

Chapter

Flushed and Forgotten: Antimicrobial Resistance from Wastewater Perspective

Sophia Karen Bakon and Zuraifah Asrah Mohamad

Abstract

Antimicrobial resistance (AMR) is a major global health threat, reducing the effectiveness of antibacterial treatments. Understanding its spread across different environments is crucial for improving surveillance and guiding policies. Wastewater is an often overlooked but significant contributor to the AMR crisis. It collects bacteria from human, industrial, and agricultural sources, making it both a potential mitigator through treatment and a pathway for antimicrobial-resistant bacteria (ARB) and antimicrobial-resistant genes (ARGs) if not properly managed. The improper handling of wastewater leads to the environmental dissemination of AMR, exacerbating its spread. In many low- and middle-income countries (LMICs), wastewater treatment facilities are either non-existent or incapable of effectively removing ARB, ARGs, and antibiotic residues. The role of wastewater in the development and dissemination of AMR has been significantly underestimated in both scientific research and policy discussions because AMR research has primarily concentrated on clinical settings and agricultural practices. A major challenge in addressing AMR in wastewater systems is the lack of global monitoring and policy frameworks, which limits coordinated efforts. Additionally, wastewater surveillance remains an underutilized tool for tracking AMR trends, missing a critical opportunity for early intervention. Raising awareness about the role of wastewater in AMR is essential for safeguarding global health. Strengthening wastewater treatment practices, implementing standardized monitoring systems, and integrating wastewater-based AMR surveillance into public health strategies can help mitigate the crisis.

Keywords: antimicrobial resistance, antibiotics, wastewater, bacteria, horizontal gene transfer, environment, global policy

1. Introduction

Nearly a century ago, in the year 1928, the *Penicillium* was observed on a contaminated petri dish by Alexander Fleming [1]. It was later purified by Norman Heatley, Howard Florey, Ernst Chain, and colleagues at Oxford, in the development of penicillin as a drug [2]. The development of antibiotics has been a key milestone in medicine. The use of antibiotics following patient diagnosis enabled the commencement of a treatment-focused approach to patient care to kill the bacteria that caused the illness.

Besides focusing on treating infectious diseases, antibiotics made modern medical procedures such as organ transplant, cancer treatment, and open-heart surgery possible to perform. This breakthrough significantly contributed to the reduction of hospitalization time and ensured the survival of more lives. However, bacteria may develop resistance and survive exposure to the antimicrobial that is designed to kill them and to stop their growth, thereby causing the ineffectiveness of the antimicrobial. It has become a problem in recent years because misuse of antimicrobials has accelerated the growth and spread of resistance, but there are no novel drugs to combat these new superbugs. AMR is a critical global health challenge that poses a threat to the effective treatment of infectious diseases and challenges decades of medical advancement. The review on AMR reported by Jim O'Neill predicted that based on the increasing pattern without urgent action in tackling the drug-resistant infection globally, there will be 10 million deaths attributable to AMR in the year 2050 [3]. AMR has resulted in patients staying in the hospital for extended periods of time to treat common illnesses and surgery procedures, which raises medical costs [4]. It is widely acknowledged and understood that overuse and misuse of antibiotics can lead to the phenomenon of resistance. AMR has been linked to increasing morbidity, mortality, and economic burden worldwide. According to the World Health Organization (WHO), AMR is responsible for an estimated 1.27 million deaths annually, with the highest burden observed in LMICs where access to quality healthcare and sanitation is limited [5].

Antibiotics have certainly been a blessing to civilization; they not only have medicinal uses, but they are also used in various applications, including animal husbandry and animal production as preventive measures and feeding in many low-income and developing countries for decades [6]. The consequences of AMR extend far beyond healthcare settings, posing risks to food security, agriculture, water sources, and environmental sustainability [7–9]. The environment, particularly wastewater systems, has emerged as a significant reservoir and conduit for the dissemination of antimicrobial-resistant bacteria (ARB) and antimicrobial-resistant genes (ARGs). Untreated or inadequately treated wastewater from hospitals, pharmaceutical industries, and agricultural runoff facilitates the proliferation of AMR, creating a feedback loop that amplifies the spread of resistance globally [10–12].

Wastewater is increasingly recognized as a significant but often overlooked contributor to the global AMR crisis. Wastewater receives bacteria from various human, industrial, and agricultural activities. These bacteria, including both harmless and pathogenic types, often carry antimicrobial ARGs, making wastewater a critical reservoir and mixing ground for resistance. It plays a dual role: on the one hand, as a potential mitigator through treatment processes; on the other hand, if not properly managed, it can serve as a pool and pathway for ARB and ARGs [13]. While significant attention has been paid to the misuse of antimicrobials in human health and agriculture, wastewater's role as a key contributor to the development and spread of AMR has been relatively underexplored by researchers until recent years. Wastewater acts as a critical interface where resistant microorganisms, antimicrobial residues, and environmental factors converge, thus creating hotspots for resistance evolution. In this context, the roles and limitations of the wastewater in the AMR crisis will be discussed in this chapter, **Figure 1**.

2. The roles of wastewater to the ecosystems

Wastewater has emerged in many new developments in urban and suburban areas. Effluents from various anthropogenic activities, such as agricultural activities, animal

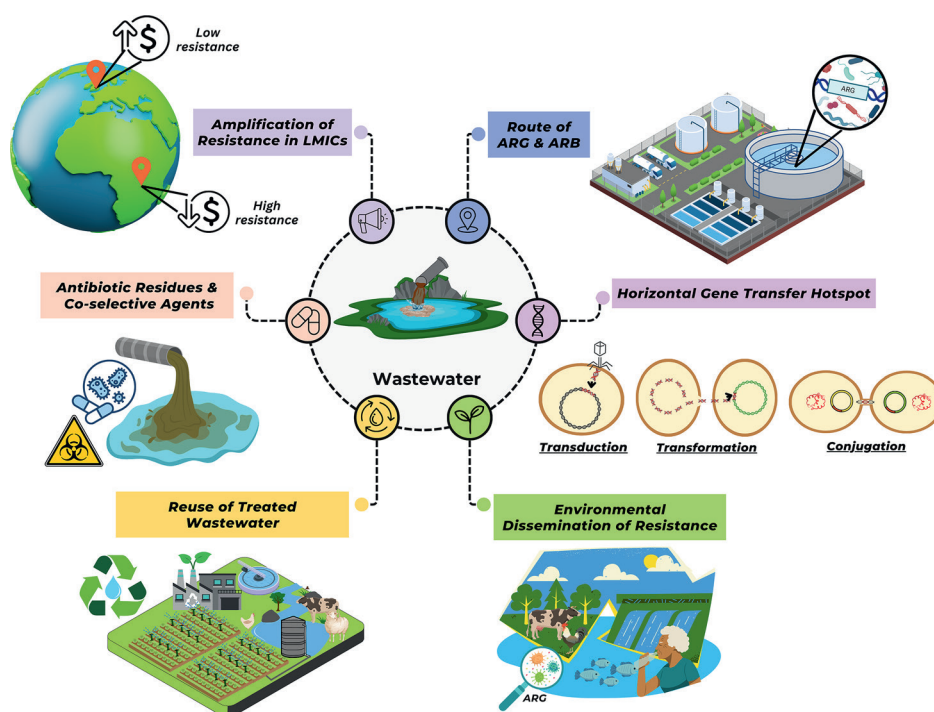


Figure 1.
 Role of wastewater in the AMR crisis in the environment.

farming, the pharmaceutical industry, domestic sewage, and hospital effluents, contribute to environmental pollution and the dissemination of antimicrobial resistance. Effluents from these sectors often contain a mixture of organic and inorganic matter, as well as heavy metals, which can significantly impact the environment [14]. Organic matter typically includes biodegradable substances like plant or animal waste, while inorganic matter may include non-biodegradable compounds such as salts and minerals. Heavy metals like mercury, lead, cadmium, and arsenic are particularly concerning due to their toxicity, persistence in the environment, and potential to bioaccumulate in living organisms.

These effluents also carry a variety of microorganisms, including bacteria, some of which can play a dual role in environmental impact. While certain bacteria contribute to bioremediation by breaking down organic matter or immobilizing heavy metals, others may harbor ARGs, posing a significant public health threat. Effluent discharge can act as a reservoir and vehicle for the spread of ARB into natural ecosystems, exacerbating the global challenge of AMR [15]. The persistence and proliferation of AMR in effluent-affected environments highlight the need for stringent monitoring, advanced treatment technologies, and sustainable management practices to mitigate ecological and health risks.

2.1 A route for antimicrobial-resistant genes and antimicrobial-resistant bacteria

Wastewater serves as a critical reservoir and mixing ground for ARB and antimicrobial residues, collecting contaminants from diverse sources. Hospitals contribute significantly through effluents containing high concentrations of resistant pathogens

due to the use of antibiotics in treating infections. Similarly, households release residual antibiotics into wastewater systems, often as a result of improper disposal or excretion of medications [16]. Agriculture also plays a role, with runoff from farms introducing antibiotic residues and resistant bacteria into the system. These diverse inputs converge in wastewater treatment plants (WWTPs), creating an environment where bacteria from different origins interact. This environment facilitates the exchange of resistance genes through horizontal gene transfer (HGT), a key mechanism driving the spread of antimicrobial resistance.

Unfortunately, standard wastewater treatment processes are often inadequate in completely removing ARGs and ARB. Despite improvements in technology, many treatment plants fail to fully neutralize these contaminants, leading to their release into natural water bodies or recycled water used for irrigation or industrial purposes. This incomplete elimination not only perpetuates the environmental dissemination of resistance but also creates a feedback loop where resistant bacteria re-enter the human and animal populations through water reuse [17].

Due to a lack of adequate treatment infrastructure, wastewater including ARGs, ARBs, and antibiotic residues has been released directly into rivers, lakes, or agricultural land. This unregulated release amplifies the spread of antimicrobial resistance and underscores the need for global investment in wastewater management. Moreover, the presence of co-selective agents such as heavy metals, biocides, and disinfectants in wastewater can further enhance the persistence and evolution of resistance. These agents create selective pressure, allowing resistant bacteria to thrive and increasing the likelihood of resistance spreading within microbial communities [18].

2.2 Wastewater as horizontal gene transfer (HGT) hotspot

Wastewater treatment plants (WWTPs) and untreated sewage act as critical hotspots for HGT, facilitating the exchange and proliferation of AMR genes among microorganisms. This process is driven by the remarkable genome plasticity of bacteria, enabled by mechanisms such as conjugation, transformation, and transduction, which allow microbes to adapt, evolve, and survive in antibiotic-contaminated environments, **Figure 2** [19].

In wastewater systems, several factors amplify the development and spread of AMR. The high bacterial density in these environments brings diverse microbial communities into close contact, enhancing genetic exchange, including the transfer of plasmids carrying resistance genes. Selective pressures from contaminants such as antibiotic residues, heavy metals, and disinfectants further accelerate this process by promoting the survival and proliferation of resistant strains [20].

Biofilms, common in wastewater systems, provide a protective matrix for bacteria, fostering HGT and shielding microbes from harsh conditions, while also trapping contaminants that create microenvironments with elevated selective pressure [21]. Additionally, the dynamic environmental stressors in WWTPs, such as fluctuating pH, temperature, and oxygen levels, can activate mobile genetic elements like integrons and transposons, further enhancing HGT [22]. Opportunistic pathogens such as *Pseudomonas aeruginosa*, *Escherichia coli*, and *Acinetobacter baumannii*, often present in wastewater, are particularly adept at acquiring and spreading resistance genes.

Unfortunately, standard wastewater treatment processes frequently fail to completely eliminate ARB and ARGs, allowing their release into natural water bodies [18]. This incomplete treatment perpetuates AMR dissemination and creates a dangerous

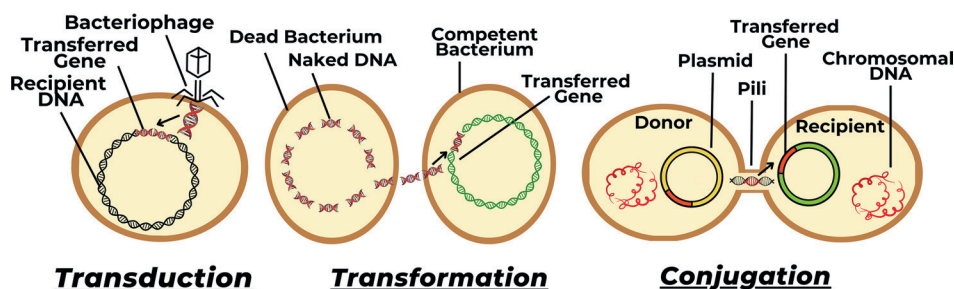


Figure 2.
 Horizontal gene transfer (HGT) is the process by which genetic information is transferred between organisms. The three main mechanisms of HGT in bacteria are transduction, transformation, and conjugation.

feedback loop where resistant bacteria re-enter human and animal populations, exacerbating the global AMR crisis.

2.3 Wastewater leading to environmental dissemination of resistance

The environmental dissemination of AMR is a critical consequence of wastewater management practices. Treated and untreated wastewater often acts as a vector for the release of ARB and ARGs into natural ecosystems. These microorganisms and genetic elements can persist in the environment, where they interact with native microbial communities and spread to humans, animals, and plants through multiple pathways.

From the wastewater treatment plant outlet, the treated or untreated wastewater is released into the natural ecosystems [23]. One of the primary ways wastewaters contribute to AMR dissemination is through its discharge into natural water bodies, including rivers, lakes, and oceans. While WWTPs aim to remove contaminants, standard processes are often insufficient to eliminate ARB and ARGs completely. As a result, these resistant microorganisms are introduced into aquatic ecosystems, where they can survive and proliferate. Wildlife, such as fish and birds, that rely on these water sources can become carriers of resistant bacteria, further extending the spread of AMR. Additionally, when untreated or partially treated wastewater is used for irrigation, it directly contaminates agricultural soil, where resistance genes can persist and propagate in the soil microbiota. Over time, these ARGs can integrate into microbial populations in the environment, creating long-term reservoirs of resistance that are challenging to control [24].

The wastewater that enters the ecosystems has become the exposure pathway for humans. The release of ARB and ARGs into the environment poses significant risks to human health through various exposure pathways. Direct exposure can occur during recreational activities such as swimming or fishing in contaminated water bodies. Similarly, individuals working in agriculture may come into contact with resistant bacteria while using reclaimed wastewater for irrigation or handling soil amended with wastewater-derived biosolids [25].

Indirect exposure also poses a major concern. Crops irrigated with reclaimed wastewater may absorb resistant bacteria or ARGs, introducing them into the food chain. This contamination is particularly problematic for raw or minimally processed foods, such as fruits and vegetables, which may be consumed without sufficient cooking to neutralize the resistant bacteria [26]. Groundwater contamination is another critical pathway. Resistant bacteria and ARGs can leach from wastewater discharge

sites or agricultural fields irrigated with reclaimed water, infiltrating groundwater systems that serve as drinking water sources for many communities. This indirect route exposes populations to AMR through a resource that is essential for daily life [27].

The broader implications of the dissemination of AMR through wastewater highlight the interconnectedness of human, animal, and environmental health in line with the “One Health” perspective. Addressing these pathways requires a multidisciplinary approach that includes strengthening wastewater treatment processes, regulating the use of reclaimed water for agriculture, and improving surveillance of resistant bacteria and genes in the environment. Without intervention, the environmental spread of AMR will continue to pose significant risks to ecosystems, food security, and public health on a global scale.

2.4 Reuse of wastewater: A double-edged sword

In water-scarce regions, the reuse of treated wastewater is becoming a critical solution to address water shortages. Treated wastewater is commonly repurposed for agricultural irrigation and in some cases, even as a source of drinking water. While this practice alleviates water scarcity and supports sustainable water management, it also introduces significant public health concerns due to the incomplete removal of ARGs, ARB, and antibiotic residues during wastewater treatment. These contaminants can persist in reclaimed water, increasing human exposure to resistance pathways [28].

The reuse of treated wastewater for agricultural irrigation represents a significant pathway for human exposure to ARGs and ARB. Crops irrigated with reclaimed water can become contaminated with resistant bacteria, particularly on their surfaces or in the soil where they grow. These resistant microorganisms can subsequently be transferred to humans through the consumption of raw or undercooked produce. In some cases, ARGs can persist in soil microbiota, creating a long-term reservoir of resistance genes that can further propagate through the food chain [27].

In regions where treated wastewater is used for drinking water, the risks are even more direct. Despite treatment, trace levels of ARGs, ARB, and antibiotic residues may remain, posing a potential threat to public health. Chronic exposure to these contaminants through drinking water, even at low concentrations, can exert selection pressure on human microbiota, promoting the emergence and persistence of resistant strains [29].

The practice of balancing sustainability and safety from the reuse of treated wastewater is undeniably crucial for addressing water scarcity, especially in arid regions where freshwater resources are limited [30, 31]. However, the current gaps in treatment processes necessitate urgent attention to minimize the risks associated with AMR. To strike a balance between sustainability and safety, it is essential to invest in advanced and cost-effective wastewater treatment technologies that specifically target ARGs and antibiotic residues. Additionally, robust monitoring systems should be implemented to assess the quality of reclaimed water and its potential impact on human and environmental health.

Public awareness campaigns and policy interventions are also needed to promote safe practices in wastewater reuse. By addressing the challenges associated with AMR in reclaimed water, water-scarce regions can continue to benefit from wastewater reuse while safeguarding public health and the environment [32].

Due to the limitations of current treatment technologies, conventional wastewater treatment processes primarily focus on the removal of organic pollutants, pathogens,

and suspended solids to meet safety and regulatory standards. However, these systems often fail to effectively eliminate ARGs, ARB, and antibiotic residues. ARGs, which are encoded on mobile genetic elements such as plasmids and transposons, are particularly challenging to remove because they can persist in bacterial DNA even after treatment [33]. Similarly, ARBs often survive the disinfection process, while low concentrations of antibiotics remain in treated water due to their stability and resistance to degradation.

Advanced treatment technologies, such as membrane filtration, advanced oxidation processes, and ozonation, have shown promise in targeting ARGs and antibiotic residues [34]. However, these methods are often prohibitively expensive for widespread implementation, especially in LMICs. Consequently, treated wastewater that retains these contaminants is frequently reused in agriculture or aquifer recharge, inadvertently contributing to the dissemination of AMR [31].

2.5 Pool of antibiotic residues and co-selective agents

Antibiotic residues and co-selective agents play a significant role in the persistence and proliferation of AMR in wastewater environments. These agents, often present in wastewater from diverse sources, create selective pressures that promote the survival and spread of ARB and ARGs. Their influence extends beyond the direct effects of antibiotics, encompassing other contaminants like heavy metals, biocides, and disinfectants that contribute to the evolution of resistance [35].

Wastewater systems frequently contain sub-inhibitory concentrations of antibiotics, which, although insufficient to kill bacteria outright, are capable of selecting for resistant strains. These residual antibiotics originate from various sources, including hospitals, pharmaceutical manufacturing facilities, agricultural runoff, and even household waste [14]. Antibiotics excreted by humans and animals often enter wastewater systems unchanged and maintain their biologically active properties. Even in the absence of direct therapeutic use, the presence of these residual antibiotics exerts selection pressure on microbial populations. Sub-inhibitory concentrations encourage bacterial survival mechanisms, such as efflux pump activation or mutations that confer resistance [36]. Over time, this environment fosters the persistence of resistant bacteria, increasing the prevalence of ARGs within the wastewater microbiome.

In addition to antibiotics, other co-selective agents in wastewater environments contribute to the proliferation of AMR. These agents, including heavy metals, biocides, and disinfectants, create additional selective pressures that indirectly promote resistance [35]. Heavy metals, such as mercury, copper, and zinc, frequently enter wastewater systems through industrial discharges, mining activities, and agricultural runoff. Importantly, resistance to heavy metals is often genetically linked to ARGs on the same mobile genetic elements, such as plasmids or integrons. This genetic co-localization allows the selective pressure exerted by heavy metals to co-select for antibiotic resistance, even in environments where antibiotic concentrations are minimal or absent [37].

Biocides and disinfectants used in wastewater treatment plants, such as chlorine, quaternary ammonium compounds, and hydrogen peroxide, can also contribute to the selection of resistant strains [38]. These agents, while intended to eliminate harmful pathogens, create a selective environment for bacteria that possess intrinsic or acquired resistance mechanisms [39]. For instance, bacteria that can withstand oxidative stress caused by disinfectants are more likely to survive and propagate [40]. Moreover, exposure to biocides can induce cross-resistance, where resistance

to biocides also enhances resistance to antibiotics and further complicates the efforts to control AMR in wastewater systems. Traditional wastewater treatment processes are often inadequate in addressing these contaminants, underscoring the importance of advanced treatment technologies such as adsorption, advanced oxidation, and membrane filtration to target residual antibiotics and heavy metals effectively [41].

Additionally, stricter regulations on industrial and agricultural discharges, coupled with improved waste management practices, can help reduce the influx of co-selective agents into wastewater systems [42]. Monitoring and quantifying the levels of antibiotics, heavy metals, and other co-selective agents in wastewater are also essential for understanding their role in AMR dynamics and developing targeted interventions. By addressing the combined impact of antibiotic residues and co-selective agents, it is possible to reduce the selective pressures that drive AMR in wastewater systems and minimize the risks to public health and the environment.

2.6 Wastewater causing the amplification of resistance in low- and middle-income countries (LMICs)

The issue of AMR is particularly pronounced in LMICs, where numerous factors converge to amplify resistance and accelerate its spread. Limited access to advanced wastewater treatment technologies, inadequate healthcare infrastructure, and unregulated antibiotic use have created an environment where wastewater acts as a significant driver of AMR [43]. This burden disproportionately affects vulnerable populations, posing severe risks to public health and the environment.

In many LMICs, wastewater treatment facilities are either non-existent or incapable of effectively removing ARB, ARGs, and antibiotic residues [43]. Untreated or inadequately treated wastewater is often discharged directly into rivers, lakes, and other natural water bodies, which then become reservoirs for AMR. This untreated discharge not only spreads resistance but also contaminates drinking water supplies, irrigation systems, and aquatic ecosystems, creating feedback loops of resistance amplification [44]. For example, in some urban areas of LMICs, sewer systems directly release untreated sewage into the environment, creating hotspots for HGT among microorganisms. This scenario is particularly problematic in densely populated regions where microbial densities in wastewater are high, increasing the likelihood of gene exchange and the proliferation of resistance traits [45].

The widespread misuse and overuse of antibiotics in LMICs significantly contribute to AMR amplification. Antibiotics are frequently used not only to treat human infections but also as prophylactic agents in livestock and aquaculture, as growth promoters in animal feed, and as disease preventatives in crops. In many cases, these practices are carried out without proper veterinary or medical oversight, leading to the unchecked use of sub-therapeutic doses of antibiotics, which are known to be selected for resistant bacterial strains [46].

In addition, inadequate pharmaceutical waste management exacerbates the problem. Many pharmaceutical manufacturing plants in LMICs discharge untreated or partially treated wastewater containing high concentrations of antibiotics and other pharmaceutical residues [47]. These effluents create localized hotspots of extreme selective pressure, promoting the emergence and spread of highly resistant bacterial strains.

Poor sanitation and hygiene practices in LMICs further compound the problem by facilitating the spread of resistant bacteria. Open defecation, improper waste disposal, and inadequate access to clean water create conditions where human and

animal waste mix freely with the environment, enhancing the dissemination of AMR [48]. Additionally, limited access to healthcare infrastructure means infections are often treated inappropriately, with patients receiving suboptimal antibiotic regimens or counterfeit medications. These systemic challenges are exacerbated by a lack of public awareness regarding AMR. In many LMICs, antibiotics are sold over the counter without a prescription, leading to self-medication and improper use [49]. Combined with limited government oversight and enforcement of antibiotic use regulations, these practices accelerate the development and spread of AMR in both human and environmental settings.

The agricultural sector in LMICs is another major contributor to the amplification of AMR. Runoff from farms that use antibiotics for livestock or crop protection carries residues and resistant bacteria into nearby water bodies [50]. This agricultural runoff mixes with human and industrial waste in wastewater systems, creating environments rich in selective pressures and co-selective agents, such as heavy metals and biocides, which further enhance resistance evolution. Additionally, irrigation systems in water-scarce regions often rely on untreated or partially treated wastewater, inadvertently introducing ARGs and ARB into the soil and crops. Consumption of these contaminated crops provides another pathway for resistance to enter human and animal populations, creating a cyclical pattern of resistance dissemination.

Effectively addressing AMR in LMICs requires a comprehensive and multi-faceted approach that targets the root causes and unique challenges faced by these regions. One of the key strategies is improving wastewater treatment infrastructure, which is often inadequate or non-existent in LMICs. Investments in affordable and efficient technologies, such as constructed wetlands, decentralized treatment systems, and advanced filtration methods, are critical. These solutions should be tailored to the specific environmental, economic, and technical contexts of LMICs to ensure sustainability and effectiveness in curbing the spread of antimicrobial-resistant bacteria and genes.

Another essential component is regulating antibiotic use across human health, veterinary, and agricultural sectors. Strict enforcement of prescription policies and the reduction of over-the-counter antibiotic sales can minimize misuse and overuse, which are major drivers of resistance. In parallel, public awareness campaigns are vital to educate communities about the risks of inappropriate antibiotic use and promote responsible practices. This dual approach of regulation and education can help shift behaviors and reduce the development of resistance.

Improving sanitation and hygiene is equally important in limiting the spread of AMR. Access to clean water and proper sanitation facilities, coupled with hygiene education, can significantly reduce the transmission of resistant bacteria within communities. Strengthening these basic public health measures not only addresses AMR but also improves overall health outcomes, particularly in regions where infectious diseases are prevalent.

Finally, encouraging global collaboration is imperative to support AMR mitigation efforts in LMICs. International funding and technical assistance are needed to build capacity, enhance infrastructure, and implement effective surveillance systems. Global policies and agreements, such as the WHO's Global Action Plan on AMR, should emphasize the unique challenges faced by LMICs, ensuring that these regions are prioritized in global AMR initiatives. By fostering cooperation and resource-sharing, the international community can help bridge the gap between LMICs and high-income countries in the fight against AMR. Through these integrated strategies, LMICs can make significant strides in combating AMR, reducing its impact on public health, and contributing to global efforts to address this growing crisis.

3. Challenges in tackling AMR from wastewater system

3.1 Lack of global monitoring and policy frameworks

One of the most significant barriers to addressing AMR in wastewater systems is the lack of robust global monitoring and policy frameworks. Despite growing evidence that wastewater plays a pivotal role in the emergence, persistence, and dissemination of ARB and ARGs, regulatory efforts have lagged behind scientific understanding. This regulatory gap weakens the potential to mitigate AMR effectively and leaves a critical pathway for resistance largely unaddressed [51].

The insufficient regulation of wastewater-related AMR has been a major limitation for the monitoring to be done. Globally, there are few binding policies or guidelines that specifically mandate the monitoring and management of ARGs, ARB, or residual antibiotics in wastewater [52]. While some countries have introduced regulations to control antibiotic use in clinical and agricultural settings, wastewater has largely been overlooked as a significant contributor to AMR dissemination [53]. For example, while many nations enforce standards for removing organic pollutants and pathogens in treated wastewater, these standards rarely account for ARGs, ARB, or co-selective agents like heavy metals and biocides. This lack of regulation is further complicated by disparities in resources and infrastructure across countries [54]. High-income countries may have the capacity to invest in advanced wastewater treatment technologies and monitoring systems, but LMICs often struggle to meet even basic wastewater treatment standards. Without clear international guidelines or incentives, the global effort to tackle AMR remains fragmented and ineffective.

Research on AMR has historically focused on clinical and agricultural settings, as these are well-known sources of antibiotic use and resistance. This is known knowledge gaps in wastewater research that need to be filled by scientists. Hospitals and farms are recognized hotspots for AMR, and significant resources have been allocated to understanding and mitigating resistance in these contexts. However, wastewater systems, which act as convergence points for clinical, agricultural, and municipal waste, have not received comparable attention.

The lack of global monitoring and policy frameworks for wastewater-related AMR has far-reaching consequences. Without standardized monitoring, it is difficult to assess the scale of the problem or measure the effectiveness of interventions. This absence of data also hampers international collaboration, as countries lack a common baseline for comparison or coordinated action. Moreover, the failure to recognize wastewater as a key player in AMR dissemination allows resistant bacteria and genes to persist in treated water, re-enter natural ecosystems, and spread through food and water supplies. This unregulated dissemination poses a direct threat to human and animal health, particularly in vulnerable communities that rely on untreated or inadequately treated water sources.

Moving toward global action to combat the AMR crisis in the context of wastewater requires a comprehensive, coordinated, multi-sectoral approach that bridges regulatory and knowledge gaps. Governments, international organizations, and research institutions must collaborate to develop policies that integrate wastewater monitoring into broader AMR management strategies. These policies should establish clear standards for the presence of ARGs, ARB, and antibiotic residues in treated wastewater. They should also include enforceable guidelines for the effective removal of these contaminants through appropriate treatment processes. Standardization of these regulations across countries is essential to ensure consistency and enable global comparisons of wastewater-related AMR data.

In tandem, increased funding and resources are critical for advancing research on AMR dynamics in wastewater systems. Research should explore the interactions between resistant bacteria, antimicrobial residues, and co-selective agents such as heavy metals and disinfectants, which may accelerate resistance development. Studies must also focus on identifying hotspots for HGT in wastewater environments, a key mechanism by which resistance spreads between bacterial populations. Furthermore, evaluating the efficacy of advanced treatment technologies, such as membrane bioreactors, advanced oxidation processes, and biochar filters in diverse settings, will help establish best practices for wastewater management tailored to local needs and capacities.

Equally important are public awareness campaigns and capacity-building initiatives aimed at empowering local governments, public health officials, and communities. These efforts are especially critical in LMICs, where infrastructure and expertise for managing wastewater-related AMR are often limited. Educational programs can help communities understand the risks of AMR and the role of wastewater in its spread, fostering support for policy changes and infrastructure investments. Capacity-building initiatives should include training programs for wastewater treatment operators and public health professionals, as well as the provision of tools and resources to implement and monitor AMR mitigation strategies effectively [55].

A major barrier to progress is the lack of international and national regulations specifically targeting AMR in wastewater. While the role of wastewater as a reservoir and dissemination pathway for AMR is well-documented, policies mandating the monitoring of ARGs, ARB, or antibiotic residues in wastewater remain scarce. Similarly, knowledge gaps persist, as research has predominantly focused on clinical and agricultural sources of AMR, often neglecting wastewater's critical role in the AMR crisis [56]. Expanding the scope of research to include wastewater as a key ecological reservoir and conduit for resistance is crucial to understanding and addressing the full scale of the problem. By bridging regulatory and knowledge gaps and fostering international collaboration, the global community can make significant strides in mitigating wastewater-related AMR. Such efforts will safeguard both environmental and public health, ensuring a more sustainable approach to combating one of the most pressing health challenges of our time.

3.2 Missed surveillance opportunity

Wastewater-based epidemiology (WBE) has emerged as a promising tool for monitoring public health trends, including the spread of infectious diseases such as COVID-19. By analyzing sewage samples, researchers can track the prevalence of pathogens and assess public health risks on a community-wide scale. Despite its demonstrated success in infectious disease surveillance, the application of WBE for tracking AMR remains significantly underutilized [57]. Considering the potential of wastewater for AMR monitoring is another opportunity to be explored. Wastewater contains a wealth of information about the ARB and ARGs present within a population [58]. It provides a non-invasive, cost-effective means of monitoring resistance trends at the community level, offering insights into emerging resistance hotspots, the prevalence of specific ARGs, and the effectiveness of public health interventions [59].

Unlike traditional clinical surveillance methods that rely on patient samples from healthcare facilities, wastewater sampling captures data from entire communities, including individuals who do not seek medical care. This broad coverage makes wastewater an invaluable resource for identifying resistance patterns that might otherwise go unnoticed [60]. Furthermore, WBE can be used to detect resistance in pathogens of environmental and agricultural origin, providing a more comprehensive

understanding of AMR dynamics across sectors. Despite its potential as a powerful tool for AMR monitoring, the adoption of WBE faces several significant challenges that limit its widespread application. These challenges span technical, infrastructural, and policy-related domains, each requiring targeted interventions to unlock the full potential of WBE in addressing the AMR crisis.

One of the primary challenges is the lack of standardized protocols for detecting and quantifying ARGs and ARB in wastewater. Current methodologies vary widely across studies and regions, leading to inconsistencies in the data collected. This lack of standardization not only hinders the comparability of results but also affects the reliability and reproducibility of findings. Establishing globally recognized guidelines and protocols for sample collection, analysis, and reporting is essential to overcome this barrier and facilitate collaboration among researchers, public health agencies, and policymakers.

Another major hurdle is the complexity of wastewater samples. Wastewater is a highly heterogeneous matrix, containing a mixture of human, animal, and environmental microbiota, along with various chemical contaminants such as antibiotics, heavy metals, and other pollutants. Isolating and identifying specific ARGs or ARBs within this complex environment require advanced analytical tools, such as next-generation sequencing and quantitative Polymerase Chain Reaction (qPCR), along with highly trained personnel [61, 62]. The technical expertise and sophisticated equipment needed for these processes can be a significant barrier, particularly in regions with limited resources. The challenge of limited infrastructure is particularly acute in LMICs. Many of these regions lack the laboratory facilities, equipment, and trained personnel required to implement and sustain wastewater surveillance programs [62]. Even where infrastructure exists, it may not be equipped to handle the specific demands of AMR monitoring. Addressing this gap requires substantial investments in capacity-building, including the provision of funding, the development of accessible technologies, and the establishment of training programs to build local expertise.

In addition to technical and infrastructural challenges, policy and regulatory gaps have also hindered the integration of WBE into public health surveillance systems. Few policies mandate the use of wastewater monitoring for AMR, and the absence of clear regulatory frameworks means that its implementation is often left to isolated research initiatives rather than being incorporated into coordinated public health strategies. Without strong policy support, including funding mechanisms and enforcement strategies, the potential of WBE to inform AMR mitigation efforts remains largely untapped.

Overcoming these challenges requires a coordinated and multidisciplinary approach. Collaboration between governments, academic institutions, public health organizations, and the private sector is essential to develop standardized methods, improve infrastructure, and establish robust policies for wastewater-based AMR monitoring. By addressing these barriers, WBE can become an invaluable tool in the global fight against antimicrobial resistance.

Benefits of integrating WBE into AMR surveillance frameworks have the potential to transform how resistance is monitored and managed. One significant advantage of WBE is its ability to enable early detection of emerging threats. By analyzing wastewater, it is possible to identify new resistance genes or strains in a community before they become widespread, allowing for timely and proactive interventions to contain their spread. This early warning capability is critical in mitigating the risks associated with rapidly evolving AMR.

Another major benefit is the cost-effectiveness of WBE as a public health tool. Collecting and analyzing wastewater samples is far less resource-intensive compared

to traditional methods that rely on individual clinical or agricultural samples. This makes WBE an efficient option for large-scale surveillance, particularly in resource-limited settings where extensive individual testing may be impractical or cost-prohibitive [57]. Additionally, WBE supports real-time monitoring of AMR trends. By providing near real-time data, WBE enables health authorities to track the impact of interventions, monitor resistance trends, and adjust antibiotic usage policies accordingly. This dynamic and timely feedback is invaluable for designing effective public health responses to AMR.

Finally, the integration of WBE into global surveillance networks could create a unified platform for tracking AMR across regions and borders. Such networks would facilitate international collaboration, enabling coordinated efforts to combat resistance on a global scale [63]. By pooling data and resources, countries could address AMR more effectively, particularly in areas where resistance poses a significant threat to public health. Overall, WBE offers a promising, scalable, and impactful approach to revolutionizing AMR surveillance and supporting global efforts to combat this critical health challenge.

The way forward to harness the full potential of WBE for AMR surveillance, concerted efforts are needed to overcome existing challenges. Investments in research and technology development are critical for advancing analytical techniques and standardizing methodologies [64]. Policymakers should recognize the value of wastewater monitoring and integrate it into national and international AMR action plans.

Additionally, cross-sectoral collaboration between public health agencies, environmental scientists, and wastewater management authorities is essential for building robust surveillance systems. Public-private partnerships could play a pivotal role in funding and implementing these initiatives, particularly in resource-limited settings. By leveraging the untapped potential of wastewater as a surveillance tool, we can gain deeper insights into the spread of AMR, identify resistance hotspots, and implement targeted interventions to mitigate the global threat of antimicrobial resistance.

4. Why scientists have overlooked wastewater's role in AMR?

The role of wastewater in the development and dissemination of AMR has been significantly underestimated in both scientific research and policy discussions. This oversight stems from a combination of historical focus, technical challenges, and fragmented policy frameworks that have marginalized the environmental dimensions of AMR.

For decades, historically AMR research has primarily concentrated on clinical settings and agricultural practices. In healthcare, the misuse and overuse of antibiotics in treating human infections have been identified as major contributors to the emergence of resistant strains [46]. Similarly, in agriculture, the use of antibiotics for livestock growth promotion and disease prevention has received significant attention due to its role in driving resistance in pathogens [8]. This narrow focus has inadvertently sidelined the environmental dimension of AMR, particularly wastewater's role as a reservoir and dissemination pathway. Wastewater systems, which collect contaminants from hospitals, households, and farms, are critical interfaces for the convergence of ARB, ARGs, and co-selective agents. However, their significance has been largely overlooked in favor of more traditionally studied sources.

The highly complex and heterogeneous nature of wastewater systems poses significant challenges to studying their role in AMR. Wastewater contains a diverse

mix of microbial communities, residual antibiotics, heavy metals, biocides, and other pollutants, all of which interact in ways that are difficult to disentangle. This complexity makes it challenging to isolate and quantify the specific contributions of wastewater to the development and spread of AMR [22]. For instance, distinguishing between resistance genes originating from human pathogens versus environmental bacteria requires advanced analytical tools and expertise. Additionally, wastewater's dynamic nature, characterized by fluctuations in temperature, pH, and contaminant concentrations, adds another layer of complexity to studying its impact on AMR [22, 65].

Until recently, the tools and methodologies required to study AMR in wastewater were either unavailable or insufficiently advanced. Traditional microbiological techniques were limited in their ability to detect and quantify ARGs and ARB in complex environmental samples. The advent of modern technologies, such as next-generation sequencing (NGS) and metagenomics, has revolutionized our ability to analyze the microbial composition of wastewater and identify resistance genes [66]. However, these technologies are resource-intensive and require specialized expertise, which limits their accessibility, particularly in low-resource settings. The historical lack of such capabilities contributed to the underrepresentation of wastewater in AMR research.

AMR mitigation efforts have traditionally been siloed across different domains, including healthcare, agriculture, and environmental management. This fragmentation has led to the exclusion of wastewater from many AMR action plans. For example, while healthcare policies often focus on improving antibiotic stewardship and surveillance within hospitals, agricultural regulations target the use of antibiotics in livestock. These policies rarely address the environmental pathways through which resistance can spread, such as wastewater discharge into natural ecosystems. The lack of integration between these sectors has perpetuated the oversight of wastewater's critical role in the AMR crisis [67].

To rectify this oversight, it is essential to adopt a more holistic and integrated approach to AMR research and mitigation [68]. Policymakers must recognize wastewater systems as key contributors to the resistance cycle and include them in national and international AMR action plans. Investments in advanced technologies for monitoring and treating wastewater are crucial for mitigating its impact on resistance dissemination. Additionally, fostering interdisciplinary collaboration between microbiologists, environmental scientists, and public health experts can help bridge the knowledge gaps and create a more comprehensive understanding of wastewater's role in AMR [68]. By addressing these challenges, the scientific community can unlock the untapped potential of wastewater research in combating the global threat of antimicrobial resistance.

5. Conclusions

The role of wastewater in the AMR crisis is critical yet underappreciated and “*forgotten*” acting as a reservoir, amplifier, and dissemination pathway for ARB and ARGs. It bridges the gap between environmental, agricultural, and clinical sources of resistance, creating hotspots where resistance can evolve and spread. This overlooked contributor plays a dual role, both as a potential mitigator when effectively treated and as a risk multiplier when treatment is inadequate or absent.

Addressing this challenge requires a multi-pronged approach. First, improved wastewater treatment technologies must be developed and implemented, incorporating advanced methods such as membrane filtration, advanced oxidation processes, and biological treatments specifically designed to remove ARB and ARGs. Second, stricter

regulations should be established to control the release of untreated or partially treated wastewater from hospitals, pharmaceutical industries, and agricultural sources, with enforceable standards for antimicrobial residues and resistant organisms. Third, expanded surveillance systems are necessary to monitor the prevalence and spread of AMR within wastewater and the environment, enabling early detection and targeted interventions.

Furthermore, raising awareness about the role of wastewater in the AMR crisis is essential to drive investment and innovation in this area. Collaborative efforts between governments, industries, researchers, and public health organizations are required to prioritize wastewater management within broader AMR mitigation strategies. Recognizing wastewater's central role is not only vital for addressing the current crisis but also for safeguarding global health, environmental sustainability, and food security in the face of growing antimicrobial resistance.

Acknowledgements

We would like to express our gratitude to the Institute for Medical Research, National Institutes of Health, Malaysia for providing access to the resources necessary for this book chapter writing. The financial support provided by the National Institutes of Health is gratefully acknowledged. The author acknowledges the use of Grammarly for language polishing of the manuscript.

Conflict of interest

The authors declare no conflict of interest.

Notes/thanks/other declarations

We thank the Director General of the Ministry of Health Malaysia for the permission to publish this book chapter.

Author details

Sophia Karen Bakon and Zuraifah Asrah Mohamad*
Environmental Health Research Centre, Institute for Medical Research, National Institutes of Health, Shah Alam, Malaysia

*Address all correspondence to: asrahz@moh.gov.my

IntechOpen

© 2025 The Author(s). Licensee IntechOpen. This chapter is distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/4.0>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited. 

References

- [1] Fleming A. Classics in infectious diseases: On the antibacterial action of cultures of a penicillium, with special reference to their use in the isolation of *B. Influenzae* by Alexander Fleming, reprinted from the British Journal of Experimental Pathology 10:226-236, 1929. Reviews of Infectious Diseases. 1980;2(1):129-139
- [2] Abraham EP, Chain E, Fletcher CM, Florey HW, Gardner AD, Heatley NG, et al. Further observations on penicillin. 1941. European Journal of Clinical Pharmacology. 1992;42(1):3-9
- [3] O'Neill J. Tackling Drug-Resistant Infections Globally: Final Report and Recommendations. The Review on Antimicrobial Resistance. United Kingdom: United Kingdom Government and Wellcome Trust; 2016
- [4] Wozniak TM, Dyda A, Lee X. The increased length of hospital stay and mortality associated with community-associated infections in Australia. Open Forum Infectious Diseases. 2022;9(5):ofac133
- [5] Collaborators GBDAR. Global burden of bacterial antimicrobial resistance 1990-2021: A systematic analysis with forecasts to 2050. Lancet. 2024;404(10459):1199-1226
- [6] Castro-Sanchez E, Moore LS, Husson F, Holmes AH. What are the factors driving antimicrobial resistance? Perspectives from a public event in London, England. BMC Infectious Diseases. 2016;16(1):465
- [7] Mohamad ZA, Bakon SK, Jamilan MAJ, Daud N, Ciric L, Ahmad N, et al. Prevalence of antibiotic-resistant bacteria and antibiotic-resistant genes and the quantification of antibiotics in drinking water treatment plants of Malaysia: Protocol for a cross-sectional study. JMIR Research Protocols. 2022;11(11):e37663
- [8] Thongratsakul S, Amavisit P, Poolkhet C. Antimicrobial resistance in poultry farming: A look back at environmental *Escherichia coli* isolated from poultry farms during the growing and resting periods. Veterinary Medicine International. 2023;2023:8354235
- [9] Razavi M, Marathe NP, Gillings MR, Flach CF, Kristiansson E, Joakim Larsson DG. Discovery of the fourth mobile sulfonamide resistance gene. Microbiome. 2017;5(1):160
- [10] Bakon SK, Mohamad ZA, Jamilan MA, Hashim H, Kuman MY, Shaharudin R, et al. Prevalence of antibiotic-resistant pathogenic bacteria and level of antibiotic residues in hospital effluents in Selangor, Malaysia: Protocol for a cross-sectional study. JMIR Research Protocols. 2023;12:e39022
- [11] Osinska A, Harnisz M, Korzeniewska E. Prevalence of plasmid-mediated multidrug resistance determinants in fluoroquinolone-resistant bacteria isolated from sewage and surface water. Environmental Science and Pollution Research International. 2016;23(11):10818-10831
- [12] Hou J, Wang C, Mao D, Luo Y. The occurrence and fate of tetracyclines in two pharmaceutical wastewater treatment plants of northern China. Environmental Science and Pollution Research International. 2016;23(2):1722-1731
- [13] Mao D, Yu S, Rysz M, Luo Y, Yang F, Li F, et al. Prevalence and

proliferation of antibiotic resistance genes in two municipal wastewater treatment plants. *Water Research*. 2015;**85**:458-466

[14] Kandie FJ, Krauss M, Beckers LM, Massei R, Fillinger U, Becker J, et al. Occurrence and risk assessment of organic micropollutants in freshwater systems within the Lake Victoria South Basin, Kenya. *Science of the Total Environment*. 2020;**714**:136748

[15] Fouz N, Pangesti KNA, Yasir M, Al-Malki AL, Azhar EI, Hill-Cawthorne GA, et al. The contribution of wastewater to the transmission of antimicrobial resistance in the environment: Implications of mass gathering settings. *Tropical Medicine Infectious Diseases*. 2020;**5**(1):25

[16] Marathe NP, Berglund F, Razavi M, Pal C, Dröge J, Samant S, et al. Sewage effluent from an Indian hospital harbors novel carbapenemases and integron-borne antibiotic resistance genes. *Microbiome*. 2019;**7**(1):97

[17] Macrì M, Bonetta S, Di Cesare A, Sabatino R, Corno G, Catozzo M, et al. Antibiotic resistance and pathogen spreading in a wastewater treatment plant designed for wastewater reuse. *Environmental Pollution*. 2024;**363**:125051

[18] Lira F, Vaz-Moreira I, Tamames J, Manaia CM, Martínez JL. Metagenomic analysis of an urban resistome before and after wastewater treatment. *Scientific Reports*. 2020;**10**(1):8174

[19] Li B, Qiu Y, Song Y, Lin H, Yin H. Dissecting horizontal and vertical gene transfer of antibiotic resistance plasmid in bacterial community using microfluidics. *Environment International*. 2019;**131**:105007

[20] Brown CL, Maile-Moskowitz A, Lopatkin AJ, Xia K, Logan LK, Davis BC, et al. Author correction: Selection and horizontal gene transfer underlie microdiversity-level heterogeneity in resistance gene fate during wastewater treatment. *Nature Communications*. 2024;**15**(1):6166

[21] Luo T, Dai X, Wei W, Xu Q, Ni B-J. Microplastics enhance the prevalence of antibiotic resistance genes in anaerobic sludge digestion by enriching antibiotic-resistant bacteria in surface biofilm and facilitating the vertical and horizontal gene transfer. *Environmental Science & Technology*. 2023;**57**(39):14611-14621

[22] Sharma P, Pal N, Kumawat M, Singh S, Das D, Tilwari A, et al. Investigating the antibiotic resistance genes and mobile genetic elements in water systems impacted with anthropogenic pollutants. *Environmental Research*. 2025;**269**:120814

[23] Jia S, Zhang X-X, Miao Y, Zhao Y, Ye L, Li B, et al. Fate of antibiotic resistance genes and their associations with bacterial community in livestock breeding wastewater and its receiving river water. *Water Research*. 2017;**124**:259-268

[24] Lorenzo P, Adriana A, Jessica S, Carles B, Marinella F, Marta L, et al. Antibiotic resistance in urban and hospital wastewaters and their impact on a receiving freshwater ecosystem. *Chemosphere*. 2018;**206**:70-82

[25] Moazeni M, Nikaeen M, Hadi M, Moghim S, Mouhebat L, Hatamzadeh M, et al. Estimation of health risks caused by exposure to enteroviruses from agricultural application of wastewater effluents. *Water Research*. 2017;**125**:104-113

- [26] Patra M, Dubey SK. Understanding the spread of antibiotic resistance in vegetables cultivated with sewage sludge: Implications for food safety and human health. *Environmental Systems Research*. 2024;**13**(1):21
- [27] Dungan RS, McKinney CW, Leytem AB. Tracking antibiotic resistance genes in soil irrigated with dairy wastewater. *Science of the Total Environment*. 2018;**635**:1477-1483
- [28] Barreiro A, Cela-Dablanca R, Nebot C, Rodríguez-López L, Santás-Miguel V, Arias-Estévez M, et al. Occurrence of nine antibiotics in different kinds of sewage sludge, soils, corn and grapes after sludge spreading. *Spanish Journal of Soil Science*. 2022;**12**:13
- [29] Gudda F, Odinga ES, Tang L, Waigi MG, Wang J, Abdalmegeed D, et al. Tetracyclines uptake from irrigation water by vegetables: Accumulation and antimicrobial resistance risks. *Environmental Pollution*. 2023;**338**:122696
- [30] Yin A, Duan J, Xue L, Feng Y, Petropoulos E, Yang L. High yield and mitigation of N-loss from paddy fields obtained by irrigation using optimized application of sewage tail water. *Agriculture, Ecosystems & Environment*. 2020;**304**:107137
- [31] Abd-Elhamid HF, Abd-Elmoneem SM, Abdelaal GM, Zelenáková M, Vranayova Z, Abd-Elaty I. Investigating and managing the impact of using untreated wastewater for irrigation on the groundwater quality in arid and semi-arid regions. *International Journal of Environmental Research and Public Health* [Internet]. 2021;**18**(14):17
- [32] Akhkha A, Al-Radaddi ES, Al-Shoaibi AK. The impact of treated and untreated municipal sewage water on growth and physiology of the desert plant *Calotropis procera*. *Journal of Taibah University for Science*. 2019;**13**(1):746-754
- [33] Gupta S, Mittal Y, Panja R, Prajapati KB, Yadav AK. Chapter 3 - Conventional wastewater treatment technologies. In: Kumar S, Kumar R, Pandey A, editors. *Current Developments in Biotechnology and Bioengineering*. Amsterdam: Elsevier; 2021. p. 47-75
- [34] Sabri NA, van Holst S, Schmitt H, van der Zaan BM, Gerritsen HW, Rijnaarts HHM, et al. Fate of antibiotics and antibiotic resistance genes during conventional and additional treatment technologies in wastewater treatment plants. *Science of the Total Environment*. 2020;**741**:140199
- [35] Chukwu KB, Abafe OA, Amoako DG, Essack SY, Abia ALK. Antibiotic, heavy metal, and biocide concentrations in a wastewater treatment plant and its receiving water body exceed pnc limits: Potential for antimicrobial resistance selective pressure. *Antibiotics* [Internet]. 2023;**12**(7):14
- [36] Lv L, Wan M, Wang C, Gao X, Yang Q, Partridge Sally R, et al. Emergence of a plasmid-encoded resistance-nodulation-division efflux pump conferring resistance to multiple drugs, including tigecycline, in *klebsiella pneumoniae*. *mBio*. 2020;**11**(2):15
- [37] Kim M, Weigand Michael R, Oh S, Hatt Janet K, Krishnan R, Tezel U, et al. Widely used benzalkonium chloride disinfectants can promote antibiotic resistance. *Applied and Environmental Microbiology*. 2018;**84**(17):e01201-e01218
- [38] Skovgaard S, Nielsen LN, Larsen MH, Skov RL, Ingmer H,

- Westh H. *Staphylococcus epidermidis* isolated in 1965 are more susceptible to triclosan than current isolates. *PLoS One*. 2013;**8**(4):e62197
- [39] Voumard M, Venturelli L, Borgatta M, Croxatto A, Kasas S, Dietler G, et al. Adaptation of *Pseudomonas aeruginosa* to constant sub-inhibitory concentrations of quaternary ammonium compounds. *Environmental Science: Water Research & Technology*. 2020;**6**(4):1139-1152
- [40] Vulin C, Leimer N, Huemer M, Ackermann M, Zinkernagel AS. Prolonged bacterial lag time results in small colony variants that represent a sub-population of persisters. *Nature Communications*. 2018;**9**(1):4074
- [41] Colella M, Ripa M, Coccozza A, Panfilo C, Ulgiati S. Challenges and opportunities for more efficient water use and circular wastewater management. The case of Campania Region, Italy. *Journal of Environmental Management*. 2021;**297**:113171
- [42] Kardos MK, Patziger M, Jolánkai Z, Clement A. The new urban wastewater treatment directive from the perspective of the receiving rivers' quality. *Environmental Sciences Europe*. 2025;**37**(1):10
- [43] Gao P, Munir M, Xagorarakis I. Correlation of tetracycline and sulfonamide antibiotics with corresponding resistance genes and resistant bacteria in a conventional municipal wastewater treatment plant. *Science of the Total Environment*. 2012;**421-422**:173-183
- [44] Bagnis S, Boxall A, Gachanja A, Fitzsimons M, Murigi M, Snape J, et al. Characterization of the Nairobi River catchment impact zone and occurrence of pharmaceuticals: Implications for an impact zone inclusive environmental risk assessment. *Science of the Total Environment*. 2020;**703**:134925
- [45] Lumabao JPD, Otero MCB, Acaso JT, Alviola PA, Jaraula CMB, Murao LAE. An antimicrobial resistance gene situationer in the backyard swine industry of a Philippine City. *Scientific Reports*. 2024;**14**(1):26193
- [46] Gebretekle GB, Haile Mariam D, Abebe W, Amogne W, Tenna A, Fenta TG, et al. Opportunities and barriers to implementing antibiotic stewardship in low and middle-income countries: Lessons from a mixed-methods study in a tertiary care hospital in Ethiopia. *PLoS One*. 2018;**13**(12):e0208447
- [47] Musoke D, Namata C, Lubega GB, Kitutu FE, Mugisha L, Amir S, et al. Access, use and disposal of antimicrobials among humans and animals in Wakiso district, Uganda: A qualitative study. *Journal of Pharmaceutical Policy and Practice*. 2021;**14**(1):69
- [48] Taviani E, van den Berg H, Nhassengo F, Nguluve E, Paulo J, Pedro O, et al. Occurrence of waterborne pathogens and antibiotic resistance in water supply systems in a small town in Mozambique. *BMC Microbiology*. 2022;**22**(1):243
- [49] Miller R, Goodman C. Performance of retail pharmacies in low- and middle-income Asian settings: A systematic review. *Health Policy and Planning*. 2016;**31**(7):940-953
- [50] Gudda F, Muloi D, Nganga F, Nolari C, Gao Y, Moodley A. Antibiotic ecotoxicity and resistance risks in resource-constrained chicken and pig farming environments. *npj Antimicrobials and Resistance*. 2024;**2**(1):51

- [51] Lien LT, Hoa NQ, Chuc NT, Thoa NT, Phuc HD, Diwan V, et al. Antibiotics in wastewater of a rural and an urban hospital before and after wastewater treatment, and the relationship with antibiotic use—a one year study from vietnam. *International Journal of Environmental Research and Public Health* [Internet]. 2016;**13**(6):13
- [52] Bloomer E, McKee M. Policy options for reducing antibiotics and antibiotic-resistant genes in the environment. *Journal of Public Health Policy*. 2018;**39**(4):389-406
- [53] Maron DF, Smith TJS, Nachman KE. Restrictions on antimicrobial use in food animal production: An international regulatory and economic survey. *Globalization and Health*. 2013;**9**(1):48
- [54] Guetiya Wadoum RE, Zambou NF, Anyangwe FF, Njimou JR, Coman MM, Verdenelli MC, et al. Abusive use of antibiotics in poultry farming in Cameroon and the public health implications. *British Poultry Science*. 2016;**57**(4):483-493
- [55] Chaw PS, Höpner J, Mikolajczyk R. The knowledge, attitude and practice of health practitioners towards antibiotic prescribing and resistance in developing countries—A systematic review. *Journal of Clinical Pharmacy and Therapeutics*. 2018;**43**(5):606-613
- [56] McCubbin KD, Anholt RM, de Jong E, Ida JA, Nóbrega DB, Kastelic JP, et al. Knowledge gaps in the understanding of antimicrobial resistance in Canada. *Frontiers in Public Health*. 2021;**9**:726484
- [57] Karkman A, Johnson TA, Lyra C, Stedtfeld RD, Tamminen M, Tiedje JM, et al. High-throughput quantification of antibiotic resistance genes from an urban wastewater treatment plant. *FEMS Microbiology Ecology*. 2016;**92**(3):7
- [58] Xu L, Zang J, Cong W, Holton E, Jiang L, Sheppard SK, et al. Assessment of community-wide antimicrobials usage in eastern China using wastewater-based epidemiology. *Water Research*. 2022;**222**:118942
- [59] Kwak YK, Colque P, Byfors S, Giske CG, Möllby R, Kühn I. Surveillance of antimicrobial resistance among *Escherichia coli* in wastewater in Stockholm during 1 year: Does it reflect the resistance trends in the society? *International Journal of Antimicrobial Agents*. 2015;**45**(1):25-32
- [60] Rytönen A, Tiwari A, Hokajärvi AM, Uusheimo S, Vepsäläinen A, Tulonen T, et al. The use of ribosomal RNA as a microbial source tracking target highlights the assay host-specificity requirement in water quality assessments. *Frontiers in Microbiology*. 2021;**12**:673306
- [61] Paulshus E, Kühn I, Möllby R, Colque P, O'Sullivan K, Midtvedt T, et al. Diversity and antibiotic resistance among *Escherichia coli* populations in hospital and community wastewater compared to wastewater at the receiving urban treatment plant. *Water Research*. 2019;**161**:232-241
- [62] Helmy M, Awad M, Mosa KA. Limited resources of genome sequencing in developing countries: Challenges and solutions. *Applied Translational Genomics*. 2016;**9**:15-19
- [63] Hutinel M, Larsson DGJ, Flach CF. Antibiotic resistance genes of emerging concern in municipal and hospital wastewater from a major Swedish city. *Science of the Total Environment*. 2022;**812**:151433

[64] Hultman J, Tamminen M, Pärnänen K, Cairns J, Karkman A, Virta M. Host range of antibiotic resistance genes in wastewater treatment plant influent and effluent. *FEMS Microbiology Ecology*. 2018;**94**(4):10

[65] Sun C, Li W, Chen Z, Qin W, Wen X. Responses of antibiotics, antibiotic resistance genes, and mobile genetic elements in sewage sludge to thermal hydrolysis pre-treatment and various anaerobic digestion conditions. *Environment International*. 2019;**133**:105156

[66] Mao Y, Shisler JL, Nguyen TH. Enhanced detection for antibiotic resistance genes in wastewater samples using a CRISPR-enriched metagenomic method. *Water Research*. 2025;**274**:123056

[67] Buschhardt T, Günther T, Skjerdal T, Torpdahl M, Gethmann J, Filippitzi M-E, et al. A one health glossary to support communication and information exchange between the human health, animal health and food safety sectors. *One Health*. 2021;**13**:100263

[68] Falzon LC, Lechner I, Chantziaras I, Collineau L, Courcoul A, Filippitzi M-E, et al. Quantitative outcomes of a one health approach to study global health challenges. *EcoHealth*. 2018;**15**(1):209-227