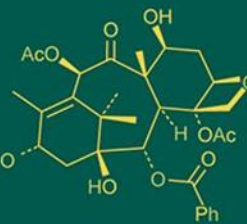
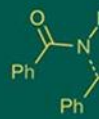
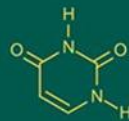
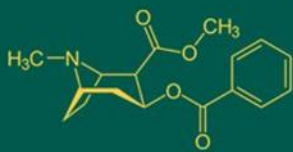


International Journal of Advanced Biochemistry Research



ISSN Print: 2617-4693
ISSN Online: 2617-4707
NAAS Rating (2026): 5.29
IJABR 2026; 10(7): 248-262
www.biochemjournal.com
Received: 27-04-2026
Accepted: 30-05-2026

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Antimicrobial resistance in aquatic ecosystems: Sources, transmission pathways, ecological impacts, and mitigation strategies

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DOI: <https://www.doi.org/10.33545/26174693.2026.v10.i7d.8986>

Abstract

Antimicrobial resistance (AMR) has emerged as one of the most critical global health and environmental challenges of the twenty-first century, with aquatic ecosystems increasingly recognized as important reservoirs and dissemination pathways for antibiotic-resistant bacteria (ARB), antibiotic resistance genes (ARGs), and mobile genetic elements (MGEs). Aquatic environments receive continuous inputs of antibiotics and resistant microorganisms from anthropogenic activities, including municipal wastewater discharge, hospital effluents, agricultural runoff, livestock production, aquaculture practices, and pharmaceutical manufacturing. The persistence of these contaminants in water columns, sediments, and biofilms creates selective pressure that promotes the maintenance, evolution, and horizontal transfer of resistance determinants among diverse microbial communities. Furthermore, the coexistence of antibiotics with heavy metals, disinfectants, microplastics, and other environmental pollutants enhances co-selection processes and accelerates the emergence of multidrug-resistant microorganisms. Aquatic ecosystems therefore function not only as passive reservoirs but also as active evolutionary hotspots facilitating resistance dissemination across environmental, animal, and human interfaces. Transmission of resistant bacteria and ARGs may occur through drinking water, seafood consumption, recreational activities, and occupational exposure, highlighting the importance of the One Health perspective in understanding environmental AMR dynamics. This review comprehensively examines the major sources, transmission mechanisms, ecological consequences, public health implications, and mitigation strategies associated with AMR in aquatic ecosystems. Strengthening environmental surveillance, improving wastewater management, promoting sustainable aquaculture practices, and implementing integrated One Health interventions are essential for mitigating the global spread of antimicrobial resistance and protecting ecosystem and public health.

Keywords: Antimicrobial resistance, aquatic ecosystems, antibiotic resistance genes, antibiotic-resistant bacteria, horizontal gene transfer, One Health, wastewater, aquaculture

1. Introduction

Antimicrobial resistance (AMR) has emerged as one of the most pressing global public health challenges of the 21st century, threatening the effective prevention and treatment of infectious diseases caused by bacteria, viruses, parasites, and fungi. The rapid increase in multidrug-resistant (MDR) pathogens has been driven primarily by the excessive and inappropriate use of antibiotics in human medicine, veterinary practices, agriculture, and aquaculture systems (World Health Organization, 2014; Murray *et al.*, 2022). While clinical settings have traditionally been considered the primary focus of AMR studies, increasing evidence suggests that environmental compartments, particularly aquatic ecosystems, play a crucial role in the evolution, maintenance, and dissemination of antimicrobial resistance (Berendonk *et al.*, 2015; Pruden *et al.*, 2013). Aquatic ecosystems, including rivers, lakes, estuaries, groundwater, coastal waters, and aquaculture systems, act as dynamic reservoirs for antibiotics, antibiotic-resistant bacteria (ARB), and antibiotic resistance genes (ARGs). These systems continuously receive inputs from municipal wastewater, hospital effluents, agricultural runoff, livestock production, and pharmaceutical industries, leading to the accumulation of selective agents that promote the persistence of resistant microbial populations [41, 29, 6, 31]

(Rizzo *et al.*, 2013; Waseem *et al.*, 2017). Wastewater treatment plants, although designed to reduce microbial load, are not fully efficient in removing antibiotics and ARGs, resulting in their continuous release into receiving water bodies (Michael *et al.*, 2013). Once introduced into aquatic environments, ARGs and ARB can persist for extended periods and spread through horizontal gene transfer mechanisms such as conjugation, transformation, and transduction. Mobile genetic elements, including plasmids, integrons, and transposons, play a central role in the dissemination of resistance determinants among phylogenetically diverse bacterial communities (Gillings, 2014; Stalder *et al.*, 2012). The aquatic environment thus acts not only as a passive reservoir but also as an active evolutionary hotspot where genetic exchange and selection of resistance traits occur continuously. The concept of the environmental resistome highlights the presence of a vast collection of resistance genes in natural environments, many of which predate the clinical use of antibiotics (D'Costa *et al.*, 2011). However, anthropogenic activities have significantly amplified the abundance and mobility of these genes, accelerating their transfer into clinically relevant pathogens. Aquatic ecosystems, due to their connectivity and mobility, facilitate the long-range transport of resistant microorganisms, making them key nodes in the global AMR dissemination network (Martí *et al.*, 2014). Different aquatic compartments contribute uniquely to AMR propagation. Freshwater systems act as primary conduits for terrestrial pollution, while marine and coastal ecosystems receive cumulative inputs from river discharge and anthropogenic waste. Sediments serve as long-term reservoirs of antibiotics and ARGs, whereas biofilms provide protective niches that enhance microbial survival and horizontal gene transfer efficiency (Balcázar *et al.*, 2015; Flores-Vargas *et al.*, 2021). Aquaculture environments further intensify selective pressure due to the frequent application of antibiotics for disease prevention and growth promotion, thereby increasing the risk of resistance development in aquatic bacteria such as *Aeromonas*, *Vibrio*, and *Pseudomonas* spp. (Cabello *et al.*, 2013). The environmental dimension of AMR has gained increasing attention under the One Health framework, which recognizes the interconnectedness of human, animal, and environmental health. Understanding the role of aquatic ecosystems in AMR dissemination is essential for developing effective surveillance systems, risk assessment models, and mitigation strategies. Despite significant progress, major knowledge gaps still exist regarding the fate, transport, and ecological impacts of ARGs in complex aquatic environments. Therefore, this review aims to (i) analyze the major sources of antimicrobial resistance in aquatic ecosystems, (ii) describe transmission pathways of ARGs and ARB, (iii) evaluate ecological and public health impacts, and (iv) discuss mitigation strategies for controlling the spread of AMR in aquatic environments. This review is a narrative review based on published literature retrieved from major scientific databases, including PubMed, Scopus, Web of Science, and Google Scholar. Relevant peer-reviewed articles, reviews, and reports related to antimicrobial resistance in aquatic ecosystems were critically evaluated to summarize current knowledge regarding sources, transmission pathways, ecological impacts, public health implications, and mitigation strategies within the One Health framework [40, 27, 28, 32, 26, 3, 1, 24, 38, 15, 6, 27, 5, 1, 2, 10, 5, 14, 7, 8].

2. Aquatic ecosystems as reservoirs of antimicrobial resistance: Aquatic ecosystems are increasingly recognized as major environmental reservoirs and dissemination hubs for antimicrobial resistance (AMR) (Martí *et al.*, 2014; Waseem *et al.*, 2017). Rivers, lakes, estuaries, coastal waters, groundwater, aquaculture ponds, and marine environments receive continuous inputs of antibiotics, resistant bacteria, antibiotic resistance genes (ARGs), and mobile genetic elements (MGEs) from anthropogenic activities (Martí *et al.*, 2014). These contaminants originate from municipal wastewater, hospital effluents, pharmaceutical industries, agricultural runoff, livestock production, and aquaculture operations (Waseem *et al.*, 2017). Once introduced into aquatic environments, resistant microorganisms and ARGs can persist for extended periods and spread among diverse microbial communities through horizontal gene transfer, thereby contributing to the environmental resistome (Martí *et al.*, 2014; Balcázar *et al.*, 2015). Unlike terrestrial ecosystems, aquatic environments provide interconnected habitats where microorganisms from multiple sources converge and interact under dynamic physicochemical conditions (Martí *et al.*, 2014). Water currents facilitate the transport of resistant bacteria over long distances, while sediments and suspended particles act as sinks that accumulate antibiotics and ARGs (Waseem *et al.*, 2017). The coexistence of antibiotics, heavy metals, disinfectants, and other environmental pollutants creates selective pressure that promotes the maintenance and evolution of multidrug-resistant microorganisms (Martí *et al.*, 2014; Waseem *et al.*, 2017). Consequently, aquatic ecosystems function not only as repositories of antimicrobial resistance but also as active environments where resistance determinants are amplified and redistributed across ecological boundaries (Martí *et al.*, 2014). The major aquatic reservoirs contributing to the persistence and dissemination of AMR are summarized in Table 1 [27, 40, 14].

Environmental bacteria naturally possess a wide diversity of resistance determinants that collectively constitute the environmental resistome (Martí *et al.*, 2014). Human activities substantially increase the abundance and diversity of these genes by introducing antibiotics and resistant microorganisms into aquatic habitats (Waseem *et al.*, 2017). Mobile genetic elements, including plasmids, integrons, transposons, and bacteriophages, facilitate the exchange of ARGs among phylogenetically distinct bacterial species (Martí *et al.*, 2014; Balcázar *et al.*, 2015). This genetic exchange enables environmental bacteria to transfer clinically relevant resistance genes to opportunistic and pathogenic microorganisms, thereby strengthening the link between environmental and clinical antimicrobial resistance (Martí *et al.*, 2014). Freshwater ecosystems, particularly rivers and lakes located near densely populated or agricultural regions, are among the most significant reservoirs of AMR (Waseem *et al.*, 2017). Wastewater treatment plants, although effective at reducing microbial loads, often fail to completely eliminate antibiotics, ARGs, and resistant bacteria before treated effluents are discharged into receiving waters (Waseem *et al.*, 2017). As a result, downstream aquatic environments frequently contain elevated concentrations of resistant microorganisms compared with upstream locations (Martí *et al.*, 2014). Agricultural runoff carrying animal manure and veterinary antibiotics further increases the abundance of ARGs in freshwater ecosystems, making these environments important hotspots for resistance dissemination

(Waseem *et al.*, 2017). Marine and coastal ecosystems also contribute significantly to the environmental dissemination of antimicrobial resistance (Martí *et al.*, 2014). Coastal waters receive untreated sewage, industrial discharges, stormwater runoff, and riverine inputs containing antibiotics and resistant bacteria (Waseem *et al.*, 2017). Shellfish-growing areas and coastal sediments can accumulate ARGs because filter-feeding organisms concentrate microorganisms from surrounding waters (Martí *et al.*, 2014). The extensive exchange of water masses facilitates regional dissemination of resistance determinants, increasing the possibility of transmission through seafood consumption and recreational water exposure (Martí *et al.*, 2014; Waseem *et al.*, 2017).

Aquaculture environments represent another critical reservoir of antimicrobial resistance because antibiotics are frequently used for disease prevention and treatment in fish and shellfish production (Martí *et al.*, 2014). Continuous or repeated exposure to antimicrobial agents creates strong selective pressure, favoring resistant bacterial populations in water, sediments, cultured animals, and associated microbial biofilms (Martí *et al.*, 2014; Balcázar *et al.*, 2015). Important aquatic bacterial genera such as *Aeromonas*, *Vibrio*, *Pseudomonas*, and *Shewanella* commonly harbor multiple ARGs and may act as reservoirs from which resistance determinants spread to other environmental or pathogenic bacteria (Martí *et al.*, 2014). Sediments and biofilms are particularly important ecological niches for the persistence of antimicrobial resistance (Balcázar *et al.*, 2015; Flores-Vargas *et al.*, 2021). Sediments accumulate organic matter,

antibiotics, metals, and microbial biomass, allowing resistant bacteria and ARGs to persist even when concentrations decline in the overlying water column (Balcázar *et al.*, 2015). Biofilms provide a protective extracellular matrix that enhances bacterial survival under environmental stress while maintaining high cell density and close physical contact among microorganisms (Flores-Vargas *et al.*, 2021). These conditions significantly increase the frequency of horizontal gene transfer through conjugation, transformation, and bacteriophage-mediated transduction (Balcázar *et al.*, 2015; Flores-Vargas *et al.*, 2021). Consequently, aquatic biofilms are regarded as hotspots for the maintenance, evolution, and dissemination of antimicrobial resistance within aquatic ecosystems (Balcázar *et al.*, 2015). Overall, aquatic ecosystems represent dynamic reservoirs where environmental, veterinary, and human-associated bacteria continuously interact (Martí *et al.*, 2014). The convergence of antibiotics, resistant microorganisms, ARGs, and mobile genetic elements under favorable ecological conditions facilitates the persistence and dissemination of antimicrobial resistance across environmental compartments and ultimately contributes to the transmission of resistance to humans, animals, and food production systems (Martí *et al.*, 2014; Waseem *et al.*, 2017). Understanding the ecological role of aquatic ecosystems is therefore fundamental for developing effective surveillance programs and implementing mitigation strategies within the One Health framework (Martí *et al.*, 2014; Flores-Vargas *et al.*, 2021)^[27, 40, 14].

Table 1: Aquatic ecosystems as reservoirs of antimicrobial resistance (AMR)

Aquatic ecosystem	Major AMR sources	Predominant resistant bacteria	Major ARGs	Ecological significance
Rivers	Municipal wastewater, agricultural runoff	<i>Aeromonas</i> , <i>E. coli</i> , <i>Pseudomonas</i>	tet, sul, bla	Major dissemination pathway
Lakes	Domestic sewage, livestock runoff	<i>Aeromonas</i> , <i>Enterococcus</i>	tet, sul	Long-term ARG accumulation
Estuaries	River discharge, urban runoff	<i>Vibrio</i> , <i>E. coli</i>	qnr, sul	Mixing zone for freshwater and marine bacteria
Marine waters	Sewage, aquaculture	<i>Vibrio</i> , <i>Pseudomonas</i>	bla, qnr	Seafood-associated AMR
Aquaculture ponds	Antibiotic use	<i>Aeromonas</i> , <i>Vibrio</i> , <i>Edwardsiella</i>	tet, sul, qnr	High selective pressure
Sediments	Antibiotic deposition	Diverse bacteria	All major ARGs	Long-term environmental reservoir
Biofilms	All aquatic habitats	Mixed microbial community	Multiple ARGs	Hotspot of horizontal gene transfer

3. Sources of antimicrobial resistance in aquatic environments: The increasing prevalence of antimicrobial resistance (AMR) in aquatic ecosystems is primarily driven by continuous anthropogenic inputs of antibiotics, antibiotic-resistant bacteria (ARB), antibiotic resistance genes (ARGs), and other selective contaminants. Multiple environmental sources contribute to the dissemination of resistance determinants into freshwater, estuarine, and marine ecosystems, creating interconnected pathways that facilitate the emergence and persistence of resistant microbial populations. Understanding these sources is essential for developing effective strategies to reduce environmental AMR under the One Health framework (Berendonk *et al.*, 2015). The principal sources of antimicrobial resistance in aquatic ecosystems, together with their associated contaminants and environmental dissemination pathways, are summarized in Table 2.

3.1 Municipal wastewater

Municipal wastewater is considered one of the largest contributors of antimicrobial resistance to aquatic environments. Domestic sewage contains antibiotics excreted by humans, resistant bacteria from the intestinal microbiota, disinfectants, and numerous resistance genes. Although wastewater treatment plants (WWTPs) substantially reduce microbial loads, conventional treatment processes cannot completely remove antibiotics, ARGs, or mobile genetic elements. Consequently, treated effluents discharged into rivers and lakes continue to introduce resistant microorganisms into receiving waters (Michael *et al.*, 2013; Rizzo *et al.*, 2013). Furthermore, activated sludge systems provide nutrient-rich conditions with high microbial density, promoting horizontal gene transfer among environmental bacteria. Plasmids, integrons, and transposons carrying clinically relevant resistance genes may spread rapidly within WWTP microbial communities before being released into natural aquatic ecosystems (Berendonk *et al.*, 2015)^[28, 32, 6].

3.2 Hospital effluents

Hospitals represent concentrated point sources of antimicrobial resistance because of the extensive use of broad-spectrum antibiotics for treating infectious diseases. Hospital wastewater frequently contains elevated concentrations of antibiotics, multidrug-resistant pathogens, disinfectants, and resistance genes, often exceeding those found in municipal sewage. Clinically important bacteria such as *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, and *Enterococcus* spp. have been detected in untreated hospital effluents along with carbapenemase and extended-spectrum β -lactamase (ESBL) genes (Hocquet *et al.*, 2016) ^[18]. When hospital wastewater is discharged without advanced treatment, these resistant microorganisms and ARGs enter wastewater treatment systems and subsequently spread into downstream aquatic environments, increasing the risk of environmental dissemination.

3.3 Agricultural runoff and livestock production

The extensive use of antibiotics in livestock production contributes significantly to environmental AMR. Antibiotics administered to cattle, poultry, pigs, and other food-producing animals are often incompletely metabolized, with a large proportion excreted through urine and feces. Animal manure therefore contains residual antibiotics, resistant bacteria, and ARGs, which can be transported into nearby rivers, ponds, and groundwater through rainfall, irrigation, and surface runoff (Heuer *et al.*, 2011) ^[17]. Repeated manure application also enriches agricultural soils with resistance determinants, creating secondary sources of contamination for adjacent aquatic ecosystems. In addition to antibiotics, heavy metals commonly added to animal feed may co-select for antimicrobial resistance by maintaining resistance plasmids within environmental bacterial populations (Seiler & Berendonk, 2012) ^[35].

3.4 Aquaculture activities

Aquaculture is recognized as one of the fastest-growing sectors contributing to antimicrobial resistance in aquatic ecosystems. Antibiotics are frequently administered for disease treatment, prophylaxis, and, in some regions, growth promotion in fish and shellfish production. Because many antibiotics are directly applied to culture water or medicated feed, a substantial proportion remains in the surrounding environment, where it exerts selective pressure on aquatic microbial communities (Cabello, 2006) ^[8]. Sediments

beneath aquaculture facilities accumulate antibiotic residues and resistant bacteria, serving as long-term reservoirs of ARGs. Opportunistic aquatic pathogens such as *Aeromonas*, *Vibrio*, *Edwardsiella*, and *Pseudomonas* often acquire multidrug resistance through horizontal gene transfer, increasing the possibility of transmission to cultured animals, wild aquatic organisms, and ultimately humans through seafood consumption (Cabello *et al.*, 2013) ^[8].

3.5 Pharmaceutical manufacturing effluents

Pharmaceutical manufacturing plants may release wastewater containing exceptionally high concentrations of active antimicrobial compounds if treatment processes are inadequate. Such industrial discharges create intense selective pressure that favors the survival of highly resistant bacterial populations. Studies conducted near pharmaceutical manufacturing facilities have reported antibiotic concentrations several thousand times higher than those normally detected in municipal wastewater, making these environments important hotspots for the evolution and dissemination of resistance genes (Larsson, 2014) ^[22].

3.6 Urban stormwater and surface runoff

Urban runoff transports antibiotics, resistant bacteria, disinfectants, microplastics, heavy metals, and other contaminants from residential, commercial, and industrial areas into nearby water bodies. Combined sewer overflows during heavy rainfall events further increase the direct release of untreated sewage into aquatic ecosystems. Stormwater retention ponds and urban drainage systems may therefore become reservoirs where resistant bacteria accumulate and persist before entering rivers and coastal waters (Taylor *et al.*, 2011) ^[39].

3.7 Wildlife and natural environmental reservoirs

Wild birds, aquatic mammals, reptiles, amphibians, and migratory fish contribute to the natural dissemination of antimicrobial resistance across geographically distant aquatic habitats. These animals may acquire resistant bacteria from contaminated environments and subsequently transport them through migration and ecological interactions. Although antimicrobial resistance naturally exists within environmental microbial communities, anthropogenic activities substantially amplify the abundance and diversity of ARGs beyond natural background levels (Allen *et al.*, 2010) ^[2].

Table 2: Major sources and pathways of antimicrobial resistance in aquatic environments

Source	Major contaminants	Entry pathway into aquatic systems	Representative references
Municipal wastewater	Antibiotics, antibiotic-resistant bacteria (ARB), ARGs	Wastewater treatment plant (WWTP) effluent discharge	Michael <i>et al.</i> , 2013
Hospital wastewater	Multidrug-resistant pathogens, high antibiotic loads	Direct or partially treated discharge	Hocquet <i>et al.</i> , 2016
Livestock production runoff	Veterinary antibiotics, manure-associated ARGs	Surface runoff and drainage systems	Heuer <i>et al.</i> , 2011
Aquaculture systems	Therapeutic and prophylactic antibiotics	Direct water application and sediment deposition	Cabello <i>et al.</i> , 2013
Pharmaceutical industries	High-concentration antibiotic residues	Industrial effluent discharge	Larsson, 2014

4. Antibiotics commonly detected in aquatic systems

Antibiotics are among the most frequently detected pharmaceutical contaminants in aquatic ecosystems due to

their extensive use in human medicine, veterinary practice, livestock production, and aquaculture. Following administration, a considerable proportion of antibiotics is

excreted either unchanged or as biologically active metabolites, which subsequently enter wastewater treatment plants through domestic sewage, hospital discharges, and agricultural runoff. Since conventional wastewater treatment processes are not specifically designed to remove these micropollutants completely, residual antibiotics are continuously released into rivers, lakes, estuaries, coastal waters, and groundwater (Michael *et al.*, 2013; Maghsodian *et al.*, 2022). Their persistent presence exerts selective pressure on environmental microorganisms, facilitating the development and dissemination of antibiotic-resistant bacteria (ARB) and antibiotic resistance genes (ARGs) (Kümmerer, 2009). Among the numerous antibiotics detected in aquatic environments, tetracyclines, sulfonamides, fluoroquinolones, macrolides, β -lactams, aminoglycosides, and glycopeptides are reported most frequently. Their occurrence, persistence, and environmental fate depend on physicochemical properties such as water solubility, adsorption coefficients, degradation rates, and susceptibility to photolysis or biodegradation (Maghsodian *et al.*, 2022). While some antibiotics remain dissolved in the water column and are transported over long distances, others strongly adsorb to sediments where they may persist for months or years [28, 25, 21].

4.1 Tetracyclines

Tetracyclines are among the most widely used broad-spectrum antibiotics in both human and veterinary medicine. Common representatives include tetracycline, oxytetracycline, chlortetracycline, and doxycycline. According to Xu *et al.* (2021), more than half of administered tetracyclines are excreted without complete metabolism, allowing significant quantities to enter aquatic ecosystems through wastewater discharge and agricultural runoff. Due to their strong affinity for clay minerals and organic matter, tetracyclines readily accumulate in sediments, where they persist and continue exerting selective pressure on microbial communities. Numerous studies have demonstrated that the presence of tetracyclines is strongly associated with the enrichment of tetracycline resistance genes, including *tetA*, *tetM*, *tetO*, and *tetX* (Xu *et al.*, 2021) [42].

4.2 Sulfonamides

Sulfonamides are frequently detected in freshwater environments because of their high water solubility and widespread use in livestock production and human healthcare. Sulfamethoxazole, sulfadiazine, sulfamethazine, and sulfathiazole are among the most commonly reported compounds. As highlighted by Duan *et al.* (2022), sulfonamides exhibit relatively low adsorption to sediments compared with tetracyclines, enabling them to persist in surface water and groundwater over extended periods. Their continuous environmental occurrence contributes to the maintenance and dissemination of sulfonamide resistance genes such as *sul1*, *sul2*, and *sul3*, particularly within class 1 integrons and other mobile genetic elements (Duan *et al.*, 2022) [13].

4.3 Fluoroquinolones

Fluoroquinolones, including ciprofloxacin, norfloxacin, ofloxacin, and enrofloxacin, are broad-spectrum antibiotics extensively prescribed for bacterial infections in both humans and animals. According to Maghsodian *et al.* (2022), these compounds exhibit high environmental persistence due to

their chemical stability and strong adsorption to suspended particles and sediments. Fluoroquinolones have been detected in rivers, lakes, estuaries, wastewater effluents, and marine sediments worldwide. Their prolonged persistence promotes the selection of quinolone resistance genes (*qnrA*, *qnrB*, and *qnrS*) as well as chromosomal mutations affecting DNA gyrase and topoisomerase IV, thereby accelerating the evolution of resistant bacterial populations (Maghsodian *et al.*, 2022) [25].

4.4 Macrolides

Macrolide antibiotics, including erythromycin, clarithromycin, azithromycin, and roxithromycin, are commonly used for the treatment of respiratory and gastrointestinal infections. Michael *et al.* (2013) reported that macrolides are frequently detected in municipal wastewater treatment plant effluents because conventional treatment technologies achieve only partial removal. Their continuous release into aquatic ecosystems promotes the enrichment of macrolide resistance genes such as *erm* and *mef*, which are widely distributed among environmental bacteria. Clarithromycin and erythromycin have been consistently reported as dominant macrolides in rivers and wastewater across Europe and Asia (Michael *et al.*, 2013) [28].

4.5 β -Lactam antibiotics

β -Lactam antibiotics, including amoxicillin, ampicillin, penicillin, and cephalosporins, represent the most extensively prescribed antimicrobial agents worldwide. Although β -lactams generally undergo rapid hydrolysis because of the instability of their β -lactam ring, Kümmerer (2009) emphasized that their continuous discharge through municipal and hospital wastewater results in their frequent detection in aquatic environments. Persistent exposure to β -lactam residues facilitates the maintenance and dissemination of clinically important β -lactamase genes such as *blaTEM*, *blaSHV*, *blaCTX-M*, and carbapenemase genes among environmental microorganisms [21].

4.6 Aminoglycosides and glycopeptides

Aminoglycosides (gentamicin, kanamycin, streptomycin, and amikacin) and glycopeptides (primarily vancomycin) are detected less frequently than tetracyclines or sulfonamides but remain environmentally significant because of their extensive use in clinical settings. According to Maghsodian *et al.* (2022), these antibiotics mainly enter aquatic environments through hospital wastewater and pharmaceutical manufacturing effluents. Continuous environmental exposure supports the persistence of aminoglycoside-modifying enzyme genes and glycopeptide resistance determinants such as *vanA* and *vanB*, which pose significant concerns for public health [25].

5. Antibiotic resistance genes (ARGs) in aquatic ecosystems: Antibiotic resistance genes (ARGs) are genetic determinants that enable bacteria to survive exposure to antimicrobial agents by encoding various resistance mechanisms, including antibiotic degradation, target modification, reduced membrane permeability, active efflux, and metabolic bypass. In aquatic ecosystems, ARGs have emerged as environmental contaminants because they can persist independently of their bacterial hosts and be transferred among environmental and pathogenic microorganisms. As emphasized by Berendonk *et al.* (2015),

aquatic environments are not merely passive reservoirs but active evolutionary hotspots where antibiotics, resistant bacteria, and mobile genetic elements interact, promoting the emergence and dissemination of antimicrobial resistance. Aquatic environments receive ARGs from multiple anthropogenic sources, including municipal wastewater, hospital effluents, pharmaceutical industries, agricultural runoff, livestock manure, and aquaculture facilities. These environments provide favorable ecological conditions for bacterial growth and horizontal gene transfer because microorganisms occur at high densities within water columns, sediments, and biofilms. Consequently, ARGs can persist for prolonged periods and spread among phylogenetically diverse bacterial populations, increasing the environmental resistome and creating potential pathways for transmission to clinically important pathogens. Martí *et al.* (2014) and Berendonk *et al.* (2015) highlighted that rivers, wastewater treatment plants, and aquaculture systems serve as major reservoirs facilitating the environmental circulation of resistance genes [6, 27].

5.1 Major Antibiotic resistance genes

Numerous ARGs have been identified in aquatic ecosystems, with their distribution largely reflecting the antibiotics commonly used in human medicine, veterinary practice, and aquaculture.

Tetracycline resistance genes (tet genes)

Tetracycline resistance genes are among the most abundant ARGs detected in aquatic environments. Common members include *tetA*, *tetB*, *tetM*, *tetO*, *tetW*, and *tetX*. According to Roberts (2012), these genes confer resistance through three principal mechanisms: efflux pumps, ribosomal protection proteins, and enzymatic antibiotic inactivation. Environmental monitoring studies have consistently detected *tet* genes in rivers, wastewater, sediments, aquaculture ponds, and marine ecosystems, reflecting the widespread use of tetracyclines in both agriculture and aquaculture [33].

Sulfonamide resistance genes (sul genes)

The genes *sul1*, *sul2*, and *sul3* encode altered forms of dihydropteroate synthase that reduce susceptibility to sulfonamide antibiotics. Among these, *sul1* is particularly important because it is commonly associated with Class 1 integrons, which facilitate the capture and dissemination of multiple resistance genes. Gillings (2014) described *sul1* as an important molecular indicator of anthropogenic pollution because of its strong association with wastewater and human activities. High abundances of *sul* genes have been reported in wastewater treatment plants, rivers, aquaculture sediments, and estuarine environments [15].

β-Lactam resistance genes (bla genes)

β-Lactam resistance is primarily mediated by β-lactamase enzymes encoded by genes such as *blaTEM*, *blaSHV*, *blaCTX-M*, *blaOXA*, *blaNDM*, and *blaKPC*. These enzymes hydrolyze the β-lactam ring, rendering penicillins, cephalosporins, and carbapenems ineffective. Berendonk *et al.* (2015) [6] reported that *bla* genes are increasingly detected in aquatic environments impacted by hospital wastewater and municipal sewage, indicating the environmental dissemination of clinically significant resistance determinants. Carbapenemase genes such as *blaNDM* and *blaKPC* are of particular concern because they confer

resistance to last-line antibiotics used in critical healthcare settings.

Quinolone resistance genes (qnr genes)

Plasmid-mediated quinolone resistance genes, including *qnrA*, *qnrB*, *qnrC*, *qnrD*, and *qnrS*, protect bacterial DNA gyrase and topoisomerase IV from inhibition by quinolone antibiotics. These genes are widely distributed among aquatic bacteria and frequently coexist with β-lactam resistance genes on transferable plasmids. Their widespread occurrence in wastewater and surface waters highlights the important role of aquatic ecosystems in disseminating multidrug resistance.

Macrolide resistance genes (erm genes)

Macrolide resistance is primarily mediated by methylase genes such as *ermA*, *ermB*, *ermC*, and *ermF*, which methylate bacterial ribosomal RNA and reduce antibiotic binding. Environmental studies have consistently identified *erm* genes in wastewater treatment plants, rivers, and sediments receiving municipal and hospital effluents. Because these genes are frequently associated with mobile genetic elements, they can spread rapidly among Gram-positive and Gram-negative bacteria.

Colistin resistance genes (mcr genes)

The discovery of the plasmid-mediated colistin resistance gene *mcr-1* represented a major milestone in antimicrobial resistance research because colistin is regarded as a last-resort antibiotic for multidrug-resistant Gram-negative infections. Since its first description, additional variants (*mcr-2* to *mcr-10*) have been reported in environmental waters, aquaculture systems, livestock farms, and wastewater. Their occurrence in aquatic environments raises serious concerns regarding the environmental dissemination of resistance to critically important antimicrobials.

Vancomycin resistance genes (van genes)

Vancomycin resistance genes (*vanA*, *vanB*, *vanC*, and related variants) are primarily associated with *Enterococcus* species but have also been detected in environmental bacteria inhabiting wastewater and aquatic ecosystems. These genes modify the bacterial cell wall precursor, thereby reducing vancomycin binding and conferring high-level resistance. Their environmental occurrence demonstrates that clinically important resistance determinants are no longer confined to healthcare settings.

5.2 Mobile genetic elements (MGEs)

The dissemination of ARGs in aquatic ecosystems is largely driven by mobile genetic elements (MGEs), including plasmids, integrons, transposons, insertion sequences, and bacteriophages. These genetic platforms facilitate horizontal gene transfer among unrelated bacterial species and accelerate the spread of multidrug resistance. According to Berendonk *et al.* (2015) [6], MGEs represent one of the principal mechanisms linking environmental bacteria with clinically important pathogens. Plasmids are self-replicating extrachromosomal DNA molecules that frequently carry multiple resistance genes, enabling simultaneous resistance to several antibiotic classes. Integrons, particularly Class 1 integrons carrying the *intI1* gene, capture and express resistance gene cassettes and are widely recognized as indicators of anthropogenic pollution. Transposons facilitate

movement of ARGs between plasmids and bacterial chromosomes, while bacteriophages can mediate ARG transfer through transduction. Environmental biofilms further enhance these transfer processes by maintaining dense microbial communities in close physical contact, thereby increasing opportunities for genetic exchange.

5.3 Environmental resistome

The environmental resistome comprises the complete collection of antibiotic resistance genes present within environmental microbial communities, including both naturally occurring and anthropogenically enriched genes. As described by Allen *et al.* (2010), natural ecosystems have long contained resistance genes; however, human activities have dramatically increased their abundance, diversity, and mobility. Wastewater discharge, agricultural runoff, aquaculture practices, and pharmaceutical pollution continuously enrich the aquatic resistome, transforming aquatic ecosystems into major reservoirs of clinically relevant ARGs. Monitoring the environmental resistome therefore provides valuable information regarding emerging resistance threats and supports One Health surveillance strategies [2].

6. Transmission pathways of antimicrobial resistance in aquatic ecosystems

The dissemination of antimicrobial resistance (AMR) in aquatic ecosystems is a complex ecological process involving the continuous movement of antibiotic-resistant bacteria (ARB), antibiotic resistance genes (ARGs), and mobile genetic elements (MGEs) among environmental compartments. Rivers, lakes, estuaries, marine environments, wastewater treatment plants, and aquaculture systems provide ideal conditions for the persistence and transmission of resistance determinants because they receive continuous inputs of antibiotics, nutrients, heavy metals, and diverse microbial communities. As emphasized by Berendonk *et al.* (2015), aquatic ecosystems should be regarded as dynamic transmission networks rather than passive reservoirs, where resistance determinants are exchanged among environmental, animal, and human-associated bacteria. The spread of AMR occurs through two principal mechanisms: vertical gene transmission, in which resistance genes are inherited from parent to daughter cells, and horizontal gene transfer (HGT), which enables unrelated bacterial species to exchange genetic material. Among these mechanisms, HGT plays the dominant role in accelerating the environmental dissemination of antimicrobial resistance because it allows rapid acquisition of resistance genes without bacterial reproduction (Aminov, 2011; Berendonk *et al.*, 2015) [3, 6].

6.1 Horizontal gene transfer

Horizontal gene transfer (HGT) is considered the primary mechanism responsible for the rapid dissemination of ARGs in aquatic ecosystems. Through HGT, bacteria acquire resistance genes carried on plasmids, integrons, transposons, bacteriophages, and other mobile genetic elements. According to Aminov (2011) and Berendonk *et al.* (2015), HGT enables environmental microorganisms to transfer clinically important resistance genes to pathogenic bacteria, thereby linking environmental and clinical antimicrobial resistance. Three classical mechanisms of HGT dominate in aquatic environments: conjugation, transformation, and transduction, while membrane vesicle-mediated transfer has

recently emerged as an additional mechanism. Jiang *et al.* (2022) reviewed evidence that environmental contaminants can stimulate all four pathways, further enhancing ARG dissemination in water environments (Fig. 1) [3, 6, 20].

6.1.1 Conjugation

Conjugation is the most efficient and widely reported mechanism of ARG dissemination in aquatic ecosystems. During conjugation, DNA-containing conjugative plasmids or integrative conjugative elements are transferred directly from a donor bacterium to a recipient cell through a conjugative pilus. Because many plasmids carry multiple resistance genes, a single conjugation event may simultaneously confer resistance to several antibiotic classes. According to Aminov (2011) and Jiang *et al.* (2022), conjugation occurs at particularly high frequencies within wastewater treatment plants, river sediments, and aquatic biofilms where bacterial densities are high and cells remain in close contact. Plasmids carrying *bla*, *tet*, *sul*, *qnr*, and *mcr* genes have been frequently transferred through conjugation in aquatic environments [3, 20].

6.1.2 Natural transformation

Natural transformation involves the uptake of extracellular DNA released from lysed bacterial cells. Competent bacteria incorporate this free DNA into their genomes or maintain it as plasmids if homologous recombination or plasmid replication is successful. As described by Lu *et al.* (2015), aquatic sediments and biofilms contain abundant extracellular DNA originating from dead microorganisms, making these environments favorable for transformation-mediated dissemination of ARGs. Environmental DNA carrying resistance genes can remain stable for extended periods because adsorption to sediment particles protects it from enzymatic degradation. Consequently, transformation contributes significantly to the environmental persistence of resistance genes [24].

6.1.3 Transduction

Transduction is mediated by bacteriophages, viruses that infect bacteria and accidentally package fragments of bacterial DNA during replication. These DNA fragments, including ARGs, are subsequently transferred into new bacterial hosts during phage infection. Ngando. (2014) demonstrated that bacteriophages are abundant in freshwater and marine environments and may transport clinically relevant resistance genes between environmental bacteria. Because aquatic ecosystems contain extremely high viral abundances, phage-mediated transduction represents an important but often underestimated mechanism of ARG dissemination [36].

6.1.4 Emerging transfer mechanisms

Recent studies have identified additional pathways of genetic exchange, including membrane vesicle-mediated transfer (vesiduction) and bacterial nanotubes. Membrane vesicles released by Gram-negative bacteria may transport DNA, plasmids, RNA, proteins, and quorum-sensing molecules between bacterial cells. According to Jiang *et al.* (2022), environmental contaminants such as heavy metals, disinfectants, and nanoparticles can stimulate membrane vesicle production, potentially enhancing ARG dissemination in aquatic environments [20].

6.2 Vertical gene transmission

Unlike HGT, vertical transmission occurs during bacterial cell division when resistance genes are inherited by daughter cells. Once an ARG becomes established within a bacterial population, continuous exposure to antibiotics or other environmental stressors favors the survival and multiplication of resistant clones. Although vertical inheritance does not generate new resistance mechanisms, it plays a crucial role in maintaining resistant bacterial populations in rivers, wastewater treatment plants, aquaculture ponds, and marine sediments. The persistence of multidrug-resistant *Aeromonas*, *Vibrio*, *Escherichia coli*, and *Pseudomonas* populations in aquatic environments is largely sustained through vertical transmission combined with periodic horizontal gene acquisition.

6.3 Biofilms as hotspots for AMR transmission

Biofilms represent one of the most important ecological niches for antimicrobial resistance transmission in aquatic ecosystems. These structured microbial communities are embedded within extracellular polymeric substances that protect microorganisms against environmental stress while maintaining exceptionally high cell densities. According to Abe *et al.* (2020) [1], biofilms significantly increase the frequency of conjugation, transformation, and transduction because bacterial cells remain in close proximity and extracellular DNA accumulates within the biofilm matrix. Wastewater treatment plants, aquaculture facilities, river sediments, drinking water distribution systems, and microplastic surfaces commonly harbor mature biofilms that function as reservoirs and dissemination centers for ARGs.

6.4 Food chain transmission

Aquatic organisms may accumulate antibiotic-resistant bacteria through continuous exposure to contaminated water and sediments. Fish, shrimp, oysters, mussels, and other filter-feeding organisms can harbor resistant bacteria within their gastrointestinal tracts and tissues. Consumption of raw or inadequately cooked seafood therefore provides a potential route for transmission of resistant microorganisms to humans. Cabello *et al.* (2013) emphasized that aquaculture contributes to food-chain dissemination of antimicrobial resistance because resistant bacteria originating from production systems may contaminate seafood products entering international markets. The transfer of resistance genes from aquatic bacteria to human pathogens through the food chain remains an important public health concern [8].

6.5 Human exposure pathways

Humans are exposed to aquatic antimicrobial resistance through several interconnected pathways. These include consumption of contaminated drinking water, recreational activities in polluted rivers and coastal waters, occupational exposure during aquaculture and wastewater treatment, and ingestion of contaminated seafood. Berendonk *et al.* (2015) concluded that wastewater treatment plants constitute critical interfaces linking environmental, animal, and human microbial communities. Because resistance genes continuously circulate among these compartments, aquatic ecosystems represent an integral component of the One Health transmission cycle of antimicrobial resistance [6].

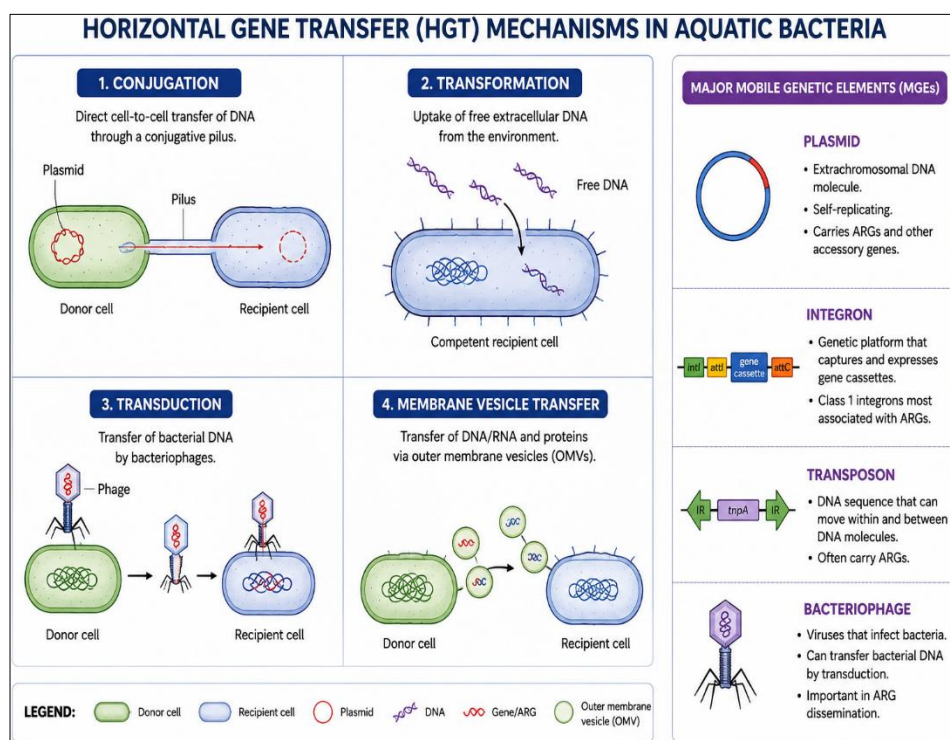


Fig 1: Schematic representation of horizontal gene transfer mechanisms (conjugation, transformation, transduction, and membrane vesicle-mediated transfer) and the major mobile genetic elements (plasmids, integrons, transposons, and bacteriophages) responsible for the dissemination of antibiotic resistance genes in aquatic ecosystems.

7. Major antimicrobial-resistant bacteria in aquatic ecosystems: Aquatic ecosystems harbor a wide diversity of antimicrobial-resistant bacteria (ARB), many of which are recognized as opportunistic or clinically important

pathogens. Continuous contamination from municipal wastewater, hospital effluents, agricultural runoff, and aquaculture activities has transformed aquatic environments into reservoirs where resistant bacteria persist, multiply, and

exchange antibiotic resistance genes (ARGs). These bacteria not only threaten aquatic animal health but also pose significant risks to human health through contaminated drinking water, seafood consumption, and recreational water exposure. Among the most frequently reported AMR bacteria in aquatic ecosystems are *Aeromonas* spp., *Vibrio* spp., *Escherichia coli*, *Pseudomonas aeruginosa*, *Enterococcus* spp., *Salmonella* spp., *Acinetobacter* spp., and *Klebsiella pneumoniae*.

7.1 *Aeromonas* spp.

Members of the genus *Aeromonas* are among the most important aquatic bacteria because they are indigenous to freshwater ecosystems and are frequently isolated from rivers, lakes, estuaries, wastewater, aquaculture ponds, drinking water, fish, and shellfish. Janda and Abbott (2010) described *Aeromonas* as almost synonymous with aquatic environments due to its ubiquitous distribution and ecological adaptability. The genus includes important pathogenic species such as *A. hydrophila*, *A. caviae*, and *A. veronii*, which cause disease in fish, amphibians, and humans. *Aeromonas* has become an important indicator organism for environmental AMR surveillance because it readily acquires resistance genes through horizontal gene transfer. Environmental isolates frequently harbor bla, tet, sul, qnr, erm, and int1 genes, conferring resistance to β -lactams, tetracyclines, sulfonamides, fluoroquinolones, and macrolides. Wastewater treatment plants and aquaculture facilities are recognized as major hotspots for multidrug-resistant *Aeromonas* populations, facilitating the dissemination of ARGs into natural waters.

7.2 *Vibrio* spp.

Species of *Vibrio* are naturally associated with marine and estuarine environments where they play important ecological roles but also include significant pathogens affecting humans and aquatic animals. Important species include *Vibrio cholerae*, *V. parahaemolyticus*, *V. vulnificus*, and *V. anguillarum*. According to Lee *et al.* (2018), intensive aquaculture practices and excessive antibiotic application have accelerated the emergence of multidrug-resistant *Vibrio* strains, particularly in coastal aquaculture systems. Environmental *Vibrio* isolates frequently exhibit resistance to tetracyclines, quinolones, aminoglycosides, and β -lactam antibiotics and commonly possess resistance genes including tet, qnr, bla, and sul. Because shellfish concentrate *Vibrio* through filter feeding, antimicrobial-resistant strains may enter the human food chain via seafood consumption, representing an important One Health concern.

7.3 *Escherichia coli*

Escherichia coli is widely used as a fecal indicator organism for assessing microbial water quality and environmental antimicrobial resistance. Although many strains are harmless commensals, pathogenic variants such as enterohemorrhagic and enterotoxigenic *E. coli* can cause severe human disease. The widespread detection of multidrug-resistant *E. coli* in wastewater, rivers, lakes, and coastal waters reflects continuous fecal contamination from human and animal sources. Recent systematic reviews identified *E. coli* as one of the most commonly monitored antimicrobial-resistant bacteria in freshwater, wastewater, and seawater. Environmental *E. coli* frequently carries extended-spectrum β -lactamase (ESBL) genes including blaCTX-M, blaTEM,

and blaSHV, together with tetA, sul1, qnr, and mcr genes. These resistance determinants are often located on transferable plasmids, facilitating dissemination to other Gram-negative bacteria.

7.4 *Pseudomonas aeruginosa*

Pseudomonas aeruginosa is an opportunistic pathogen commonly isolated from rivers, wastewater, sediments, drinking water distribution systems, and aquaculture environments. Its remarkable metabolic versatility enables survival under diverse environmental conditions. In addition to intrinsic resistance mediated by low membrane permeability and multidrug efflux pumps, environmental isolates frequently acquire plasmid-mediated resistance genes through horizontal gene transfer. Wastewater treatment plants are considered important reservoirs for multidrug-resistant *P. aeruginosa*, contributing to environmental dissemination of carbapenem resistance genes.

7.5 *Enterococcus* spp.

Species of *Enterococcus*, particularly *E. faecalis* and *E. faecium*, are common inhabitants of the gastrointestinal tract of humans and animals but are increasingly detected in wastewater, rivers, recreational waters, and aquaculture environments. Their ability to survive harsh environmental conditions allows prolonged persistence after wastewater discharge. Environmental isolates frequently possess vancomycin resistance genes (vanA, vanB) together with resistance to aminoglycosides, tetracyclines, and macrolides. Because *Enterococcus* readily exchanges plasmids and transposons carrying ARGs, it serves as an important indicator of environmental dissemination of clinically relevant resistance.

7.6 *Salmonella* spp.

Salmonella species are zoonotic pathogens transmitted through contaminated food and water. Agricultural runoff, livestock manure, untreated sewage, and wastewater effluents introduce *Salmonella* into aquatic ecosystems, where resistant strains may persist in sediments and biofilms. Environmental isolates frequently exhibit multidrug resistance involving bla, tet, sul, and qnr genes. Aquatic contamination therefore represents an important route for foodborne transmission and environmental persistence of resistant *Salmonella*.

7.7 *Acinetobacter* spp.

Members of the genus *Acinetobacter*, particularly *Acinetobacter baumannii*, are increasingly recognized as environmental reservoirs of antimicrobial resistance. Although primarily associated with healthcare settings, multidrug-resistant *Acinetobacter* has been recovered from wastewater treatment plants, rivers, estuaries, and coastal waters receiving hospital effluents. Environmental isolates frequently carry carbapenemase genes such as blaOXA, blaNDM, and blaIMP, making them important reservoirs of resistance to critically important antibiotics. Their persistence in wastewater facilitates dissemination to other aquatic microorganisms through mobile genetic elements.

7.8 *Klebsiella pneumoniae*

Klebsiella pneumoniae is an opportunistic pathogen commonly detected in wastewater, surface waters, and aquaculture environments influenced by anthropogenic

activities. Environmental isolates often produce extended-spectrum β -lactamases and carbapenemases, including blaCTX-M, blaKPC, blaNDM, and blaOXA-48, conferring resistance to multiple β -lactam antibiotics. Wastewater discharge has been identified as a major pathway introducing multidrug-resistant *K. pneumoniae* into aquatic ecosystems, increasing opportunities for horizontal gene transfer with indigenous environmental bacteria.

8. Detection and monitoring of antimicrobial resistance in aquatic ecosystems: The effective surveillance of antimicrobial resistance (AMR) in aquatic ecosystems is essential for understanding the distribution of antibiotic-resistant bacteria (ARB), antibiotic resistance genes (ARGs), and mobile genetic elements (MGEs). Because aquatic environments contain both culturable and non-culturable microorganisms, no single analytical method is sufficient for comprehensive AMR monitoring. Consequently, integrated surveillance strategies combining conventional microbiological techniques with molecular and genomic approaches are increasingly recommended. Recent advances in high-throughput sequencing, bioinformatics, and biosensor technologies have significantly improved the sensitivity and resolution of environmental AMR surveillance, enabling early detection of emerging resistance determinants and supporting One Health monitoring programs.

8.1 Culture-based detection methods

Culture-dependent methods remain the foundation of environmental microbiology because they enable the isolation of viable bacteria for phenotypic characterization. Water, sediment, wastewater, and aquaculture samples are cultured on selective and differential media to recover target bacterial species such as *Aeromonas*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Vibrio*, *Salmonella*, and *Enterococcus*. Following isolation, bacterial identification is performed using colony morphology, Gram staining, biochemical assays, automated identification systems, or matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS). Although culture-based methods cannot detect viable but non-culturable microorganisms, they remain indispensable because they provide pure isolates for downstream antimicrobial susceptibility testing, molecular characterization, and epidemiological investigations.

8.2 Antimicrobial susceptibility testing (AST)

Antimicrobial susceptibility testing (AST) is the standard phenotypic approach for determining bacterial resistance profiles. The Kirby-Bauer disk diffusion method, recommended by the Clinical and Laboratory Standards Institute (CLSI) and the European Committee on Antimicrobial Susceptibility Testing (EUCAST), is widely used because it is simple, cost-effective, and suitable for routine laboratory surveillance. Antibiotic-impregnated discs are placed on inoculated agar plates, and inhibition zone diameters are interpreted according to standardized breakpoints. For quantitative evaluation, minimum inhibitory concentration (MIC) testing is performed using broth microdilution, agar dilution, or gradient strip methods. MIC determination provides precise information regarding bacterial susceptibility and is particularly important for surveillance of multidrug-resistant pathogens and monitoring resistance trends over time. According to recent

environmental monitoring frameworks, combining AST with molecular characterization provides a more reliable assessment of environmental AMR than phenotypic testing alone.

8.3 Polymerase chain reaction (PCR)-based detection

Polymerase chain reaction (PCR) is among the most widely applied molecular techniques for detecting ARGs in aquatic environments because of its high specificity, sensitivity, and rapid turnaround time. Conventional PCR enables qualitative detection of specific resistance genes, including bla, tet, sul, qnr, erm, mcr, and van, directly from bacterial isolates or environmental DNA. Multiplex PCR further enhances efficiency by simultaneously amplifying several ARGs within a single reaction, thereby reducing analytical time and reagent consumption. PCR-based assays have become indispensable tools for confirming resistance mechanisms identified through phenotypic susceptibility testing and for investigating the distribution of clinically relevant ARGs in rivers, wastewater, sediments, and aquaculture systems.

8.4 Quantitative PCR (qPCR) and digital PCR (dPCR)

Quantitative real-time PCR (qPCR) represents one of the most widely used techniques for environmental ARG surveillance because it provides both detection and absolute or relative quantification of target resistance genes. Compared with conventional PCR, qPCR offers higher sensitivity, broader dynamic range, and improved reproducibility. Frequently monitored genes include intI1, sul1, tetA, blaCTX-M, qnrS, and mcr-1, which are often used as indicators of anthropogenic contamination. Digital PCR (dPCR) has recently emerged as an advanced quantitative method capable of absolute gene quantification without requiring standard calibration curves. By partitioning DNA samples into thousands of micro-reactions, dPCR achieves exceptional sensitivity for detecting low-abundance ARGs in environmental samples and is increasingly applied in wastewater surveillance and environmental risk assessment.

8.5 High-throughput sequencing technologies

Next-generation sequencing (NGS) has revolutionized AMR surveillance by enabling comprehensive characterization of microbial communities and resistance determinants without prior cultivation.

16S rRNA gene sequencing

16S rRNA sequencing is widely employed to characterize bacterial community composition within aquatic ecosystems. Although it cannot directly identify resistance genes, it provides valuable information regarding microbial diversity and identifies bacterial taxa potentially associated with ARG dissemination.

Whole genome sequencing (WGS): Whole genome sequencing provides complete genomic information for individual bacterial isolates, enabling identification of ARGs, virulence factors, plasmids, integrons, transposons, and phylogenetic relationships. WGS has become the gold standard for investigating multidrug-resistant pathogens because it links resistance genes with specific bacterial hosts and facilitates outbreak investigations.

Shotgun metagenomic sequencing: Shotgun metagenomics enables direct sequencing of all DNA present within

environmental samples, allowing simultaneous identification of bacterial communities, ARGs, MGEs, and metabolic pathways without cultivation. According to Hemamalini *et al.* (2022), metagenomic surveillance provides comprehensive insight into the diversity and abundance of resistance determinants in aquaculture and other aquatic environments. However, its application requires advanced sequencing platforms, computational resources, and bioinformatic expertise.

8.6 Emerging detection technologies

Rapid technological developments are transforming environmental AMR surveillance beyond conventional molecular diagnostics.

Nanopore sequencing enables real-time long-read DNA sequencing, allowing simultaneous identification of bacterial species, ARGs, and plasmids directly from environmental samples. Its portability makes it particularly attractive for field-based environmental monitoring.

CRISPR-Cas-based diagnostics have recently emerged as highly sensitive platforms capable of rapid and sequence-specific detection of resistance genes. These systems offer considerable potential for point-of-care and environmental surveillance because of their high specificity and minimal instrumentation requirements. Environmental biosensors incorporating electrochemical, optical, or nanomaterial-based detection platforms have also gained attention for rapid ARG monitoring. Recent reviews indicate that biosensors may enable continuous, real-time surveillance of aquatic environments while reducing analytical time compared with laboratory-based molecular methods.

8.7 Bioinformatics and Artificial Intelligence

Modern AMR surveillance increasingly relies on bioinformatics to analyze the large datasets generated through high-throughput sequencing. Specialized databases such as the Comprehensive Antibiotic Resistance Database (CARD), ResFinder, and ARG-ANNOT facilitate identification and annotation of resistance genes from genomic and metagenomic sequences. Artificial intelligence (AI) and machine learning algorithms are now being explored to predict resistance patterns, identify emerging ARGs, analyze temporal trends, and improve environmental risk assessment. Integration of sequencing technologies with AI-based analytical tools is expected to enhance future environmental surveillance and support evidence-based AMR management.

9. Ecological impacts of antimicrobial resistance in aquatic ecosystems: The increasing prevalence of antimicrobial resistance (AMR) in aquatic ecosystems has profound ecological consequences that extend well beyond the emergence of resistant pathogens. Continuous inputs of antibiotics, antibiotic-resistant bacteria (ARB), antibiotic resistance genes (ARGs), and other contaminants alter microbial community composition, disrupt ecosystem functions, interfere with biogeochemical cycling, and threaten aquatic biodiversity. Aquatic environments act as interconnected ecological networks where resistance determinants circulate among microorganisms, aquatic organisms, wildlife, and humans. As highlighted by Singh *et al.* (2024)^[37], the persistence and dissemination of antibiotics and ARGs in aquatic environments can fundamentally alter

ecosystem functioning while increasing environmental and public health risks.

9.1 Alteration of microbial community structure

Microbial communities are essential for maintaining ecosystem stability because they regulate nutrient cycling, organic matter decomposition, and primary productivity. Chronic exposure to antibiotics in aquatic environments imposes selective pressure that suppresses susceptible microorganisms while favoring resistant populations. Consequently, microbial diversity decreases, community composition shifts, and resistant taxa become dominant. According to Berendonk *et al.* (2015), environmental antibiotic contamination promotes the enrichment of resistant bacterial communities capable of carrying multiple ARGs and mobile genetic elements. Similarly, Singh *et al.* (2024) reported that long-term antibiotic exposure alters microbial succession and reduces functional diversity in rivers, lakes, and wastewater-impacted aquatic ecosystems. These ecological shifts may decrease ecosystem resilience and reduce the ability of microbial communities to recover following environmental disturbances^[37].

9.2 Disruption of biogeochemical cycles

Aquatic microorganisms play indispensable roles in carbon, nitrogen, phosphorus, and sulfur cycling. Antibiotic pollution and AMR can impair these microbial processes by inhibiting sensitive functional groups involved in nitrification, denitrification, methanogenesis, and organic matter mineralization. For example, suppression of ammonia-oxidizing bacteria reduces nitrification efficiency, whereas disruption of denitrifying microorganisms affects nitrogen removal and contributes to eutrophication. Likewise, changes in decomposer communities influence carbon turnover and organic matter degradation, ultimately affecting energy flow throughout aquatic food webs. Singh *et al.* (2024) emphasized that widespread antibiotic contamination has the potential to disrupt ecosystem functions by altering microbial metabolism and nutrient recycling processes.

9.3 Biofilms as ecological hotspots of antimicrobial resistance: Biofilms are structured microbial communities embedded within extracellular polymeric substances that colonize natural and artificial surfaces in aquatic environments. These structures provide protection against environmental stress while creating ideal conditions for microbial interaction and horizontal gene transfer. According to Abe *et al.* (2020), biofilms function as hotspots for ARG dissemination because bacterial cells remain in close proximity, extracellular DNA accumulates within the biofilm matrix, and conjugation frequencies are significantly enhanced. Biofilms formed in wastewater treatment plants, river sediments, aquaculture facilities, and drinking water systems therefore become long-term reservoirs of resistant microorganisms. Microplastics further increase this ecological risk by providing durable surfaces for biofilm development. Rummel *et al.* (2017) demonstrated that microbial colonization on microplastic surfaces modifies particle transport, persistence, and ecological interactions within aquatic systems. More recently, reviews have shown that microplastic-associated biofilms frequently contain pathogenic bacteria, ARB, and ARGs, facilitating their persistence and dissemination in freshwater and marine environments^[1, 34].

9.4 Impacts on aquatic organisms

Antibiotic residues and resistant bacteria influence a wide range of aquatic organisms, including phytoplankton, zooplankton, benthic invertebrates, fish, crustaceans, and mollusks. Exposure to environmentally relevant concentrations of antibiotics may induce oxidative stress, immune suppression, developmental abnormalities, reproductive impairment, and changes in gut microbial composition. In aquaculture systems, continuous antibiotic exposure alters the intestinal microbiota of fish and shrimp while increasing colonization by multidrug-resistant bacteria such as *Aeromonas*, *Vibrio*, and *Pseudomonas*. These changes reduce disease resistance and increase the frequency of bacterial outbreaks, ultimately affecting aquaculture productivity and food security. Furthermore, filter-feeding organisms such as oysters and mussels can accumulate resistant bacteria and ARGs, thereby facilitating their transfer through aquatic food webs. Singh *et al.* (2024) highlighted that these ecological effects extend beyond individual species and may influence entire aquatic communities ^[37].

9.5 Effects on biodiversity and ecosystem functioning

Healthy aquatic ecosystems depend on diverse microbial communities that maintain ecological balance through complementary metabolic activities. Persistent antibiotic contamination reduces microbial richness and favors resistant taxa with limited ecological functions, resulting in decreased ecosystem resilience. Loss of microbial diversity may disrupt predator-prey interactions, alter food-web dynamics, reduce primary productivity, and impair ecosystem services such as water purification and nutrient recycling. Because microorganisms form the foundation of aquatic food webs, disturbances at the microbial level can propagate throughout higher trophic levels, affecting fish populations and overall biodiversity. Singh *et al.* (2024) concluded that chronic antibiotic pollution may contribute to long-term ecological degradation if effective mitigation measures are not implemented ^[37].

9.6 Co-selection by heavy metals and other environmental pollutants: Environmental contaminants other than antibiotics also contribute to the maintenance and dissemination of AMR. Heavy metals, including copper, zinc, mercury, cadmium, and arsenic, are widely used in agriculture, aquaculture, and industry. Unlike antibiotics, heavy metals do not degrade and therefore remain in sediments for prolonged periods. Seiler and Berendonk (2012) demonstrated that heavy metals promote the co-selection of antibiotic resistance because genes conferring metal tolerance and antibiotic resistance are frequently located on the same plasmids or integrons. Consequently, exposure to heavy metals may maintain ARGs even in the absence of antibiotic residues. Similar co-selection has been reported for disinfectants, pesticides, and other environmental contaminants, highlighting the complexity of AMR evolution in aquatic ecosystems ^[6].

9.7 Microplastics and the spread of antimicrobial resistance: Microplastics have emerged as important ecological drivers of AMR because they provide stable substrates for microbial colonization and biofilm formation. Their large surface area allows adsorption of antibiotics, heavy metals, and organic pollutants while simultaneously supporting diverse microbial communities collectively

referred to as the plastisphere. Recent studies indicate that microplastic-associated biofilms contain higher abundances of ARB and ARGs than surrounding water. These biofilms enhance horizontal gene transfer and facilitate long-distance transport of resistant microorganisms across freshwater, estuarine, and marine environments. As reviewed by Dhinakaran. (2023) and other recent studies, microplastics therefore act as ecological vectors connecting pollution, AMR dissemination, aquatic biodiversity loss, and food safety concerns.

10. Public health implications of antimicrobial resistance in aquatic ecosystems: Aquatic ecosystems have emerged as important reservoirs and transmission pathways for antimicrobial-resistant bacteria (ARB) and antibiotic resistance genes (ARGs), creating significant public health challenges. The continuous discharge of municipal wastewater, hospital effluents, agricultural runoff, and aquaculture wastes introduces antibiotics and resistant microorganisms into aquatic environments, facilitating the dissemination of resistance through the human-animal-environment interface (Berendonk *et al.*, 2015; Martí *et al.*, 2014). Consequently, aquatic ecosystems are now recognized as critical components of the One Health framework ^[6, 27].

10.1 Human exposure pathways: Humans are exposed to AMR through multiple routes, including contaminated drinking water, recreational water, seafood consumption, and occupational activities such as aquaculture and wastewater treatment. Inadequately treated wastewater and contaminated surface waters may contain multidrug-resistant bacteria and extracellular ARGs, increasing opportunities for colonization and infection (Manaia *et al.*, 2016). Seafood, particularly fish and shellfish produced in antibiotic-intensive aquaculture systems, may also act as vehicles for transmitting resistant bacteria to consumers (Cabello *et al.*, 2013) ^[6, 28].

10.2 Waterborne and foodborne resistant pathogens

Several clinically important pathogens have been isolated from aquatic environments, including *Aeromonas hydrophila*, *Vibrio cholerae*, *Vibrio parahaemolyticus*, *Escherichia coli*, *Salmonella enterica*, *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, and *Klebsiella pneumoniae*. These organisms frequently harbor clinically relevant resistance genes such as blaCTX-M, blaNDM, tet, sul, qnr, mcr, and van, limiting therapeutic options and increasing the risk of multidrug-resistant infections (Berendonk *et al.*, 2015) ^[6].

10.3 Clinical and socioeconomic impacts

Infections caused by antimicrobial-resistant pathogens are associated with prolonged hospitalization, treatment failure, increased healthcare costs, and higher mortality. The environmental dissemination of carbapenem- and colistin-resistant bacteria is particularly concerning because these antibiotics are considered last-line therapies for severe infections (Singh *et al.*, 2024). The growing environmental reservoir of resistance therefore represents a significant threat to global public health ^[37].

10.4 One health perspective

Mitigating aquatic AMR requires an integrated One Health approach that links environmental, veterinary, and human health surveillance. Strengthening antimicrobial stewardship,

improving wastewater treatment, regulating antibiotic use in aquaculture, and implementing genomic surveillance of aquatic environments are essential strategies to reduce the environmental spread of antimicrobial resistance (Berendonk *et al.*, 2015; Manaia *et al.*, 2016) ^[6, 26].

11. Mitigation strategies and future perspectives

The increasing prevalence of antimicrobial resistance (AMR) in aquatic ecosystems necessitates integrated mitigation strategies targeting antibiotic use, environmental contamination, wastewater management, and resistance surveillance. Because aquatic environments serve as reservoirs and transmission pathways for antibiotic-resistant bacteria (ARB) and antibiotic resistance genes (ARGs), effective mitigation requires coordinated actions under the One Health framework involving environmental, veterinary, and human health sectors (Berendonk *et al.*, 2015) ^[6].

11.1 Prudent antibiotic use

Reducing unnecessary antibiotic use in human medicine, livestock production, and aquaculture is one of the most effective approaches for limiting environmental AMR. Implementation of antimicrobial stewardship programs, prescription-based antibiotic use, vaccination, improved biosecurity, and good husbandry practices can substantially reduce selective pressure that drives the emergence of resistant bacteria (World Health Organization, 2023) ^[41].

11.2 Advanced wastewater treatment technologies

Conventional wastewater treatment plants incompletely remove antibiotics, ARGs, and resistant bacteria. Therefore, advanced treatment technologies including membrane bioreactors (MBRs), ozonation, advanced oxidation processes (AOPs), activated carbon adsorption, ultraviolet (UV) irradiation, and constructed wetlands have been investigated to improve the removal of antimicrobial contaminants before effluent discharge (Michael *et al.*, 2013) ^[28].

11.3 Sustainable aquaculture practices

Sustainable aquaculture can significantly reduce antibiotic dependence by adopting improved management practices. The use of vaccines, probiotics, prebiotics, phytobiotics, immunostimulants, and biosecurity measures enhances disease resistance in cultured fish and shrimp while minimizing antibiotic application. Regular health monitoring and good water quality management are also essential for preventing disease outbreaks (Cabello *et al.*, 2013) ^[8].

11.4 Environmental surveillance

Routine monitoring of antibiotics, ARB, ARGs, and mobile genetic elements should be incorporated into national environmental surveillance programs. Molecular tools such as qPCR, whole-genome sequencing (WGS), metagenomics, and environmental DNA (eDNA) analysis enable early detection of emerging resistance and support evidence-based risk assessment (Berendonk *et al.*, 2015) ^[6].

11.5 Policy and One health approaches: International collaboration is essential for combating environmental AMR. Organizations such as the World Health Organization, Food and Agriculture Organization, and World Organisation for Animal Health advocate integrated One Health strategies that combine antimicrobial stewardship, environmental

monitoring, improved sanitation, wastewater management, and public awareness. Harmonized regulations governing antibiotic use and environmental discharge are critical for reducing the global spread of resistance (WHO, 2023) ^[41].

11.6 Research Gaps and Future Challenges

Despite significant advances in understanding antimicrobial resistance (AMR) in aquatic ecosystems, several knowledge gaps remain. Standardized methods for detecting and quantifying antibiotic resistance genes (ARGs) are still lacking, limiting comparisons among studies. Environmental AMR surveillance remains inadequate in many developing countries, and data from aquaculture systems are relatively limited. The establishment of global databases integrating ARGs, resistant bacteria, and antibiotic residues would improve risk assessment and international monitoring efforts. Furthermore, the influence of climate change on the dissemination of AMR in aquatic environments remains poorly understood and requires further investigation. Addressing these gaps through interdisciplinary and long-term research will be essential for effective AMR management under the One Health framework.

12. Conclusion

Antimicrobial resistance (AMR) in aquatic ecosystems has emerged as a major global environmental and public health challenge driven by the widespread use of antibiotics in human medicine, veterinary practice, agriculture, and aquaculture. Continuous discharge of antibiotics, antibiotic-resistant bacteria (ARB), and antibiotic resistance genes (ARGs) into rivers, lakes, estuaries, and coastal waters has transformed aquatic ecosystems into important reservoirs and dissemination pathways for antimicrobial resistance. Horizontal gene transfer, mobile genetic elements, and biofilm formation further accelerate the persistence and spread of resistance among environmental and pathogenic microorganisms. The ecological consequences of AMR include alterations in microbial community structure, disruption of biogeochemical cycles, reduced biodiversity, and impaired ecosystem functioning. Moreover, aquatic environments facilitate the transmission of resistant pathogens to humans through contaminated water, seafood, and recreational activities, reinforcing the importance of the One Health concept. Advances in molecular diagnostics, whole-genome sequencing, metagenomics, and environmental surveillance have greatly improved our understanding of the environmental resistome and the dynamics of resistance dissemination. Addressing AMR in aquatic ecosystems requires integrated and multidisciplinary approaches that combine prudent antibiotic use, improved wastewater treatment, sustainable aquaculture practices, continuous environmental monitoring, and international policy implementation. Future research should prioritize standardized surveillance methods, long-term ecological studies, and innovative technologies for rapid detection and mitigation of antimicrobial resistance. Strengthening collaboration among researchers, policymakers, healthcare professionals, and environmental agencies will be essential to safeguard aquatic ecosystem health and reduce the global burden of antimicrobial resistance.

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