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(REVIEW ARTICLE)

Antibiotic resistance genes (ARGs) in wastewater: Environmental fate and risk mitigation strategies

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Abstract

Antibiotic resistance genes (ARGs) in wastewater systems represent a growing environmental and public health concern, fueled by the widespread and often indiscriminate use of antibiotics in medical, veterinary, and agricultural sectors. This review paper comprehensively examines the environmental fate of ARGs, elucidating their sources, persistence, transport mechanisms, and impacts across aquatic and terrestrial ecosystems. Wastewater treatment plants (WWTPs), though designed to manage conventional pollutants, frequently fail to remove ARGs effectively, thereby functioning as both reservoirs and amplifiers of resistance through microbial interactions and horizontal gene transfer. Effluents and biosolids discharged or applied post-treatment contribute to ARG dissemination in natural environments, where selective pressures from residual antibiotics and environmental conditions promote their persistence and spread. The review further evaluates current and emerging strategies for ARG mitigation, including advanced oxidation processes, membrane filtration, constructed wetlands, and synthetic biology-based interventions. Emphasis is also placed on the integration of artificial intelligence in predictive modeling and biosensing technologies for real-time monitoring. Challenges related to detection methods, regulatory inconsistencies, and infrastructure limitations, especially in low-resource settings, are discussed. Risk assessments reveal serious implications for both human and ecological health, necessitating urgent, multidisciplinary action. Global and local policy initiatives, educational efforts, and coordinated surveillance frameworks are proposed as part of a holistic approach to curbing ARG transmission. By synthesizing recent findings and highlighting future research needs, this review aims to inform and guide effective interventions against the proliferation of antibiotic resistance through wastewater systems.

Keywords: Antibiotic Resistance Genes (ARGs); Wastewater Treatment; Horizontal Gene Transfer; Public Health Risk; Environmental Fate; Advanced Treatment Technologies; Risk Mitigation Strategies

1. Introduction

Antibiotic resistance (AR) is a phenomenon where bacteria evolve to become resistant to the drugs designed to kill them or inhibit their growth. This is often driven by selective pressure caused by the overuse and misuse of antibiotics, not only in human medicine but also in veterinary and agricultural sectors [1-3]. The consequences of antibiotic resistance are far-reaching and significant. When bacteria acquire resistance to antibiotics, the treatment of infections becomes

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increasingly difficult, resulting in prolonged illness, more frequent hospitalizations, and an increased risk of mortality [4,5]. According to a study by the World Health Organization [6], antibiotic resistance is responsible for approximately 5 million deaths annually, and if left unchecked, it could lead to 10 million deaths by 2050. The growing prevalence of resistant pathogens has become a critical public health threat, and global efforts are now urgently focused on containing its spread.

The primary drivers of antibiotic resistance include both natural mechanisms and anthropogenic factors. Resistance can be inherent, arising through mutations in the bacterial genome, or acquired through horizontal gene transfer, which involves the exchange of genetic material between bacteria [7-9]. However, anthropogenic activities such as the indiscriminate use of antibiotics in humans, livestock, and agriculture exacerbate the problem. In many parts of the world, antibiotics are prescribed inappropriately, for example, for viral infections or in subtherapeutic doses, which encourages the survival of resistant strains [10,11]. Moreover, in agriculture, antibiotics are often used to promote growth in healthy animals, further increasing the risk of resistance development. Antibiotic resistance in the environment is a particularly pressing concern. Environmental reservoirs, including wastewater, agricultural runoff, and contaminated water sources, act as hotspots for the selection and spread of resistant bacteria and genes [12,13]. Environmental pathways provide an opportunity for resistant strains to reach human populations, making AR a One Health issue that links human, animal, and environmental health [12,14]. For this reason, understanding the mechanisms that contribute to the spread of antibiotic-resistant bacteria in these environments is crucial for developing strategies to mitigate their impact.

Wastewater has emerged as a critical reservoir for the spread of antibiotic resistance genes (ARGs) due to its role as a collection point for various forms of contamination [15]. These contaminants include effluents from hospitals, pharmaceuticals, households, and industries, all of which can introduce both antibiotics and resistant microorganisms into the wastewater system [7,15,16]. According to Li et al. [17], wastewater treatment plants (WWTPs) are often inadequate at completely removing ARGs from effluent, which means that even treated wastewater can still harbor significant concentrations of resistant bacteria and ARGs. This release of ARGs into the environment through treated or untreated effluent is a key pathway for the spread of resistance to surrounding water bodies and terrestrial ecosystems.

The microbial communities in wastewater treatment systems are highly diverse and include both resistant and non-resistant strains. In many cases, the dense populations of bacteria within these systems provide ideal conditions for horizontal gene transfer, where ARGs can be transferred from one bacterium to another. This process can rapidly spread resistance across a variety of bacterial species, making it difficult to control [18,19]. Wang et al. [20] highlighted that the ability of bacteria to exchange genetic material within WWTPs is a major factor in the persistence of ARGs in these environments. As such, wastewater treatment plants not only serve as conduits for the discharge of ARGs but also contribute to the amplification of resistance through microbial interactions.

The persistence of ARGs in the environment is influenced by various factors, including the types of antibiotics present, the chemical and biological conditions of the wastewater, and the microbial community composition [21,22]. According to Manoharan et al. [23], the presence of high concentrations of specific antibiotics, such as those used in hospitals and agriculture, significantly enhances the selection pressure on microorganisms, favoring the survival of resistant strains. Furthermore, researchers have found that some ARGs are more stable than others, persisting for extended periods in wastewater environments due to their ability to integrate into mobile genetic elements like plasmids, which facilitate their spread to other microorganisms [16,20,22]. As a result, wastewater systems represent not only a conduit for the spread of antibiotic resistance but also a potential source of new and more diverse resistance mechanisms.

This review critically examines the environmental fate of antibiotic resistance genes (ARGs) in wastewater systems and evaluates strategies to mitigate their spread. It synthesizes recent research to understand how ARGs are introduced, persist, and are transported through wastewater, assessing the effectiveness of treatment processes in controlling ARG dissemination. The review highlights the limitations of conventional treatment technologies and emphasizes the need for advanced solutions like membrane filtration, oxidation processes, and enhanced biological treatments. It also explores the environmental and health risks of ARG-contaminated effluents and discusses the importance of integrating technological innovations with regulatory policies. Ultimately, the review aims to provide evidence-based recommendations to curb ARG transmission and support global efforts against antimicrobial resistance.

2. The Environmental Fate of ARGs in Wastewater

2.1. ARGs in Wastewater Treatment Plants

WWTPs serve as critical infrastructure for managing urban effluents; however, they also function as significant reservoirs and conduits for the proliferation of ARGs [16,20]. These facilities receive a complex mix of influents containing antibiotics, resistant bacteria, and mobile genetic elements (MGEs), which contribute to the persistence and dissemination of ARGs [16]. Despite the primary aim of WWTPs to reduce microbial loads, studies indicate that conventional treatment processes often fail to eliminate ARGs effectively. The persistence of ARGs in WWTPs can be attributed to several factors inherent in the treatment processes. Primary treatment, which involves physical processes like sedimentation, primarily removes large particles and does not significantly affect microbial populations or ARGs. Secondary treatment, typically biological processes such as activated sludge, relies on microbial activity to degrade organic matter. However, this stage can inadvertently select for resistant strains, especially when antibiotic residues are present, providing selective pressure that favors the survival of resistant bacteria. Tertiary treatment, which may include advanced filtration or chemical disinfection, offers some improvement but is often insufficient to completely eradicate ARGs [24-26]. Research by Jia et al. [26] highlighted that even after tertiary treatment, effluents can still harbor significant levels of ARGs, posing risks to receiving environments. Understanding the composition of microbial communities is crucial for assessing the dynamics of ARGs in wastewater. Figure 1 illustrates the diversity and relative abundance of bacterial taxa in influent and effluent samples, shedding light on the microbial shifts occurring during treatment.

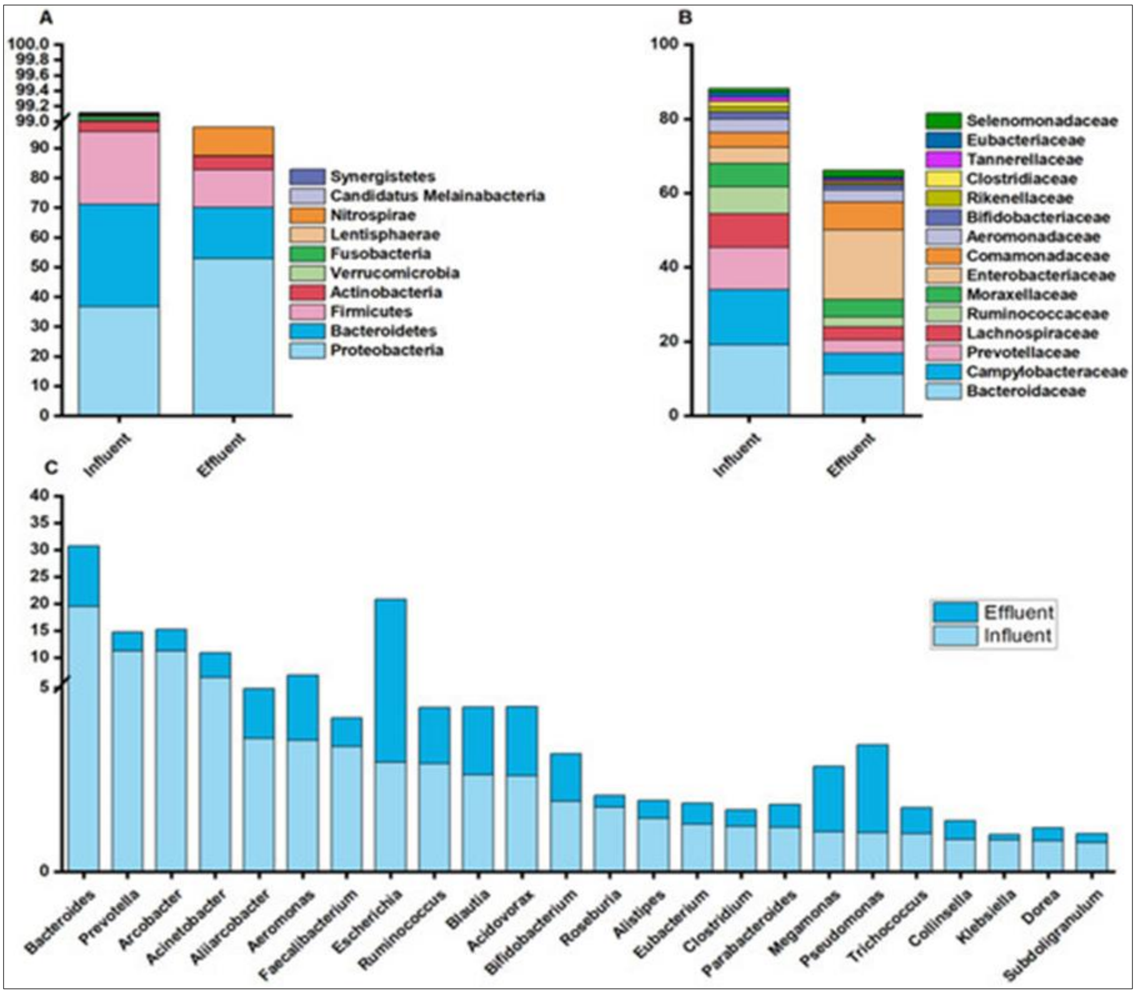

Figure 1 Diversity of Microbial Communities in Wastewater Treatment (reproduced with permission from Ref [25])

Moreover, the sludge produced during wastewater treatment represents another critical vector for ARG dissemination. Studies have shown that sludge can harbor high concentrations of ARGs, which may persist due to the complex microbial communities present and the potential for horizontal gene transfer. The application of treated sludge to land as biosolid fertilizers can facilitate the transfer of ARGs to soil ecosystems, further complicating efforts to control their spread [27,28]. According to a study by Qin et al. [29], the application of biosolids containing ARGs to agricultural lands can lead to the introduction and potential spread of these genes in terrestrial environments.

2.2. Factors Influencing ARG Persistence and Spread

The persistence and spread of ARGs in wastewater systems are influenced by a complex interplay of chemical, physical, and biological factors. Table 1 provides a concise overview of these factors, highlighting their roles in shaping ARG dynamics within wastewater environments. Chemical factors such as temperature, pH, and redox conditions can affect the stability of ARGs and the microbial communities harboring them. For instance, extreme pH levels can denature proteins and disrupt microbial cell membranes, potentially reducing the viability of resistant bacteria. Conversely, conditions that favor microbial growth, such as neutral pH and optimal temperature ranges, can enhance the survival and proliferation of resistant strains. Redox conditions also play a crucial role; environments with low oxygen levels can select for anaerobic bacteria, which may possess distinct resistance mechanisms compared to aerobic counterparts [22,30,31].

Table 1 Factors Influencing ARG Persistence in Wastewater and Receiving Environments

Factor Type	Specific Factor	Impact on ARG Persistence	Notes
Chemical	Antibiotic concentration	Increases selective pressure	Promotes survival of resistant bacteria
Physical	Temperature, pH	Affects microbial viability and ARG stability	Neutral pH and moderate temperatures favor persistence
Biological	Microbial community structure	Enables horizontal gene transfer	Involves plasmids, transposons, integrons
Operational	WWTP treatment stage	Selects or reduces ARGs	Secondary treatment often a hotspot

Biological factors, particularly microbial community interactions, are central to the dynamics of ARG persistence and spread. Microbial communities in wastewater systems are diverse and complex, providing ample opportunities for horizontal gene transfer—the process by which ARGs are exchanged between bacteria [32]. This transfer can occur through various mechanisms, including conjugation, transformation, and transduction. The presence of MGEs, such as plasmids, transposons, and integrons, facilitates the mobility of ARGs within and between bacterial populations. A study by Zhang et al. [33] emphasized the role of MGEs in the horizontal transfer of ARGs, noting that their presence in wastewater environments significantly contributes to the spread of resistance.

Additionally, anthropogenic factors, such as the use of antibiotics in healthcare and agriculture, introduce selective pressures that favor the survival of resistant bacteria in wastewater systems. The continuous influx of antibiotics into wastewater effluents maintains these selective pressures, enabling resistant strains to thrive. The concentration of antibiotics in influents correlates with the abundance of ARGs in treated effluents, highlighting the impact of antibiotic usage patterns on resistance dynamics [34,35].

2.3. Environmental Fate After Discharge

Once discharged, treated or untreated wastewater effluents containing ARGs enter receiving environments such as rivers, lakes, and oceans, where they can undergo further dissemination and impact. The transport and distribution of ARGs in these environments are influenced by hydrological factors, including water flow rates, sedimentation, and mixing patterns. Studies have shown that effluents can introduce ARGs into aquatic ecosystems, leading to increased abundance and diversity of resistant bacteria in sediments and water columns. The persistence of ARGs in these environments can vary; some studies report that ARGs can remain detectable for extended periods, while others suggest that they may degrade over time depending on environmental conditions [30-32,36].

Bioaccumulation of ARGs in aquatic organisms is a significant concern, as it can lead to the transfer of resistance through the food chain. Aquatic organisms, such as fish and invertebrates, can accumulate resistant bacteria and ARGs from contaminated water, which may then be passed on to predators, including humans. Fish exposed to ARG-contaminated water exhibited higher levels of resistant bacteria in their gut microbiota, suggesting bioaccumulation of resistance determinants [16-19,37].

Furthermore, the spread of ARGs in aquatic environments can lead to the establishment of new reservoirs of resistance in microbial communities. This can complicate efforts to manage and mitigate antibiotic resistance, as resistant strains may colonize new niches and exchange genes with indigenous microbial populations. The role of aquatic ecosystems as reservoirs for ARGs, noting that the impact of these reservoirs is exacerbated by the slow turnover of microbial communities in certain aquatic environments. As a result, once ARGs are introduced, they can persist and spread widely, making it difficult to eradicate them from the ecosystem [30,37]. Martínez et al. [38] also observed that rivers and lakes receiving treated effluents were particularly vulnerable to long-term ARG contamination due to limited dilution and slower microbial turnover rates. This makes these environments critical hotspots for the continued proliferation of resistance genes.

2.4. Emerging Insights

Recent studies on the evolution and stability of ARGs post-treatment have provided new insights into how these genes persist in the environment. While conventional wastewater treatment processes may reduce the concentrations of specific antibiotics, they do not necessarily eliminate all forms of resistance [39,40]. Specific ARGs, especially those linked to high-use antibiotics such as tetracyclines and beta-lactams, tend to be more stable in the environment. This stability is partly due to the genetic elements that harbor these ARGs, such as plasmids, which facilitate their transfer to other bacteria even under non-selective conditions. ARGs can survive post-treatment in treated effluent and sludge for extended periods, which is crucial for understanding their potential to spread in aquatic and terrestrial ecosystems [16,41]. Urban wastewater systems have been found to harbor more diverse and abundant ARGs compared to rural areas. Urban wastewater systems, with their higher load of antibiotics and industrial contaminants, create a more favorable environment for the persistence and transfer of ARGs. Wastewater systems in rural areas, with less intense anthropogenic influence, exhibit lower levels of ARG persistence.

Additionally, research into the hotspots of ARGs in wastewater treatment systems has identified critical points of amplification, where resistance genes can proliferate under specific environmental conditions [42]. Researchers such as Zhang et al. [43] have shown that certain stages of wastewater treatment, particularly secondary treatment, are key sites for the enrichment of ARGs due to the large microbial populations present and the selective pressures exerted by antibiotics in influent water. These hotspots may serve as key areas for the development of targeted strategies to limit the spread of resistance genes in the future. The study also indicated that operational factors like the residence time of wastewater and the type of microbial inoculum used in biological treatments could play significant roles in ARG dynamics within WWTPs [42,43].

3. Methods for Monitoring ARGs in Wastewater

3.1. Sampling and Analytical Techniques

The detection and quantification of ARGs in wastewater are pivotal for understanding the dissemination of antimicrobial resistance (AMR) in the environment [44-46]. Traditional molecular techniques, such as polymerase chain reaction (PCR) and quantitative PCR (qPCR), have been extensively utilized due to their specificity and sensitivity in detecting known ARGs [47-49]. PCR facilitates the amplification of specific DNA sequences, enabling the identification of target genes, while qPCR provides quantitative data on gene abundance, offering insights into the prevalence of ARGs in wastewater samples. These methods have been instrumental in monitoring the dissemination of ARGs in various WWTPs, aiding in the assessment of treatment efficacy and environmental impact [45,49]. To better understand the practical applications and limitations of different ARG detection techniques, Table 2 summarizes the key characteristics of commonly used methods in wastewater surveillance, including their detection targets, strengths, and drawbacks.

Microarray analysis represents another traditional approach, allowing for the simultaneous detection of a broad spectrum of ARGs. This technique involves hybridizing labeled DNA samples to a microarray chip containing probes for various ARGs, facilitating high-throughput screening. While microarray analysis offers comprehensive profiling capabilities, its application in wastewater monitoring is limited by factors such as the complexity of wastewater matrices and the need for specialized equipment and expertise [49,50].

Advancements in sequencing technologies have introduced next-generation sequencing (NGS) and metagenomics as powerful tools for monitoring ARGs in wastewater. NGS allows for the sequencing of entire microbial communities, providing comprehensive data on the resistome present in wastewater samples. Metagenomic approaches enable the identification of both known and novel ARGs without the need for prior knowledge of target sequences, offering a more holistic view of the resistome. However, the complexity of data analysis and the need for bioinformatics expertise pose challenges to the routine application of these methods in monitoring programs [51,52].

Table 2 Summary of ARGs Detected in Wastewater Environments by Detection Method

Detection Method	ARGs Commonly Detected	Strengths	Limitations	Reference Examples
PCR / qPCR	blaTEM, sul1, tetA	High sensitivity & specificity	Limited to known genes	[47-49]
Microarray	Multiple ARGs (e.g., ermB, aadA)	Broad screening capability	High cost, complex data interpretation	[49-50]
Next-Gene Sequencing	Full resistome	Detects known & novel ARGs	Expensive, requires bioinformatics	[51-52]
Biosensors	Specific ARGs (custom)	Real-time monitoring, rapid response	Currently limited in gene range	[53-55]

3.2. Innovative Monitoring Approaches

The integration of biosensors into wastewater monitoring represents a significant advancement in real-time detection of ARGs. Biosensors, which utilize biological elements to detect specific substances, offer rapid, sensitive, and cost-effective means of monitoring environmental contaminants. These sensors can be designed to detect specific ARGs or the presence of antibiotic-resistant bacteria, providing real-time data that can inform immediate response actions. The development of biosensors for wastewater monitoring has been facilitated by advancements in nanotechnology, which enhance the sensitivity and selectivity of these devices [53-55].

Artificial intelligence (AI) and machine learning (ML) are increasingly being applied to predict the prevalence and spread of ARGs in wastewater systems. These technologies analyze large datasets to identify patterns and make predictions about future trends. AI and ML algorithms can model the dynamics of ARGs in wastewater treatment plants, optimizing treatment processes and predicting the emergence of resistant strains. For example, AI models have been used to predict bacterial contamination levels in real-time, providing valuable information for public health management [56-58].

The combination of biosensors and AI technologies holds promise for enhancing the monitoring of ARGs in wastewater. Integrating real-time biosensor data with AI algorithms can enable dynamic risk assessment and management, allowing for timely interventions to mitigate the spread of resistance. Such integrated systems can improve the efficiency and effectiveness of wastewater surveillance programs, contributing to better management of antimicrobial resistance [54,55,58].

3.3. Challenges and Gaps

Despite the advancements in monitoring techniques, several challenges persist in the detection and quantification of ARGs in wastewater. One significant issue is the sensitivity of current methods. Traditional PCR-based techniques may not detect low-abundance ARGs, leading to underestimation of their prevalence. While qPCR offers improved sensitivity, it still has limitations in detecting low-abundance genes, particularly in complex environmental samples like wastewater [47,48]. The cost of advanced analytical methods, such as NGS and microarray analysis, poses another barrier to widespread implementation. These techniques require specialized equipment and expertise, making them less accessible to many laboratories, especially in low-resource settings. The high cost and technical complexity associated with these methods can limit their routine use in monitoring programs, hindering efforts to track ARGs effectively [59].

Sampling protocols also present challenges in monitoring ARGs in wastewater. Variability in sampling locations, times, and methods can lead to inconsistent data, complicating the interpretation of results. Establishing standardized sampling protocols is crucial to ensure reliable and comparable data across different studies and monitoring programs. Standardization of sampling methods and analytical techniques is essential for developing effective surveillance systems and informing public health interventions [6,59,60].

4. Risk Assessment of ARGs in Wastewater

4.1. Public Health Risk

The dissemination of ARGs through wastewater systems poses a significant threat to public health, particularly in low-income and underdeveloped regions. In these areas, inadequate sanitation infrastructure and limited access to clean water exacerbate the risk of exposure to ARGs. Human exposure routes include direct contact with contaminated water during activities such as bathing, washing, or recreational use, as well as indirect exposure through the consumption of crops irrigated with untreated or inadequately treated wastewater. Studies have shown that WWTPs often fail to completely remove ARGs, leading to their persistence in effluents discharged into the environment . This continuous release contributes to the proliferation of antibiotic-resistant bacteria in natural water bodies, increasing the likelihood of human exposure [61,62].

Vulnerable populations, such as immunocompromised individuals, are at heightened risk of infections caused by antibiotic-resistant bacteria. The presence of ARGs in wastewater can lead to the colonization of resistant bacteria in these individuals, resulting in infections that are difficult to treat and manage. Moreover, the spread of ARGs in communities with limited healthcare resources can lead to outbreaks of resistant infections, placing additional strain on already overburdened healthcare systems. The lack of effective antibiotics to treat these infections further complicates treatment options and can lead to increased morbidity and mortality rates [61,63,64]. The global nature of antibiotic resistance necessitates a comprehensive approach to mitigate its spread through wastewater. International collaboration is essential to develop and implement standardized guidelines for wastewater treatment and management, particularly in regions lacking adequate infrastructure. Public health initiatives should focus on improving sanitation, promoting responsible antibiotic use, and enhancing surveillance systems to monitor the prevalence of ARGs in wastewater. By addressing these factors, it is possible to reduce the public health risks associated with ARGs and prevent the further spread of antibiotic resistance [60,64].

4.2. Ecological Risks

The presence of ARGs in wastewater not only threatens human health but also poses significant ecological risks. Aquatic ecosystems receiving effluents containing ARGs can experience disruptions in microbial communities, leading to alterations in nutrient cycling and energy flow. The introduction of antibiotic-resistant bacteria into these environments can outcompete native microbial populations, resulting in reduced biodiversity and compromised ecosystem function . Furthermore, the horizontal transfer of ARGs among microbial communities can facilitate the spread of resistance throughout the ecosystem, affecting a wide range of organisms. [62,64]

Wildlife and aquatic organisms are also susceptible to the impacts of ARGs in wastewater. Fish and other aquatic species can accumulate antibiotic-resistant bacteria through direct contact with contaminated water or through the food chain. This accumulation can lead to infections that are difficult to treat, affecting the health and survival of these organisms. Additionally, the presence of ARGs in aquatic environments can have cascading effects on predator-prey relationships and overall ecosystem stability. The ecological consequences of ARG dissemination underscore the need for effective wastewater management practices to protect aquatic biodiversity [63,65].

Addressing the ecological risks associated with ARGs requires a multifaceted approach that includes monitoring, regulation, and public awareness. Regular monitoring of ARGs in wastewater and receiving water bodies can help identify hotspots of resistance and inform targeted interventions. Implementing stricter regulations on the discharge of wastewater and promoting the use of advanced treatment technologies can reduce the release of ARGs into the environment. Public education campaigns can also raise awareness about the ecological impacts of antibiotic resistance and encourage responsible antibiotic use and disposal [61-64].

4.3. Risk Characterization Models

To effectively assess and manage the risks associated with ARGs in wastewater, various risk characterization models have been developed. These models aim to quantify the potential human and ecological health risks posed by the presence of ARGs in the environment. Quantitative microbial risk assessment (QMRA) is one such model that estimates the probability of infection resulting from exposure to antibiotic-resistant bacteria in water sources. By incorporating data on pathogen concentrations, exposure scenarios, and dose-response relationships, QMRA provides a framework for evaluating the risks associated with ARGs in wastewater [66,67].

In addition to QMRA, other tools and frameworks have been employed to assess the risks of ARGs in wastewater systems. These include ecological risk assessments that evaluate the potential impacts of ARGs on aquatic ecosystems, as well as integrated models that consider both human and environmental health outcomes. For instance, the development of risk assessment frameworks grounded in scientific evidence and vetted by experts has been instrumental in informing water utilities and policymakers about the health and environmental risks of antibiotic resistance. These models facilitate the identification of critical control points and the implementation of effective mitigation strategies [66].

5. Mitigation Strategies for ARGs in Wastewater

5.1. Preventive Measures

Reducing the prevalence of ARGs in wastewater begins with addressing the root causes of antibiotic overuse in both agriculture and healthcare. In the agricultural sector, antibiotics have traditionally been employed not only for therapeutic purposes but also as growth promoters. This practice has led to the accumulation of antibiotic residues in animal waste, which subsequently enters wastewater systems. A study by Kumar et al. [68] highlighted that the non-therapeutic use of antibiotics in livestock contributes significantly to the proliferation of resistant bacteria, which can be transmitted to humans through various pathways, including water sources. Implementing stringent regulations to limit antibiotic use in agriculture is therefore crucial.

In the healthcare sector, the overprescription and misuse of antibiotics have been identified as major contributors to the emergence of resistant strains. According to Saleem et al., [69], a significant proportion of antibiotic prescriptions are unnecessary, often given for viral infections where they have no efficacy. This misuse not only affects patient health but also leads to increased levels of antibiotics in wastewater through human excretion. Educational programs aimed at both healthcare providers and patients about the judicious use of antibiotics can play a pivotal role in mitigating this issue [69,70]. Industrial effluents, particularly from pharmaceutical facilities, are significant sources of ARGs. Figure 2 illustrates the prevalence of resistance genes in wastewater samples from Nigerian pharmaceutical plants, emphasizing the need for stringent waste management.

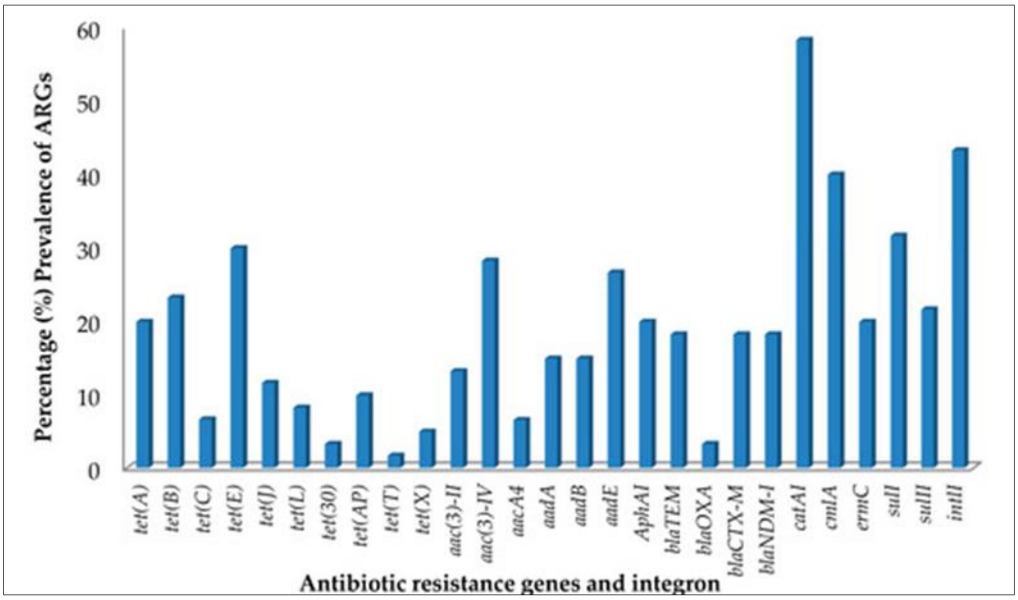

Figure 2 ARGs in Pharmaceutical Wastewaters in Nigeria (reproduced with permission from Ref [70])

On a global scale, the establishment of regulatory frameworks and policies is essential to standardize antibiotic usage and control the spread of ARGs. The World Health Organization (WHO) has developed a Global Action Plan on Antimicrobial Resistance, which emphasizes the need for coordinated efforts across sectors to reduce antibiotic misuse [71]. Countries adopting and enforcing such policies have reported a decline in antibiotic consumption and a corresponding decrease in resistance levels. For instance, the Netherlands implemented a national policy to reduce antibiotic use in livestock, resulting in a 56% reduction over five years [72].

5.2. Wastewater Treatment Modifications

Traditional wastewater treatment plants are not specifically designed to eliminate ARGs, necessitating modifications to existing processes. Advanced oxidation processes (AOPs), such as ozonation, ultraviolet (UV) irradiation, and Fenton reactions, have shown promise in degrading antibiotic compounds and ARGs [73]. A study by Foroughi et al. [74] demonstrated that ozonation could effectively reduce the concentration of antibiotics and ARGs in wastewater effluents. Similarly, UV irradiation has been found to disrupt the DNA of resistant bacteria, thereby inhibiting their replication [75]. Membrane filtration technologies, including ultrafiltration and nanofiltration, offer another approach to removing ARGs from wastewater. These systems can physically separate bacteria and genetic materials from the water. Membrane bioreactors (MBRs) could achieve higher removal efficiencies for ARGs compared to conventional activated sludge systems. However, challenges such as membrane fouling and high operational costs need to be addressed for widespread implementation [76,77].

Numerous advanced treatment strategies have been developed to improve the removal of ARGs from wastewater systems. Table 3 compares these technologies, detailing their mechanisms, relative effectiveness, and practical considerations for implementation.

Table 3 Comparison of Advanced Wastewater Treatment Strategies for ARG Removal

Treatment Technology	**Mechanism**	**ARG Removal Efficiency**	**Pros**	**Cons**	**References**
Ozonation / UV	Oxidative degradation of DNA	High	Effective against broad ARGs	High energy cost	[74-75]
Membrane Filtration (MBR)	Physical separation of microbes	Moderate–High	Removes pathogens & ARGs	Fouling issues, expensive	[76-77]
Constructed Wetlands	Bio-physico-chemical remediation	Variable	Low-cost, eco-friendly	Seasonal variability	[79-80]
Synthetic Biology	Engineered microbial ARG degraders	Emerging	Highly specific, customizable	Regulatory & ecological concerns	[60,81-82]

Bioaugmentation and bioremediation techniques involve introducing specific microorganisms or consortia into WWTPs to enhance the degradation of pollutants, including ARGs. For example, the addition of bacteria capable of degrading antibiotics can reduce the selective pressure that fosters resistance. Bioaugmentation with specific strains improved the removal of sulfamethoxazole and its associated resistance genes. These biological approaches offer sustainable solutions but require careful selection of microbial strains and monitoring to prevent unintended ecological impacts [68,78].

5.3. Novel and Green Technologies

Constructed wetlands (CWs) represent an eco-friendly alternative for wastewater treatment, utilizing natural processes involving wetland vegetation, soils, and associated microbial assemblages to treat contaminants. CWs could effectively reduce concentrations of antibiotics and ARGs in wastewater. The combination of physical filtration, microbial degradation, and plant uptake contributes to the removal of these contaminants. However, the efficiency of CWs can be influenced by factors such as plant species, hydraulic retention time, and seasonal variations [79,80].

Phytoremediation, the use of plants to absorb, accumulate, and detoxify pollutants, has been explored for ARG removal. Certain plant species have shown the ability to uptake antibiotics and associated resistance genes from contaminated water. The potential of using aquatic plants like duckweed in reducing ARG levels in wastewater. While promising, the scalability and long-term sustainability of phytoremediation require further investigation [81,82].

Advancements in synthetic biology have opened avenues for engineering microbes specifically designed to target and degrade ARGs. These engineered organisms can be programmed to express enzymes that break down antibiotic compounds or to outcompete resistant bacteria. For instance, the use of genetically modified bacteria to reduce ARGs in wastewater has been demonstrated. While this approach holds significant potential, concerns regarding the release of genetically modified organisms into the environment necessitate stringent containment and regulatory measures [60].

5.4. Sludge Management

The management of sludge, a byproduct of wastewater treatment processes, is a critical component in mitigating the dissemination of ARGs into the environment. One prevalent method of sludge management is the land application of biosolids, which involves using treated sewage sludge as fertilizer in agricultural settings. While this practice recycles nutrients and organic matter back into the soil, it also poses risks associated with the introduction of ARGs and mobile genetic elements (MGEs) into terrestrial ecosystems. Research has indicated that biosolids can serve as a significant source of ARGs in agricultural soils, potentially facilitating the horizontal gene transfer of resistance traits among soil microbiota [83,84]. To mitigate these risks, various sludge treatment processes have been explored to reduce the ARG load before land application. Composting, an aerobic process that decomposes organic matter, has been shown to decrease the abundance of certain ARGs in biosolids. The elevated temperatures achieved during composting can inactivate pathogens and degrade extracellular DNA, thereby reducing the potential for ARG dissemination. However, the effectiveness of composting in ARG reduction can vary depending on factors such as composting duration, temperature profiles, and the initial microbial composition of the sludge [85].

Anaerobic digestion (AD) is another widely employed sludge treatment method that stabilizes organic matter and produces biogas as a renewable energy source. The impact of AD on ARGs is complex and influenced by operational parameters such as temperature, retention time, and sludge characteristics. Studies have demonstrated that thermophilic AD conditions can lead to a significant reduction in ARG abundance, whereas mesophilic conditions may be less effective . Additionally, pre-treatment processes like thermal hydrolysis have been found to enhance ARG removal during subsequent AD by disrupting microbial cells and extracellular polymeric substances, thereby facilitating the degradation of resistance genes [86].

Despite these treatment strategies, the application of treated biosolids to land still necessitates careful consideration and monitoring. The persistence of residual ARGs in soils post-application underscores the need for comprehensive risk assessments and the development of guidelines to manage biosolid use in agriculture. Implementing best management practices, such as restricting biosolid application rates, timing applications to avoid periods of heavy rainfall, and incorporating biosolids into the soil promptly, can help minimize the environmental dissemination of ARGs . Continued research into advanced treatment technologies and their integration into existing wastewater management frameworks is essential to address the challenges posed by ARGs in sludge and to safeguard public and environmental health [66,87].

5.5. Global and Local Strategies

Addressing the proliferation of ARGs in wastewater requires a multifaceted approach that encompasses both global initiatives and localized strategies tailored to specific contexts. Internationally, organizations such as the World Health Organization have recognized antimicrobial resistance as a pressing public health concern and have advocated for coordinated actions to mitigate its spread. The WHO's Global Action Plan on Antimicrobial Resistance emphasizes the importance of improving sanitation, hygiene, and wastewater management to reduce the environmental dissemination of ARGs [51,54,60] .

At the national level, countries have implemented various policies and regulations to control antibiotic usage and manage wastewater treatment processes. For instance, some nations have established stringent guidelines for the discharge of antibiotics and ARGs into the environment, mandating advanced treatment technologies in wastewater treatment plants. These regulations often include monitoring requirements to assess the effectiveness of treatment processes in removing ARGs and to ensure compliance with environmental standards [88]. On a local scale, community-based interventions play a crucial role in mitigating ARG dissemination. In regions lacking centralized wastewater treatment infrastructure, decentralized systems such as constructed wetlands and biofiltration units have been employed to treat domestic and agricultural wastewater [89]. These systems leverage natural processes involving plants, microbes, and substrates to remove contaminants, including ARGs, from wastewater. Studies have demonstrated the efficacy of such systems in reducing ARG abundance, highlighting their potential as sustainable solutions for rural and peri-urban areas [89,90].

Furthermore, public awareness campaigns and educational programs are essential components of local strategies to combat antibiotic resistance. By informing communities about the prudent use of antibiotics, proper disposal of medications, and the importance of sanitation, these initiatives can reduce the selective pressure that drives the emergence and spread of ARGs. Engaging stakeholders across sectors—including healthcare providers, farmers, policymakers, and the general public—is vital to fostering a collaborative environment conducive to effective ARG mitigation. Collectively, the integration of global directives with context-specific local actions forms a comprehensive framework to address the complex challenge of antibiotic resistance in wastewater systems [5,19,60,71].

6. Future Directions and Research Needs

The integration of artificial intelligence (AI) and machine learning (ML) into environmental monitoring has opened new avenues for predicting and managing the risks associated with ARGs in wastewater systems. Recent studies have demonstrated the efficacy of ML algorithms in analyzing complex datasets to identify patterns and predict the occurrence of ARGs. For instance, supervised and unsupervised ML tools have been successfully employed to forecast antibiotic resistance trends, thereby aiding in the development of targeted mitigation strategies [56,58]. These technologies facilitate real-time surveillance and risk assessment, enabling prompt interventions to curb the spread of resistance.Advancements in microbe-based solutions and bioreactor technologies have also shown promise in mitigating ARG proliferation in wastewater. Nature-based bioreactors, which utilize benthic and planktonic microbial communities, have been effective in reducing antibiotic resistance from actual wastewater [91]. These systems leverage the natural capabilities of microbial consortia to degrade contaminants and ARGs, offering a sustainable approach to wastewater treatment. Additionally, anaerobic electrochemical membrane bioreactors have demonstrated the ability to effectively mitigate ARG proliferation under high antibiotic concentrations, highlighting the potential of integrating electrochemical processes with biological treatment methods. The convergence of AI-driven predictive models with advanced bioreactor technologies presents a synergistic approach to managing ARGs in wastewater. By harnessing the analytical power of AI and the degradative capabilities of specialized microbial communities, it is possible to develop adaptive treatment systems that respond dynamically to fluctuations in ARG concentrations. Such integrated solutions could significantly enhance the efficacy of wastewater treatment processes, thereby reducing the environmental dissemination of antibiotic resistance [76,77,91].

The global nature of antibiotic resistance necessitates stronger international collaboration and the development of cohesive policy frameworks to address the dissemination of ARGs through wastewater [92]. Wastewater-based surveillance (WBS) has emerged as a valuable tool in monitoring antimicrobial resistance within communities and healthcare settings, providing comprehensive data on AMR prevalence. However, the effectiveness of WBS is contingent upon the establishment of standardized protocols and data-sharing mechanisms across borders. International cooperation is essential to harmonize monitoring efforts, facilitate the exchange of information, and implement coordinated interventions to mitigate the spread of resistance [92,93]. Standardization of wastewater management practices is critical in controlling ARG proliferation. The lack of uniform guidelines and regulatory standards for wastewater treatment contributes to the inconsistent removal of ARGs, thereby allowing their persistence and dissemination in the environment. Establishing standardized methods for monitoring and treating wastewater can enhance the comparability of data across regions and improve the overall effectiveness of ARG mitigation strategies [57,60]. Moreover, incorporating ARG monitoring into existing regulatory frameworks can ensure that wastewater treatment plants are held accountable for their role in controlling the spread of antibiotic resistance. Policy development should also focus on incentivizing the adoption of advanced treatment technologies and promoting research into innovative solutions for ARG mitigation. Providing financial and technical support to wastewater treatment facilities can facilitate the implementation of effective treatment methods, such as AI-integrated bioreactors. Furthermore, policies that encourage the prudent use of antibiotics in human medicine, agriculture, and aquaculture can reduce the influx of antibiotics and resistant bacteria into wastewater systems, thereby addressing the issue at its source [56,58].

Despite significant advancements in understanding the dynamics of ARGs in wastewater, several research gaps persist that hinder the development of comprehensive mitigation strategies. One critical area requiring further investigation is the long-term fate of ARGs in various ecosystems. While studies have documented the presence of ARGs in wastewater and receiving environments, there is a paucity of longitudinal data tracking their persistence, transformation, and potential for horizontal gene transfer over extended periods. Addressing this gap is essential for assessing the long-term risks associated with ARGs and for developing strategies to disrupt their environmental transmission pathways. Research on the global spread of ARGs and their environmental and health consequences is also limited. Understanding the mechanisms by which ARGs disseminate across different geographical regions and ecosystems is crucial for predicting potential hotspots of resistance emergence. Furthermore, studies examining the impact of environmental ARGs on human and animal health are necessary to elucidate the full extent of the risks posed by environmental reservoirs of resistance. Such research can inform risk assessments and guide the implementation of targeted interventions to protect public health. Additionally, there is a need for interdisciplinary research that integrates microbiology, environmental science, engineering, and data analytics to develop holistic approaches to ARG mitigation [94,95]. Collaborative efforts can facilitate the development of predictive models that account for the complex interactions between microbial communities, environmental factors, and human activities. Investing in such research endeavors can lead to the creation of innovative, scalable, and sustainable solutions to combat the proliferation of antibiotic resistance in wastewater systems and beyond.

7. Conclusion

Antibiotic resistance genes in wastewater environments represent a profound environmental and public health challenge. WWTPs serve as convergence points for various sources of ARGs, including domestic sewage, hospital effluents, and agricultural runoff. These facilities, while designed to treat conventional pollutants, often lack the capacity to effectively eliminate ARGs. Consequently, treated effluents discharged into natural water bodies can act as vectors for the dissemination of resistance genes, facilitating their integration into indigenous microbial communities. This integration not only alters the microbial ecology of these environments but also poses risks to human health through potential exposure to resistant pathogens.

Research has demonstrated that ARGs can persist through conventional treatment processes. For instance, studies have shown that standard chlorination methods may not sufficiently degrade ARGs, leading to their continued presence in treated water. Furthermore, the co-occurrence of heavy metals and antibiotics in wastewater can promote the co-selection of resistance genes, exacerbating the spread of multidrug-resistant organisms. The environmental persistence of these genes underscores the need for a comprehensive understanding of their behavior in wastewater systems and the development of targeted strategies to mitigate their impact. The ecological implications of ARG dissemination are significant. The introduction of resistance genes into aquatic ecosystems can disrupt microbial diversity and function, potentially leading to the emergence of novel resistance mechanisms. Additionally, the transfer of ARGs to pathogenic bacteria can result in infections that are more difficult to treat, increasing the burden on healthcare systems. The environmental dimension of antibiotic resistance, therefore, necessitates an integrated approach that considers the interconnectedness of human, animal, and environmental health.

Addressing the challenge of ARGs in wastewater requires a multidisciplinary approach that integrates technological innovation, policy development, and public engagement. Enhancing the efficacy of wastewater treatment processes is paramount. Advanced treatment technologies, such as ozonation, advanced oxidation processes, and membrane filtration, have shown promise in reducing ARG concentrations in effluents. However, the implementation of these technologies must be accompanied by rigorous monitoring to assess their effectiveness and adapt strategies as needed. Policy frameworks play a crucial role in mitigating the spread of ARGs. Establishing stringent regulations on antibiotic usage in agriculture and healthcare settings can reduce the influx of resistance genes into wastewater systems. Moreover, investing in infrastructure upgrades for WWTPs, particularly in regions with outdated facilities, can enhance their capacity to remove ARGs effectively. International cooperation is also essential, as antibiotic resistance is a global issue that transcends national boundaries. Collaborative efforts can facilitate the sharing of best practices and resources, fostering a unified response to this pressing challenge.

Public awareness and education are integral components of any comprehensive strategy to combat antibiotic resistance. Informing communities about the risks associated with improper antibiotic use and the importance of proper disposal can reduce the prevalence of resistance genes in wastewater. Additionally, promoting research into alternative treatments and the development of new antibiotics can provide long-term solutions to the problem. By adopting a holistic approach that encompasses technological, regulatory, and societal dimensions, it is possible to curtail the spread of ARGs in wastewater and safeguard environmental and public health.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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