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ANTIBIOTIC RESISTANCE GENES IN FRESHWATER ENVIRONMENT: SOURCES, FATE, ECOLOGICAL IMPACT AND CLINICAL RELEVANCE

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ABSTRACT

Global public health is seriously threatened by antibiotic resistance, making the identification of environmental reservoirs of antibiotic resistance genes (ARGs) a crucial area of study. Freshwater ecosystems are particularly important for the spread of ARGs because of the intricate interactions between diverse microbial populations, human activities, and various antibiotic contamination sources. This review aims to offer a thorough understanding of the origin, progression, environmental impact, and medical significance of ARGs in freshwater environment. ARGs enter freshwater systems through various means, including the release of treated and untreated wastewater, agricultural drainage, and discharge of antibiotics and their byproducts. Once introduced, ARGs can endure freshwater environments through processes, such as horizontal gene transfer, co-selection, and biofilm formation. Moreover, the presence of ARGs in freshwater ecosystems has significant ecological consequences, affecting microbial diversity, ecosystem functions, and biogeochemical processes. The clinical relevance of ARGs in freshwater environments is a matter of concern. These genes can be transmitted to disease-causing bacteria, resulting in treatment failure and proliferation of infections that are resistant to multiple drugs. Additionally, the potential for ARGs to contaminate drinking water supplies raises concerns regarding human health. To develop effective strategies for combating the proliferation of antibiotic resistance, it is crucial to understand the behavior of ARGs in freshwater ecosystems. This review emphasizes the necessity of enhanced wastewater treatment technologies, responsible antibiotic usage, and sustainable farming practices to decrease the release and persistence of ARGs in freshwater ecosystems, ultimately protecting public health and maintaining the ecological balance of these vital habitats.

Keywords: Antibiotic resistance genes, Freshwater environment, Microbial communities

INTRODUCTION

Overuse of antibiotics results in their release into the environment. This environmental contamination by antibiotics is a significant issue, as it promotes the development of antibiotic-resistant microorganisms and genetic material. This affects the efficiency of antibiotics in battling disease-causing organisms that harm both human and animal health (Kumar and Pal , 2018). The prevalence of antibiotics in the environment is creating widespread alarms for both the scientific community and general population. Freshwater habitats are particularly vulnerable to contamination by antibiotics released via numerous routes, including agricultural runoff, sewage outflows, and leaching from surrounding farms. The prevalence of antibiotics in natural environments, together with a larger density of active bacterial populations endemic to freshwater, offers favorable conditions for the evolution of antibiotic resistance genes. Antibiotic resistance genes (ARGs) have been found to undergo horizontal gene transfer (HGT) in freshwater ecosystems, contributing to the development of resistance. These aquatic environments have become important locations for this process. The rise of bacteria resistant to antibiotics (ARBs) and antibiotic resistance genes (ARGs) in freshwater environments has heightened the likelihood of contracting resistant illnesses. Antibiotic resistance genes (ARGs) can survive in the environment and may eventually be transmitted to humans and animals. Freshwater basins that accept wastewater also operate as supplies for drinking water reservoirs and recreational areas. Additionally, the expansion of ARBs and ARGs may result in a significant pool of ARGs in environmental bacteria, thereby promoting the transmission of resistance to both existing and developing diseases. This is in contrast to clinical settings, which contain common indicator organisms, such as enterococci and coliforms, as well as bacterial pathogens responsible for challenging-to-treat illnesses in people. However, research on the incidence and type of antibiotic resistance in freshwater settings remains limited. This restricted focus may be because of the belief that antibiotic concentrations in these situations are normally low. However, despite their low numbers, selection of resistant bacteria can still occur in freshwater settings. Studies examining the presence and characteristics of antibiotic-resistant bacteria (ARBs) and antibiotic resistance genes (ARGs) in freshwater environments such as streams, lakes, and rivers have primarily focused on the areas where wastewater treatment plants (WWTPs) release their effluent.

The concentrations of ARBs and ARGs often increased near the effluent discharge locations of WWTPs into streams, but tended to gradually drop further downstream from the discharge spot. The reduction in the concentrations of ARGs and ARBs downstream of the WWTP effluent discharge locations might be attributed to several mechanisms, such as dilution, degradation, adsorption, and transport. Dilution and degradation have a crucial impact on the fate of ARGs in freshwater environment (Henriot *et al.,* 2021).

The worldwide health dilemma of antibiotic resistance is increasingly related to freshwater habitats, which are recognized as key conduits for the propagation of antibiotic resistance genes. A thorough analysis by the Antimicrobial Resistance Collaborators (2022) analyzed the worldwide effect of drug-resistant illnesses in 2019, investigating 88 pathogen-drug combinations. The analysis indicated that these illnesses were connected with around 4.95 million fatalities, including 1.27 million directly caused by treatment resistance (Murray *et al.,* 2022; Collaborators, 2022). From a geographic perspective, the highest drug resistance-related mortality rates across all age groups were observed in western sub-Saharan Africa, with 27.3 fatalities per 100,000 residents. In contrast, Australasia experienced the lowest rate, with 6.5 deaths per 100,000 individuals (Collaborators, 2022). Despite these results, there remains a dearth of understanding of the origins, consequences, environmental implications, and medicinal importance of resistance genes in freshwater systems. The rising risk of antibiotic resistance to public health highlights the need to understand the transmission of these genes in freshwater ecosystems and their possible influence on human health. This investigation can help to our knowledge of how antibiotic resistance genes influence freshwater environments ecologically, which may have far-reaching effects on overall ecosystem health. Gaining insights into the therapeutic importance of these genes in freshwater settings may lead to better techniques for treating and controlling illnesses caused by antibiotic-resistant bacteria. Furthermore, this review has the potential to inform policy decisions regarding antibiotic usage and its environmental effects, resulting in more effective techniques for preventing the development of antibiotic resistance.

Overview of Antibiotics and Antibiotic Resistance

Antibiotics are compounds produced by microorganisms that are either fully or partially synthesized and primarily used to combat or prevent bacterial infections. These compounds operate via diverse mechanisms to combat target bacteria and are typically classified as either bactericidal (able to eliminate bacteria) or bacteriostatic (able to suppress bacterial proliferation) when administered at appropriate dosages. Different antibiotics target various aspects of bacterial cells; some interfere with protein synthesis, others disrupt nucleic acid synthesis, and certain types attack cell membranes. Examples of these include β-lactam antibiotics, tetracyclines, and fluoroquinolones. Beyond their medical applications, antibiotics have become crucial in animal husbandry as feed supplements and growth promoters (Nicolaou *et al.,* 2018). While this practice is discouraged in the European Union, many nations, including the United States, continue to extensively use antibiotics as growth enhancers. Consequently, this application has become a significant source of antibiotics in soil- and land-based ecosystems enriched with animal waste (Rahman and Mohsin, 2019).

Antibiotics have emerged as a revolutionary medical innovation in the 20th century, becoming crucial in the treatment of infectious diseases in humans. These drugs are commonly used to combat infections in both humans and animals and have been extensively utilized to promote animal growth, even in the absence of bacterial infections. Global antibiotic consumption has increased by 35% during the first ten years of the 21st century. In 2013, China's antibiotic use reached approximately 162,000 tons, of which 52% was attributed to animal applications. This widespread use has resulted in the buildup of antibiotic residues and resistant bacteria in the digestive tract of animals, which are subsequently excreted in their feces. Antibiotics are used globally to enhance productivity and economic efficiency (Anand *et al.,* 2019). The National Institute of Animal Health reports that a significant number of livestock, including 104-110 million chickens and 275-292 million pigs, and turkeys, receive varying levels of antibiotics, contributing to worldwide antibiotic pollution (Jian *et al.,*2021). This contamination has resulted in the global proliferation of diverse antibiotic resistance genes (ARGs) in livestock manure, posing a considerable threat to public health. Annually, over 30,000 deaths in the European Union are attributed to antibiotic-resistant infections, with Italy and Greece experiencing the most severe impact. Additionally, countries with low and middle incomes in Asia, Africa, and South America experience higher rates of illness and death related to infections resistant to multiple drugs. Forecasts suggest that by 2050, global deaths from antibiotic-resistant infections will reach 10 million annually, with an estimated cumulative cost of $100 trillion in healthcare expenses and reduced productivity.

Antibiotic resistance genes (ARGs) are genetic elements that can be native or foreign DNA segments. The proteins and enzymes that enable bacteria to resist antibiotics are encoded by these genes. Antibiotic resistance genes (ARGs) typically exist as mobile genetic elements, including conjugative plasmids, chromosomally integrated conjugative elements, and transposons. These ARGs frequently propagate through horizontal gene transfer (HGT). ARGs are a subset of environmental DNA (eDNA), which comprise DNA fragments found outside or released from intact cells into the surrounding environment.

In aquatic ecosystems, environmental DNA (eDNA) can be classified into two types: soluble DNA (s-DNA) and non-soluble DNA (ns-DNA). Non-soluble DNA encompasses intracellular DNA present in both living and dead cells, as well as DNA attached to insoluble organic and inorganic matrices and aggregates. Conversely, soluble DNA refers to free-floating or reversibly adsorbed eDNA that can be easily displaced by phosphate groups, including orthophosphates, nucleotide triphosphates, metaphosphates, and pyrophosphates. Antibiotic resistance genes (ARGs) fall under this category of environmental DNA (Liu *et al.,* 2020).

Antibiotic Resistance Genes and Their Effects on Human and Animal Health

The rapid spread of antibiotic resistance genes (ARGs) in recent times has heightened the threat of microbial infections to both human and animal well-being (Zhang *et al.,* 2022). While numerous genes can confer resistance, evaluating the relative health risks associated with ARGs is a complex issue. The widespread use of antibiotics in human, animal, and environmental settings, along with the presence of antibiotic residues, can exert selective pressure on antibiotic-resistant bacteria (ARB) and antibiotic resistance genes (ARG), thereby accelerating the spread of antibiotic resistance (Zhang *et al.,* 2022).

Antibiotic resistance poses a major challenge to global health, diminishing the effectiveness of antimicrobial treatments. Although much research has focused on ARG dissemination in clinical settings, there is increasing attention on the presence and persistence of ARGs in natural environments, particularly freshwater ecosystems. Multiple studies have identified various factors contributing to ARG presence in aquatic environments. Pan *et al.* (2018) investigated ARGs in China's Pearl River and found that sewage discharge was a primary source, leading to elevated levels of genes conferring resistance to fluoroquinolones and sulfonamides. Likewise, Chen *et al.* (2019) discovered a wide range of ARGs in Beijing's urban rivers, emphasizing the significance of urban wastewater as a reservoir for resistance genes.

A notable example of research in this field is a study by Young *et al.* (2022), which examined the occurrence of antimicrobial resistance (AMR) genes in humans, animals, and water sources within an urban informal settlement in Nepal experiencing intensified livestock production. This investigation identified specific resistance genes linked to antibiotics of global health importance, spanning various drug categories including aminoglycosides, beta-lactams, tetracyclines, macrolides, and fluoroquinolones (Young *et al.,* 2022).

Another relevant investigation by Zhang *et al.* (2022) offers a quantitative evaluation of human health risks associated with 2561 antibiotic resistance genes (ARGs). This assessment considered factors such as human accessibility, mobility, pathogenicity, and clinical availability. The findings revealed that 23.78% of the analyzed ARGs posed a health risk, with those related to multidrug resistance being of particular concern (Zhang *et al.,* 2022).

Significance of Understanding Antibiotic Resistance Genes (ARGs) In Freshwater

Investigating antibiotic resistance genes (ARGs) in freshwater ecosystems is crucial due to their potential effects on environmental and human health. Antibiotics can enter aquatic environments through various means, such as wastewater discharge, runoff from land, and aquaculture practices, promoting the proliferation of ARGs among resistant microbes. Artificial freshwater reservoirs, created by dam construction to address water demands, may act as significant conduits for ARG transfer from the environment to humans. The transmission of ARGs through food chains or direct contact can enhance antibiotic resistance in human populations, enabling genetic exchange across different species (Zhao-Feng *et al.,*2023).

A study utilizing data analysis techniques investigated the occurrence of common ARGs in freshwater reservoirs. The researchers adopted a data-driven methodology to assess ARG distribution, factors influencing their presence, and pollution hotspots. Their results demonstrated geographical variations in ARG types and quantities within reservoirs, highlighting the important influence of reservoir characteristics on ARG contamination. The study also revealed that ARGs in reservoir waters and sediments exhibited distinct pollution hotspots (Zhao-Feng *et al.,* 2023).

Amarasiri *et al.* (2020) performed a comprehensive analysis of the current knowledge and unanswered questions concerning the health hazards linked to antibiotic-resistant bacteria (ARB) and ARGs in water-based ecosystems. The researchers pointed out that these environments are believed to facilitate the emergence and proliferation of antibiotic resistance, and human contact with ARB and ARGs in water systems could lead to additional health issues. To evaluate and quantify this health risk, quantitative microbial risk assessment (QMRA) has been proposed. However, there is insufficient information on ARB and ARG exposure in various water-related contexts, and specific dose-response models for ARB infections have yet to be established.

Cai *et al.* (2022) explored the mechanisms behind the formation of viable but non-culturable (VBNC) bacteria through light-based disinfection methods and their capacity to disseminate antibiotic resistance genes (ARGs). The research emphasized that disinfection techniques, particularly those using light, can trigger bacteria to enter the VBNC state. These VBNC bacteria exhibit enhanced resistance to disinfection, making their complete elimination difficult and potentially enabling the spread of ARGs. The key processes involved in VBNC bacteria formation include stringent response, general stress response system, and toxin-antitoxin (TA) system. Additionally, the study examined the horizontal gene transfer (HGT) of ARGs during and after VBNC bacteria formation induced by light-based disinfection. The findings revealed that ARGs can be spread through conjugation, transformation, and transduction processes.

Keenum *et al.*(2022) developed a standardized approach for quantifying antibiotic resistance in various water sources using quantitative polymerase chain reaction (qPCR). They conducted an extensive review to identify the most commonly measured gene targets for quantifying antibiotic resistance in surface, recycled, and wastewater, and evaluated the corresponding protocols. The research identified sul1, tetA, and intI1 as gene targets with high prevalence and association with human-related inputs, while vanA and blaCTX-M were found to be less common but clinically significant. The authors analyzed 117 peer-reviewed studies that employed these assays on relevant water matrices. They systematically assessed various aspects of the protocols, including sample collection and concentration methods, DNA extraction techniques, primer/probe specificity, amplification conditions, amplicon length, PCR inhibition assessment, and detection and quantification limits. Furthermore, they compared the reported gene copy numbers across studies based on the assay used and type of water matrix. Following this comprehensive analysis, the researchers suggested specific assays, standardized workflows, and reporting guidelines for the five target genes (Keenum *et al.,* 2022).

Research by Sun *et al.* (2020) examined the dynamics of antibiotic resistance genes (ARGs) in China's Haihe River, revealing that both physical and biological elements influence ARG fate. The researchers observed that ARGs in sediment showed greater longevity compared to those in the water column, indicating that sediment serves as a repository for antibiotic resistance.

Ohore *et al.* (2022) conducted a study on the ecological consequences of ARGs in freshwater ecosystems, with a focus on Lake Taihu in China. Their findings uncovered a significant presence and wide variety of ARGs, which were associated with alterations in bacterial community composition, potentially disrupting ecosystem functions.

Hooban *et al.* (2021) demonstrated the significant health risk posed by the movement of ARGs from freshwater to clinical settings in their research on drinking water. They detected clinically relevant resistance genes, including extended-spectrum beta-lactamase (ESBL) genes, in drinking water samples, highlighting the importance of considering freshwater sources when studying the dissemination of antibiotic resistance.

The endurance of antibiotic-resistance genes in freshwater environments presents considerable ecological and clinical obstacles. As bacteria develop resistance mechanisms, the efficacy of antibiotics against bacterial infections has diminished. Antibiotic resistance is a critical health concern that leads to increased morbidity, mortality, and healthcare expenses. According to Dadgostar (2019), drug-resistant infections could result in 10 million deaths per year by 2050. Studies conducted by Tacconelli *et al.* (2019) suggest that drug-resistant infections in ICUs are linked to a notably higher risk of mortality compared to susceptible infections.

The global proliferation of multidrug-resistant organisms (MDROs), particularly carbapenem-resistant Klebsiella pneumoniae, has complicated treatment strategies, as noted by Wyres *et al.* (2019). The use of antibiotics in livestock farming significantly contributes to veterinary antibiotic resistance, necessitating a reduction in antibiotic usage in animal agriculture to address this problem. Research by Sirichokchatchawan *et al.* (2021) on Danish pig farms revealed that policies limiting antibiotic use led to a decrease in antibiotic-resistant bacteria in pigs, showcasing the efficacy of targeted interventions in reducing antibiotic resistance in animal populations.

The transfer of antibiotic-resistant bacteria from animals to humans through food consumption and direct contact poses additional challenges. Rousham *et al.* (2021) illustrated that human exposure to antibiotic-resistant bacteria from poultry sources facilitates the spread of antibiotic resistance. The One Health approach, which promotes collaboration among human health, veterinary medicine, and environmental sectors, has emerged as a valuable strategy for comprehensively tackling antibiotic resistance.

Hernando-Amado *et al.* (2020) underscored the effectiveness of the One Health approach in combating antibiotic resistance in *Enterobacteriaceae*. Their study demonstrated that stringent antibiotic stewardship in both human and veterinary medicine, combined with improved hygiene practices, can substantially reduce the prevalence of antibiotic-resistant *Enterobacteriaceae* in humans and animals.

Antibiotic resistance genes (ARGs) are crucial in the spread and endurance of resistance within natural settings, including freshwater ecosystems. Examining the occurrence and dynamics of ARGs in freshwater is vital for understanding environmental resistance gene reservoirs, assessing their health implications, and formulating strategies to address antibiotic resistance. Research by Gu *et al.* (2019) established freshwater environments as key ARG reservoirs. Their examination of rivers, lakes, and reservoirs uncovered a diverse range of ARGs, such as those resistant to beta-lactams, tetracyclines, and sulfonamides, indicating the widespread distribution of resistance genes in these ecosystems.

Huang *et al.* (2019) demonstrated that human activities significantly contribute to the introduction and proliferation of ARGs in freshwater environments. Their study revealed that sewage discharge substantially increased ARG abundance and diversity in a river system, emphasizing the role of anthropogenic activities in spreading antibiotic resistance.

The potential transfer of ARGs from environmental bacteria to human pathogens is crucial for health risk evaluation. Hooban *et al.* (2020) explored ARG transferability and identified a mobile genetic element with a resistance gene in Escherichia coli from a freshwater lake, suggesting the possibility of ARG transfer to human pathogens.

Zhang *et al.* (2021) investigated how environmental factors influence ARG abundance and diversity in freshwater. Their research showed that temperature, dissolved oxygen, and nutrients significantly affected ARG distribution in rivers, underscoring the importance of ecological factors in understanding antibiotic resistance dynamics.

The presence of ARGs in freshwater environments has ecological consequences beyond human health. Zhao *et al.* (2019) discovered that ARGs impact microbial community structure in river systems, affecting ecosystem functions and biodiversity.

Addressing antimicrobial resistance (AMR) necessitates a One Health approach, given that over half of animal antimicrobials are medically significant for humans, and AMR can persist in the environment. This is particularly important in low- and middle-income countries and community settings, where approximately 80% of antibiotics are used (Zhang *et al.*, 2022).

Sources of antibiotic resistance genes in freshwater environments.

Antibiotic resistance genes (ARGs), which enable bacteria to withstand antibiotics, are becoming increasingly common in environments affected by human activity and are now considered significant emerging pollutants. Aquatic ecosystems are particularly vulnerable to ARG contamination through various sources, including wastewater discharge, land runoff, and fish farming practices. Research by Guo *et al.* (2023) examined ARG pollution in freshwater reservoirs, uncovering their distribution patterns, contributing factors, and areas of high concentration. Their findings revealed geographical variations in ARG types and prevalence, with reservoir characteristics playing a significant role in determining pollution levels in both water and sediment.

Larsson and Flach (2021) conducted a review of environmental elements, such as antibiotic contamination, that influence the development and spread of antibiotic resistance. They noted that despite existing obstacles, disease-causing organisms often acquire new resistance mechanisms from other species, making infection control and treatment more challenging. While the emergence of novel resistance factors is infrequent and hard to predict, the transmission of widely distributed resistant strains is more common and measurable, albeit with limited impact per occurrence. Addressing the resistance crisis requires a comprehensive understanding of these pathways, drivers, and limiting factors. Hendriksen *et al.* (2019) investigated the worldwide dissemination of ARGs and their effects on public health and food safety, highlighting the globalization of antibiotic resistance as a major concern. The World Health Organization (WHO) considers this spread a crisis that threatens global health security and the foundations of modern medicine, as increased ARG prevalence can enhance virulence, pathogenicity, and mortality rates. Adenaya *et al.* (2023) explored the relationship between human activities and ARG proliferation in aquatic ecosystems, finding a correlation between increased human activity and higher ARG exposure. The "One Health" approach emphasizes ARG pollution as a threat to human, animal, and environmental well-being, with globalization accelerating the transfer of risks among these interconnected domains.

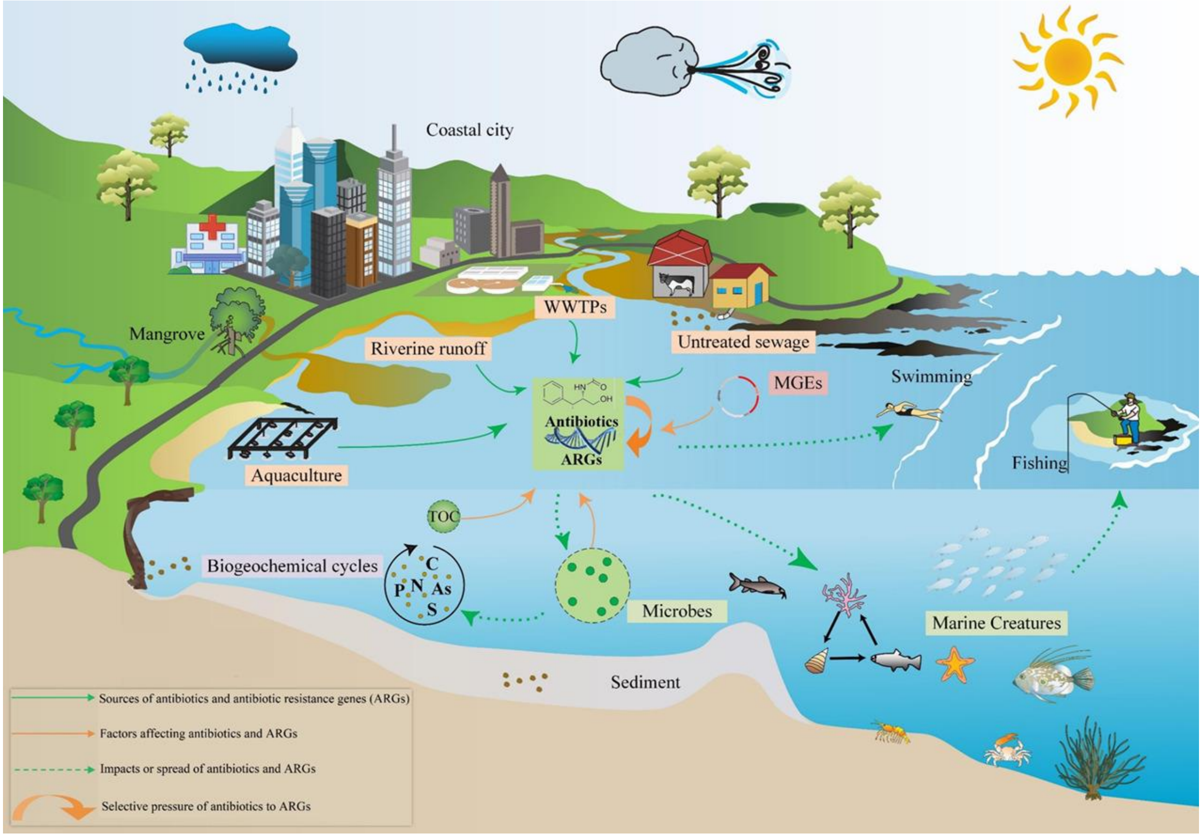

Figure 1: Different Sources of antibiotic resistance genes (Guo *et al.*,2023)

Wastewater Treatment Plants (WWTPs)

Bacteria resistant to antibiotics contain genetic components called antibiotic resistance genes (ARGs) that enable them to withstand antibiotic treatments. These genes are considered emerging pollutants commonly found in environments affected by human activity. Wastewater treatment plants (WWTPs) are recognized as major repositories and disseminators of ARGs. The introduction of antibiotics through sewage, surface water runoff, and fish farming operations contributes to the emergence and dissemination of ARGs within antibiotic-resistant bacteria.

Research conducted by Alexander *et al.* (2020) in Germany investigated the dissemination of antibiotic resistance through WWTP effluents from various catchment areas. They employed quantitative polymerase chain reaction (qPCR) to quantify ARGs, facultative pathogenic bacteria (FPB), and a mobile genetic element in DNA extracted from 23 WWTP effluents across Germany.

Gao *et al.* (2022) conducted a review of WWTPs as reservoirs and sources of ARGs, concluding that these facilities contain numerous mobile genetic elements (MGEs), heavy metals, and emerging contaminants that contribute to ARG proliferation. Chen *et al.* (2020) identified wastewater as a crucial reservoir for antibiotic resistance in diverse environments. Considering that most WWTPs process substantial volumes of wastewater daily, they routinely handle significant quantities of pollutants.

Osińska *et al.* (2020) examined the role of WWTPs in managing pollutants and discovered that these facilities receive discharges from household, medical, industrial, and agricultural sources, containing both living and non-living materials. WWTPs significantly contribute to the spread of ARGs in freshwater ecosystems because treated effluents introduce various contaminants, including antibiotics, antibiotic-resistant bacteria, and ARGs. Comprehending the origins and dynamics of ARGs within WWTPs is essential for developing strategies to mitigate the spread of antibiotic resistance.

Multiple research studies have consistently shown that wastewater treatment plants (WWTPs) serve as reservoirs and sources of antibiotic resistance genes (ARGs). For instance, Chen *et al.* (2018) investigated ARGs in a WWTP in China, discovering various resistance genes, including those conferring resistance to beta-lactams, aminoglycosides, and tetracyclines. In a similar vein, Zhang *et al.* (2019) explored ARG profiles across several WWTPs, noting a high prevalence and diversity of resistance genes, with significant variations based on different treatment processes.

WWTPs have been found to promote the selection and enrichment of ARGs. A study by Zhang *et al.* (2020) investigated the influence of WWTPs on ARG distribution in receiving water bodies and revealed that treated effluents contained higher concentrations of ARGs compared to river water. This finding suggests that the treatment processes contribute to the selection and persistence of ARGs. The presence of residual antibiotics and other selective pressures in WWTPs likely encourages ARG enrichment. The transfer of resistance genes between environmental and pathogenic bacteria is facilitated by horizontal gene transfer (HGT). Zhang *et al.* (2021) examined HGT-mediated ARG dissemination in a WWTP and observed gene transfer via transposons, underscoring the potential for ARG transmission among bacterial populations in WWTPs.

Agricultural Runoff

The presence of antibiotic resistance genes (ARGs) in freshwater ecosystems is significantly influenced by agricultural runoff. The use of antibiotics in farming practices, coupled with manure application techniques, introduces ARGs into various freshwater bodies. These antibiotics, employed for promoting animal growth and managing infections, contribute to the selection and proliferation of antibiotic-resistant bacteria and ARGs. A study by Rahman *et al.* (2018) examined the effects of swine manure application on ARG abundance and diversity in agricultural soil, revealing a substantial increase in ARGs, particularly those resistant to tetracyclines, sulfonamides, and beta-lactams, following manure application.

The transport of ARGs from fields to freshwater systems can occur through runoff from manure applications. Research conducted by Meyers *et al.* (2020) demonstrated significantly higher concentrations of ARGs, especially those associated with mobile genetic elements, in downstream surface water compared to upstream samples, emphasizing the role of agricultural runoff in ARG dissemination into freshwater. ARGs can maintain their viability and persist in runoff, posing risks for spread and transfer to human pathogens. Nappier *et al.* (2020) observed the stability and detectability of certain ARGs in agricultural runoff and downstream waters, suggesting their potential for long-distance dissemination and horizontal gene transfer.

The introduction of ARGs into freshwater systems through agricultural runoff has implications for human health. A comprehensive review by Ondon *et al.* (2021) analyzed studies connecting ARGs in water bodies affected by agricultural activities to the occurrence of antibiotic-resistant bacteria in humans. The review provided evidence supporting the transmission of ARGs from agricultural sources to human pathogens, underscoring the potential risk to human health.

Agricultural runoff plays a crucial role in the spread of antibiotic resistance genes (ARGs) from farmlands to aquatic environments. A 2021 investigation explored the impact of agricultural land-use changes on ARG patterns in runoff and associated health risks. The results showed that orchard runoff exhibited more diverse ARG patterns and higher gene abundance compared to conventional cropland runoff. Furthermore, co-occurrence network analysis between mobile genetic elements and ARGs indicates an increased threat of ARG dissemination following land-use changes (Zhang *et al.*, 2021).

A 2023 study examined the effects of antibiotic interactions on the emergence of antimicrobial resistance in wastewater, a major reservoir of antimicrobial resistance due to the accumulation of antibiotic residues from industrial and agricultural runoff. The research revealed that populations exposed to synergistic and antagonistic antibiotic conditions deviated significantly from expected behavior. Notably, *E. coli* populations exposed to synergistic antibiotics demonstrated lower resistance development than predicted, suggesting a suppressive effect of such antibiotics on the progression of resistance (Sutradhar *et al.*, 2023).

Mitigation strategies to reduce the impact of agricultural runoff on ARG dissemination are essential. Sorinolu and Munir (2022) examined the efficacy of vegetative filter strips (VFS) in reducing ARG transport in agricultural runoff. They observed that VFS notably lowered ARG concentrations in the surface runoff, demonstrating the potential of this practice to curb the spread of ARGs into freshwater systems.

Hospital Wastewater (HWW)

Antibiotic-resistant bacteria and antibiotic resistance genes (ARGs) are significantly present in hospital wastewater (HWW), making it a major environmental concern. A meta-analysis conducted in 2020 revealed a high relative abundance of genes resistant to various antibiotics and mobile genetic elements in HWW (Zhang *et al.*, 2020). In 2021, a study employing high-throughput sequencing-based metagenomics examined bacterial communities and ARGs in untreated wastewater from different hospital types, uncovering distinct ARG profiles for each (Guo *et al.*, 2021).

Hospital effluents, containing diverse pharmaceuticals and antibiotics, have been recognized as ARG sources. A study by Adhikari *et al.* (2023) detected high concentrations of ARGs, including those resistant to fluoroquinolones and β-lactam antibiotics, in wastewater samples from a U.S. hospital. This research emphasized the crucial role of hospital effluents in environmental antibiotic resistance dissemination.

The discharge of hospital effluent into freshwater bodies can result in water pollution and antibiotic resistance proliferation. Raza *et al.* (2021) explored the impact of hospital wastewater on river antibiotic resistomes, identifying a wide range of ARGs, including multi-antibiotic resistant ones, downstream from hospital discharge points. This investigation highlights the potential risks associated with hospital effluents in spreading antibiotic resistance genes throughout freshwater ecosystems.

Multiple studies have investigated ARGs in hospital effluents and their transmission to freshwater environments. Wang *et al.* (2020) examined sulfonamide resistance genes in hospital wastewater treatment plants and downstream rivers, concluding that hospital effluent significantly contributes to the spread of these genes in freshwater. The implementation of effective wastewater treatment strategies is essential for reducing ARG release from hospital effluents. Zhang *et al.* (2021) assessed the ARG removal efficiency in a hospital wastewater treatment plant using various technologies. The study found that conventional treatments were insufficient to fully eliminate ARGs, underscoring the need for advanced methods to reduce ARG discharge into freshwater ecosystems.

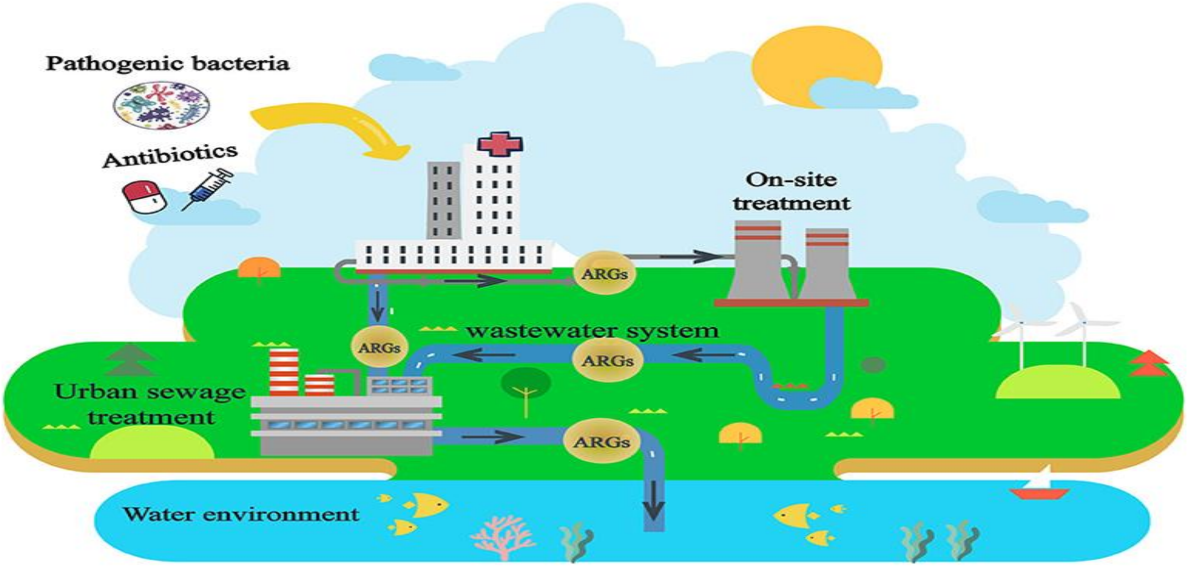

Figure 2: Flow of ARGs from HWW to water environment (Zhang *et al.*,2021)

Antibiotic Residues

The presence of antibiotic residues in environmental settings can contribute to the emergence of antibiotic resistance genes (ARGs) in freshwater ecosystems. Research conducted in 2021 examined how various environmental factors, including antibiotic contamination, influenced the evolution and spread of resistance. The findings revealed that the introduction of antibiotics as medical treatments significantly altered the conditions for resistance development and dissemination, creating unprecedented selective pressures. These pressures particularly affected the microbiota of humans and domestic animals, as well as environments heavily polluted with antibiotics (Larsson and Flach, 2021).

A subsequent study in 2023 examined the effects of antibiotic interactions on the development of antimicrobial resistance in wastewater, which serves as a crucial reservoir for resistance due to the accumulation of antibiotic residues from industrial and agricultural origins. The research demonstrated that bacterial populations exposed to synergistic and antagonistic antibiotic conditions exhibited significant deviations from expected behavior. Notably, *E. coli* populations subjected to synergistic antibiotic conditions developed lower levels of resistance than anticipated, suggesting that such antibiotics might inhibit the evolution of resistance (Sutradhar *et al.*, 2023).

Aquaculture Effluent

The presence of antibiotic resistance genes (ARGs) in freshwater ecosystems poses a significant threat to public health. Aquaculture, particularly fish and shrimp farming, is a major contributor to this issue. A study by Zhang *et al.* (2020) examined water and sediment samples from aquaculture ponds, revealing high concentrations of ARGs such as tetM and ermB, which are associated with resistance to tetracycline and macrolides. This research underscored the substantial impact of aquaculture on the proliferation of ARGs in freshwater environments.

Cabello *et al.* (2019) explored the relationship between antibiotic use in aquaculture and the development of antibiotic resistance. Their findings showed a greater abundance of ARGs in the intestines of farmed fish compared to wild fish, with genes conferring resistance to antibiotics like tetracyclines and quinolones being identified. This study further emphasized aquaculture's role in the spread of antibiotic resistance.

Research conducted by Ning *et al.* (2022) focused on the occurrence and potential transmission of antibiotic resistance from aquaculture systems to surrounding freshwater ecosystems. By analyzing water and sediment samples from aquaculture farms and nearby rivers, they discovered elevated levels of ARGs, including sul1, tetG, and ermB, in aquaculture locations. These results indicate that aquaculture activities can introduce and disseminate ARGs to adjacent aquatic ecosystems.

Ferri *et al.* (2022) investigated antibiotic use and resistance development in shrimp farms by examining ARGs in water, sediment, and shrimp samples from various facilities. They detected high levels of ARGs, such as sul1 and tetO, suggesting that shrimp farming contributes to the dissemination of antibiotic-resistance genes in freshwater. The extensive use of antibiotics in aquaculture for disease prevention and growth promotion creates selective pressure on bacteria, promoting the emergence and spread of resistance genes. The discharge of resistant bacteria and ARGs into freshwater environments risks transferring resistance to native microorganisms, while antibiotic residues in wastewater further facilitate the spread of resistance genes in surrounding waters. Consequently, aquaculture plays a significant role in the proliferation of antibiotic-resistance genes in freshwater ecosystems.

Fate of Antibiotic Resistance Genes in Fresh Water Environment

Horizontal Gene Transfer (HGT)

The primary mechanism for antibiotic resistance genes (ARGs) dissemination is horizontal gene transfer (HGT). This process has been suggested to stabilize public goods cooperation by transforming non-cooperative cells into cooperators. HGT facilitates the spread of ARGs through the exchange of genetic material between bacteria, often via plasmids. Research by Bai *et al.* (2021) on freshwater sediments revealed that multiple plasmids carrying ARGs, including sul1 and tetM, were associated with resistance to sulfonamide and tetracycline, underscoring the significance of plasmids in ARG transmission in aquatic environments.

Another contributor to HGT is integrons, which capture and express resistance genes. Gao *et al.* (2018) identified various integron classes and resistance gene cassettes for aminoglycosides and beta-lactams in freshwater, emphasizing the role of integrons in ARG transfer. Wang *et al.* (2023) discovered that cooperative antibiotic resistance enhances HGT, with KPC-2 β-lactamase expression being higher in plasmids, resulting in increased rescue of sensitive non-producers and efficient plasmid transfer, thus expanding the pool of ARG recipients. Wu *et al.* (2022) showed that biochar impedes HGT by restricting the energy supply for conjugative plasmid transfer. Biochar produced at higher temperatures more effectively inhibited RP4 plasmid transfer by downregulating transfer-related genes due to limited ATP energy supply and reduced cell membrane permeability.HGT plays a crucial role in the proliferation of ARGs in freshwater ecosystems, enabling rapid gene transfer among bacteria and leading to multidrug-resistant bacteria and fewer treatment options. The exchange of genetic material between environmental bacteria and pathogens exacerbates public health concerns related to antibiotic resistance.

Mobile Genetic Elements (MGEs)

The global public health crisis of antibiotic resistance is exacerbated by mobile genetic elements (MGEs), which play a crucial role in the spread and expression of antibiotic resistance genes (ARGs) within and across species.

A study by Jeon *et al.* (2023) examined the prevalence and distribution of MGE-linked ARGs in Acinetobacter baumannii, a highly antibiotic-resistant hospital-acquired pathogen. The research adopted a comprehensive approach, considering human, animal, and environmental aspects. To combat ARG dissemination via MGEs, four strategies were suggested: utilizing semi-permeable membrane-covered thermophilic composting to prevent airborne ARG spread; employing nanomaterials for removing emerging contaminants and pathogens; implementing tertiary treatment in wastewater facilities; and applying advanced oxidation methods to eliminate ARGs.

Partridge *et al.* (2018) provided an overview of the primary MGEs involved in acquiring and spreading antibiotic resistance in both Gram-negative and Gram-positive bacteria. Their review concentrated on the ESKAPEE group—*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, *Enterobacter spp*., and *Escherichia coli*—which are notable hospital-associated pathogens.

MGEs, including plasmids, transposons, and integrons, are vital in disseminating ARGs through horizontal gene transfer (HGT). Zhao *et al.* (2021) investigated plasmid-mediated antibiotic resistance in freshwater environments, uncovering various plasmids carrying ARGs for tetracyclines, beta-lactams, and aminoglycosides, thus highlighting plasmids' role in maintaining and transferring ARGs in freshwater ecosystems.

Li *et al.* (2022) explored transposons in freshwater settings, identifying those harboring ARGs for tetracyclines and sulfonamides, emphasizing transposons as key vectors for ARG proliferation in aquatic ecosystems. Nnadozie *et al.* (2019) examined integrons in freshwater ecosystems, discovering integrons with ARGs for fluoroquinolones, macrolides, and beta-lactams, underscoring integrons' function as ARG reservoirs and their contribution to resistance persistence.

MGEs significantly influence the fate of ARGs in freshwater environments by enabling HGT, fostering ARG spread among bacterial populations, boosting bacterial adaptability to antibiotic pressure, and resulting in multidrug-resistant strains. Additionally, MGEs facilitate the co-transfer of multiple resistance genes, complicating the treatment of antibiotic-resistant infections in both clinical and environmental settings.

Biofilm

Antibiotic resistance genes (ARGs) can accumulate in freshwater biofilms, which act as repositories for these genetic elements. Research by Flores-Vargas *et al.* (2021) utilized metagenomic techniques to examine ARG prevalence in river biofilms, demonstrating that treated wastewater substantially introduces ARGs into aquatic ecosystems, with biofilms facilitating their spread. A subsequent study by the same researchers in 2021 emphasized biofilms' function as antimicrobial resistance reservoirs, noting that persistent low-level antibiotic exposure can promote resistance development. Freshwater biofilms and areas affected by agriculture have been recognized as crucial sites for maintaining and disseminating antibiotic resistance.

Biofilms, which are intricate microbial aggregations adhering to surfaces, are integral to ARG dynamics. Conco *et al.* (2022) identified a substantial abundance and variety of ARGs in freshwater biofilms, including resistance to beta-lactams, tetracyclines, and sulfonamides, emphasizing biofilms' significance as ARG reservoirs. These structures also enable horizontal gene transfer (HGT) of ARGs, enhancing their distribution among bacterial populations. An additional study by Flores-Vargas *et al.* (2021) showed HGT of ARGs, such as those conferring resistance to fluoroquinolones and aminoglycosides, within biofilm communities; biofilms displayed enhanced antibiotic resistance compared to free-floating bacteria, contributing to ARG persistence.

Miao *et al.* (2022) investigated biofilms' adaptive responses to antibiotics in freshwater, observing increased expression of ARGs associated with efflux pumps and target site modification under antibiotic stress, highlighting biofilms' role in sustaining and promoting ARG expression. Biofilms exert a considerable influence on ARG fate in freshwater environments. Their complex structure supports ARG persistence and transfer by protecting bacteria from antibiotics, thus enabling resistant strains to survive and multiply. The close proximity of diverse bacterial species within biofilms promotes HGT, contributing to the emergence of multidrug-resistant strains and posing potential risks to both human and environmental health.

Adsorption onto Media Surfaces

The process of surface attachment, known as adsorption, plays a crucial role in determining the destiny of antibiotic resistance genes (ARGs). Sediment and suspended particles, which are types of particulate matter, can serve as storage sites for ARGs through adsorption. Research by Chen *et al.* (2018) explored ARG adsorption to sediments in freshwater ecosystems, demonstrating a strong link between ARGs and sediment particles, with sediments containing higher concentrations of ARGs compared to the water column. This investigation highlighted how particulate matter adsorption contributes to ARG persistence and accumulation in freshwater settings.

ARG adsorption also occurs on microbial surfaces, including those of bacteria and algae. A study by Wang *et al.* (2023) examined ARG adsorption to microbial biofilms in freshwater, revealing that ARGs tend to adsorb to biofilm matrices, particularly extracellular polymeric substances (EPS). These results emphasize the importance of microbial surfaces in the fate and transfer of ARGs within aquatic ecosystems.

Environmental conditions can impact ARG adsorption in freshwater. Wang *et al.* (2023) studied how pH and temperature affect ARG adsorption to sediments, discovering that both factors significantly influenced the sediments' capacity to adsorb ARGs. This research underscored the importance of considering environmental factors when evaluating ARG fate and transport in freshwater environments.

Adsorption has a substantial impact on the fate of ARGs in freshwater ecosystems. By attaching to particulate matter and microbial surfaces, ARGs can persist and potentially transfer to other organisms. This process can enhance the survival of antibiotic-resistant bacteria by offering protection against antibiotics. Furthermore, under certain conditions, adsorbed ARGs may be released, contributing to the spread and dissemination of antibiotic resistance.

Persistence in The Environment

The endurance of antibiotic-resistance genes (ARGs) in freshwater ecosystems presents a major public health issue. Sediments in freshwater bodies act as enduring storage sites for these genes. Research by Wu *et al.* (2022) explored ARG persistence in sediments from diverse freshwater settings, discovering a high concentration and variety of ARGs, including those associated with resistance to multiple antibiotics. Their study emphasized the crucial function of sediments as ARG reservoirs in freshwater systems.

Environmental conditions also influence ARG persistence. A study by Li *et al.* (2022) investigated how temperature and organic matter content affect ARG persistence in lake sediments. They found that elevated temperatures and higher organic matter levels promote ARG persistence, potentially increasing antibiotic resistance. These results stress the need to consider environmental factors when evaluating ARG fate and persistence.

Microorganisms play a significant role in ARG persistence within freshwater environments. Zhang *et al.* (2021) examined the impact of microbial communities on ARG persistence in sediments, identifying Proteobacteria and Firmicutes as key groups linked to high ARG abundance. This research highlighted the importance of microbial communities in determining ARG fate and persistence.

The long-term presence of ARGs in freshwater has significant consequences for the emergence and spread of antibiotic resistance. Persistent ARGs can be transferred to pathogenic bacteria, leading to the development of multidrug-resistant strains. The prolonged existence of ARGs in the environment can enable susceptible bacteria to acquire resistance traits, further propagating resistance. Additionally, ARG persistence complicates water treatment processes, as conventional methods may not effectively eliminate these genes. Understanding the factors that influence ARG persistence is crucial for developing strategies to mitigate the spread of antibiotic resistance in freshwater ecosystems (Li *et al.*,2022).

Degradation

The fate of antibiotic resistance genes (ARGs) in freshwater ecosystems is largely determined by degradation processes. A study by Wang *et al.*(2021) investigated the ARG degradation potential of microbial communities in lake sediments, identifying bacterial groups such as Actinobacteria and Proteobacteria as highly active in ARG breakdown. The degradation of ARGs in freshwater environments is significantly influenced by environmental conditions. Zhang *et al.*(2020) explored the effects of temperature and organic matter on ARG degradation in river sediment, demonstrating that higher temperatures and increased organic matter content accelerated ARG breakdown. These investigations highlight the crucial role of environmental factors in ARG fate and degradation, while innovative treatment methods show promise for ARG elimination in freshwater settings. Liu *et al.*(2022) assessed the efficacy of ozonation in degrading ARGs at wastewater treatment plants, discovering that this technique significantly reduced ARG abundance, suggesting its potential for mitigating resistance genes in freshwater systems.

The decomposition of ARGs in freshwater environments is crucial for reducing the prevalence and spread of antibiotic resistance. Microbial communities and environmental factors contribute to ARG removal and inactivation through degradation processes. Efficient ARG breakdown can impede the transfer of resistance traits among bacteria and reduce the risk of environmental dissemination. Furthermore, the implementation of advanced treatment technologies can enhance ARG elimination from wastewater and lessen the release of resistance genes into freshwater ecosystems. In conclusion, degradation processes are instrumental in diminishing the persistence and impact of ARGs in freshwater environments.

Ecological Implications of Antibiotic Resistance Genes In Fresh Water Environments

The global health crisis of antibiotic resistance is worsened by the use of antibiotics, which promotes the growth of resistant bacteria in individuals and the environment. Despite extensive research, the ecological consequences of this relationship remain uncertain. A study by Rahman *et al.* (2023) examined 11 years of data on antibiotic use and resistance across 26 Antibiotic–bacteria combinations in 26 European nations. Their findings showed that the occurrence of resistant bacteria increases immediately following antibiotic use and continues to rise for at least four years. In contrast, decreasing antibiotic use had little impact on resistance within the same period. The study also found that antibiotic use in neighboring countries increased resistance, regardless of local usage patterns.

Shi *et al.* (2022) investigated the swift proliferation of antibiotic resistance genes (ARGs) in non-antibiotic conditions. While the worldwide spread of ARGs has been associated with excessive antibiotic use, this research emphasized two crucial factors: residual antibiotic concentrations in the environment and alternative functions of ARGs. The study revealed that environmental antibiotic levels are typically much lower than those expected to select for antibiotic-resistant bacteria (ARB), with the exception of heavily contaminated industrial areas, such as those connected to antibiotic production.

Alteration of Community Composition

The presence of antibiotic resistance genes (ARGs) can significantly impact the structure and composition of microbial communities in freshwater ecosystems. Research by Lee *et al.* (2018) examined the influence of ARGs on bacterial diversity in river systems, demonstrating a correlation between ARGs and changes in bacterial community makeup, as well as the proliferation of certain taxa. This investigation emphasized the capacity of ARGs to shape microbial community structure and diversity in freshwater environments.

Selection of Resistant Microorganisms

In freshwater environment, ARGs contribute to the emergence and proliferation of resistant microorganisms. A study conducted by Wang *et al.* (2020) investigated how ARGs affect the quantity and variety of antibiotic-resistant bacteria in lake ecosystems. Their findings revealed a positive relationship between ARG prevalence and the occurrence of resistant bacteria, suggesting that the existence of ARGs encourages the development of resistant microorganisms. These results underscore the significance of ARGs in the establishment and continuance of antibiotic resistance within microbial populations.

Disruption of Microbial Processes

ARGs interfere with vital microbial processes in freshwater ecosystems. Niu *et al.* (2019) examined the impact of ARGs on nitrogen-cycling bacteria in river systems and discovered that ARGs diminished the abundance and activity of nitrogen-fixing bacteria, thereby influencing nitrogen cycling. This research highlights the potential ramifications of ARGs on crucial ecosystem functions facilitated by microbial communities. The occurrence of ARGs in microbial populations substantially affects the functioning of freshwater ecosystems. Alterations in community composition and the selection of resistant microorganisms can impact nutrient cycling, energy flow, and overall ecosystem stability. Disturbances in microbial processes, such as nitrogen cycling, can have far-reaching effects on ecosystem dynamics and productivity. Moreover, changes in microbial community diversity and composition may influence ecosystem resilience and vulnerability to disturbances.

Influence on the Ecosystem Processes

The presence of antibiotic resistance genes (ARGs) in freshwater ecosystems has significant ecological consequences, particularly in influencing ecosystem processes. Research by Dang *et al.* (2021) revealed that ARGs reduced the quantity and variety of nitrogen-fixing bacteria in lakes, thereby modifying nitrogen cycling and impacting ecosystem functionality. In a similar vein, Zhang *et al.* (2021) showed that ARGs influence microbial metabolic profiles in rivers, changing metabolic pathways and affecting processes like the breakdown of organic matter and energy transfer. Ohore *et al.* (2022) emphasized that ARGs diminish predation efficiency in aquatic organisms, disturbing species interactions and potentially destabilizing ecosystems. Furthermore, Zhang *et al.* (2021) demonstrated that ARGs modify trophic interactions and decrease microbial biomass in river food webs, potentially affecting higher trophic levels. These disturbances in nutrient cycling, microbial metabolism, and trophic interactions have substantial implications for freshwater ecosystem functioning, influencing primary productivity, resource availability, and food web stability.

Impact on Biodiversity

ARGs in freshwater environments have ecological ramifications, especially concerning biodiversity. These genes can modify the diversity and composition of microbial communities. Liu *et al.* (2021) discovered that ARGs are associated with shifts in bacterial community composition, leading to reduced microbial diversity in lakes. This study underscored the potential of ARGs to shape microbial taxa, affecting the overall biodiversity of freshwater ecosystems. ARGs can also favor resistant organisms, influencing species diversity. Wang *et al.* (2021) noted a correlation between ARG presence and increased antibiotic-resistant invertebrates, suggesting ARGs impact higher trophic levels. Moreover, ARGs can disrupt keystone species, which are vital for ecosystem balance. Liu *et al.* (2022) reported that ARGs decrease the abundance of key macroinvertebrate species, altering community composition and potentially causing ecological consequences. This disruption can affect ecosystem processes, such as nutrient cycling and energy flow, with cascading effects on higher trophic levels, potentially reducing overall biodiversity. Disturbances in keystone species can destabilize food webs and impact ecosystem resilience to environmental disturbances.

Increased Resistance

The presence of ARGs in freshwater environments has ecological implications, particularly in contributing to increased resistance. Comprehending the mechanisms underlying increased antibiotic resistance is essential for evaluating the ecological relevance of antibiotic resistance in freshwater ecosystems. ARGs play a crucial role in promoting the spread of resistance determinants in freshwater environments. Han *et al.* (2022) investigated ARG dissemination in river systems and found a correlation between ARG presence and an increased prevalence of antibiotic-resistant bacteria. The study highlighted ARGs as vectors for horizontal gene transfer, facilitating resistance dissemination among microbial populations.

ARGs can promote co-selection, resulting in multiple resistance in microbial populations. Chen *et al.* (2019) identified a positive correlation between ARGs and heavy metal resistance genes in freshwater sediments, indicating co-selection processes. This suggests the potential of ARGs to contribute to multidrug-resistant strains in freshwater ecosystems and to the persistence of resistant phenotypes in freshwater environments. Yu *et al.* (2022) discovered that ARGs are stable over time in lakes and correlated with persistent antibiotic resistance in bacterial populations, emphasizing their role in maintaining resistant phenotypes and long-term resistance prevalence.

Increased resistance mediated by ARGs significantly affects human and environmental health in freshwater ecosystems. The spread of resistance determinants can reduce antibiotic treatment efficacy in humans and animals, leading to higher treatment failure rates and emergence of multidrug-resistant pathogens. The co-selection of multiple resistances amplifies the environmental risks from pollutants and contaminants, compromising ecosystem integrity. The persistence of resistant phenotypes creates a long-term reservoir for the transfer of resistance to other microbial populations. Thus, ARGs in freshwater environments contribute to increased resistance through the spread of resistance determinants, the co-selection of multiple resistances, and the persistence of resistant phenotypes.

Contamination of the Food Chain

Antibiotic resistance genes (ARGs) in freshwater ecosystems pose significant ecological risks, particularly in their ability to enter the food chain. These genes can be transmitted to bacteria within the food supply, leading to antibiotic-resistant microorganisms in consumables. Research by Amato *et al.* (2021) explored the movement of ARGs from contaminated irrigation water to vegetables, revealing the presence of these genes in the produce and emphasizing the contamination risk. This finding underlines the danger of ingesting foods containing antibiotic-resistant bacteria, which may propagate resistance to human pathogens.

Freshwater ARGs can also promote horizontal gene transfer to the gut microbiota of organisms within the food chain. A study by Johnson *et al.* (2020) investigated the transmission of ARGs from environmental sources to fish gut microbiomes, identifying these genes in fish gut samples and suggesting potential resistance gene transfer through the food supply. These results emphasize the role of ARGs in influencing antibiotic resistance profiles of gut microbiota and their potential spread within the food chain.

The application of antibiotics in livestock farming exacerbates ARG proliferation and contaminates the food supply. EFSA (2021) observed higher ARG concentrations in freshwater environments downstream from livestock farms, demonstrating contamination potential and subsequent food chain transmission. This research highlights the necessity for sustainable farming practices to reduce ARG contamination in food chains.

The infiltration of ARGs into the food supply threatens both human health and ecosystem balance. Consuming contaminated food can transfer resistance to human pathogens, compromising the effectiveness of antibiotic treatments. ARGs in the food chain can also spread to other organisms, disrupt ecological relationships, and jeopardize ecosystem stability. This interconnection between environmental and human health calls for comprehensive approaches to curb antibiotic resistance spread in freshwater ecosystems, where ARGs worsen the occurrence of antibiotic-resistant bacteria in food products.

Clinical Relevance of Antibiotic Resistance Genes (ARGs) In Fresh Water Ecosystems

The proliferation of antibiotic resistance genes (ARGs) in freshwater reservoirs poses a significant threat to both environmental stability and public health, garnering considerable scientific interest. Man-made reservoirs, created through damming to address water needs, can serve as conduits for ARGs to move from the environment to human populations.

A study by Zhao-Feng *et al.* (2023) employed a data-driven approach to examine the distribution, influencing factors, and pollution hotspots of ARGs in freshwater reservoirs. Their findings revealed notable geographical variations in ARG types and quantities, indicating that reservoir characteristics play a crucial role in ARG contamination. The research identified specific pollution hotspots for ARGs in both the water and sediment of reservoirs.

Leff *et al.* (2023) explored the occurrence of ARGs in streams and rivers affected by human activities. Their research demonstrated that these water bodies are extensively impacted by various factors, including hydrological alterations and pollution. The presence of pharmaceuticals and personal care products, antibiotics among them, contributes to the prevalence of ARGs in these aquatic ecosystems.

In a review by Zhang *et al.* (2020), the potential health risks associated with antibiotic-resistant bacteria (ARB) and ARGs in aquatic environments were examined. The study highlighted that numerous uncertainties persist regarding the health implications of ARB and ARGs in these water systems.

Increased Risk of Infections

The presence of antibiotic resistance genes (ARGs) in freshwater ecosystems has significant clinical ramifications, especially in terms of heightening infection risks. These genes can enable the spread of pathogens that are resistant to human antibiotics. Research by Zhang *et al.* (2020) demonstrated that water containing ARGs is more prone to harboring antibiotic-resistant bacteria, suggesting a potential for resistant pathogens to infect those who consume such water. Additionally, Chen *et al.* (2021) discovered a positive link between ARG levels in aquatic environments and antibiotic-resistant wound infections, indicating that exposure to ARGs in freshwater may result in infections that are difficult to treat. A study by Li *et al.* (2022) uncovered high concentrations of ARGs in hospital wastewater, pointing to the potential for antibiotic resistance to spread to healthcare-associated pathogens. This finding emphasizes the role of freshwater environments as ARG reservoirs, contributing to challenging nosocomial infections. The existence of ARGs in freshwater systems substantially affects public health by reducing the efficacy of antibiotic treatments, resulting in more severe and longer-lasting infections. This situation restricts treatment options for various infections and creates challenges for healthcare systems, increasing the strain on providers and the costs associated with patient care.In conclusion, the presence of ARGs in freshwater environments elevates the risk of infections, including the transmission of resistant pathogens, the development of antibiotic-resistant wounds, and the occurrence of nosocomial infections.

Spreading of Resistance

The presence of antibiotic resistance genes (ARGs) in freshwater ecosystems presents substantial clinical risks due to their role in resistance propagation. Research by Shao *et al.* (2018) examined ARG transmission among aquatic microorganisms, revealing that mobile genetic elements facilitated ARG transfer between pathogenic and non-pathogenic bacteria. This finding emphasizes the capacity of freshwater ARGs to amplify antibiotic resistance across bacterial communities.

Contribution to Multi-Drug Resistance

Freshwater ARGs can foster multi-drug resistance in bacteria. Zhang *et al.* (2020) investigated the influence of ARGs on bacterial resistance in river ecosystems, uncovering a positive relationship between ARG prevalence and multi-drug resistance in bacterial samples. These findings indicate that freshwater ARGs may contribute to broad-spectrum resistance, thereby reducing the efficacy of current treatments for various infections.

Potential for Resistance Gene Transfer to Human Pathogens

ARGs in freshwater environments have the potential to be transferred to human pathogens, thus intensifying resistance spread. Wang *et al.* (2021) explored ARG transferability from environmental microorganisms to clinically significant pathogens, identifying genetic components that enable this transfer. This research highlights the risk of resistance gene transmission from freshwater to human pathogens, raising important clinical concerns.

The spread of ARGs in freshwater ecosystems significantly impacts antibiotic treatment and public health by diminishing antibiotic effectiveness and restricting infection treatment options. The emergence of multi-drug resistance hinders the success of combination therapy and complicates infection management. The possible transfer of resistance genes to human pathogens enhances the resistance capabilities of clinically relevant bacteria, resulting in increased morbidity and mortality rates, higher healthcare costs, and extended hospital stays.

This trend is concerning to healthcare professionals, as treating infections caused by resistant pathogens has become more challenging, often leading to longer hospitalizations and increased medical expenses (Centers for Disease Control and Prevention 2018; European Center for Disease Prevention and Control 2019). These challenges particularly affect immunocompromised individuals and those undergoing invasive procedures, such as chemotherapy, dialysis, joint replacement, and surgery (Centers for Disease Control and Prevention, 2018).

The Burden of Antibiotic Resistance Genes

The global public health is significantly threatened by antimicrobial resistance (AMR), with aquatic ecosystems playing a vital role in its dissemination. Research by Amarasiri *et al.* (2022) revealed that about 28% of the world's antibiotic resistance genes (ARGs) are found in marine environments, underscoring the importance of water bodies in AMR proliferation. To facilitate the creation of internationally comparable databases, Liguori *et al.* (2022) proposed a framework in Environmental Science and Technology for standardized methods and quality control in monitoring AMR in aquatic settings.

In 2019, drug-resistant infections were associated with roughly 4.95 million deaths worldwide, with 1.27 million directly attributed to AMR, according to the Antimicrobial Resistance Collaborators (2022) (Murray *et al.,* 2022; Collaborators, 2022). Western sub-Saharan Africa experienced the highest all-age death rate due to resistance at 27.3 deaths per 100,000, while Australasia had the lowest at 6.5 deaths per 100,000 (Collaborators, 2022). The study employed predictive statistical modeling to estimate AMR burden, even in areas with limited data. Their methodology involved estimating infection-related deaths, identifying syndrome-related deaths caused by specific pathogens, determining the percentage of resistant pathogens, and evaluating the excess mortality risk or extended infection duration due to resistance (Collaborators, 2022).

A review of molecular mechanisms of antibiotic resistance, including enzyme-mediated antibiotic inactivation, was conducted in a BioMed Research International study (2023). If current trends persist, AMR-related deaths could reach 10 million annually, with yearly economic costs of $100 trillion by 2050 (Ahmed *et al.,* 2023).

Strategies for Addressing Antibiotic Resistance Genes in Fresh Water Environments

In the last ten years, antibiotic resistance genes (ARGs) have become an increasing threat to human health. While numerous genes confer resistance, assessing the relative health risks of ARGs remains challenging (Zhang *et al.,* 2022). ARGs, recognized as an emerging environmental health concern, can modify the human microbiome and foster antibiotic-resistant bacteria, contributing to global health issues. Tackling ARGs in freshwater requires managing antimicrobial contaminant discharge and reducing their accumulation. Strategies to combat antimicrobial resistance (AMR) include decreasing antibiotic residues in water systems, enhancing surveillance, and developing effective treatment, removal, or decomposition methods (Nava *et al.,* 2022). Expanding the World Bank's (2021) 'One Health Approach to AMR crises' to encompass reducing environmental antimicrobial contamination could strengthen AMR mitigation efforts.

Monitoring ARGs and antibiotics in wastewater treatment plants (WWTPs) is vital as these facilities create environments conducive to ARG transfer and antibiotic-resistant bacterial (ARB) growth. Molecular biology techniques enable ARG quantification, ARB identification in wastewater, and mapping of keystone species in the AMR network. Understanding ARG-pathogen host relationships is crucial for risk assessment and monitoring (Nguyen *et al.,* 2021).

Nava *et al.* (2022) emphasized the absence of standardized methods for monitoring ARGs and antibiotics in terrestrial and aquatic environments, as well as the lack of protocols for their removal. Nguyen *et al.* (2021) observed that advanced technologies, such as metagenomic sequencing and fluorescence-activated cell sorting, have enhanced ARG/ARB databases, identified key species in antimicrobial resistance networks, and improved AMR dissemination models. Zhao-Feng *et al.* (2023) employed a data-driven framework to illustrate ARG distribution, factors, and pollution hotspots in freshwater reservoirs, revealing significant geospatial variations and identifying distinct pollution hotspots in both reservoir waters and sediments.

Zhang *et al.* (2022) evaluated the global health risks associated with antibiotic resistance genes. This study identified 2,561 ARGs conferring resistance to 24 different antibiotic classes. It quantitatively assessed human health risks, defined as the potential for ARGs to complicate clinical infection treatment, by incorporating factors such as human accessibility, mobility, pathogenicity, and clinical availability. The findings showed that 23.78% of the identified ARGs presented a health risk, particularly those associated with multidrug resistance.

Singh *et al.* (2022) investigated the role of water bodies in spreading antimicrobial resistance (AMR), including antimicrobial-resistant bacteria (ARB) and antimicrobial resistance genes (ARGs), among pathogens, animals, and humans. They discovered that reservoirs collecting rainwater and wastewater for drinking purposes facilitate ARG transfer from the environment to aquatic organisms and humans. This process can lead to antibiotic resistance in humans through the food chain or direct contact, promoting cross-species gene sharing.

Gillings *et al.* (2021) examined the dynamics of antibiotic resistance in environmental contexts and highlighted its global health significance. They noted that antibiotic resistance involves the transfer of bacteria and resistance genes between humans, animals, and environmental sources. Despite barriers limiting this exchange, pathogens continually acquire new resistance factors from other species.

Future perspectives

Recent insights into antibiotic resistance genes (ARGs) in freshwater ecosystems underscore the urgent need for comprehensive approaches to address this escalating public health issue. This overview explores their origins, endurance, environmental consequences, and implications for human well-being. ARGs originate from natural environments, agricultural activities, and human actions, particularly through wastewater release. Antibiotics utilized in human and veterinary medicine enter freshwater bodies via runoff and treated effluents. Agricultural methods, including manure application and irrigation with polluted water, introduce ARGs into aquatic settings. These contaminants persist in freshwater ecosystems through various mechanisms. Resistance transfer among different bacterial species occurs via horizontal gene transfer, while mobile genetic elements, such as plasmids and integrons, enable ARG dissemination within microbial communities. ARGs in freshwater ecosystems have substantial ecological impacts, influencing microbial populations and their functions, which in turn affect nutrient cycling and organic matter decomposition. These genes also contribute to the development of antibiotic-resistant bacteria, increasing the risk of infection in aquatic organisms. The presence of ARGs in freshwater environments poses human health risks through multiple pathways, including water-based recreational activities, consumption of contaminated food or water, and direct contact with polluted water. Such exposure may lead to ARG acquisition, potentially resulting in antibiotic resistance in human pathogens and complicating medical treatments.

CONCLUSION

Addressing the issue of antibiotic resistance genes (ARGs) in freshwater systems demands a multifaceted strategy. This includes improving sewage treatment methods to remove antibiotics and ARGs, adopting best practices in agriculture to minimize antibiotic usage and pollution, and investigating novel antimicrobial approaches. The worldwide health threat posed by antibiotic resistance is intricately connected to freshwater ecosystems, which act as significant ARG repositories. To successfully tackle environmental and health issues, it is crucial to gain insights into the sources, longevity, and impacts of ARGs.

To combat antibiotic resistance in freshwater ecosystems, several strategies can be employed. These include enhancing wastewater treatment methods, adopting cutting-edge technologies like membrane filtration and activated carbon adsorption, encouraging prudent antibiotic usage, and establishing suitable regulations. It is crucial to set up comprehensive monitoring systems to detect antibiotic resistance genes (ARGs) in freshwater environments and regularly evaluate ARGs and antibiotic residues in water sources to identify problem areas and monitor changes. Raising public awareness about antimicrobial resistance and its impact on freshwater ecosystems and human health is essential. The general public, healthcare professionals, and agricultural workers should be educated on responsible antibiotic use, proper medication disposal, and the importance of water quality.

Promoting agricultural practices that minimize antibiotic use and reduce ARG release into the environment is vital. This includes supporting environmentally friendly farming methods such as organic farming, crop rotation, and integrated pest management to decrease the agricultural sector's dependence on antibiotics. Developing green infrastructure, such as constructed wetlands, bioretention systems, and permeable surfaces, can effectively manage stormwater runoff and reduce antibiotic and ARG levels in freshwater habitats. Supporting research on ARG dynamics in freshwater ecosystems, including their origins, evolution, and environmental effects, is crucial. Additionally, encouraging the development of innovative techniques for monitoring, detecting, and eliminating ARGs from aquatic systems is necessary.

Employing these approaches together can help reduce the prevalence and spread of antibiotic resistance genes (ARGs) in freshwater environments, thus protecting both ecological and human health.

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