11.356	57.253	586.945	3009.452	-	-
Flumequine	River water	7	0.796	1.745	-1.932	0.594	1.461	5.256	12.799	31.167	122.143	275.622	250	0 (0/7)
Azithromycin	River water	16	0.940	1.547	-1.945	0.567	1.563	6.626	18.083	49.348	209.183	576.836	250	0 (0/16)
	Lake water	5	0.982	2.698	-2.969	1.731	3.096	7.087	12.602	22.410	51.298	91.769	250	0 (0/5)
	Sea water	8	0.899	0.804	-0.642	0.008	0.057	0.911	6.288	43.394	698.788	4920.112	250	12.5 (1/8)
	WWTPs influent	5	0.873	3.844	-11.356	223.370	335.98	600.826	899.942	1347.969	2410.549	3625.799	250	100 (5/5)
	WWTPs effluent	6	0.682	1.392	-3.259	4.677	14.441	71.893	219.399	669.550	3333.369	10291.118	250	66.66 (4/6)

Clarithromycin	River water	20	0.951	1.476	-1.497	0.274	0.794	3.608	10.333	29.594	134.471	389.354	250	0 (0/20)
	Lake water	5	0.879	1.586	-1.577	0.337	0.906	3.707	9.870	26.279	107.506	289.153	250	0 (0/5)
	Sea water	9	0.846	1.155	-0.283	0.017	0.066	0.458	1.758	6.745	46.681	181.625	250	0 (0/9)
	WWTPs influent	5	0.949	0.729	-1.551	0.086	0.743	15.935	134.144	1129.271	24203.992	208315.394	250	40 (2/5)
	WWTPs effluent	6	0.754	0.968	-1.916	0.377	1.906	19.167	95.354	474.379	4770.543	24131.859	250	33.33 (2/6)
Erythromycin	River water	42	0.944	1.261	-2.574	1.572	5.455	32.089	109.961	376.810	2216.365	7692.735	1000	14.28 (6/42)
	Lake water	13	0.982	0.938	-1.510	0.135	0.718	7.776	40.720	213.246	2308.784	12300.510	1000	15.38 (2/13)
	Drinking water	7	0.985	1.332	-1.659	0.315	1.025	5.484	17.599	56.477	302.253	981.755	1000	0 (0/7)
	Ground water	7	0.933	1.082	-1.740	0.287	1.224	9.655	40.563	170.414	1343.787	5730.300	1000	0 (0/7)
	Sea water	11	0.968	1.034	-0.829	0.036	0.163	1.411	6.335	28.448	246.893	1126.143	1000	0 (0/11)
	Other water compartments	5	0.911	0.694	-1.272	0.030	0.290	7.260	68.054	637.882	15957.289	153087.348	1000	20 (1/5)
	WWTPs influent	14	0.648	0.657	-1.507	0.057	0.617	18.499	196.680	2091.126	62710.736	683319.567	1000	21.42 (3/14)
	WWTPs effluent	21	0.887	1.094	-2.299	0.944	3.962	30.544	126.317	522.394	4027.032	16901.416	1000	4.76 (1/21)
Roxithromycin	River water	39	0.936	1.209	-1.781	0.354	1.296	8.227	29.725	107.401	681.751	2496.376	1000	2.56 (1/39)

	Lake water	12	0.915	1.258	-1.155	0.117	0.408	2.410	8.282	28.463	168.128	585.285	1000	0 (0/12)
	Drinking water	6	0.941	1.844	-1.608	0.408	0.955	3.208	7.448	17.290	58.079	136.017	1000	0 (0/6)
	Sea water	14	0.956	0.858	-0.930	0.024	0.147	1.985	12.132	74.137	1002.293	6241.331	1000	7.14 (1/14)
	WWTPs influent	10	0.822	1.091	-2.585	1.726	7.273	56.384	234.092	971.889	7534.297	31746.346	1000	20 (2/10)
	WWTPs effluent	15	0.925	1.068	-2.242	0.834	3.623	29.357	125.676	538.017	4358.891	18944.331	1000	13.33 (2/15)
Tylosin	River water	7	0.930	1.286	-1.351	0.174	0.591	3.358	11.234	37.586	213.595	723.642	4000	0 (0/7)
	Drinking water	7	0.822	1.695	-0.467	0.080	0.202	0.754	1.886	4.715	17.617	44.462	4000	0 (0/7)
	WWTPs effluent	7	0.892	1.181	-1.364	0.153	0.578	3.836	14.287	53.220	352.953	1332.801	4000	0 (0/7)
Tetracycline	River water	31	0.943	1.060	-1.850	0.355	1.561	12.852	55.627	240.765	1981.672	8708.650	1000	9.67 (3/31)
	Lake water	8	0.849	1.329	-2.055	0.625	2.035	10.933	35.178	113.186	608.048	1980.274	1000	0 (0/8)
	Ground water	6	0.939	1.771	-2.859	1.999	4.848	17.119	41.147	98.900	349.231	847.073	1000	0 (0/6)
	Sea water	9	0.906	1.138	-1.908	0.429	1.703	12.132	47.493	185.919	1324.389	5258.575	1000	11.11 (1/9)
	Other water compartments	6	0.92	1.042	-2.182	0.727	3.277	27.974	124.179	551.255	4705.483	21214.336	1000	16.66 (1/6)
	Wastewater	5	0.907	0.574	-2.246	0.724	11.150	546.793	8182.613	122450.690	6004690.66	92419336.60	1000	80 (4/5)

	WWTPs influent	8	0.973	2.583	-6.179	31.013	56.935	135.224	246.706	450.097	1069.004	1962.536	1000	0 (0/8)
	WWTPs effluent	13	0.751	1.061	-1.674	0.243	1.065	8.751	37.823	163.481	1342.898	5893.267	1000	7.69 (1/13)
Chlortetracycline	River water	26	0.909	1.162	-1.852	0.391	1.508	10.312	39.247	149.369	1021.740	3942.978	-	-
	Lake water	8	0.961	1.138	-1.758	0.317	1.257	8.956	35.060	137.250	977.699	3882.021	-	-
	Ground water	6	0.657	0.547	-1.193	0.008	0.149	8.870	151.701	2594.526	154181.485	2715866.149	-	-
	Sea water	5	0.803	0.964	-1.101	0.054	0.273	2.770	13.871	69.470	705.336	3592.031	-	
	Wastewater	5	0.963	0.809	-3.688	48.209	335.36	5308.62	36200.49	246857.972	3907581.70	27183099.76	-	-
	WWTPs influent	7	0.844	1.633	-2.841	2.066	5.401	21.218	54.922	142.161	558.472	1459.917	-	-
	WWTPs effluent	8	0.856	1.416	-2.103	0.695	2.106	10.206	30.561	91.517	443.393	1342.983	-	-
Oxytetracycline	River water	35	0.931	1.199	-2.345	1.036	3.837	24.732	90.323	329.868	2126.430	7871.121	500	14.28 (5/35)
	Lake water	7	0.846	1.077	-1.779	0.310	1.332	10.606	44.855	189.705	1510.308	6483.902	500	14.28 (1/7)
	Drinking water	7	0.887	1.877	-2.942	2.128	4.910	16.145	36.931	84.477	277.789	640.902	500	0 (0/7)
	Ground water	6	0.684	0.669	-1.403	0.042	0.435	12.273	125.072	1274.576	35961.270	375414.482	500	16.66 (1/6)
	Sea water	8	0.911	0.889	-1.890	0.323	1.887	23.296	133.655	766.815	9467.020	55309.344	500	25 (2/8)

	Other water compartments	5	0.959	0.846	-1.876	0.294	1.876	26.316	165.004	1034.578	14513.278	92750.076	500	40 (2/5)
	Wastewater	5	0.967	0.885	-4.439	243.881	1436.2	17934.2	103709.6	599728.268	7488776.64	44102262.98	500	100 (5/5)
	WWTPs influent	8	0.969	1.748	-4.680	22.207	54.495	195.651	475.714	1156.669	4152.765	10190.797	500	50 (4/8)
	WWTPs effluent	13	0.966	1.406	-2.657	1.719	5.247	25.706	77.582	234.143	1147.202	3502.226	500	23.07 (3/13)
Doxycycline	River water	15	0.961	1.858	-2.023	0.687	1.598	5.318	12.269	28.302	94.208	219.218	2000	0 (0/15)
	Lake water	5	0.677	1.245	-2.271	0.903	3.184	19.157	66.695	232.196	1397.217	4927.753	2000	0 (0/5)
	Drinking water	5	0.800	1.226	-1.987	0.529	1.901	11.764	41.756	148.208	916.981	3297.830	2000	0 (0/5)
	WWTPs effluent	6	0.809	0.503	-1.017	0.002	0.056	4.797	105.164	2305.699	195864.406	4434195.725	2000	16.66 (1/6)
Penicillin	Drinking water	7	0.925	0.950	-1.421	0.111	0.581	6.107	31.318	160.612	1687.386	8801.906	64	57.14 (4/7)
Lincomycin	River water	10	0.968	1.411	-2.747	1.987	6.041	29.432	88.480	265.992	1295.932	3940.660	2000	0 (0/10)
	Lake water	7	0.917	0.911	-1.271	0.069	0.389	4.516	24.841	136.633	1587.514	8887.717	2000	0 (0/7)
	Drinking water	6	0.962	1.540	-1.536	0.307	0.850	3.626	9.940	27.251	116.276	322.120	2000	0 (0/6)
Sulfamethoxazole	River water	55	0.956	1.561	-2.907	2.355	6.435	26.926	72.823	196.950	824.111	2251.962	16000	0 (0/55)
	Lake water	17	0.979	1.292	-2.474	1.301	4.383	24.706	82.198	273.471	1541.584	5193.254	16000	0 (0/17)

	Drinking water	11	0.900	1.846	-2.486	1.220	2.855	9.579	22.218	51.532	172.875	404.488	16000	0 (0/11)
	Ground water	7	0.936	1.147	-1.443	0.170	0.667	4.678	18.116	70.164	492.168	1933.155	16000	0 (0/7)
	Sea water	16	0.945	1.338	-1.615	0.294	0.950	5.046	16.107	51.420	273.126	882.472	16000	0 (0/16)
	Other water compartments	8	0.948	1.601	-2.373	1.069	2.850	11.506	30.353	80.074	323.288	861.505	16000	0 (0/8)
	Wastewater	6	0.956	0.468	-1.176	0.003	0.100	11.792	325.702	8995.761	1065283.04	30454347.81	16000	33.33 (2/6)
	WWTPs influent	19	0.948	1.047	-2.482	1.408	6.303	53.256	234.738	1034.652	8741.752	39129.145	16000	0 (0/19)
	WWTPs effluent	25	0.981	1.173	-2.472	1.331	5.072	34.073	128.61	481.315	3233.547	12321.488	16000	0 (0/25)
Sulfacetamide	River water	6	0.994	1.333	-1.126	0.126	0.408	2.181	6.994	22.424	119.856	388.963	-	-
Sulfachloropyridazine	River water	11	0.910	1.839	-1.414	0.319	0.749	2.524	5.873	13.667	46.060	108.119	-	-
	WWTPs effluent	6	0.965	1.121	-1.019	0.068	0.277	2.029	8.110	32.411	237.859	964.391	-	-
Sulfadiazine	River water	49	0.974	1.349	-2.203	0.810	2.593	13.585	42.960	135.848	711.828	2278.031	-	-
	Lake water	14	0.924	1.089	-1.659	0.244	1.030	8.018	33.375	138.926	1081.040	4567.094	-	-
	Drinking water	9	0.908	1.992	-2.533	1.270	2.792	8.570	18.689	40.755	125.117	275.063	-	-
	Sea water	15	0.940	1.100	-0.406	0.018	0.075	0.570	2.339	9.600	73.183	304.756	-	-

	Other water compartments	8	0.983	1.136	-1.766	0.321	1.278	9.138	35.857	140.708	1005.801	4003.307	-	-
	WWTPs influent	13	0.974	0.829	-1.644	0.134	0.893	13.220	86.072	560.376	8298.568	55089.777	-	-
	WWTPs effluent	20	0.917	0.805	-1.430	0.077	0.541	8.680	59.758	411.408	6602.280	46373.583	-	-
Sulfadimethoxine	River water	9	0.958	2.033	-0.943	0.209	0.452	1.355	2.910	6.246	18.747	40.564	-	-
	Sea water	5	0.948	1.204	0.0914	0.010	0.036	0.231	0.840	3.050	19.509	71.824	-	-
	WWTPs influent	5	0.984	1.166	-0.837	0.053	0.203	1.378	5.222	19.784	134.437	516.407	-	-
	WWTP effluent	5	0.885	1.109	0.0222	0.008	0.031	0.235	0.955	3.874	29.051	119.586	-	-
Sulfadimidine	River water	9	0.964	1.234	-1.975	0.519	1.852	11.321	39.855	140.305	857.888	3059.815	-	-
Sulfamerazine	River water	9	0.847	0.678	-0.599	0.003	0.029	0.774	7.647	75.562	2039.476	20638.227	-	-
	WWTPs influent	7	0.976	1.541	-1.162	0.176	0.486	2.072	5.676	15.551	66.290	183.523	-	-
	WWTPs effluent	10	0.880	0.966	-0.380	0.010	0.049	0.496	2.474	12.348	124.773	633.290	-	-
Sulfameter	River water	11	0.946	1.103	-1.112	0.079	0.329	2.493	10.190	41.655	315.801	1309.994	-	-
Sulfamethazine	River water	38	0.968	1.192	-2.053	0.590	2.200	14.337	52.761	194.161	1265.395	4720.082	-	-
	Lake water	12	0.789	1.417	-0.865	0.093	0.282	1.363	4.078	12.202	59.053	178.725	-	-

	Drinking water	9	0.953	1.500	-1.773	0.428	1.217	5.339	15.205	42.821	189.919	540.628	-	-
	Ground water	5	0.873	1.314	-0.912	0.084	0.277	1.516	4.944	16.120	88.279	291.404	-	-
	Sea water	9	0.957	1.226	-0.952	0.076	0.272	1.684	5.977	21.216	131.266	472.084	-	-
	WWTPs influent	13	0.853	1.197	-1.621	0.257	0.955	6.176	22.606	82.737	535.015	1984.727	-	-
	WWTPs effluent	19	0.978	0.975	-1.154	0.063	0.314	3.103	15.261	75.054	742.368	3711.826	-	-
Sulfamethizole	WWTPs influent	5	0.891	0.796	-0.512	0.005	0.038	0.625	4.398	30.943	512.407	3679.287	-	-
	WWTPs effluent	5	0.970	1.599	-0.199	0.047	0.125	0.504	1.332	3.518	14.228	37.960	-	-
Sulfamonomethoxine	River water	12	0.986	1.591	-2.622	1.534	4.113	16.752	44.465	118.021	480.694	1288.880	-	-
	Lake water	6	0.818	1.206	-1.800	0.366	1.345	8.575	31.084	112.672	718.505	2639.463	-	-
	Drinking water	6	0.895	1.368	-2.177	0.778	2.449	2.541	39.028	121.457	621.946	1958.488	-	-
Sulfapyridine	River water	21	0.991	1.272	-1.630	0.284	0.973	5.639	19.118	64.820	375.466	1289.248	-	-
	Lake water	8	0.962	1.184	-0.692	0.042	0.157	1.035	3.841	14.260	94.123	354.226	-	-
	Drinking water	6	0.951	2.290	-0.769	0.209	0.415	1.100	2.167	4.269	11.326	22.474	-	-
	WWTPs effluent	6	0.929	0.920	-1.661	0.189	1.041	11.811	63.890	345.602	3920.299	21581.110	-	-

Sulfaquinoxaline	River water	12	0.969	1.884	-2.014	0.683	1.570	5.140	11.722	26.731	87.512	201.277	-	-
Sulfathiazole	River water	16	0.876	1.275	-1.148	0.119	0.408	2.352	7.950	26.878	155.051	530.858	-	-
	Lake water	6	0.950	1.953	-1.838	0.562	1.256	3.942	8.732	19.341	60.720	135.607	-	-
	WWTPs effluent	6	0.844	0.997	-0.454	0.013	0.064	0.601	2.853	13.548	127.400	614.772	-	-
Trimethoprim	River water	38	0.901	1.124	-2.033	0.548	2.215	16.167	64.375	256.330	1871.164	7558.287	500	18.42 (7/38)
	Lake water	8	0.922	1.657	-1.384	0.270	0.696	2.680	6.843	17.470	67.284	173.459	500	0 (0/8)
	Drinking water	7	0.844	2.127	-2.022	0.719	1.504	4.301	8.926	18.524	52.961	110.753	500	0 (0/7)
	Sea water	10	0.992	1.226	-1.509	0.215	0.775	4.794	17.015	60.393	373.658	1343.824	500	0 (0/10)
	Other water compartments	5	0.727	1.510	-2.102	0.710	2.008	8.818	24.663	68.982	302.942	856.408	500	0 (0/5)
	WWTPs influent	15	0.909	1.255	-2.814	2.447	8.543	50.674	174.675	602.106	3571.671	12470.812	500	40 (6/15)
	WWTPs effluent	21	0.931	0.994	-1.946	0.414	2.009	19.019	90.729	432.820	4097.593	19867.201	500	23.80 (5/21)
Thiamphenicol	River water	7	0.949	0.944	-1.084	0.255	0.618	2.715	14.070	72.913	777.562	4098.799	1000	0 (0/7)
Florfenicol	River water	12	0.952	1.065	-1.996	0.490	2.137	17.412	74.848	321.738	2622.056	11443.071	2000	8.33 (1/12)
	Lake water	10	0.898	1.334	-2.942	2.894	9.384	50.094	160.471	514.058	2744.233	8897.874	2000	0 (0/10)

	Sea water	5	0.912	1.517	-1.399	0.245	0.689	3.003	8.360	23.272	101.507	285.584	2000	0 (0/5)
Chloramphenicol	River water	16	0.914	1.396	-1.543	0.275	0.845	4.189	12.744	38.767	192.116	591.206	8000	0 (0/16)
	WWTPs influent	5	0.943	5.905	-9.76	18.150	23.675	34.563	44.961	58.487	85.387	111.378	8000	0 (0/5)
	WWTPs effluent	8	0,967	1.609	-1.657	0.384	1.018	4.080	10.711	28.121	112.750	298.997	8000	0 (0/8)

Abbreviations: n: the total number of data points or studies, a: slope, b: intercept, PNEC: predicted not effect concentration for the development of antibiotic resistance, WWTPs: waste water treatment plants.

Appendix 5. Values of equations for regression analysis and values corresponding to various centiles for measured environmental concentration distributions of the maximum reported antibiotic concentrations (ng/L) in aquatic compartments of India, and percentage exceeding a predicted no effect concentration (PNEC) for the development of antibiotic resistance (Study III)

India														
Antibiotic	**Compartment**	**n**	**R^2**	**a**	**b**	**Centile value (ng/L)**							**PNEC (ng/L)**	**Percentage Exceedance PNEC**
						1%	**5%**	**25%**	**50%**	**75%**	**95%**	**99%**		
Ofloxacin	Waste water	3	1.000	1.014	-3.933	38.412	180.53	1635.012	7563.072	34984.486	316841.340	1489110.402	500	100 (3/3)
Levofloxacin	Waste water	3	0.989	1.219	-4.929	136.48	494.46	3091.465	11052.95	39517.754	247072.813	895127.807	250	100 (3/3)

Abbreviations: n: the total number of data points or studies, a: slope, b: intercept, PNEC: predicted not effect concentration for the development of antibiotic resistance.

Appendix 6. Ranking of antibiotics in various aquatic environmental compartments of China, based on descending orders of overall, criterion, and attribute risk utility scores (Study IV)

Priority	Overall risk	Environmental risk			Human health risk		
		Resistance risk on environment	Ecotoxicity risk	Overall environmental risk	Resistance risk on human health	Toxicity risk on human health	Overall human health risk
1	Ciprofloxacin	Ciprofloxacin	Norfloxacin	Ciprofloxacin	Ciprofloxacin	Ciprofloxacin	Ciprofloxacin
2	Norfloxacin	Oxytetracyclin	Tetracyclin	Oxytetracyclin	Penicillin	Norfloxacin	Norfloxacin
3	Oxytetracyclin	Enrofloxacin	Roxithromycin	Norfloxacin	Enrofloxacin	Oxytetracyclin	Penicillin
4	Enrofloxacin	Azithromycin	Azithromycin	Azithromycin	Norfloxacin	Erythromycin	Oxytetracyclin
5	Tetracyclin	Norfloxacin	Oxytetracyclin	Enrofloxacin	Oxytetracyclin	Penicillin	Erythromycin
6	Erythromycin	Ofloxacin	Chlortetracyclin	Tetracyclin	Ofloxacin	Doxycycline	Enrofloxacin
7	Penicillin	Tetracyclin	Erythromycin	Ofloxacin	Azithromycin	Sulfamethoxazole	Doxycycline
8	Ofloxacin	Trimethoprim	Sulfamethoxazole	Roxithromycin	Tetracyclin	Tetracyclin	Sulfamethoxazole
9	Azithromycin	Erythromycin	Clarithromycin	Erythromycin	Roxithromycin	Sulfamonomethoxine	Ofloxacin
10	Sulfamethoxazole	Clarithromycin	Ofloxacin	Clarithromycin	Erythromycin	Ofloxacin	Tetracyclin

11	Chlortetracyclin	Roxithromycin	Lincomycin	Chlortetracyclin	Clarithromycin	Lomefloxacin	Sulfamonomethoxine
12	Roxithromycin	Sulfamethoxazole	Ciprofloxacin	Sulfamethoxazole	Chlortetracyclin	Enrofloxacin	Lomefloxacin
13	Doxycycline	Moxifloxacin	Enrofloxacin	Trimethoprim	Sulfamethoxazole	Sulfamethazine	Chlortetracyclin
14	Clarithromycin	Doxycycline	Moxifloxacin	Lincomycin	Trimethoprim	Sulfadiazine	Sulfamethazine
15	Sulfamonomethoxine	Florfenicol	Tylosin	Moxifloxacin	Lincomycin	Chlortetracyclin	Sulfadiazine
16	Lomefloxacin	Penicillin	Lomefloxacin	Doxycycline	Moxifloxacin	Azithromycin	Azithromycin
17	Trimethoprim	Chlortetracyclin	Sulfadiazine	Florfenicol	Doxycycline	Roxithromycin	Roxithromycin
18	Lincomycin	Sulfamonomethoxine	Trimethoprim	Tylosin	Florfenicol	Clarithromycin	Clarithromycin
19	Sulfadiazine	Lomefloxacin	Doxycycline	Lomefloxacin	Tylosin	Trimethoprim	Trimethoprim
20	Sulfamethazine	Lincomycin	Florfenicol	Sulfadiazine	Lomefloxacin	Lincomycin	Lincomycin
21	Moxifloxacin	Sulfadiazine	Penicillin	Penicillin	Sulfadiazine	Moxifloxacin	Moxifloxacin
22	Florfenicol	Sulfamethazine	Sulfamonomethoxine	Sulfamonomethoxine	Sulfamonomethoxine	Florfenicol	Florfenicol
23	Tylosin	Tylosin	Sulfamethazine	Sulfamethazine	Sulfamethazine	Tylosin	Tylosin
24	Fleroxacin	Fleroxacin	Fleroxacin	Fleroxacin	Fleroxacin	Fleroxacin	Fleroxacin

25	Enoxacin	Enoxacin	Enoxacin	Enoxacin	Enoxacin	Enoxacin	Enoxacin
26	Nalidixic acid	Nalidixic acid	Nalidixic acid	Nalidixic acid	Nalidixic acid	Nalidixic acid	Nalidixic acid
27	Sarafloxacin	Sarafloxacin	Sarafloxacin	Sarafloxacin	Sarafloxacin	Sarafloxacin	Sarafloxacin
28	Difloxacin	Difloxacin	Difloxacin	Difloxacin	Difloxacin	Difloxacin	Difloxacin
29	Flumequine	Flumequine	Flumequine	Flumequine	Flumequine	Flumequine	Flumequine
30	Sulfacetamide	Sulfacetamide	Sulfacetamide	Sulfacetamide	Sulfacetamide	Sulfacetamide	Sulfacetamide
31	Sulfachloropyridazine	Sulfachloropyridazine	Sulfachloropyridazine	Sulfachloropyridazine	Sulfachloropyridazine	Sulfachloropyridazine	Sulfachloropyridazine
32	Sulfadimethoxine	Sulfadimethoxine	Sulfadimethoxine	Sulfadimethoxine	Sulfadimethoxine	Sulfadimethoxine	Sulfadimethoxine
33	Sulfadimidine	Sulfadimidine	Sulfadimidine	Sulfadimidine	Sulfadimidine	Sulfadimidine	Sulfadimidine
34	Sulfamerazine	Sulfamerazine	Sulfamerazine	Sulfamerazine	Sulfamerazine	Sulfamerazine	Sulfamerazine
35	Sulfameter	Sulfameter	Sulfameter	Sulfameter	Sulfameter	Sulfameter	Sulfameter
36	Sulfamethizole	Sulfamethizole	Sulfamethizole	Sulfamethizole	Sulfamethizole	Sulfamethizole	Sulfamethizole
37	Sulfapyridine	Sulfapyridine	Sulfapyridine	Sulfapyridine	Sulfapyridine	Sulfapyridine	Sulfapyridine
38	Sulfaquinoxaline	Sulfaquinoxaline	Sulfaquinoxaline	Sulfaquinoxaline	Sulfaquinoxaline	Sulfaquinoxaline	Sulfaquinoxaline

39	Sulfathiazole	Sulfathiazole	Sulfathiazole	Sulfathiazole	Sulfathiazole	Sulfathiazole	Sulfathiazole
40	Thiamphenicol	Thiamphenicol	Thiamphenicol	Thiamphenicol	Thiamphenicol	Thiamphenicol	Thiamphenicol
41	Chloramphenicol	Chloramphenicol	Chloramphenicol	Chloramphenicol	Chloramphenicol	Chloramphenicol	Chloramphenicol

Appendix 7. Ranking of data gap of antibiotics in various aquatic environmental compartments of China, based on descending orders of overall, criterion, and attribute data gap scores (Study IV)

Priority	Overall risk	Environmental risk			Human health risk		
		Resistance risk on environment	Ecotoxicity risk	Overall environmental risk	Resistance risk on human health	Toxicity risk on human health	Overall human health risk
1	Fleroxacin	Fleroxacin	Fleroxacin	Fleroxacin	Fleroxacin	Fleroxacin	Fleroxacin
2	Enoxacin	Enoxacin	Enoxacin	Enoxacin	Enoxacin	Enoxacin	Enoxacin
3	Sarafloxacin	Sarafloxacin	Sarafloxacin	Sarafloxacin	Sarafloxacin	Sarafloxacin	Sarafloxacin
4	Difloxacin	Difloxacin	Difloxacin	Difloxacin	Difloxacin	Difloxacin	Difloxacin
5	Sulfacetamide	Sulfacetamide	Sulfacetamide	Sulfacetamide	Sulfacetamide	Sulfacetamide	Sulfacetamide
6	Sulfachloropyridazine	Sulfachloropyridazine	Sulfachloropyridazine	Sulfachloropyridazine	Sulfachloropyridazine	Sulfachloropyridazine	Sulfachloropyridazine
7	Sulfamerazine	Sulfamerazine	Sulfamerazine	Sulfamerazine	Sulfamerazine	Sulfamerazine	Sulfamerazine
8	Sulfameter	Sulfameter	Sulfameter	Sulfameter	Sulfameter	Sulfameter	Sulfameter
9	Sulfamethizole	Sulfamethizole	Sulfamethizole	Sulfamethizole	Sulfamethizole	Sulfamethizole	Sulfamethizole
10	Sulfaquinoxaline	Sulfaquinoxaline	Sulfaquinoxaline	Sulfaquinoxaline	Sulfaquinoxaline	Sulfaquinoxaline	Sulfaquinoxaline

11	Sulfadimidine	Sulfadimidine	Sulfapyridine	Sulfapyridine	Sulfapyridine	Sulfadimidine	Sulfadimidine
12	Nalidixic acid	Sulfathiazole	Penicillin	Penicillin	Sulfamethazine	Nalidixic acid	Nalidixic acid
13	Flumequine	Sulfadimethoxine	Sulfamethazine	Sulfamethazine	Sulfadimidine	Flumequine	Flumequine
14	Sulfathiazole	Sulfapyridine	Nalidixic acid	Sulfadimidine	Nalidixic acid	Sulfathiazole	Sulfathiazole
15	Moxifloxacin	Sulfamonomethoxine	Doxycycline	Nalidixic acid	Sulfamonomethoxine	Moxifloxacin	Moxifloxacin
16	Thiamphenicol	Lomefloxacin	Flumequine	Sulfamonomethoxine	Flumequine	Thiamphenicol	Thiamphenicol
17	Sulfadimethoxine	Sulfadiazine	Moxifloxacin	Flumequine	Sulfathiazole	Sulfadimethoxine	Sulfadimethoxine
18	Sulfapyridine	Chlortetracyclin	Sulfadimidine	Sulfathiazole	Lomefloxacin	Florfenicol	Florfenicol
19	Florfenicol	Penicillin	Thiamphenicol	Lomefloxacin	Moxifloxacin	Chloramphenicol	Chloramphenicol
20	Chloramphenicol	Sulfamethazine	Tylosin	Moxifloxacin	Thiamphenicol	Azithromycin	Azithromycin
21	Sulfamonomethoxine	Nalidixic acid	Sulfamonomethoxine	Thiamphenicol	Sulfadimethoxine	Clarithromycin	Clarithromycin
22	Lomefloxacin	Flumequine	Lincomycin	Sulfadimethoxine	Sulfadiazine	Sulfapyridine	Sulfapyridine
23	Sulfadiazine	Moxifloxacin	Sulfathiazole	Tylosin	Chlortetracyclin	Sulfamonomethoxine	Sulfamonomethoxine
24	Azithromycin	Thiamphenicol	Lomefloxacin	Doxycycline	Florfenicol	Lomefloxacin	Lomefloxacin

25	Clarithromycin	Tylosin	Florfenicol	Lincomycin	Chloramphenicol	Sulfadiazine	Sulfadiazine
26	Chlortetracyclin	Lincomycin	Chloramphenicol	Sulfadiazine	Azithromycin	Chlortetracyclin	Chlortetracyclin
27	Penicillin	Florfenicol	Sulfadimethoxine	Chlortetracyclin	Clarithromycin	Penicillin	Penicillin
28	Sulfamethazine	Chloramphenicol	Azithromycin	Florfenicol	Penicillin	Tylosin	Tylosin
29	Tylosin	Doxycycline	Clarithromycin	Chloramphenicol	Tylosin	Doxycycline	Doxycycline
30	Doxycycline	Azithromycin	Roxithromycin	Azithromycin	Doxycycline	Lincomycin	Lincomycin
31	Lincomycin	Clarithromycin	Trimethoprim	Clarithromycin	Lincomycin	Roxithromycin	Roxithromycin
32	Roxithromycin	Roxithromycin	Norfloxacin	Roxithromycin	Roxithromycin	Trimethoprim	Trimethoprim
33	Trimethoprim	Trimethoprim	Sulfadiazine	Trimethoprim	Trimethoprim	Enrofloxacin	Enrofloxacin
34	Enrofloxacin	Norfloxacin	Ofloxacin	Norfloxacin	Enrofloxacin	Tetracyclin	Tetracyclin
35	Tetracyclin	Ofloxacin	Erythromycin	Ofloxacin	Tetracyclin	Sulfamethazine	Sulfamethazine
36	Norfloxacin	Erythromycin	Chlortetracyclin	Erythromycin	Norfloxacin	Norfloxacin	Norfloxacin
37	Ofloxacin	Enrofloxacin	Enrofloxacin	Enrofloxacin	Ofloxacin	Ofloxacin	Ofloxacin
38	Erythromycin	Tetracyclin	Tetracyclin	Tetracyclin	Erythromycin	Erythromycin	Erythromycin

39	Ciprofloxacin	Ciprofloxacin	Ciprofloxacin	Ciprofloxacin	Ciprofloxacin	Ciprofloxacin	Ciprofloxacin
40	Oxytetracyclin	Oxytetracyclin	Oxytetracyclin	Oxytetracyclin	Oxytetracyclin	Oxytetracyclin	Oxytetracyclin
41	Sulfamethoxazole	Sulfamethoxazole	Sulfamethoxazole	Sulfamethoxazole	Sulfamethoxazole	Sulfamethoxazole	Sulfamethoxazole