Review Article

Macrolide Contamination in Aquatic Ecosystems Driving Multidrug Resistance and Horizontal Gene Transfer in Pathogens

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Abstract

The presence of macrolide antibiotics in aquatic environments has become a growing concern due to their role in promoting antimicrobial resistance. These antibiotics, frequently discharged from hospital effluents, pharmaceutical manufacturing, and agricultural runoff, persist in water systems and create favorable conditions for the selection and spread of resistant bacteria. Among the most affected species, Enterococcus faecium, Staphylococcus aureus, and Pseudomonas aeruginosa have demonstrated increasing resistance, largely driven by the horizontal transfer of ermB and msrA genes. This review examines the pathways through which macrolide contamination influences bacterial resistance and facilitates gene transmission in aquatic ecosystems. It also evaluates the effectiveness of wastewater treatment strategies, including advanced oxidation processes and membrane filtration, in mitigating antibiotic pollution. Given the rising threat of antimicrobial resistance, improving wastewater management, enforcing stricter regulations on antibiotic disposal, and enhancing monitoring systems are essential steps toward controlling the spread of resistant bacterial strains and protecting public health.

Keywords: Macrolide antibiotics, Enterococcus faecium, Staphylococcus aureus, Pseudomonas aeruginosa, Antibiotic resistance, Environmental microbiology

INTRODUCTION

The growing presence of antibiotics in the environment has raised significant concerns about the emergence and spread of antimicrobial resistance (AMR)[1]. Among them, macrolide antibiotics have received particular attention due to their widespread use in human and veterinary medicine and their persistence in natural water systems. These antibiotics are extensively prescribed for respiratory, skin, and soft tissue infections, as well as for livestock disease management[2]. However, their incomplete metabolism in humans and animals, combined with improper disposal practices, leads to their continuous release into aquatic environments. The primary pathways of macrolide contamination include hospital and municipal wastewater, pharmaceutical industry effluents, and agricultural runoff from livestock farms where these antibiotics are commonly used as growth promoters. Their prolonged presence in water bodies creates selective pressure that favors the survival and proliferation of resistant bacterial strains, intensifying the global challenge of antibiotic resistance[3]. One of the primary concerns associated with macrolide pollution is its role in driving resistance evolution through horizontal gene transfer (HGT). Unlike intrinsic resistance, which occurs through spontaneous mutations, HGT allows bacteria to rapidly acquire resistance determinants from other microbes via plasmids, transposons, or integrative conjugative elements (ICEs). The ermB and msrA genes, which confer resistance to macrolides through ribosomal methylation and active efflux mechanisms, respectively, are among the most frequently detected resistance determinants in aquatic ecosystems. Their widespread distribution has been documented in various multidrug-resistant (MDR) pathogens, including Enterococcus faecium, Staphylococcus aureus, and Pseudomonas aeruginosa, all of which pose serious threats to human health due to their ability to cause persistent and hard-to-treat infections[4].Aquatic environments, particularly those receiving untreated or partially treated wastewater, serve as hotspots for bacterial

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How to cite this article: Najafi Kalyani F. Macrolide Contamination in Aquatic Ecosystems: Driving Multidrug Resistance and Horizontal Gene Transfer (ermB/msrA) in E. faecium, S. aureus, and P. aeruginosa: A Review. Arch Pharm Pract. 2025;16(S):A0625403.

adaptation and resistance gene dissemination. Studies have shown that antibiotic residues at sub-inhibitory concentrations can enhance genetic exchange between bacterial populations, even in non-clinical settings. Wastewater treatment plants (WWTPs), while effective at reducing bacterial loads, often fail to completely remove antibiotic contaminants, allowing resistant bacteria and resistance genes to persist and be discharged into rivers, lakes, and coastal waters. This continuous cycle of contamination reinforces the global AMR crisis and highlights the urgent need for improved water management strategies[3]. Despite increasing awareness of antibiotic resistance in environmental microbiology, significant knowledge gaps remain regarding the long-term ecological consequences of macrolide pollution and the efficiency of various wastewater treatment technologies in mitigating its effects. While advanced treatment methods, such as advanced oxidation processes (AOPs) and membrane filtration technologies[3, 5], have shown promise in degrading antibiotic residues, their practical application remains limited due to high operational costs and variability in treatment efficiency across different water matrices. Additionally, standard monitoring protocols for tracking antibiotic contamination and resistance gene prevalence in aquatic environments are still lacking in many regions, making it difficult to assess the full extent of the problem[6, 7].This review aims to provide a comprehensive analysis of the impact of macrolide antibiotics on bacterial resistance in aquatic ecosystems, with a focus on resistance gene transfer mechanisms and the role of environmental reservoirs in sustaining AMR. Furthermore, it evaluates existing wastewater treatment approaches, their limitations, and emerging strategies for mitigating macrolide pollution. Addressing these challenges is essential for protecting water quality, reducing the spread of antibiotic-resistant bacteria, and safeguarding public health in the face of a rapidly evolving resistance crisis.

Materials and Methods

Characteristics and Uses of Macrolide Antibiotics

Macrolide antibiotics (MCLs) are a class of antimicrobial agents characterized by a macrocyclic lactone ring, typically containing 12 to 16 atoms, with attached deoxy sugar moieties such as desosamine and cladinose. These antibiotics function by binding to the 23S rRNA of the 50S ribosomal subunit, thereby inhibiting bacterial protein synthesis by preventing peptide chain elongation[7]. Unlike β-lactam antibiotics, which target bacterial cell walls, macrolides act intracellularly and exhibit bacteriostatic activity, though at higher concentrations, they may exert bactericidal effects against certain pathogens[8, 9]. They are particularly effective against Gram-positive bacteria, atypical pathogens such as *Mycoplasma pneumoniae* and *Chlamydia trachomatis*, and some Gram-negative species. Due to their broad-spectrum activity, macrolides are widely prescribed in human medicine for treating respiratory tract infections, skin and soft tissue infections, sexually transmitted diseases, and gastrointestinal infections, particularly those caused by *Helicobacter pylori*. Their immunomodulatory properties have also led to their use in chronic inflammatory conditions such as cystic fibrosis and chronic obstructive pulmonary disease (COPD)[10]. The pharmacokinetics of macrolides vary based on structural modifications that influence acid stability, tissue penetration, and half-life. Erythromycin, the prototypical 14-membered macrolide, suffers from acid instability, leading to poor bioavailability and gastrointestinal side effects. Semi-synthetic derivatives such as clarithromycin and azithromycin have been developed to overcome these limitations. Clarithromycin demonstrates improved acid stability and tissue penetration, making it more effective for eradicating *Helicobacter pylori*. Azithromycin, a 15-membered azalide, has a longer intracellular half-life, allowing for once-daily dosing and enhanced activity against intracellular pathogens. These modifications have expanded the clinical utility of macrolides, making them essential in modern antimicrobial therapy[11, 12]. Beyond human medicine, macrolides are extensively used in veterinary medicine and agriculture, particularly in livestock production. They are administered to poultry, swine, and cattle for therapeutic, prophylactic, and metaphylactic purposes, targeting bacterial infections such as bovine respiratory disease, swine dysentery, and mycoplasmal pneumonia in chickens. Additionally, macrolides have historically been employed as growth promoters in food-producing animals to enhance feed efficiency and weight gain. This practice, however, has been highly controversial due to its direct link to antimicrobial resistance (AMR). Sub-therapeutic exposure to macrolides in livestock promotes the selection of resistant bacterial populations, which can disseminate to humans through direct contact, contaminated food products, or environmental pathways. As a result, the use of macrolides as growth promoters has been banned or restricted in many countries, though concerns remain regarding illegal or unregulated antibiotic use in agriculture[13, 14].

A major concern regarding the widespread use of macrolides is their incomplete metabolism in humans and animals, leading to the excretion of active antibiotic residues into wastewater systems. Once excreted, macrolides enter municipal sewage, hospital effluents, and agricultural runoff, eventually reaching aquatic environments. Due to their moderate hydrophobicity, macrolides can bind to organic matter and sediments, prolonging their environmental persistence. Their degradation is influenced by pH, photodegradation, sorption, and microbial metabolism. Although macrolides undergo acid-catalyzed hydrolysis, they remain relatively stable in neutral and alkaline waters. Photodegradation, primarily mediated by sunlight exposure, is a significant removal pathway in surface waters, yet its efficiency is limited in turbid or polluted environments where light penetration is reduced. Certain bacterial species, including Actinobacteria and Proteobacteria, can degrade macrolides enzymatically, but this process is slow and inconsistent across different water matrices. As a result, macrolides persist in aquatic systems, contributing to chronic antibiotic exposure in microbial communities[15, 16]. One of the most pressing issues associated with macrolide contamination in aquatic environments is its role in selecting for antimicrobial resistance (AMR) through horizontal gene transfer (HGT). Prolonged exposure to low, sub-inhibitory antibiotic concentrations creates selective pressure that favors the survival of resistant bacterial strains. Macrolide-resistant bacteria commonly harbor ribosomal methylation genes such as *ermB*, which confer target-site modification-based resistance, and efflux pump genes such as *msrA*, which actively transport macrolides out of bacterial cells. These resistance determinants can be horizontally transferred via mobile genetic elements, including plasmids, transposons, and integrative conjugative elements (ICEs), facilitating the rapid spread of macrolide resistance across diverse bacterial populations. Aquatic environments, particularly wastewater treatment plants (WWTPs), river sediments, and agricultural runoff sites, serve as hotspots for resistance gene exchange, where antibiotic residues and bacterial communities coexist under conditions that drive genetic adaptation[17, 18]. Wastewater treatment plants are often ineffective in fully removing macrolides, leading to their continuous release into surface and groundwater systems. Conventional treatment processes, such as activated sludge systems and biological filtration, are not designed to degrade antibiotics efficiently, allowing residual macrolides to persist in effluent discharge. Advanced treatment technologies, including ozonation, advanced oxidation processes (AOPs), and membrane filtration, have shown promise in reducing antibiotic contamination, yet their large-scale implementation remains cost-prohibitive and operationally complex. Given these limitations, macrolides remain ubiquitous contaminants in the environment, contributing to the global AMR crisis by perpetuating resistance gene dissemination. The persistent presence of macrolides in aquatic ecosystems not only threatens environmental microbial communities but also poses direct risks to human health. Resistant bacterial strains originating from aquatic environments can colonize the human microbiome through exposure to contaminated water sources, food chains, or recreational activities. The increasing detection of macrolide-resistant pathogens, such as multidrug-resistant *Staphylococcus aureus*, *Enterococcus faecium*, and *Pseudomonas aeruginosa*, in clinical settings highlights the urgency of addressing antibiotic contamination at its environmental source. Strategies for mitigating macrolide pollution must involve a multifaceted approach, including improved wastewater treatment infrastructure, stricter regulations on antibiotic disposal, and surveillance programs for monitoring resistance trends in environmental reservoirs. As shown in **Table 1**, the Comparison of Macrolide Resistance Mechanisms in *Enterococcus faecium*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa* and the Role of Aquatic Environments in Their Dissemination is presented[19, 20].

Table1. Comparison of Macrolide Resistance Mechanisms in Enterococcus faecium, Staphylococcus aureus, and Pseudomonas aeruginosa and the Role of Aquatic Environments in Their Dissemination

Bacterium	Macrolide Susceptibility	Resistance Mechanism	Genetic Mechanism (Key Resistance Genes)	Environments Prone to Increased Resistance	Role of Macrolides in Resistance Enhancement
Enterococcus faecium	Moderate to resistant	Ribosomal methylation (reducing drug binding), increased efflux pump expression, altered membrane permeability	ermB, msrC, mefA, vatD	Hospital wastewater, wastewater treatment plants, contaminated surface water	Selective pressure promotes survival of resistant strains and dissemination of HGT genes in aquatic environments
Staphylococcus aureus	Variable (MRSA is resistant)	Ribosomal target modification (methylation of 23S rRNA), increased efflux pump activity, restricted drug influx, macrolide-degrading enzymes	ermA, ermC, msrA, mphC, vgaA	Hospital environments, medical devices, contaminated equipment	Enhanced survival of MRSA and spread of resistance genes in clinical and community settings
Pseudomonas aeruginosa	Typically resistant	Reduced membrane permeability, multidrug efflux pumps (MDR efflux pumps), macrolide enzymatic degradation, stress-induced resistance gene regulation	mexAB-oprM, mexXY-oprM, macA, macB, arnB	Industrial wastewater, pharmaceutical effluents, low-oxygen zones in wastewater systems	Macrolides become ineffective, facilitating widespread resistance gene exchange in bacterial communities

Sources of Macrolide Contamination in Water Systems

The contamination of aquatic environments with macrolide antibiotics originates from multiple anthropogenic sources, each contributing to the persistence of these compounds in surface and groundwater systems. One of the most significant pathways is through hospital and municipal wastewater. Hospitals discharge substantial quantities of macrolides due to their widespread use in treating bacterial infections, particularly respiratory and soft tissue infections[21]. Patients excrete unmetabolized antibiotics through urine and feces, introducing these compounds into sewage networks. In urban settings, household use of macrolides further amplifies the contamination, as many individuals improperly dispose of unused medications by flushing them down toilets or sinks. Wastewater treatment plants (WWTPs), which are not specifically designed to eliminate pharmaceuticals, fail to fully degrade macrolides, resulting in their continuous release into natural water bodies. Conventional treatment methods, such as activated sludge processes, may remove some fraction of these antibiotics, but significant concentrations persist in treated effluent, often at levels sufficient to exert selective pressure on microbial communities[22]. Studies have detected macrolides in effluent discharges from WWTPs at concentrations ranging from nanograms to micrograms per liter, levels that, although low, are still capable of promoting the proliferation of resistant bacterial strains[23].

Industrial pharmaceutical effluents are another major contributor to macrolide contamination. Manufacturing plants that produce antibiotics often generate wastewater containing high concentrations of active pharmaceutical ingredients (APIs), including macrolides and their degradation byproducts. Inadequate treatment of these industrial discharges has been documented in several regions, where water samples collected near pharmaceutical production sites have shown alarmingly high levels of macrolides, sometimes exceeding environmental safety thresholds by several orders of magnitude. This contamination is particularly concerning because industrial effluents can introduce not only antibiotics but also resistance genes harbored by antibiotic-producing microbes, further accelerating the spread of antimicrobial resistance (AMR) in aquatic ecosystems. Unlike hospital wastewater, which undergoes partial treatment before being released into municipal sewage systems, industrial pharmaceutical discharges are often released directly into rivers and lakes, creating localized hotspots of antibiotic pollution[23, 24]. In addition to human healthcare and pharmaceutical industries, agriculture is a major source of macrolide contamination in water systems. These antibiotics are widely used in livestock farming for both therapeutic and non-therapeutic purposes. In many regions, macrolides are routinely administered to poultry, swine, and cattle, not only to treat bacterial infections but also as prophylactic agents to prevent disease outbreaks in high-density animal farming operations. Moreover, subtherapeutic doses of macrolides have historically been used as growth promoters to enhance feed efficiency, though this practice has been banned or restricted in many countries due to its role in accelerating antibiotic resistance. Once administered to animals, a significant proportion of macrolides is excreted in urine and feces in an unmetabolized or partially metabolized form, leading to their accumulation in manure. This manure is frequently applied to agricultural fields as fertilizer, where rainfall and irrigation facilitate the leaching of antibiotics into nearby water sources. The diffuse nature of agricultural runoff makes it particularly difficult to control, as macrolides introduced into surface waters through this pathway can persist over long distances, affecting ecosystems far beyond their original point of release[24, 25]. The persistence of macrolides in aquatic environments is exacerbated by their physicochemical properties, which influence their mobility, bioavailability, and degradation pathways. Macrolides exhibit moderate hydrophobicity, allowing them to adsorb onto organic matter and sediments, reducing their immediate bioavailability in the water column but creating long-term reservoirs of contamination. In sediments, macrolides may undergo slow desorption back into the water, prolonging their environmental impact. Their degradation is influenced by several abiotic and biotic factors, including pH, light exposure, and microbial activity. While macrolides are susceptible to acid-catalyzed hydrolysis, they remain relatively stable under neutral and alkaline conditions, meaning that in most surface and groundwater systems, they persist for extended periods. Photodegradation can contribute to their breakdown in sunlit surface waters, but this process is highly dependent on water clarity and the presence of dissolved organic matter, which can shield antibiotics from direct photolysis. Biodegradation by aquatic microorganisms is another potential removal pathway, but macrolides are inherently recalcitrant to microbial breakdown. Only specific bacterial taxa, such as certain Actinobacteria and Proteobacteria, possess enzymatic systems capable of degrading macrolides, and even in these cases, the process is often slow and incomplete[3, 8, 26].

Compounding these challenges is the fact that even when macrolides degrade, their transformation products (TPs) can retain biological activity and contribute to antimicrobial resistance selection. Some TPs have been found to exhibit similar or even higher toxicity than their parent compounds, raising concerns about their long-term ecological impact. Because conventional monitoring efforts often focus on detecting the parent macrolide compounds, the presence of bioactive transformation products is frequently overlooked, leading to an underestimation of the true extent of antibiotic pollution in aquatic environments[27-29]. **Table 2** shows the sources of macrolide contamination in aquatic systems and their relationship to antimicrobial resistance.

Table 2. Sources of Macrolide Contamination in Aquatic Systems and Their Link to Antimicrobial Resistance

Source of Contamination	Pathway to Water Systems	Common Macrolides	Target Bacteria Affected	Identified Resistance Genes (erm/msr)	Type of Gene Transfer	Environmental Persistence Level	Resistance Spread Risk
Hospital Wastewater	Excretion of unmetabolized drugs via urine/feces	Azithromycin, Clarithromycin	E. faecium, S. aureus	ermB, msrA, mefA	Plasmid, ICE	High	High
Domestic Sewage	Flushing or sink disposal of	Clarithromycin, Azithromycin	S. aureus	msrA, ermF	Plasmid	Medium	Medium

	unused antibiotics						
Pharmaceutical Industry Effluents	Direct discharge of API-containing wastewater	Erythromycin, Tylosin	P. aeruginosa	ermX, msrD	ICE, Transposon	Very High	Critical
Agricultural Runoff	Animal manure runoff from rainfall/irrigation	Tylosin, Tilmicosin	E. faecium, S. aureus	ermB, ermC	Plasmid	High	High
Aquaculture Effluents	Use of antibiotics in fish/shrimp farming	Azithromycin, Erythromycin	E. faecium	ermB	ICE	High	Medium
Landfill Leachate	Decomposition of discarded medications	Mixed macrolides	P. aeruginosa, environmental species	Limited data	Unknown	Medium	Under-monitored
Urban Stormwater	Rainwater carrying residuals from streets	Mixed	Various environmental bacteria	msrA, ermB	Plasmid	Low	Low

Beyond direct contamination, macrolides also contribute to the selective pressure that drives antimicrobial resistance evolution in environmental microbiomes. The continuous, low-level presence of macrolides in water bodies creates conditions that favor the survival of resistant bacterial populations while suppressing susceptible ones. Resistance genes such as *ermB*, which mediates ribosomal methylation-based resistance, and *msrA*, which encodes an efflux pump that expels macrolides from bacterial cells, are frequently detected in environmental samples from contaminated water bodies. These genes are often carried on mobile genetic elements, enabling their horizontal transfer between bacterial species. Aquatic environments, particularly those receiving untreated or partially treated wastewater, serve as hubs for genetic exchange, where bacteria from diverse ecological niches come into contact and facilitate the spread of resistance determinants. Wastewater treatment plants, rather than eliminating resistance genes, often act as selective environments where bacteria undergo adaptation to antibiotic exposure, leading to the enrichment of multidrug-resistant (MDR) populations. Once released into the environment, these resistant bacteria can be transported through interconnected water systems, eventually infiltrating drinking water supplies, agricultural irrigation sources, and marine ecosystems[30-32]. The contamination of water systems with macrolides is a complex, multifaceted issue driven by human activity across multiple sectors. The interplay between hospital discharges, industrial emissions, and agricultural runoff ensures that these antibiotics remain an ongoing source of environmental exposure. Conventional wastewater treatment technologies are insufficient to fully remove macrolides and their transformation products, allowing these compounds to persist in aquatic ecosystems and contribute to the broader crisis of antibiotic resistance. Without comprehensive intervention strategies, including stricter regulations on antibiotic disposal, improved treatment technologies, and enhanced environmental surveillance, the accumulation of macrolides in water systems will continue to fuel the emergence and dissemination of resistant bacterial strains, posing a significant threat to both ecosystem health and global public health[33, 34]. **Figure 1** shows potential sources and pathways of antibiotic exposure in the environment.

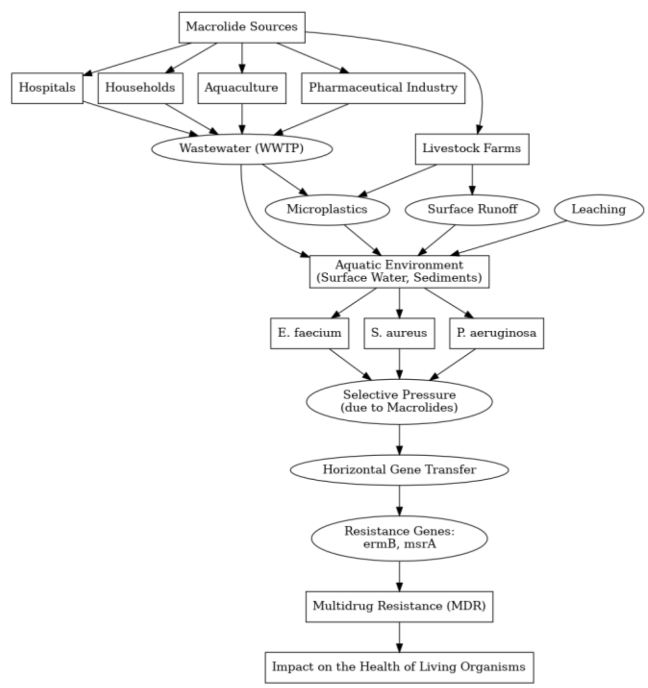

Figure 1. Sources and potential pathways of antibiotic exposure in the environment

Mechanisms of Macrolide Resistance in Bacteria

Macrolide resistance in bacteria arises through various biochemical and genetic mechanisms that enable bacterial survival in the presence of these antibiotics. Macrolides inhibit bacterial protein synthesis by binding to the 23S rRNA of the 50S ribosomal subunit, preventing peptide chain elongation. However, bacteria have evolved multiple strategies to evade this inhibition, including ribosomal target modification, active efflux systems, and enzymatic inactivation of macrolides[35, 36]. Some bacterial species exhibit intrinsic resistance to macrolides due to their low membrane permeability, the presence of native efflux pumps, or the lack of a high-affinity ribosomal binding site. For example, *Pseudomonas aeruginosa* possesses an impermeable outer membrane, limiting macrolide penetration, while members of the *Enterobacteriaceae* family naturally show reduced susceptibility to macrolides due to their ribosomal structure and innate efflux systems. In contrast, acquired resistance occurs through horizontal gene transfer (HGT), facilitated by plasmids, transposons, and integrative conjugative elements (ICEs), leading to the dissemination of resistance genes among bacterial populations[37, 38]. One of the most prevalent mechanisms of macrolide resistance is ribosomal target modification, primarily mediated by erm (erythromycin ribosomal methylase) genes, which encode enzymes that methylate adenine at position A2058 of the 23S rRNA, thereby preventing macrolide binding. This modification results in high-level resistance and is frequently associated with cross-resistance to lincosamides and streptogramins (MLS resistance phenotype). The *ermB* gene, commonly found in *Streptococcus pneumoniae*, *Enterococcus faecium*, and *Staphylococcus aureus*, is one of the most widespread determinants of this resistance mechanism[39, 40]. Another major resistance mechanism involves active efflux pumps, which function by expelling macrolide molecules from the bacterial cytoplasm, reducing intracellular antibiotic concentration to sub-inhibitory levels. The most well-characterized efflux-related genes include *msrA*, which encodes an ATP-binding cassette (ABC) transporter found in *Staphylococcus aureus*, and *mefA*/*mefE*, which encode efflux pumps responsible for moderate macrolide resistance in *Streptococcus pneumoniae*. Unlike target modification, efflux-mediated resistance often confers lower levels of resistance and is primarily effective against 14- and 15-membered macrolides, such as erythromycin and azithromycin, but has reduced activity against 16-membered macrolides like spiramycin.

A less common but significant mechanism of resistance is enzymatic inactivation of macrolides, in which bacterial enzymes chemically modify or degrade macrolide molecules, rendering them ineffective. This mechanism is primarily found in Gram-negative bacteria, where specific enzymes, such as macrolide phosphotransferases (mph) and esterases (ereA, ereB), catalyze the phosphorylation or hydrolysis of macrolides, leading to antibiotic inactivation. The *mphA* gene, frequently identified in *Escherichia coli*, encodes a phosphotransferase that modifies macrolides, whereas *ereB* in *Pseudomonas aeruginosa* hydrolyzes the macrolide lactone ring. Although enzymatic inactivation is less commonly observed compared to target modification and efflux, it plays a crucial role in macrolide resistance within multidrug-resistant (MDR) pathogens, particularly those associated with hospital-acquired infections[41, 42]. The persistence of macrolides in clinical, agricultural, and environmental settings exerts selective pressure, favoring resistant bacterial strains and accelerating the spread of resistance genes. Wastewater treatment plants (WWTPs), hospital effluents, and agricultural runoff act as hotspots for macrolide resistance evolution, where sub-inhibitory concentrations of these antibiotics promote the maintenance and exchange of resistance determinants. The increasing prevalence of macrolide-resistant pathogens, such as multidrug-resistant *Staphylococcus aureus*, *Enterococcus faecium*, and *Pseudomonas aeruginosa*, poses significant challenges for antimicrobial therapy, necessitating improved surveillance and regulatory strategies to mitigate the environmental and clinical impact of macrolide resistance[42, 43].

Strategies for Reducing Macrolide Contamination in Water

To effectively mitigate the impact of macrolide contamination in aquatic environments, a multifaceted approach is necessary. This approach should integrate advanced wastewater treatment technologies, sustainable natural remediation systems, regulatory frameworks, and responsible antibiotic use in healthcare and agriculture. Below, the key strategies for reducing macrolide pollution are categorized into technological solutions, policy interventions, agricultural management, and monitoring efforts.

Advanced Wastewater Treatment Technologies

The persistence of macrolide antibiotics in aquatic environments poses a serious challenge due to their incomplete removal in conventional wastewater treatment plants (WWTPs). Biological treatment processes, such as activated sludge systems, are often ineffective in degrading macrolides because of their low biodegradability and chemical stability. As a result, advanced wastewater treatment technologies have been developed to enhance macrolide removal before effluent discharge[19, 44]. These technologies include advanced oxidation processes (AOPs), membrane filtration techniques, activated carbon adsorption, and bioaugmentation strategies. Each of these methods operates through distinct physicochemical and biological mechanisms to break down or remove macrolides from wastewater, reducing their environmental impact.

One of the most effective methods for macrolide degradation is the use of advanced oxidation processes (AOPs), which rely on the generation of highly reactive hydroxyl radicals (•OH) to break down antibiotic molecules into smaller, less toxic compounds. Ozonation is a widely employed AOP that utilizes ozone (O_3) to degrade macrolides through direct electrophilic oxidation and indirect radical-mediated reactions. The effectiveness of ozonation depends on factors such as ozone dose, reaction time, pH, and the composition of the water matrix. Studies have shown that ozonation can achieve over 90% degradation of erythromycin and clarithromycin within minutes under optimal conditions. However, the formation of oxidation byproducts (OBPs) must be carefully assessed, as some intermediate compounds may retain antimicrobial properties and contribute to antibiotic resistance selection. Another major limitation of ozonation is its high operational cost and energy demand, making it less feasible for large-scale implementation in developing regions[5, 6, 45]. The Fenton and photo-Fenton processes are additional AOPs that utilize hydrogen peroxide (H_2O_2) in the presence of ferrous ions (Fe^{2+}) to generate hydroxyl radicals. The oxidative degradation of macrolides via Fenton reactions occurs through hydroxylation, lactone ring cleavage, and demethylation reactions, which disrupt antibiotic activity. The Fenton process has been reported to remove over 80% of azithromycin and erythromycin within 30 minutes under acidic conditions (pH ~3). However, pH adjustment is necessary for real-world applications, as neutral and alkaline conditions significantly reduce treatment efficiency. Moreover, sludge formation due to iron precipitation is a major drawback, necessitating additional handling and disposal steps[46, 47].

Photocatalysis, particularly titanium dioxide (TiO_2)-mediated UV processes, has also been widely studied for macrolide degradation. In this approach, TiO_2 acts as a semiconductor photocatalyst, generating electron-hole pairs upon exposure to UV light. These reactive species initiate radical-driven degradation of macrolides, breaking them down into harmless compounds. Photocatalysis has demonstrated the ability to eliminate up to 95% of erythromycin and clarithromycin within 60 minutes of UV exposure, making it an attractive alternative to chemical oxidation. However, its practical application is limited by high energy demands, potential catalyst deactivation due to particle aggregation, and the need for optimized reactor designs to enhance photon absorption[48, 49]. Membrane filtration technologies offer a different approach by physically removing macrolide molecules based on size exclusion, charge interactions, and adsorption mechanisms. Reverse osmosis (RO) and nanofiltration (NF) are two of the most effective membrane processes for pharmaceutical removal. RO utilizes high-pressure semi-permeable membranes to reject antibiotic molecules based on their molecular weight, hydrophobicity, and charge, achieving removal efficiencies of over 99%. However, the high operational cost and susceptibility to membrane fouling make RO less practical for widespread use. Nanofiltration, while slightly less effective (70-95% removal efficiency), requires lower energy input and is more suitable for applications where complete removal is not necessary[50-52].Activated carbon adsorption is another widely used method for removing pharmaceuticals from wastewater. Granular activated carbon (GAC) and powdered activated carbon (PAC) are effective in adsorbing macrolides due to their high surface area and porous structure. The adsorption process is influenced by factors such as pH, contact time, and carbon dosage. PAC has been reported to remove 85-95% of erythromycin and azithromycin from wastewater within 24 hours. However, adsorption is a non-destructive process, meaning that macrolides remain intact within the carbon matrix, requiring periodic regeneration or disposal of spent carbon, adding to operational costs[3, 53].

Biological approaches such as bioaugmentation and enzyme-mediated degradation are being explored as environmentally friendly alternatives to chemical and physical treatment methods. Some microorganisms, including genetically engineered bacteria and fungi, have demonstrated the ability to degrade macrolides under controlled conditions. Specific enzymes, such as laccases and peroxidases, catalyze the oxidative breakdown of macrolides, converting them into less toxic metabolites. Immobilized enzyme reactors have shown promising results for continuous antibiotic degradation in wastewater treatment plants. However, bioaugmentation strategies are often limited by slow degradation rates and the challenge of maintaining bacterial survival and activity in real wastewater environments[3]. Each of these advanced treatment technologies offers specific advantages and limitations, necessitating an integrated approach that combines oxidation, filtration, adsorption, and biological degradation for optimal macrolide removal. While ozonation and Fenton-based processes provide rapid and effective degradation, they require high energy input and generate potential byproducts. Membrane filtration ensures near-complete removal but is costly and prone to operational issues such as membrane fouling. Adsorption using activated carbon is relatively simple and effective but does not degrade macrolides, requiring additional treatment steps. Biological approaches hold promise for sustainable degradation but require further optimization to improve efficiency and scalability. In **Table 3**. A comparative analysis of advanced technologies for the removal of macrolides from wastewater is reviewed. Given the complexity of macrolide contamination, the selection of an appropriate treatment method depends on multiple factors, including wastewater composition, economic feasibility, and environmental sustainability. Future research should focus on optimizing hybrid treatment systems that integrate multiple technologies, enhancing removal efficiency while minimizing environmental impact. Additionally, policy interventions and regulatory measures should encourage the adoption of advanced wastewater treatment technologies to mitigate the risks associated with macrolide pollution and antimicrobial resistance development.

Table 3. Comparative Analysis of Advanced Technologies for Macrolide Removal from Wastewater

Technology	Macrolide Removal (%)	Reaction Time	Operational Cost	Advantages	Limitations	Integration Potential	REF
Ozonation	>90%	Few minutes	High	High speed, efficient degradation	Byproduct formation, energy intensive	High	[7, 54, 55]
Fenton / Photo-Fenton	~80%	~30 minutes	Medium	Simple, effective under acidic conditions	Requires low pH, sludge formation	High	[47, 56]

Photocatalysis	~95%	~60 minutes	High	Eco-friendly, complete degradation	Requires UV, catalyst deactivation	Medium	[57, 58]
Reverse Osmosis (RO)	>99%	<5 minutes	Very High	Near-complete removal	High cost, membrane fouling	Low	[59]
Nanofiltration (NF)	70–95%	~10 minutes	Medium	Lower energy than RO	Partial removal, requires pretreatment	Medium	[60, 61]
Activated Carbon (GAC/PAC)	85–95%	~24 hours	Medium	Simple, cost-effective	Non-destructive, carbon regeneration required	High	[62]
Bioaugmentation / Enzymatic	60–80%	≥48 hours	Low	Environmentally friendly, promising	Slow rate, microbial stability issues	High	[63-65]

Environmental Fate and Degradation of Macrolides

The persistence of macrolide antibiotics in aquatic environments is governed by their physicochemical properties, environmental interactions, and degradation pathways. Once introduced into water bodies through pharmaceutical discharges, hospital effluents, and agricultural runoff, macrolides undergo a series of sorption, transformation, and transport processes that determine their long-term fate. Due to their structural stability and resistance to microbial degradation, macrolides can persist in aquatic ecosystems for extended periods, posing risks related to antimicrobial resistance (AMR), bioaccumulation, and ecological toxicity. Understanding these processes is essential for predicting environmental exposure and designing effective mitigation strategies. The hydrophobicity, molecular weight, and ionization potential of macrolides play a crucial role in their fate in aquatic environments. Most macrolides exhibit moderate hydrophobicity, with log Kow values between 2 and 4, allowing them to partition between aqueous and solid phases. Additionally, macrolides contain dimethylamino groups, giving them a cationic nature at neutral pH, which promotes electrostatic interactions with negatively charged soil particles and organic matter. These properties influence their mobility, bioavailability, and potential for long-term environmental persistence. One of the primary mechanisms governing macrolide behavior in water systems is sorption to sediments and organic matter. Sorption plays a dual role by reducing macrolide mobility but also prolonging their environmental residence time. The extent of sorption is influenced by factors such as organic carbon content, sediment composition, and pH fluctuations. Higher organic matter levels enhance sorption, effectively trapping macrolides within sediments and slowing their degradation. However, sorption is a reversible process, and changes in environmental conditions—such as pH shifts or redox fluctuations—can lead to desorption, reintroducing macrolides into the water column. This dynamic equilibrium complicates predictions regarding macrolide persistence and transport in aquatic systems. Abiotic degradation processes such as photodegradation and hydrolysis contribute to macrolide breakdown, albeit at varying rates depending on environmental conditions and molecular structure. Photodegradation primarily occurs in surface waters where macrolides are exposed to solar radiation. While direct photolysis is generally slow due to the weak UV absorbance of macrolides, indirect photolysis mediated by reactive oxygen species (ROS), hydroxyl radicals (•OH), and singlet oxygen (O_2) plays a more significant role in degradation. The efficiency of photodegradation is influenced by turbidity, dissolved organic matter (DOM), and water depth. In clear, shallow waters with high UV penetration, macrolide degradation rates increase, whereas in turbid environments, photodegradation is significantly hindered. Experimental studies indicate that erythromycin has a photodegradation half-life of 4–10 days, while clarithromycin and azithromycin degrade more slowly due to their structural modifications[3, 54, 66, 67]. Hydrolysis is another potential degradation pathway, but most macrolides exhibit chemical stability in neutral and alkaline pH, resulting in prolonged persistence in environmental waters. Hydrolysis rates are significantly influenced by temperature, pH, and the presence of catalytic ions, with degradation accelerating in strongly acidic or basic conditions. Erythromycin, for example, undergoes acid-catalyzed hydrolysis in wastewater treatment plants, producing anhydroerythromycin, a metabolite with reduced antimicrobial activity. However, under environmentally relevant conditions, hydrolysis half-lives often exceed several weeks to months, rendering this process ineffective as a major attenuation pathway[68, 69]. Biodegradation is a critical factor in determining the long-term persistence of macrolides, yet these antibiotics are poorly biodegradable due to their complex lactone ring structure, which resists enzymatic breakdown. Under aerobic conditions, macrolide degradation is limited, as most microbial communities lack the enzymatic capacity to metabolize these compounds efficiently. In contrast, anaerobic degradation in sediments and sludge environments may be more effective, as certain obligate and facultative anaerobic bacteria can partially metabolize macrolides through reductive pathways. However, incomplete degradation often results in the formation of bioactive transformation products (TPs), which may retain antimicrobial properties and continue to exert selective pressure on microbial communities. Specific bacterial strains, such as *Pseudomonas putida* and *Actinobacteria*, have demonstrated co-metabolic degradation of macrolides when alternative carbon sources are available[3, 8, 70]. The persistence of macrolides varies significantly across different environmental compartments. In surface waters, half-lives typically range from a few days to several weeks, whereas in sediments and soils, macrolides can persist for months to years. Experimental data suggest that erythromycin exhibits a half-life of 5–12 days in water, but when adsorbed to sediments, its persistence can extend beyond 100 days. Clarithromycin and azithromycin, due to their increased stability, demonstrate even longer half-lives, often exceeding 200 days in sedimentary environments. This extended persistence raises concerns about bioaccumulation and trophic transfer, particularly in aquatic organisms exposed to chronic low-dose contamination[71, 72].Beyond their persistence, macrolides pose significant ecotoxicological risks to aquatic life and microbial ecosystems. Low concentrations of these antibiotics in water bodies exert sub-lethal effects on non-target microorganisms, disrupting microbial community structures and altering nutrient cycling processes. One of the most critical consequences of macrolide contamination is the promotion of antimicrobial resistance (AMR). Prolonged exposure to sub-inhibitory macrolide concentrations selects for resistant bacterial strains, facilitating horizontal gene transfer (HGT) of resistance determinants such as *ermB* and *msrA*. This phenomenon has been observed in wastewater treatment plants, river sediments, and coastal marine environments, where antibiotic residues create selective hotspots for resistance evolution. Additionally, macrolide accumulation in aquatic organisms has raised concerns regarding biomagnification and potential human exposure through food chains[3]. **Table 4**. Environmental fate, resistance mechanisms, and AMR potential of macrolides in E. faecium, S. aureus, and P. aeruginosa are shown. Given the persistence and ecological risks associated with macrolides, mitigation strategies must focus on enhanced wastewater treatment, stricter regulatory controls, and improved environmental monitoring. Conventional wastewater treatment plants are often ineffective in fully removing macrolides, necessitating the adoption of advanced treatment technologies such as ozonation, activated carbon adsorption, and membrane filtration. Policy-driven interventions, including pharmaceutical take-back programs, restrictions on veterinary antibiotic use, and stricter discharge regulations for pharmaceutical industries, are essential for reducing environmental macrolide loads. Understanding the environmental fate of macrolides is crucial for designing targeted remediation strategies and predicting long-term ecological impacts. Future research should focus on developing more efficient biodegradation pathways, exploring novel enzymatic degradation techniques, and improving predictive models for macrolide transport and persistence in aquatic ecosystems. Addressing these challenges requires a multidisciplinary approach, integrating expertise from environmental chemistry, microbiology, wastewater engineering, and policy regulation to mitigate the risks associated with macrolide contamination and ensure sustainable water resource management.

Table 4. Environmental Fate, Resistance Mechanisms, and AMR Potential of Macrolides in E. faecium, S. aureus, and P. aeruginosa

Process	Sensitive Structure	Molecular Mechanism	Aerobic/Anaerobic Conditions	Half-life (Water/Sediment)	AMR Potential	Resistance Genes	Involved Bacterial Species	Transformation Product Activity
Sorption	Dimethylamino group, lactone ring	Electrostatic binding with organic matter and soil particles	Both; enhanced in anaerobic sediment	Up to 100–250 days (sediment)	Moderate	Indirect	E. faecium, S. aureus, P. aeruginosa	Generally inactive but can be remobilized
Photodegradation	Chromophores, π bonds	Degradation via •OH and ^1O2 under UV exposure	Aerobic only (surface waters)	4–10 days (erythromycin)	Low	Incomplete inhibition	Mainly S. aureus, weak in P. aeruginosa	Some TPs retain partial activity
Hydrolysis	Lactone, ester bonds	Chemical cleavage under acidic/basic pH	Both; requires non-neutral pH	Weeks to months	Low	Very slow	Reported in S. aureus	TPs like anhydroerythromycin may retain mild activity
Biodegradation (Aerobic)	Methyl, lactone	Non-specific enzymes from aerobic bacteria	Aerobic	Poor (>30–60 days)	Moderate	msrA	E. faecium, S. aureus	Incomplete degradation; TPs may still be bioactive
Biodegradation (Anaerobic)	Carbon side chains	Reductive degradation by anaerobic microbes	Anaerobic (sludge, sediments)	Better than aerobic, still incomplete	High	ermB, msrA	E. faecium, P. aeruginosa	Partially active intermediates possible
Environmental Persistence	Macrolide core ring	Resistance to breakdown; bioaccumulation in tissues	Both; enhanced in anaerobic settings	Up to >250 days (azithromycin)	High	ermB, msrA, mphA	All three species documented	Persistent parent compounds retain antimicrobial activity

Horizontal Gene Transfer and the Spread of Macrolide Resistance

The spread of macrolide resistance among bacterial populations is largely driven by horizontal gene transfer

(HGT), a process that allows bacteria to exchange genetic material outside of traditional reproduction. Unlike vertical gene transfer, where genetic information is passed from parent to offspring, HGT enables rapid adaptation by transferring resistance genes between unrelated bacterial species. This phenomenon is particularly concerning in aquatic environments, where low but persistent concentrations of macrolides create selective pressure, promoting the acquisition and retention of resistance genes.

Macrolide resistance genes, such as *ermB*, *msrA*, and *mefA*, are frequently located on mobile genetic elements (MGEs), including plasmids, transposons, and integrative conjugative elements (ICEs). These MGEs facilitate the transfer of resistance determinants through three primary mechanisms: conjugation, transformation, and transduction. Each of these pathways plays a unique role in shaping bacterial populations and accelerating the spread of antimicrobial resistance in both clinical and environmental settings. Conjugation is one of the most effective means of HGT, involving the direct transfer of plasmids between bacterial cells via a specialized pilus. Many plasmids carrying macrolide resistance genes also encode tra (transfer) genes, which enable self-replication and dissemination across bacterial species. This mechanism has been widely documented in enterococci, streptococci, and staphylococci, where plasmid-mediated transfer of *ermB* has been linked to the rapid emergence of macrolide-resistant strains. In wastewater treatment plants, where diverse bacterial communities coexist under selective pressure from antibiotic residues, conjugation-driven resistance transfer is particularly problematic, as it allows resistance genes to spread between commensal bacteria and potential human pathogens.

Transformation, another major HGT pathway, occurs when bacteria uptake free DNA fragments from their surroundings and integrate them into their genome. This process is facilitated by natural competence, a state in which bacterial cells express specific proteins that allow them to recognize and incorporate extracellular DNA. Environmental bacteria, such as *Acinetobacter* and *Pseudomonas*, have demonstrated the ability to acquire macrolide resistance genes through transformation, particularly in polluted water bodies with high bacterial densities. The presence of antibiotic residues in these environments enhances transformation efficiency, as sub-inhibitory macrolide concentrations can induce stress responses that increase natural competence in susceptible bacteria[73, 74]. Transduction, the third major HGT mechanism, involves the transfer of genetic material via bacteriophages (viruses that infect bacteria). In this process, bacteriophages accidentally package host bacterial DNA, including resistance genes, and introduce it into new bacterial hosts during subsequent infections. While transduction is less common than conjugation or transformation, it plays a crucial role in the long-term maintenance and evolution of resistance genes. Studies have shown that phage-mediated transfer of macrolide resistance genes can occur in sewage systems, hospital effluents, and surface waters, further complicating efforts to control the spread of antimicrobial resistance.

Beyond these primary HGT mechanisms, macrolide resistance genes often reside on integrative conjugative elements (ICEs) and transposons, which act as genetic vehicles that can mobilize resistance determinants across bacterial species. These elements integrate into the bacterial chromosome and can excise themselves under selective pressure, allowing for their dissemination via conjugation or transformation. The Tn916 transposon family, for example, has been widely identified in Gram-positive bacteria, carrying resistance genes for macrolides, tetracyclines, and aminoglycosides. The presence of such transposons in wastewater treatment plants and agricultural runoff sites highlights the environmental dimension of macrolide resistance spread[75]. The persistence of macrolides in aquatic environments not only promotes HGT but also facilitates co-selection for multidrug resistance (MDR). Many macrolide resistance genes are co-located with resistance determinants for β-lactams, fluoroquinolones, and sulfonamides, meaning that exposure to a single antibiotic can select for bacteria resistant to multiple drug classes. This phenomenon has been observed in river sediments and municipal wastewater, where bacteria carrying multidrug-resistant plasmids have been isolated from samples containing macrolide residues. Such findings emphasize the need for comprehensive environmental monitoring programs to track resistance gene dissemination and mitigate its public health impact. Given the complexity of HGT and its role in macrolide resistance, addressing this issue requires a multi-pronged approach. Reducing environmental antibiotic contamination through improved wastewater treatment, stricter pharmaceutical discharge regulations, and sustainable agricultural practices is critical for minimizing selective pressure. Additionally, advancing surveillance efforts using whole-genome sequencing (WGS) [76] and metagenomics can provide deeper insights into HGT dynamics and resistance gene hotspots, guiding targeted intervention strategies. The rapid and uncontrolled spread of macrolide resistance via horizontal gene transfer poses a significant threat to global health, necessitating coordinated efforts between microbiologists, environmental scientists, and public health policymakers. Understanding the mechanisms driving this dissemination is key to developing effective containment strategies and preserving the efficacy of macrolides and other antibiotics for future generations[76-78].

Conclusion

The presence of macrolide antibiotics in aquatic environments, particularly in municipal and hospital wastewater, poses a significant challenge due to their chemical stability and persistence. Even at low concentrations, these compounds exert selective pressure on microbial communities, accelerating the evolution and horizontal transfer of antibiotic resistance genes (ARGs). As a result, effective removal of macrolides from wastewater is a critical priority in addressing pharmaceutical pollution and mitigating the risks associated with antimicrobial resistance

(AMR). A comprehensive review of macrolide removal methods indicates that no single technology can achieve complete and risk-free elimination of these compounds. Advanced oxidation processes (AOPs), including ozonation, photocatalysis, and hydroxyl radical-based treatments, have demonstrated high efficiency (>90%) in degrading macrolides. However, concerns remain regarding the formation of potentially toxic transformation products and the high operational costs associated with these methods. Similarly, adsorption-based techniques using activated carbon and biochar provide a cost-effective and environmentally friendly approach, though their long-term sustainability depends on efficient regeneration and disposal strategies. Natural treatment systems, such as constructed wetlands and microalgal bioreactors, have shown promise in gradual macrolide degradation through microbial metabolism, photodegradation, and root uptake. These approaches are particularly suitable for decentralized wastewater treatment, but their efficiency is subject to seasonal variations and hydraulic retention time. Meanwhile, membrane filtration technologies, such as nanofiltration (NF) and reverse osmosis (RO), offer near-complete removal of macrolides, but membrane fouling, high energy demands, and the management of concentrated brine reject streams pose practical challenges for large-scale application. Ultimately, achieving optimal macrolide removal requires a multi-stage, integrated approach that combines physicochemical, biological, and adsorption-based methods to maximize efficiency while minimizing environmental impact. Additionally, enhanced regulatory frameworks, stricter pharmaceutical disposal policies, and continuous environmental monitoring are essential to reducing macrolide contamination at its source and preventing the further spread of antibiotic resistance in aquatic ecosystems. A holistic strategy that incorporates technological innovation, environmental policy, and responsible antibiotic stewardship is necessary to effectively manage macrolide pollution and safeguard water quality for future generations.

ACKNOWLEDGMENTS: None
CONFLICT OF INTEREST: The author declares no conflict of interest
FINANCIAL SUPPORT: None
ETHICS STATEMENT: Not applicable

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