

# Serious Threat of Antibiotic Resistance in Fish Microflora: A Critical Review

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**Abstract:** Antimicrobial resistance (AMR) has emerged as one of the most serious biological threats affecting aquatic ecosystems, aquaculture sustainability, food security, and public health. The extensive use of antibiotics in aquaculture for therapeutic, prophylactic, and metaphylactic purposes has accelerated the selection of resistant microorganisms within fish-associated microbiota. Fish microflora, particularly the gut, gill, skin, and environmental microbial communities associated with culture systems, serve as reservoirs of antibiotic-resistant bacteria (ARB) and antibiotic resistance genes (ARGs). These resistance determinants can spread through horizontal gene transfer, facilitating the emergence of multidrug-resistant pathogens with implications extending beyond aquatic environments. Recent studies have demonstrated the widespread occurrence of tetracycline, sulfonamide, quinolone,  $\beta$ -lactam, and aminoglycoside resistance genes in aquaculture systems worldwide. The dissemination of ARGs through water, sediments, feeds, fish products, and aquatic wildlife has strengthened concerns regarding the role of aquaculture in the global AMR crisis. Modern metagenomic investigations have further revealed the complexity of fish-associated resistomes and their connectivity with environmental and human-associated microbial communities. This review critically examines the development of antibiotic resistance within fish microflora, the mechanisms underlying resistance acquisition, global distribution patterns, ecological and public health implications, and emerging mitigation strategies. Emphasis is placed on the One Health framework, which recognizes the interconnectedness of human, animal, and environmental health in addressing AMR. Current evidence indicates that antibiotic resistance in fish microflora is no longer a localized aquaculture problem but a global environmental and public health challenge requiring coordinated international intervention.

**Keywords:** Antimicrobial resistance, Aquaculture, Fish microflora, Antibiotic resistance genes (ARGs) and One Health

## I. INTRODUCTION

Antimicrobial resistance (AMR) has emerged as one of the most significant global health challenges of the twenty-first century. The increasing prevalence of antibiotic-resistant bacteria (ARB) and antibiotic resistance genes (ARGs) threatens the effectiveness of antimicrobial therapies in human and veterinary medicine. Although clinical misuse of antibiotics remains a major driver of resistance, environmental reservoirs are now recognized as critical contributors to the global dissemination of AMR (Salam et al., 2023).

Aquaculture is the fastest-growing animal food production sector and contributes substantially to global fish consumption. Intensification of aquaculture practices has been accompanied by increased disease outbreaks caused by bacterial pathogens such as *Aeromonas*, *Vibrio*, *Edwardsiella*, and *Flavobacterium* (Gutiérrez-Pacheco et al., 2026). To control these infections and reduce economic losses, antibiotics have been widely used for therapeutic and prophylactic purposes in many aquaculture systems (Afroze et al., 2025). Unlike terrestrial livestock production, a considerable proportion of administered antibiotics in aquaculture enters the surrounding environment through uneaten feed, fish excretion, and water exchange, resulting in prolonged exposure of aquatic microbial communities to antimicrobial compounds.

Fish-associated microflora constitute a diverse microbial ecosystem inhabiting the gastrointestinal tract, skin mucus, gills, and other external surfaces. These microbial communities play essential roles in nutrient metabolism, immune regulation,



pathogen exclusion, and host health maintenance. However, continuous exposure to antibiotic residues can alter microbial composition and select for resistant bacterial populations. Consequently, fish microflora have become important reservoirs of ARGs and multidrug-resistant bacteria within aquatic ecosystems (Miranda et al., 2026).

Recent advances in molecular biology and metagenomic sequencing have revealed the widespread occurrence of resistance determinants in fish-associated microbiota. Genes conferring resistance to tetracyclines, sulfonamides, quinolones,  $\beta$ -lactams, and aminoglycosides have been frequently detected in fish intestines, gills, skin mucus, aquaculture water, and sediments (Zvezdanova et al., 2026). Furthermore, many of these genes are associated with mobile genetic elements, facilitating horizontal gene transfer among environmental bacteria, fish pathogens, and potentially human-associated microorganisms.

The growing recognition of aquaculture environments as reservoirs and dissemination pathways for antibiotic resistance has generated significant scientific concern. Therefore, understanding the occurrence, mechanisms, ecological consequences, and public health implications of antibiotic resistance in fish microflora is essential for developing sustainable disease-management strategies and mitigating the spread of resistance. This review critically examines current knowledge regarding fish-associated resistomes, major resistant bacterial taxa, mechanisms of resistance acquisition, environmental dissemination pathways, public health risks, and emerging alternatives to antibiotic-dependent aquaculture practices.

## **II. FISH MICROFLORA: DIVERSITY, COMPOSITION, AND FUNCTIONAL IMPORTANCE**

### **2.1 Concept and Definition of Fish Microflora**

Fish microflora refers to the diverse assemblage of microorganisms associated with different tissues and body surfaces of fish, including the gastrointestinal tract, skin, gills, and reproductive organs. These microbial communities comprise bacteria, archaea, fungi, protozoa, and viruses that coexist with the host and contribute to various physiological functions. Among these groups, bacteria represent the most extensively studied component due to their significant roles in nutrition, immunity, and disease resistance (Aggarwal et al., 2023).

The composition of fish-associated microbiota is highly dynamic and influenced by host genetics, developmental stage, feeding habits, environmental conditions, water quality, salinity, temperature, and farming practices. Unlike terrestrial animals, fish are continuously exposed to surrounding water, creating constant interactions between environmental microorganisms and host-associated microbial communities. Consequently, the fish microbiome is strongly shaped by the aquatic environment and often reflects changes occurring within culture systems (Huang et al., 2020).

### **2.2 Gut Microbiota of Fish**

The gastrointestinal tract harbors the most abundant and metabolically active microbial community in fish. The gut microbiota contributes significantly to digestion, nutrient absorption, immune development, and maintenance of intestinal homeostasis. Studies employing high-throughput sequencing technologies have revealed that the fish gut microbiome is dominated by bacterial phyla such as Proteobacteria, Firmicutes, Bacteroidetes, Actinobacteriota, and Fusobacteriota, although their relative abundance varies among species and environmental conditions (Huang et al., 2025).

Several bacterial genera commonly reported in fish intestines include *Aeromonas*, *Pseudomonas*, *Vibrio*, *Photobacterium*, *Cetobacterium*, *Lactobacillus*, *Bacillus*, and *Shewanella*. Herbivorous, omnivorous, and carnivorous fish often possess distinct microbial communities reflecting differences in dietary composition and digestive physiology. For example, *Cetobacterium* species are frequently dominant in freshwater fishes and contribute to vitamin B<sub>12</sub> synthesis, whereas marine fishes often harbor higher proportions of *Vibrio* and *Photobacterium* species (Yajima et al., 2023).

Recent metagenomic studies have demonstrated that intensive aquaculture practices can alter gut microbial composition and reduce microbial diversity. Such changes may disrupt intestinal homeostasis and increase susceptibility to opportunistic pathogens. Antibiotic exposure represents one of the major factors responsible for these alterations, frequently leading to enrichment of resistant bacterial populations and associated resistance genes (Galgano et al., 2025).



### **2.3 Gill-Associated Microbiota**

Fish gills represent a critical interface between the host and the surrounding aquatic environment. In addition to their primary roles in respiration, osmoregulation, and excretion, gills support diverse microbial communities adapted to oxygen-rich conditions. Because gills are continuously exposed to waterborne microorganisms, their microbiota is often more variable than intestinal microbial communities.

Gill-associated bacteria commonly include members of the genera *Pseudomonas*, *Flavobacterium*, *Aeromonas*, *Acinetobacter*, and *Vibrio*. These microorganisms contribute to mucosal health and may provide protection against pathogen colonization through competitive exclusion mechanisms. However, gills also serve as important entry points for pathogenic bacteria and environmental contaminants. Exposure to antibiotic residues and polluted water can promote the selection of resistant microbial populations within gill-associated communities, thereby contributing to the dissemination of antibiotic resistance in aquaculture systems (Mohammed et al., 2025).

### **2.4 Skin and Mucosal Microbiota**

The skin mucus layer constitutes the first line of defense against environmental stressors and pathogenic microorganisms. Fish skin hosts a diverse microbiota that interacts closely with mucus components, antimicrobial peptides, immunoglobulins, and innate immune cells. These microbial communities play a protective role by occupying ecological niches and preventing colonization by pathogenic bacteria.

Proteobacteria, Bacteroidetes, Firmicutes, and Actinobacteria are frequently reported as dominant bacterial groups on fish skin surfaces. Common genera include *Pseudomonas*, *Aeromonas*, *Shewanella*, *Flavobacterium*, and *Bacillus*. Environmental conditions, stocking density, water quality, and disease status significantly influence skin microbial composition. Disturbances caused by antibiotic exposure may alter the balance between beneficial and opportunistic microorganisms, resulting in microbial dysbiosis and increased disease susceptibility (Li et al., 2025a).

### **2.5 Environmental Microbial Communities Associated with Fish**

Fish-associated microbiota are closely connected with microbial populations present in water columns, sediments, biofilms, feeds, and aquaculture infrastructure. These environmental communities act as sources of microbial recruitment and play a crucial role in shaping host-associated microbiomes. Consequently, the microbiological quality of aquaculture environments directly influences fish health and microbial stability.

Aquaculture sediments deserve particular attention because they function as reservoirs of organic matter, antibiotic residues, resistant bacteria, and antibiotic resistance genes. Numerous studies have reported higher abundances of ARGs in sediments compared with surrounding water, highlighting their importance as long-term resistance reservoirs (Li et al., 2025b). Continuous exchange of microorganisms between fish and environmental habitats facilitates the circulation of resistance determinants throughout aquaculture ecosystems.

### **2.6 Functional Roles of Fish Microflora**

Fish-associated microbial communities perform numerous physiological functions that are essential for host survival and health. One of their primary roles involves nutrient metabolism and digestion. Gut microorganisms produce enzymes capable of degrading complex dietary compounds, thereby enhancing nutrient utilization and energy extraction. Certain bacterial taxa also synthesize essential vitamins, amino acids, and other bioactive metabolites that contribute to host growth and development.

Another important function of fish microflora is immune modulation. Microbial communities stimulate the maturation of mucosal immune tissues and influence both innate and adaptive immune responses. Beneficial microorganisms can induce the production of antimicrobial peptides, cytokines, and immunoglobulins that enhance host defense mechanisms against pathogens (Lyu, 2023).

Fish microbiota additionally contribute to disease resistance through competitive exclusion. By occupying ecological niches and competing for nutrients and attachment sites, beneficial microorganisms can suppress colonization by



opportunistic and pathogenic bacteria. This phenomenon has become the basis for the development of probiotic applications in aquaculture aimed at improving fish health while reducing antibiotic dependence.

### **2.7 Microbial Homeostasis and Dysbiosis**

Under healthy conditions, fish-associated microbial communities exist in a relatively stable state known as microbial homeostasis. This balanced microbial ecosystem supports optimal physiological function and resistance to disease. However, environmental stressors such as poor water quality, nutritional deficiencies, temperature fluctuations, pathogen exposure, and antibiotic treatment can disrupt microbial equilibrium.

Dysbiosis refers to alterations in microbial composition, diversity, and functional activity that negatively affect host health. Antibiotic administration is one of the most significant causes of dysbiosis in aquaculture systems because it reduces susceptible bacterial populations while selectively enriching resistant microorganisms. Such disturbances may decrease microbial diversity, impair immune function, facilitate pathogen colonization, and promote the accumulation of antibiotic resistance genes within fish-associated microbiota (Yu et al., 2025).

The increasing recognition of fish microflora as both a functional component of host health and a reservoir of antibiotic resistance highlights its central role in modern aquaculture research. Understanding the diversity, ecology, and functional significance of these microbial communities is therefore essential for evaluating how antibiotic use influences resistance development and dissemination within aquatic ecosystems.

## **III. ANTIBIOTIC USAGE IN AQUACULTURE: HISTORICAL AND CONTEMPORARY PERSPECTIVES**

### **3.1 Evolution of Antibiotic Application in Aquaculture**

The use of antibiotics in aquaculture began in the 1950s and 1960s following the rapid expansion of intensive fish farming systems. As aquaculture production increased, disease outbreaks caused by bacterial pathogens emerged as a major constraint to productivity. Antibiotics were initially regarded as highly effective tools for controlling infectious diseases and minimizing economic losses. Their successful application in terrestrial livestock production further encouraged their adoption in aquaculture. Over time, antimicrobial agents became integrated into routine disease management practices in many fish-producing countries (Afroze et al., 2025).

During the early stages of aquaculture development, antibiotics were frequently used without comprehensive knowledge of their ecological consequences. In many regions, antimicrobial agents were applied not only for disease treatment but also for prophylactic purposes to prevent infections before disease outbreaks occurred. Such practices significantly increased the exposure of aquatic microbial communities to antibiotics, creating favorable conditions for the emergence of resistant bacterial populations. Subsequent research demonstrated that extensive antibiotic usage could alter aquatic microbial ecology and contribute to the dissemination of antibiotic resistance genes throughout aquaculture environments (Lajqi Berisha et al., 2024).

In recent decades, increasing awareness of antimicrobial resistance has prompted stricter regulations and surveillance programs in several countries. Nevertheless, antibiotic use remains substantial in many aquaculture-producing regions, particularly where disease pressure is high and alternative disease-control strategies are limited.

### **3.2 Therapeutic, Prophylactic, and Metaphylactic Uses**

Antibiotics in aquaculture are generally employed for three primary purposes: therapeutic treatment, prophylactic prevention, and metaphylactic control of disease outbreaks.

Therapeutic use involves the administration of antibiotics to clinically diseased fish following diagnosis of bacterial infections. This approach represents the most scientifically justified application because treatment targets specific pathogens responsible for disease outbreaks. Common bacterial diseases treated with antibiotics include motile aeromonad septicemia, vibriosis, enteric septicemia, columnaris disease, and edwardsiellosis.

Prophylactic use refers to the administration of antibiotics to healthy fish populations before any signs of disease are observed. Historically, this practice was widely adopted in intensive aquaculture systems where disease outbreaks were



anticipated during stressful periods such as transportation, grading, or environmental fluctuations. However, prophylactic antibiotic use has become increasingly controversial because it exposes large microbial populations to antimicrobial agents without confirmed infection, thereby accelerating resistance selection.

Metaphylactic treatment occupies an intermediate position between therapeutic and prophylactic applications. In this approach, antibiotics are administered to an entire population when disease has been diagnosed in a subset of individuals, with the objective of preventing further spread of infection. Although metaphylaxis may reduce mortality during severe outbreaks, it also increases antimicrobial exposure within aquaculture environments and contributes to selective pressure favoring resistant microorganisms (Gomes et al., 2026).

### **3.3 Major Antibiotic Classes Used in Fish Farming**

Several classes of antibiotics have been utilized in aquaculture worldwide. Their selection depends on pathogen susceptibility, regulatory approval, availability, and production practices.

#### **3.3.1 Tetracyclines**

Tetracyclines are among the most extensively used antibiotics in aquaculture. Oxytetracycline is particularly important because of its broad-spectrum activity against numerous Gram-positive and Gram-negative bacteria. The antibiotic functions by inhibiting bacterial protein synthesis through binding to the 30S ribosomal subunit. Despite its effectiveness, prolonged use has resulted in the widespread occurrence of tetracycline resistance genes such as *tetA*, *tetB*, *tetM*, and *tetO* in aquaculture environments (Yuan et al., 2023 and Semenova et al., 2025).

#### **3.3.2 Sulfonamides**

Sulfonamides are synthetic antimicrobial compounds frequently administered in combination with trimethoprim to enhance antibacterial activity. These agents inhibit folic acid synthesis, thereby disrupting bacterial growth and replication. Resistance to sulfonamides is commonly associated with the *sul1*, *sul2*, and *sul3* genes, which have been widely detected in fish-associated microbiota and aquaculture sediments (Yuan et al., 2023).

#### **3.3.3 Fluoroquinolones**

Fluoroquinolones such as enrofloxacin, ciprofloxacin, and norfloxacin possess potent activity against a broad range of bacterial pathogens. These antibiotics inhibit bacterial DNA gyrase and topoisomerase IV, preventing DNA replication. Their high efficacy has contributed to widespread use in some aquaculture sectors; however, increasing reports of quinolone-resistant bacteria and plasmid-mediated *qnr* genes have generated significant concern regarding their continued application (Ruiz, 2024).

#### **3.3.4 $\beta$ -Lactams**

$\beta$ -Lactam antibiotics, including ampicillin and amoxicillin, interfere with bacterial cell wall synthesis by targeting peptidoglycan formation. Although their use in aquaculture is generally more limited than tetracyclines or sulfonamides,  $\beta$ -lactam resistance genes, particularly those encoding  $\beta$ -lactamases, have been frequently identified in aquatic bacteria. The emergence of extended-spectrum  $\beta$ -lactamase (ESBL)-producing microorganisms has further increased public health concerns (Ribeiro et al., 2024).

#### **3.3.5 Phenicol**

Florfenicol is currently one of the most important phenicol antibiotics used in modern aquaculture. It is effective against numerous fish pathogens and is often considered an alternative to chloramphenicol, whose use has been restricted in many countries due to safety concerns. Resistance to florfenicol is primarily associated with the *flor* gene, which has been detected in both pathogenic and environmental aquatic bacteria (Zhang et al., 2022).



### **3.3.6 Aminoglycosides**

Aminoglycosides such as streptomycin, neomycin, and gentamicin have historically been used to treat bacterial infections in fish. These antibiotics inhibit protein synthesis by binding to the bacterial ribosome. Resistance is commonly mediated by aminoglycoside-modifying enzymes encoded by genes such as *aadA* and *aphA*. Although aminoglycoside use has declined in several regions, resistance determinants remain widespread in aquatic environments (Solanki & Das, 2024).

### **3.4 Routes of Antibiotic Administration**

Antibiotics are administered in aquaculture through medicated feed, immersion treatments, and direct water application. Medicated feed is the most common route because it allows targeted delivery to cultured fish while minimizing labor requirements. However, incomplete feed consumption often results in antibiotic residues entering surrounding water and sediments.

Immersion treatments involve exposing fish to antibiotic-containing water for a specified period. This method is commonly used for larval and juvenile stages when feed-based administration may be impractical. Direct water application, although less common today, has historically contributed to substantial environmental contamination because antibiotics are dispersed throughout the culture system rather than delivered specifically to infected individuals (Goswami et al., 2026).

The efficiency of antibiotic delivery depends on species, feeding behavior, environmental conditions, and disease status. Regardless of administration route, a significant proportion of antibiotics may remain unmetabolized and enter aquatic ecosystems, where they continue to influence microbial communities long after treatment has ceased.

### **3.5 Global Trends in Antibiotic Consumption**

Patterns of antibiotic usage vary considerably among countries and aquaculture sectors. Nations with highly regulated aquaculture industries, such as Norway, have achieved substantial reductions in antibiotic consumption through vaccination programs, improved biosecurity, and enhanced disease management practices. In contrast, antibiotic use remains relatively high in several regions where aquaculture production is expanding rapidly and disease challenges remain significant.

Recent studies indicate that Asia, which accounts for the majority of global aquaculture production, also represents the largest consumer of aquaculture antibiotics. The combination of intensive production systems, high disease pressure, and varying regulatory frameworks contributes to considerable regional differences in antimicrobial usage patterns (Raut et al., 2026).

The increasing globalization of seafood trade further emphasizes the importance of harmonized international approaches to antimicrobial stewardship. Resistant bacteria and resistance genes originating in one geographical region may disseminate through aquatic environments, seafood products, and international trade networks, highlighting the global nature of the AMR challenge.

### **3.6 Regulatory Frameworks and Restrictions**

Growing concerns regarding antimicrobial resistance have led to the implementation of stricter regulations governing antibiotic use in aquaculture. International organizations including the World Health Organization (WHO), the Food and Agriculture Organization (FAO), and the World Organisation for Animal Health (WOAH) have advocated responsible antimicrobial use and strengthened surveillance programs.

Many countries now require veterinary oversight for antibiotic administration, establish withdrawal periods for marketed fish products, and prohibit the use of certain critically important antimicrobials. Regulatory authorities have also increased monitoring of antibiotic residues in seafood products to protect consumer health and ensure compliance with international trade standards.

Despite these advances, substantial challenges remain. Inadequate enforcement, limited surveillance infrastructure, and disparities in regulatory capacity continue to affect antimicrobial stewardship efforts in many developing aquaculture-



producing regions. Addressing these challenges will be essential for reducing antibiotic dependence and mitigating the emergence of resistance within fish-associated microbial communities.

#### **IV. EMERGENCE AND EVOLUTION OF ANTIBIOTIC RESISTANCE IN FISH MICROFLORA**

##### **4.1 Concept of Antibiotic Resistance in Aquatic Environments**

Antibiotic resistance is the ability of microorganisms to survive, grow, or persist in the presence of antimicrobial concentrations that would normally inhibit or eliminate susceptible bacteria. In aquaculture ecosystems, resistance develops when microbial communities are repeatedly exposed to antibiotics, creating selective pressure that favors resistant populations. Over time, susceptible microorganisms decline while resistant strains become dominant components of fish-associated microflora (Krahulcová et al., 2023).

Unlike clinical environments, aquatic ecosystems contain highly diverse microbial communities that interact continuously with fish, sediments, water, aquatic plants, and other organisms. This ecological complexity provides numerous opportunities for resistance genes to emerge, persist, and spread among bacterial populations. Consequently, aquaculture environments are increasingly recognized as important hotspots for the evolution and dissemination of antimicrobial resistance.

##### **4.2 Selection Pressure and Resistance Development**

The development of antibiotic resistance is fundamentally driven by selective pressure. When antibiotics are introduced into aquaculture systems, they not only affect pathogenic bacteria but also influence commensal and environmental microorganisms. Bacteria possessing resistance determinants survive antibiotic exposure and gain a competitive advantage over susceptible populations. Repeated or prolonged exposure further enriches resistant microorganisms within the microbial community (Li et al., 2025c).

A significant proportion of administered antibiotics is released into surrounding environments through fish excretion, uneaten feed, and water exchange. These residual antibiotics often occur at sub-inhibitory concentrations that may not kill bacteria but can stimulate genetic adaptation, mutation, and horizontal gene transfer. Such conditions promote the gradual accumulation of resistance traits throughout fish-associated microbiota and surrounding environmental communities.

Studies conducted in freshwater ponds, recirculating aquaculture systems, and marine cages have consistently demonstrated higher frequencies of resistant bacteria in environments exposed to antimicrobial agents compared with untreated systems (Hassen, 2026). These findings indicate that antibiotic exposure remains one of the strongest drivers of resistance evolution in aquaculture.

##### **4.3 Role of Fish Microflora in Resistance Evolution**

Fish-associated microbial communities serve as important reservoirs and amplifiers of antibiotic resistance. The gastrointestinal tract is particularly significant because it contains dense bacterial populations exposed to both dietary antibiotics and environmental microorganisms. The high microbial density within the gut increases opportunities for genetic exchange and resistance dissemination.

Several studies have reported the presence of resistant strains of *Aeromonas*, *Pseudomonas*, *Vibrio*, *Acinetobacter*, and *Shewanella* within fish intestines. Many of these bacteria harbor resistance genes against multiple antibiotic classes, including tetracyclines, sulfonamides, quinolones, and  $\beta$ -lactams (Miao et al., 2021). Because these microorganisms are capable of surviving both within fish hosts and in external aquatic environments, they function as effective vectors for resistance transmission.

The skin and gill microbiota also contribute to resistance evolution. These microbial communities are continuously exposed to waterborne contaminants and antibiotic residues, creating favorable conditions for the selection of resistant populations. Consequently, resistance determinants are often distributed throughout multiple fish tissues rather than being confined solely to the gastrointestinal tract.



#### ***4.4 Environmental Drivers of Resistance Emergence***

##### ***4.4.1 Intensive Aquaculture Practices***

Intensive aquaculture systems typically involve high stocking densities and increased fish biomass per unit area. Although such practices improve production efficiency, they also facilitate pathogen transmission and increase disease incidence. Greater disease pressure often leads to increased antibiotic usage, thereby enhancing selective pressure on microbial communities (Salam et al., 2023).

Furthermore, intensive systems frequently accumulate organic matter, uneaten feed, and metabolic wastes that support bacterial growth. The resulting increase in microbial abundance creates favorable conditions for resistance evolution and gene exchange among bacterial populations.

##### ***4.4.2 Poor Water Quality***

Water quality is a critical determinant of microbial community structure and fish health. Elevated concentrations of ammonia, nitrite, suspended solids, and organic matter can stress fish and increase susceptibility to infections. Disease outbreaks under poor water-quality conditions often result in greater antibiotic dependence, indirectly contributing to resistance development.

Environmental stress may also alter microbial community composition, favoring opportunistic bacteria that frequently possess multiple resistance traits. Consequently, degraded water quality can accelerate both disease emergence and resistance evolution within aquaculture systems.

##### ***4.4.3 Antibiotic Residues in Sediments***

Aquaculture sediments function as major reservoirs of antibiotic residues and resistant microorganisms. Many antimicrobial compounds adsorb onto sediment particles where they persist for extended periods after treatment has ceased. These residues continue to exert selective pressure on sediment-associated microbial communities and promote the maintenance of resistance genes (Li et al., 2025d).

Numerous investigations have reported significantly higher abundances of antibiotic resistance genes in sediments compared with overlying water. Sediments therefore serve as long-term reservoirs that support the persistence and dissemination of resistance determinants throughout aquaculture ecosystems.

##### ***4.4.4 Co-selection by Heavy Metals and Environmental Pollutants***

Recent research indicates that antibiotic resistance can also be promoted by non-antibiotic pollutants. Heavy metals such as copper, zinc, mercury, and cadmium are commonly detected in aquaculture environments due to feed additives, agricultural runoff, and industrial contamination. Many resistance genes are located on the same mobile genetic elements as metal-resistance genes. Consequently, exposure to heavy metals may co-select for antibiotic resistance even in the absence of direct antibiotic pressure (Gillieatt et al., 2024).

Similarly, disinfectants, pesticides, and other environmental contaminants may contribute to the enrichment of resistant bacterial populations by selecting for microorganisms possessing broad stress-response mechanisms.

#### ***4.5 Multidrug Resistance in Fish-Associated Bacteria***

One of the most alarming developments in aquaculture microbiology is the increasing prevalence of multidrug-resistant (MDR) bacteria. MDR organisms are defined as bacteria resistant to three or more classes of antimicrobial agents. Such strains have been reported in numerous fish species and aquaculture environments worldwide.

Species belonging to *Aeromonas*, *Vibrio*, *Pseudomonas*, and *Acinetobacter* frequently exhibit resistance to tetracyclines, sulfonamides, quinolones, aminoglycosides, and  $\beta$ -lactams simultaneously. The occurrence of MDR bacteria is particularly concerning because it limits therapeutic options and increases the likelihood of treatment failure during disease outbreaks (Marino et al., 2025).



The widespread detection of MDR bacteria in fish microflora also has implications beyond aquaculture production. These microorganisms may act as reservoirs of resistance genes that can be transferred to other environmental bacteria and potentially to human pathogens, reinforcing concerns regarding the role of aquaculture in the broader antimicrobial resistance crisis.

#### **4.6 Persistence of Resistance in Aquaculture Ecosystems**

A common misconception is that antibiotic resistance disappears once antibiotic use is discontinued. However, numerous studies have demonstrated that resistance genes and resistant bacterial populations can persist in aquatic environments for prolonged periods. The persistence of resistance is facilitated by mobile genetic elements, biofilm formation, ecological fitness advantages, and continuous environmental contamination (Hossain et al., 2026).

Biofilms formed on tank surfaces, pond sediments, aquatic vegetation, and fish tissues provide protective microenvironments where resistant bacteria can survive and exchange genetic material. These biofilm-associated communities often display enhanced tolerance to antimicrobial agents and environmental stressors, making them important reservoirs of resistance.

The long-term persistence of resistance determinants indicates that reducing antibiotic usage alone may not immediately eliminate resistance from aquaculture systems. Effective management therefore requires integrated strategies combining prudent antimicrobial use, environmental monitoring, improved husbandry practices, and alternative disease-control approaches.

#### **4.7 Significance of Resistance Evolution in Fish Microflora**

The emergence of antibiotic resistance within fish microflora represents a critical challenge at the intersection of aquaculture, environmental microbiology, and public health. Fish-associated microbial communities function not only as indicators of antimicrobial exposure but also as active participants in the evolution and dissemination of resistance genes. The increasing prevalence of resistant and multidrug-resistant bacteria highlights the urgent need for sustainable management practices aimed at reducing selective pressure and limiting the spread of resistance throughout aquatic ecosystems.

Understanding the ecological and evolutionary processes that drive resistance emergence is essential for developing effective surveillance systems and mitigation strategies. Such knowledge forms the foundation for subsequent investigations into specific resistant bacterial taxa, resistance genes, and mechanisms of genetic transfer within fish-associated microbial communities.

### **V. MAJOR ANTIBIOTIC-RESISTANT BACTERIA ASSOCIATED WITH FISH MICROFLORA**

Fish-associated microbial communities comprise a diverse assemblage of bacteria, many of which contribute positively to host nutrition and immunity. However, several members of the normal fish microflora have increasingly acquired resistance to multiple antimicrobial agents and now function as reservoirs of antibiotic resistance genes (ARGs). Some of these bacteria are opportunistic pathogens capable of causing disease in fish, while others act primarily as environmental reservoirs that facilitate the persistence and dissemination of resistance determinants. The most important antibiotic-resistant bacterial groups associated with fish microflora are discussed below.

#### **5.1 *Aeromonas* Species**

Members of the genus *Aeromonas* are among the most frequently reported bacteria in freshwater fish microbiota and are considered important indicators of antimicrobial resistance in aquatic environments. Species such as *Aeromonas hydrophila*, *A. veronii*, *A. caviae*, and *A. sobria* are commonly isolated from the gastrointestinal tract, skin, and gills of cultured fish. Although these bacteria may exist as commensals under normal conditions, they can become opportunistic pathogens when fish are exposed to stress or immunosuppression (Zhao et al., 2025).

Numerous studies have documented high levels of resistance among *Aeromonas* isolates against tetracyclines, sulfonamides,  $\beta$ -lactams, aminoglycosides, and quinolones. Resistance genes frequently identified in *Aeromonas* include



*tetA*, *tetE*, *sul1*, *sul2*, *qnrS*, and various  $\beta$ -lactamase genes. The ability of *Aeromonas* species to harbor plasmids, integrons, and transposons significantly enhances their role as reservoirs of transferable resistance determinants (Li et al., 2025e).

Of particular concern is the increasing prevalence of multidrug-resistant *Aeromonas* strains in aquaculture systems. Such strains not only complicate disease treatment in fish but also raise public health concerns because several *Aeromonas* species are recognized as emerging human pathogens capable of causing gastroenteritis, wound infections, and septicemia.

### 5.2 *Vibrio* Species

The genus *Vibrio* represents one of the most important groups of bacteria associated with marine and brackish-water fish. Species including *Vibrio anguillarum*, *V. harveyi*, *V. vulnificus*, and *V. parahaemolyticus* are frequently isolated from fish intestines, skin mucus, gills, and surrounding aquatic environments.

Vibriosis remains one of the most economically significant bacterial diseases affecting marine aquaculture worldwide. The extensive use of antibiotics to control *Vibrio* infections has contributed to the emergence of resistant populations in numerous fish-producing regions. Resistance to oxytetracycline, chloramphenicol, florfenicol, sulfonamides, and quinolones has been widely reported among fish-associated *Vibrio* isolates (Mohammed et al., 2025).

Molecular investigations have revealed the presence of multiple resistance genes within *Vibrio* populations, including *tet*, *qnr*, *sul*, and *floR* gene families. Many of these genes are located on conjugative plasmids, facilitating horizontal gene transfer between *Vibrio* species and other aquatic bacteria. Consequently, *Vibrio* populations serve as important reservoirs of resistance within marine aquaculture ecosystems.

### 5.3 *Pseudomonas* Species

Species belonging to the genus *Pseudomonas* are ubiquitous inhabitants of aquatic environments and are commonly detected in fish intestines, gills, skin mucus, and aquaculture water. Their remarkable metabolic versatility allows them to survive under diverse environmental conditions, making them important components of fish-associated microbial communities.

*Pseudomonas aeruginosa*, *P. fluorescens*, and related species possess intrinsic resistance to numerous antibiotics due to low membrane permeability, efflux pump systems, and enzymatic defense mechanisms. In addition to intrinsic resistance, many isolates have acquired resistance genes conferring protection against  $\beta$ -lactams, aminoglycosides, quinolones, and tetracyclines (Hu & Chua, 2025).

The environmental persistence of *Pseudomonas* species, combined with their capacity to form biofilms, enhances their significance as reservoirs of antimicrobial resistance. Biofilm-associated populations exhibit increased tolerance to antibiotics and frequently facilitate genetic exchange among bacterial communities inhabiting fish and aquaculture environments.

### 5.4 *Edwardsiella* Species

The genus *Edwardsiella* includes important fish pathogens such as *Edwardsiella tarda*, *E. piscicida*, and *E. ictaluri*. These bacteria are responsible for edwardsiellosis, a severe systemic disease affecting numerous freshwater and marine fish species.

Historically, antibiotics have been widely used to control edwardsiellosis outbreaks, resulting in the emergence of resistant strains in several aquaculture-producing countries. Resistance to tetracyclines, sulfonamides, aminoglycosides, and quinolones has been reported in both environmental and clinical isolates of *Edwardsiella*. Several studies have identified plasmid-mediated resistance determinants that facilitate transmission of ARGs among aquatic bacterial populations (Rolbiecki et al., 2020).



Although *Edwardsiella* species are less abundant than *Aeromonas* or *Pseudomonas* within normal fish microflora, their pathogenic importance and increasing resistance levels make them significant contributors to the antimicrobial resistance burden in aquaculture.

### **5.5 *Flavobacterium* Species**

Species of *Flavobacterium* are widely distributed in freshwater and marine environments and frequently colonize fish skin, gills, and mucosal surfaces. Certain species, particularly *Flavobacterium columnare* and *F. psychrophilum*, are major fish pathogens responsible for columnaris disease and bacterial cold-water disease, respectively. The treatment of flavobacterial infections often relies on antibiotics such as oxytetracycline and florfenicol. Consequently, resistant strains have emerged in many aquaculture systems. Resistance genes associated with tetracycline and phenicol resistance have been detected in several pathogenic *Flavobacterium* isolates, indicating increasing selective pressure resulting from antimicrobial usage (Pavelquesi et al., 2021). Because flavobacterial diseases are often associated with environmental stress and poor water quality, improving husbandry practices remains an important strategy for reducing antibiotic dependence and resistance development.

### **5.6 *Shewanella* Species**

*Shewanella* species are common inhabitants of aquatic environments and are frequently detected in fish intestines and marine ecosystems. Although traditionally considered environmental bacteria, several species have recently gained attention because of their role as reservoirs of clinically relevant resistance genes. Particularly noteworthy is the detection of carbapenemase-related genes and other  $\beta$ -lactam resistance determinants in *Shewanella* populations. Evidence suggests that certain resistance genes found in clinically important pathogens may have originated from environmental bacteria closely related to *Shewanella* species (Li et al., 2022b). The ecological distribution of *Shewanella* and its capacity to exchange genetic material with other microorganisms highlight its significance in the evolution and dissemination of antimicrobial resistance across aquatic environments.

### **5.7 *Acinetobacter* Species**

Members of the genus *Acinetobacter* are increasingly reported in fish microbiota, aquaculture sediments, and aquatic environments. Species such as *Acinetobacter baumannii* and related taxa possess remarkable genetic plasticity and an exceptional capacity to acquire resistance determinants.

Aquatic isolates frequently exhibit resistance to  $\beta$ -lactams, aminoglycosides, tetracyclines, and quinolones. The presence of integrons, plasmids, and transposons contributes significantly to the accumulation and dissemination of resistance genes within *Acinetobacter* populations. Given the importance of *Acinetobacter* as a multidrug-resistant pathogen in human medicine, its occurrence in aquaculture environments raises concerns regarding environmental-human transmission pathways (Ahuatzin-Flores et al., 2024).

### **5.8 *Enterobacteriaceae* Associated with Fish**

Various members of the family Enterobacteriaceae, including *Escherichia coli*, *Klebsiella pneumoniae*, *Enterobacter spp.*, *Citrobacter spp.*, and *Serratia spp.*, have been isolated from fish intestines and aquaculture environments. These bacteria often originate from environmental contamination, agricultural runoff, sewage discharge, or contaminated feed sources.

The detection of extended-spectrum  $\beta$ -lactamase (ESBL)-producing Enterobacteriaceae in aquaculture systems has become a major concern. Resistance genes such as *bla*TEM, *bla*SHV, and *bla*CTX-M have been reported from fish-associated isolates worldwide. Their occurrence demonstrates the increasing overlap between environmental and clinically important resistance reservoirs (Goudarzi et al., 2013).



### **5.9 Multidrug-Resistant Fish Pathogens: An Emerging Threat**

The most alarming trend in recent years is the increasing prevalence of multidrug-resistant (MDR) bacteria within fish microflora. MDR strains have been identified among *Aeromonas*, *Vibrio*, *Pseudomonas*, *Edwardsiella*, *Acinetobacter*, and Enterobacteriaceae isolated from both cultured and wild fish populations. These bacteria frequently exhibit simultaneous resistance to tetracyclines, sulfonamides, quinolones, aminoglycosides, and  $\beta$ -lactams (Zhao et al., 2025b). The emergence of MDR microorganisms significantly reduces treatment options during disease outbreaks and increases the risk of therapeutic failure. Furthermore, fish-associated MDR bacteria function as reservoirs of mobile resistance genes capable of transferring to environmental and potentially human-associated microorganisms. Consequently, the increasing prevalence of MDR bacteria in fish microflora represents not only an aquaculture challenge but also a broader environmental and public health concern.

The widespread occurrence of resistant bacterial taxa within fish-associated microbial communities demonstrates that fish microflora constitute an important component of the aquatic resistome. Understanding the biology, ecology, and resistance profiles of these bacteria is essential for evaluating the mechanisms through which antibiotic resistance develops and spreads within aquaculture ecosystems.

## **VI. MOLECULAR MECHANISMS OF ANTIBIOTIC RESISTANCE**

The emergence and persistence of antibiotic resistance in fish microflora are primarily driven by genetic and physiological adaptations that enable bacteria to survive antimicrobial exposure. These mechanisms may be intrinsic, reflecting naturally occurring characteristics of bacterial species, or acquired through mutations and horizontal gene transfer. Understanding the molecular basis of resistance is essential for evaluating how fish-associated microorganisms withstand antimicrobial pressure and disseminate resistance determinants throughout aquatic ecosystems.

### **6.1 Intrinsic and Acquired Resistance**

Antibiotic resistance can be categorized into intrinsic and acquired forms. Intrinsic resistance is a naturally occurring characteristic encoded within the bacterial chromosome and is independent of prior antibiotic exposure. Examples include the low outer membrane permeability of many Gram-negative bacteria and the presence of naturally occurring efflux systems. Such mechanisms provide baseline protection against specific antimicrobial agents.

Acquired resistance develops through genetic changes resulting from mutation or acquisition of foreign DNA. This form of resistance is particularly important in aquaculture because antibiotic exposure creates selective pressure favoring bacteria that possess adaptive resistance traits. Acquired resistance genes are frequently located on plasmids, transposons, integrons, and other mobile genetic elements that facilitate transmission among bacterial populations (Pepi & Focardi, 2021).

In fish-associated microbial communities, both intrinsic and acquired resistance mechanisms often coexist, resulting in complex resistance phenotypes and increasing the likelihood of multidrug resistance.

### **6.2 Enzymatic Inactivation of Antibiotics**

One of the most effective bacterial defense strategies involves the production of enzymes capable of modifying or destroying antimicrobial compounds before they reach their cellular targets.

#### **6.2.1 $\beta$ -Lactamases**

$\beta$ -Lactam antibiotics exert their antibacterial activity by interfering with bacterial cell wall synthesis. In response, many bacteria produce  $\beta$ -lactamase enzymes that hydrolyze the  $\beta$ -lactam ring, rendering the antibiotic inactive. Genes encoding  $\beta$ -lactamases have been widely detected in fish-associated bacteria, including *Aeromonas*, *Pseudomonas*, *Acinetobacter*, and members of the Enterobacteriaceae.

Particularly concerning is the emergence of extended-spectrum  $\beta$ -lactamases (ESBLs), which can degrade a broad range of  $\beta$ -lactam antibiotics. Genes such as *blaTEM*, *blaSHV*, and *blaCTX-M* have been reported from aquaculture



environments and fish-associated microbiota worldwide (Yuan et al., 2023). The occurrence of these genes in aquatic ecosystems demonstrates increasing overlap between environmental and clinical resistance reservoirs.

### 6.2.2 Aminoglycoside-Modifying Enzymes

Aminoglycoside resistance is frequently mediated by enzymes that chemically modify antibiotic molecules through acetylation, phosphorylation, or adenylation. These modifications prevent effective binding of the antibiotic to bacterial ribosomes, thereby eliminating its antibacterial activity.

Genes encoding aminoglycoside-modifying enzymes, including *aadA*, *aphA*, and *aac* variants, have been identified in numerous aquatic bacterial species. Their widespread distribution reflects long-term exposure of aquatic microbial communities to aminoglycoside antibiotics and contributes significantly to resistance dissemination within fish microflora.

### 6.3 Modification of Antibiotic Target Sites

Many antibiotics function by binding to specific bacterial structures or enzymes. Alteration of these targets reduces antibiotic affinity and allows bacterial survival despite antimicrobial exposure.

Resistance to quinolones commonly results from mutations in genes encoding DNA gyrase and topoisomerase IV, the primary targets of these antibiotics. Such mutations decrease antibiotic binding efficiency and reduce susceptibility. In addition to chromosomal mutations, plasmid-mediated quinolone resistance genes such as *qnrA*, *qnrB*, and *qnrS* provide protective effects by shielding target enzymes from antibiotic action (Millanao et al., 2021).

Similarly, resistance to macrolides, lincosamides, and streptogramins often involves methylation of ribosomal target sites, preventing effective antibiotic binding. Although these resistance mechanisms are more frequently studied in terrestrial and clinical settings, increasing evidence indicates their presence within aquaculture-associated microbial communities.

### 6.4 Efflux Pump-Mediated Resistance

Efflux pumps are membrane-associated transport proteins that actively expel toxic compounds from bacterial cells. These systems reduce intracellular antibiotic concentrations and frequently confer resistance to multiple classes of antimicrobial agents. Efflux-mediated resistance is particularly important among aquatic bacteria because many efflux pumps possess broad substrate specificity. Members of the genera *Pseudomonas*, *Aeromonas*, *Acinetobacter*, and *Vibrio* commonly harbor multidrug efflux systems capable of removing tetracyclines, quinolones, chloramphenicol,  $\beta$ -lactams, and other antibiotics (Milligan et al., 2023). The significance of efflux pumps extends beyond resistance alone. These systems may also facilitate bacterial adaptation to environmental stressors such as heavy metals, disinfectants, and organic pollutants. Consequently, environmental contamination can indirectly select for antibiotic-resistant bacteria through co-selection mechanisms involving efflux-mediated defense systems.

### 6.5 Reduced Membrane Permeability

For many antibiotics to exert their effects, they must first penetrate bacterial cell envelopes. Alterations in membrane structure can significantly reduce antibiotic uptake and contribute to resistance development. Gram-negative bacteria possess an outer membrane containing porin proteins that regulate molecular transport. Mutations leading to reduced porin expression or structural modification of porin channels decrease antibiotic entry into bacterial cells. Such mechanisms are particularly important in resistance to  $\beta$ -lactams, quinolones, and tetracyclines. Reduced permeability often acts synergistically with other resistance mechanisms, such as efflux pumps and enzymatic degradation, producing high levels of resistance that can be difficult to overcome therapeutically.

### 6.6 Biofilm-Associated Resistance

Biofilm formation represents one of the most important survival strategies employed by aquatic microorganisms. Biofilms consist of structured microbial communities embedded within extracellular polymeric matrices attached to



surfaces such as fish skin, gills, intestinal mucosa, tank walls, nets, and sediments. Bacteria residing within biofilms exhibit substantially greater tolerance to antibiotics than their planktonic counterparts. Several factors contribute to this enhanced resistance. The extracellular matrix acts as a physical barrier that limits antibiotic penetration. Additionally, biofilms create nutrient gradients and physiological heterogeneity that result in metabolically inactive bacterial subpopulations less susceptible to antimicrobial agents (Hindieh et al., 2025).

Fish-associated bacteria such as *Aeromonas*, *Vibrio*, *Pseudomonas*, and *Flavobacterium* readily form biofilms within aquaculture environments. These biofilm communities provide ideal conditions for horizontal gene transfer and long-term persistence of resistance determinants. Consequently, biofilms function as important reservoirs of antibiotic resistance genes in aquatic ecosystems.

### **6.7 Resistance Through Genetic Mutation**

Spontaneous genetic mutations represent another major mechanism contributing to antibiotic resistance. Mutations may alter antibiotic target sites, regulatory pathways, membrane proteins, or metabolic processes, thereby reducing antimicrobial susceptibility. Although mutation rates are generally low, the enormous population sizes of aquatic microorganisms increase the likelihood that resistant mutants will arise. Exposure to sublethal antibiotic concentrations further enhances selection of these mutants by providing a competitive advantage over susceptible bacteria. Mutational resistance is particularly important for antibiotics such as quinolones and rifampicin, where single nucleotide changes may substantially reduce antibiotic efficacy. Once established, resistant mutants can proliferate rapidly within fish-associated microbial communities under continued antimicrobial pressure.

### **6.8 Mobile Genetic Elements and Resistance Dissemination**

Many resistance genes are associated with mobile genetic elements that facilitate movement within and between bacterial genomes. These elements include plasmids, transposons, insertion sequences, and integrons. Their mobility enables rapid dissemination of resistance traits throughout microbial communities. Integrons are particularly significant because they capture and express multiple resistance gene cassettes. Class 1 integrons, which are frequently detected in aquaculture environments, often contain genes conferring resistance to sulfonamides, aminoglycosides,  $\beta$ -lactams, and other antibiotics. Numerous studies have reported strong correlations between antibiotic exposure and increased abundance of integron-associated resistance genes in fish-associated microbiota (Kador et al., 2025). The widespread occurrence of mobile genetic elements within fish microflora greatly enhances the potential for resistance dissemination across ecological boundaries. Through genetic exchange, resistance determinants originating in environmental bacteria can eventually become established in fish pathogens and potentially in human-associated microorganisms.

### **6.9 Ecological Significance of Resistance Mechanisms**

The diversity of resistance mechanisms found within fish microflora demonstrates the remarkable adaptive capacity of aquatic microorganisms. Individual bacterial strains frequently possess multiple resistance mechanisms simultaneously, including enzymatic degradation, target modification, efflux systems, and biofilm formation. Such combinations increase resistance levels and contribute to the emergence of multidrug-resistant populations.

From an ecological perspective, these mechanisms facilitate the persistence of resistant bacteria even when antibiotic concentrations are low or intermittent. They also promote long-term maintenance of resistance genes within aquatic ecosystems, transforming fish-associated microbial communities into important reservoirs of antimicrobial resistance. The molecular mechanisms described above provide the biological foundation for the development and spread of antibiotic resistance within fish microflora.

However, resistance evolution cannot be fully understood without examining the specific resistance genes responsible for these phenotypes and the mechanisms through which they are exchanged among bacterial populations. Therefore, the next section focuses on the diversity, distribution, and ecological significance of antibiotic resistance genes within fish-associated microbial communities.



## **VII. ANTIBIOTIC RESISTANCE GENES (ARGS) IN FISH MICROFLORA**

Antibiotic resistance genes (ARGs) constitute the genetic foundation of antimicrobial resistance and are now recognized as major components of aquatic microbial ecosystems. These genes encode proteins or regulatory mechanisms that enable bacteria to survive exposure to antimicrobial agents. Fish-associated microbial communities harbor a diverse collection of ARGs that have accumulated through natural evolution, anthropogenic antibiotic exposure, and horizontal gene transfer. Increasing evidence indicates that fish microflora function not only as reservoirs of resistant bacteria but also as important repositories of resistance genes capable of spreading across aquatic and terrestrial environments (Wang et al., 2022). The widespread occurrence of ARGs in fish intestines, skin mucus, gills, aquaculture water, sediments, and biofilms highlights the significant role of aquaculture ecosystems in resistance dissemination. Modern molecular techniques, particularly quantitative PCR, whole-genome sequencing, and metagenomics, have greatly expanded understanding of the diversity and distribution of resistance genes within fish-associated microbiota.

### **7.1 The Aquatic Resistome and Fish Microflora**

The term “resistome” refers to the complete collection of antibiotic resistance genes present within a microbial community. In aquatic environments, the resistome includes genes found in pathogenic, commensal, and environmental microorganisms regardless of whether they are actively expressed. Fish-associated microbial communities form an integral component of the aquatic resistome because they continuously interact with environmental bacteria, antibiotic residues, and mobile genetic elements.

Aquaculture systems provide ideal conditions for resistome expansion. High microbial densities, continuous environmental exposure, nutrient-rich conditions, and periodic antibiotic application facilitate the maintenance and dissemination of ARGs. As a result, fish microflora often contain a greater diversity of resistance determinants than previously recognized through conventional culture-based methods (Rana et al., 2023). Metagenomic analyses have revealed that many ARGs persist even in environments where antibiotic use has been reduced or discontinued. This observation suggests that resistance genes may remain stable within microbial communities due to ecological fitness advantages and association with mobile genetic elements.

### **7.2 Tetracycline Resistance Genes**

Tetracycline resistance genes are among the most frequently detected ARGs in fish-associated microbiota. The widespread historical use of oxytetracycline in aquaculture has contributed significantly to the enrichment of these genes within aquatic environments. The major tetracycline resistance genes identified in fish microflora include *tetA*, *tetB*, *tetC*, *tetD*, *tetE*, *tetG*, *tetM*, *tetO*, and *tetX*. These genes confer resistance through different mechanisms, including active efflux of antibiotics, ribosomal protection, and enzymatic inactivation (Pazra et al., 2023).

Among them, *tetA* and *tetB* are commonly associated with efflux pump-mediated resistance and are frequently detected in *Aeromonas*, *Pseudomonas*, and Enterobacteriaceae isolated from fish and aquaculture environments. In contrast, *tetM* and *tetO* encode ribosomal protection proteins that prevent tetracycline binding to bacterial ribosomes. Numerous studies have reported high prevalence of these genes in fish intestines, pond sediments, and aquaculture wastewater, indicating extensive dissemination throughout production systems (Yuan et al., 2023). The persistence of tetracycline resistance genes is particularly concerning because many are located on conjugative plasmids and transposons that facilitate transfer between bacterial species.

### **7.3 Sulfonamide Resistance Genes**

Sulfonamides have historically been used extensively in combination with trimethoprim to treat bacterial infections in aquaculture. Consequently, sulfonamide resistance genes are now widely distributed in aquatic microbial communities. The most frequently reported sulfonamide resistance genes include *sul1*, *sul2*, and *sul3*. These genes encode alternative forms of dihydropteroate synthase that exhibit reduced affinity for sulfonamide antibiotics, thereby maintaining folate biosynthesis despite antimicrobial exposure.



Among these determinants, *sul1* is particularly important because of its strong association with Class 1 integrons. Numerous investigations have identified *sul1* in fish intestines, sediments, biofilms, and aquaculture effluents. The prevalence of *sul2* is similarly widespread, especially among Gram-negative bacteria associated with freshwater fish production systems (Bojarski et al., 2020). The frequent detection of sulfonamide resistance genes even in environments with limited current antibiotic use suggests long-term persistence and ecological stabilization within aquatic resistomes.

#### **7.4 Quinolone Resistance Genes**

Quinolones and fluoroquinolones are important broad-spectrum antibiotics used in aquaculture and human medicine. Resistance to these compounds was initially attributed primarily to chromosomal mutations affecting DNA gyrase and topoisomerase IV. However, the discovery of plasmid-mediated quinolone resistance genes significantly altered understanding of quinolone resistance evolution.

The most common plasmid-mediated quinolone resistance genes include *qnrA*, *qnrB*, *qnrC*, *qnrD*, and *qnrS*. These genes encode proteins that protect DNA gyrase and topoisomerase IV from quinolone inhibition. Additional resistance determinants such as *aac(6')-Ib-cr* and *qepA* contribute to quinolone resistance through antibiotic modification and active efflux mechanisms (Abdelrahim et al., 2024).

Several studies have detected *qnr* genes in fish-associated bacteria including *Aeromonas*, *Vibrio*, *Pseudomonas*, and Enterobacteriaceae. Their occurrence in both cultured fish and surrounding environmental compartments highlights the extensive circulation of quinolone resistance determinants within aquaculture ecosystems.

#### **7.5 $\beta$ -Lactam Resistance Genes**

$\beta$ -Lactam antibiotics remain among the most widely used antimicrobial agents globally. Resistance to these compounds is primarily mediated by genes encoding  $\beta$ -lactamase enzymes capable of hydrolyzing the  $\beta$ -lactam ring. Important  $\beta$ -lactam resistance genes reported in fish microflora include *blaTEM*, *blaSHV*, *blaOXA*, *blaCTX-M*, and *blaCMY*. These genes have been identified in diverse aquatic bacteria, including *Aeromonas*, *Pseudomonas*, *Acinetobacter*, and Enterobacteriaceae (Pepi & Chua, 2021).

The emergence of extended-spectrum  $\beta$ -lactamase (ESBL) genes is of particular concern because these enzymes can inactivate a broad spectrum of  $\beta$ -lactam antibiotics. Detection of ESBL-producing bacteria in fish and aquaculture environments indicates increasing convergence between environmental and clinical resistance reservoirs (Plaku et al., 2026). Recent studies have also reported carbapenemase-related genes in environmental aquatic bacteria, raising concerns regarding the potential dissemination of resistance to critically important antimicrobials.

#### **7.6 Aminoglycoside Resistance Genes**

Resistance to aminoglycosides is commonly mediated by genes encoding antibiotic-modifying enzymes. These enzymes chemically alter aminoglycoside molecules through acetylation, phosphorylation, or adenylation, thereby reducing their antibacterial activity. Frequently reported aminoglycoside resistance genes in fish-associated microbiota include *aadA*, *aphA*, *aac(3)*, and *aac(6')*. These determinants have been detected in numerous aquatic bacterial taxa and are often associated with integrons and plasmids (El-Far et al., 2023). The coexistence of aminoglycoside resistance genes with determinants conferring resistance to other antibiotic classes contributes significantly to multidrug resistance. Consequently, aminoglycoside resistance genes frequently occur within complex resistance gene clusters that facilitate co-selection and dissemination.

#### **7.7 Phenicol Resistance Genes**

Florfenicol is one of the most important antibiotics currently approved for use in aquaculture. Although it has been widely adopted as an alternative to chloramphenicol, increasing reports of phenicol resistance have emerged in recent years. The principal phenicol resistance genes identified in fish microflora are *floR*, *cmlA*, and *cat* genes. Among these, *floR* is



particularly widespread and has been detected in *Aeromonas*, *Vibrio*, *Edwardsiella*, and various environmental bacteria associated with aquaculture systems (Ortiz-Severín et al., 2025).

The increasing prevalence of phenicol resistance is noteworthy because florfenicol remains an important therapeutic option for managing bacterial diseases in fish production.

### **7.8 Distribution of ARGs in Fish Tissues**

Antibiotic resistance genes are not distributed uniformly throughout fish-associated microbial communities. The gastrointestinal tract generally contains the highest abundance and diversity of ARGs due to its dense microbial populations and direct exposure to medicated feed. Numerous studies have reported detection of tetracycline, sulfonamide, quinolone, and  $\beta$ -lactam resistance genes within fish intestines. However, ARGs are also frequently identified in gill microbiota, skin mucus communities, and internal organs. These findings suggest that resistance determinants can colonize multiple host-associated niches and may circulate continuously between fish tissues and surrounding environments (Gutiérrez-Pacheco et al., 2026). The tissue-specific distribution of ARGs may influence both disease dynamics and transmission pathways within aquaculture systems.

### **7.9 ARGs in Aquaculture Water and Sediments**

Aquaculture water and sediments represent major environmental reservoirs of resistance genes. Residual antibiotics, organic matter accumulation, and dense microbial populations create favorable conditions for ARG persistence and dissemination. Sediments are particularly important because they frequently contain higher ARG abundances than water columns. Several investigations have demonstrated accumulation of *tet*, *sul*, *qnr*, and *bla* genes within pond sediments and marine cage farming areas. These sediment-associated resistomes may persist for extended periods and function as long-term sources of resistance determinants for fish-associated microbiota (Miličević et al., 2024).

The continuous exchange of microorganisms between fish and their environment facilitates bidirectional movement of ARGs, reinforcing the ecological connectivity of aquatic resistance reservoirs.

### **7.10 Ecological and Public Health Significance of ARGs**

The growing diversity and abundance of ARGs in fish microflora have significant ecological and public health implications. Fish-associated microbial communities serve as dynamic reservoirs where resistance determinants can accumulate, persist, and spread through horizontal gene transfer. Because many ARGs are associated with mobile genetic elements, their movement is not restricted by species boundaries. From a One Health perspective, ARGs present in fish microflora may ultimately contribute to resistance development in human and veterinary pathogens. The detection of clinically relevant genes such as *bla*CTX-M, *qnrS*, *sulI*, and *tetM* within aquaculture environments underscores the interconnected nature of environmental and clinical resistomes.

Understanding the diversity, distribution, and ecological significance of ARGs is therefore fundamental to evaluating the broader consequences of antimicrobial use in aquaculture. Equally important is understanding how these genes move between bacterial populations, a process primarily mediated through horizontal gene transfer mechanisms discussed in the following section.

## **VIII. HORIZONTAL GENE TRANSFER: THE DRIVING FORCE BEHIND RESISTANCE DISSEMINATION**

The emergence of antibiotic resistance within fish microflora cannot be explained solely by mutation and natural selection. Although spontaneous genetic mutations contribute to resistance evolution, the rapid spread of resistance genes throughout aquatic microbial communities is largely driven by horizontal gene transfer (HGT). HGT refers to the movement of genetic material between organisms independent of reproduction, enabling bacteria to acquire new traits from unrelated species within relatively short periods. Aquatic environments provide highly favorable conditions for HGT because they contain dense and diverse microbial populations that interact continuously within water columns,



sediments, biofilms, and host-associated habitats. Fish-associated microbiota represent particularly active sites of gene exchange due to the close proximity of microorganisms inhabiting the gastrointestinal tract, skin mucus, and gills. Consequently, HGT has become recognized as one of the most important mechanisms responsible for the dissemination of antibiotic resistance genes (ARGs) in aquaculture ecosystems (Wang et al., 2025).

### **8.1 Importance of Horizontal Gene Transfer in Aquatic Ecosystems**

Unlike vertical gene transfer, where genetic information passes from parent cells to offspring, horizontal gene transfer allows bacteria to acquire resistance genes directly from neighboring microorganisms. This process significantly accelerates bacterial adaptation because beneficial traits can spread rapidly through entire microbial communities without requiring successive generations of mutation and selection. Aquaculture environments represent ideal settings for HGT due to several ecological factors. High microbial densities increase physical contact among bacteria, nutrient-rich conditions support bacterial growth, biofilms facilitate cellular interactions, and residual antibiotics provide selective pressure favoring bacteria that acquire resistance determinants. Together, these conditions promote the continuous circulation of ARGs among fish-associated and environmental microorganisms (Uruén et al., 2020). Studies using metagenomic approaches have revealed extensive sharing of resistance genes between fish pathogens, commensal bacteria, sediment microorganisms, and aquatic environmental microbes. Such findings highlight the interconnected nature of aquatic resistomes and emphasize the central role of HGT in resistance dissemination.

### **8.2 Conjugation: The Most Important Transfer Mechanism**

Conjugation is widely regarded as the most significant mechanism responsible for the spread of antibiotic resistance genes in aquatic environments. This process involves direct transfer of DNA between bacterial cells through specialized structures known as conjugative pili. During conjugation, donor bacteria carrying plasmids establish physical contact with recipient cells and transfer genetic material through a cytoplasmic bridge. Many resistance plasmids possess broad host ranges, enabling gene transfer between distantly related bacterial species. As a result, resistance determinants can move from environmental microorganisms to fish pathogens and vice versa.

Numerous studies have documented conjugative transfer of tetracycline, sulfonamide, quinolone, aminoglycoside, and  $\beta$ -lactam resistance genes among aquatic bacteria. Important plasmid-mediated resistance genes frequently associated with conjugation include *tetA*, *tetM*, *sul1*, *sul2*, *qnrS*, *floR*, and various  $\beta$ -lactamase genes (Gao et al., 2025). The gastrointestinal tract of fish provides particularly favorable conditions for conjugation because bacterial populations are densely packed and continuously exposed to incoming microorganisms from feed and water. Consequently, fish intestines are increasingly recognized as active hotspots for resistance gene exchange.

### **8.3 Transformation and Environmental DNA Uptake**

Transformation is another important mechanism contributing to resistance dissemination in aquatic ecosystems. During transformation, bacteria acquire free extracellular DNA released into the environment following cell death and lysis. Many aquatic bacteria are naturally competent, meaning they possess specialized systems capable of capturing and incorporating environmental DNA into their genomes. When extracellular DNA contains antibiotic resistance genes, recipient bacteria may acquire resistance traits without requiring direct cell-to-cell contact.

Aquaculture sediments and biofilms frequently contain large quantities of extracellular DNA derived from dead microbial cells. These environmental reservoirs serve as sources of resistance genes available for uptake by competent bacterial populations. Experimental studies have demonstrated successful acquisition of resistance determinants through transformation in several aquatic bacterial species, highlighting the ecological significance of this process (Yu et al., 2025). Although transformation generally occurs less frequently than conjugation, its contribution to long-term resistance persistence may be substantial because environmental DNA can remain detectable in sediments and biofilms for extended periods.



#### **8.4 Transduction and Bacteriophage-Mediated Gene Transfer**

Transduction involves the transfer of genetic material through bacteriophages, viruses that infect bacterial cells. During the bacteriophage replication cycle, fragments of bacterial DNA may occasionally become incorporated into viral particles. Subsequent infection of other bacteria allows transfer of this genetic material to new hosts. Historically, transduction received less attention than conjugation in studies of aquatic antimicrobial resistance. However, advances in metagenomics have revealed that bacteriophages frequently harbor antibiotic resistance genes and may contribute significantly to ARG dissemination within aquatic ecosystems (Mutuku et al., 2022).

Marine and freshwater environments contain enormous populations of bacteriophages, often exceeding bacterial numbers by an order of magnitude. Consequently, phage-mediated gene transfer may represent an underappreciated pathway for resistance dissemination among fish-associated microbial communities. Recent investigations have identified resistance determinants associated with bacteriophage particles isolated from aquaculture water, sediments, and wastewater, suggesting that viral vectors contribute to resistome evolution in aquatic environments.

#### **8.5 Role of Plasmids in Resistance Dissemination**

Plasmids are extrachromosomal DNA molecules capable of autonomous replication within bacterial cells. They are among the most important vehicles responsible for the dissemination of antibiotic resistance genes in fish microflora. Resistance plasmids frequently carry multiple ARGs simultaneously, allowing bacteria to acquire resistance to several antibiotic classes through a single genetic event. Plasmids may contain combinations of genes conferring resistance to tetracyclines, sulfonamides, quinolones, aminoglycosides,  $\beta$ -lactams, and phenicols.

Many plasmids also possess genes encoding conjugative machinery, facilitating their own transfer between bacterial hosts. The widespread distribution of plasmids among aquatic bacteria greatly enhances the mobility of resistance determinants throughout fish-associated microbial communities (Domingues et al., 2025). Importantly, plasmids often cross species boundaries, enabling movement of clinically relevant resistance genes from environmental bacteria into fish pathogens and potentially into human-associated microorganisms.

#### **8.6 Integrons and Gene Cassette Systems**

Integrons are genetic elements that capture, integrate, and express resistance gene cassettes. Although integrons are not independently mobile, they are frequently associated with plasmids and transposons that facilitate their dissemination. Class 1 integrons are particularly important in aquatic environments because they commonly harbor genes conferring resistance to sulfonamides, aminoglycosides,  $\beta$ -lactams, and other antimicrobial classes. Numerous studies have reported high prevalence of Class 1 integrons in fish-associated bacteria, aquaculture sediments, and aquatic biofilms (Solis et al., 2024).

The significance of integrons lies in their ability to accumulate multiple resistance genes within a single genetic structure. This capacity promotes multidrug resistance and increases bacterial adaptability under changing environmental conditions. Investigations conducted in intensive aquaculture systems frequently reveal strong correlations between antibiotic exposure and increased abundance of integron-associated resistance genes, highlighting their role in resistance evolution.

#### **8.7 Transposons and Insertion Sequences**

Transposons are mobile genetic elements capable of moving between different locations within bacterial genomes. These "jumping genes" often carry antibiotic resistance determinants and facilitate their integration into chromosomes and plasmids. Insertion sequences represent simpler mobile elements that encode enzymes required for genetic mobility. Together with transposons, they contribute significantly to genomic rearrangements and resistance gene dissemination. Many resistance genes commonly detected in fish microflora, including tetracycline and sulfonamide resistance determinants, are associated with transposons. Their mobility enhances gene transfer frequencies and promotes dissemination across bacterial populations occupying aquatic environments (Odoi et al., 2025). The interaction between



transposons, plasmids, and integrons creates highly efficient networks for resistance gene exchange, accelerating resistome evolution within aquaculture systems.

#### ***8.8 Biofilms as Hotspots for Horizontal Gene Transfer***

Biofilms are increasingly recognized as critical environments for horizontal gene transfer. Within biofilms, microorganisms exist in close physical proximity and are embedded within extracellular polymeric matrices that facilitate genetic exchange. Fish-associated biofilms occur on intestinal surfaces, skin mucus layers, gills, tank walls, nets, pipelines, and sediments. These structures create stable microenvironments that support prolonged bacterial interactions and enhance transfer frequencies of plasmids, transposons, and integrons.

Several studies have demonstrated substantially higher rates of conjugation within biofilms compared with free-living bacterial populations. Furthermore, extracellular DNA accumulates within biofilm matrices, providing opportunities for transformation-mediated gene acquisition (Azeem et al., 2025). The role of biofilms in resistance dissemination is particularly important because biofilm-associated bacteria often exhibit enhanced tolerance to antibiotics, allowing resistant populations to persist even under antimicrobial treatment.

#### ***8.9 Fish Gut as a Reservoir for Gene Exchange***

The gastrointestinal tract of fish has emerged as one of the most important reservoirs for horizontal gene transfer in aquaculture ecosystems. The gut contains dense microbial populations derived from feed, water, sediments, and resident microbiota. These microorganisms interact continuously under conditions favorable for genetic exchange.

Antibiotic exposure may further stimulate HGT by inducing bacterial stress responses, increasing plasmid transfer rates, and promoting mobilization of genetic elements. Consequently, the fish intestine functions not only as a reservoir of resistance genes but also as an active site for the generation of new resistance combinations. Recent metagenomic investigations have revealed extensive networks of gene exchange among gut-associated microorganisms, reinforcing the concept of the fish gastrointestinal tract as a critical hotspot in the ecology of antimicrobial resistance (Miranda et al., 2026).

#### ***8.10 Implications of Horizontal Gene Transfer for Public Health***

The ecological significance of horizontal gene transfer extends beyond aquaculture production. Through HGT, resistance genes originating in environmental bacteria can ultimately reach fish pathogens, animal-associated microorganisms, and human pathogens. This connectivity forms the basis of the One Health concept, which recognizes the interdependence of environmental, animal, and human health.

The widespread occurrence of transferable resistance genes within fish microflora indicates that aquaculture ecosystems may contribute to the broader global antimicrobial resistance crisis. Resistance determinants associated with critically important antimicrobials, including quinolones and  $\beta$ -lactams, have already been identified in aquatic microbial communities. Therefore, understanding the mechanisms governing horizontal gene transfer is essential for designing effective strategies to limit resistance dissemination. The development of advanced genomic technologies has greatly improved the ability to investigate these processes, leading to a new era of resistome research based on metagenomics and high-throughput sequencing approaches, which are discussed in the following section.

### **IX. METAGENOMICS AND MODERN APPROACHES FOR INVESTIGATING ANTIBIOTIC RESISTANCE IN FISH MICROFLORA**

The understanding of antibiotic resistance in fish-associated microbial communities has expanded dramatically with the advent of molecular and high-throughput sequencing technologies. Traditional culture-based microbiological methods provided valuable information regarding antibiotic-resistant bacteria; however, they were limited by the inability to cultivate a large proportion of environmental microorganisms. It is now recognized that only a fraction of aquatic



microbial diversity can be recovered through conventional laboratory cultivation, resulting in significant underestimation of both microbial diversity and resistance gene abundance (Vitorino & Bessa, 2018).

Metagenomics and other culture-independent approaches have transformed antimicrobial resistance research by enabling direct investigation of microbial communities within their natural environments. These technologies have revealed that fish microflora harbor a far greater diversity of antibiotic resistance genes (ARGs), mobile genetic elements, and resistant bacterial taxa than previously recognized. Consequently, modern molecular tools have become indispensable for understanding the ecology, evolution, and dissemination of resistance in aquaculture ecosystems.

### **9.1 Limitations of Conventional Culture-Based Methods**

Historically, studies of fish-associated microorganisms relied primarily on isolation and cultivation techniques followed by phenotypic antibiotic susceptibility testing. Although these methods remain important for identifying clinically relevant pathogens, they possess several inherent limitations.

A substantial proportion of aquatic microorganisms are difficult or impossible to cultivate under standard laboratory conditions. Consequently, culture-dependent studies capture only a limited subset of the total microbial community. Furthermore, phenotypic methods provide little information regarding the complete resistome or the diversity of resistance genes present within uncultivable microbial populations (He et al., 2026).

Another limitation is that conventional approaches often focus on specific bacterial species rather than entire microbial communities. As a result, important ecological interactions and resistance reservoirs may remain undetected. These challenges stimulated the development of culture-independent techniques capable of characterizing microbial communities at much greater resolution.

### **9.2 Polymerase Chain Reaction (PCR)-Based Detection of Resistance Genes**

Polymerase chain reaction (PCR) represented one of the earliest molecular tools used for investigating antibiotic resistance genes in aquatic environments. PCR enables highly sensitive detection of specific ARGs by amplifying target DNA sequences from environmental samples.

Researchers have successfully employed PCR to identify genes such as *tetA*, *tetM*, *sul1*, *sul2*, *qnrS*, *floR*, and *blaCTX-M* in fish intestines, aquaculture water, and sediments. The technique is relatively inexpensive, rapid, and highly specific, making it valuable for routine surveillance programs (Cheng et al., 2021). However, conventional PCR can only detect genes that are specifically targeted by primers. Consequently, it provides limited information regarding the broader diversity of ARGs within complex microbial communities. This limitation has encouraged increasing adoption of more comprehensive genomic approaches.

### **9.3 Quantitative PCR (qPCR) and High-Throughput qPCR**

Quantitative PCR (qPCR) extends the capabilities of conventional PCR by enabling simultaneous detection and quantification of resistance genes. This technique allows researchers to estimate ARG abundance and compare resistance gene concentrations across different environmental compartments.

Numerous studies have used qPCR to quantify tetracycline, sulfonamide, quinolone, and  $\beta$ -lactam resistance genes in aquaculture systems. Results consistently demonstrate elevated ARG abundances in fish intestines, sediments, and wastewater associated with intensive aquaculture operations (Jiang et al., 2025).

More recently, high-throughput qPCR platforms have enabled simultaneous quantification of hundreds of resistance genes in a single analysis. These approaches provide comprehensive insights into resistome composition and facilitate large-scale monitoring of antimicrobial resistance in aquatic environments. Despite their utility, qPCR-based methods remain limited to previously known resistance genes and cannot identify novel resistance determinants.



#### **9.4 Metagenomics: A Revolution in Resistome Analysis**

Metagenomics refers to the direct sequencing and analysis of genetic material recovered from environmental samples without prior cultivation. This approach has revolutionized understanding of microbial ecology and antimicrobial resistance by enabling comprehensive characterization of entire microbial communities. In aquaculture research, metagenomics allows simultaneous identification of microbial taxa, resistance genes, virulence factors, and mobile genetic elements within fish-associated microbiota. Because the method does not depend on cultivation, it captures both culturable and unculturable microorganisms, providing a far more complete representation of microbial diversity (Pérez-Cobas et al., 2020). Metagenomic investigations have revealed extensive reservoirs of ARGs within fish intestines, skin mucus, gills, sediments, and aquaculture water. Many of these genes were previously unknown or underestimated using conventional microbiological techniques. As a result, metagenomics has become one of the most powerful tools for resistome research.

#### **9.5 Shotgun Metagenomic Sequencing**

Shotgun metagenomic sequencing is currently considered the gold standard for comprehensive resistome analysis. In this approach, all DNA present within a sample is fragmented and sequenced without targeting specific genes. This methodology provides several important advantages. First, it enables identification of both known and novel resistance genes. Second, it facilitates analysis of microbial community composition alongside resistance gene profiles. Third, it allows investigation of genetic linkages between ARGs and mobile genetic elements such as plasmids, transposons, and integrons. Studies employing shotgun metagenomics have consistently demonstrated that aquaculture environments contain diverse ARG repertoires associated with tetracycline, sulfonamide,  $\beta$ -lactam, aminoglycoside, and quinolone resistance. Many of these genes occur within complex mobile genetic structures that promote horizontal gene transfer (Gomes-Gonçalves et al., 2024). The principal limitation of shotgun sequencing remains its relatively high cost and computational complexity, although these barriers continue to decrease as sequencing technologies advance.

#### **9.6 16S rRNA Gene Sequencing and Microbial Community Analysis**

Although 16S rRNA sequencing does not directly identify resistance genes, it remains one of the most widely used approaches for characterizing fish-associated microbial communities. This technique targets conserved regions of the bacterial 16S ribosomal RNA gene to determine microbial taxonomic composition. Understanding community structure is essential because microbial diversity strongly influences resistance dissemination. Alterations in microbial composition resulting from antibiotic exposure may favor resistant taxa and increase opportunities for horizontal gene transfer. Numerous studies have used 16S rRNA sequencing to demonstrate shifts in fish gut microbiota following antibiotic treatment. Such investigations frequently reveal reductions in microbial diversity accompanied by enrichment of opportunistic and resistant bacterial populations (Payne et al., 2021). When combined with metagenomics, 16S-based analyses provide valuable ecological context for interpreting resistome dynamics.

#### **9.7 Whole Genome Sequencing of Resistant Isolates**

Whole genome sequencing (WGS) enables complete characterization of individual bacterial genomes. Unlike metagenomics, which focuses on entire communities, WGS provides detailed information regarding specific bacterial isolates. This approach has proven particularly useful for investigating fish pathogens such as *Aeromonas hydrophila*, *Vibrio anguillarum*, *Edwardsiella tarda*, and *Flavobacterium psychrophilum*. Genome sequencing reveals the precise resistance genes present within isolates, their chromosomal or plasmid locations, and their association with mobile genetic elements (Schulz et al., 2022). WGS also facilitates evolutionary analyses that track emergence and dissemination of resistance determinants across geographical regions and production systems. Such information is invaluable for understanding transmission pathways and designing targeted intervention strategies.



### **9.8 Functional Metagenomics**

Functional metagenomics represents a complementary approach that focuses on identifying genes based on biological activity rather than sequence similarity. Environmental DNA is cloned into suitable host organisms, which are subsequently screened for resistance phenotypes. This technique is particularly valuable because it enables discovery of previously unknown resistance genes that may not be detected through conventional sequence-based analyses. Functional metagenomic studies have identified numerous novel resistance determinants in aquatic environments, highlighting the vast and largely unexplored diversity of environmental resistomes (Ibitoye et al., 2009).

The ability to uncover emerging resistance mechanisms before they become widespread provides significant advantages for resistance surveillance and risk assessment.

### **9.9 Bioinformatics and Resistance Gene Databases**

Modern resistome research depends heavily on bioinformatics tools and specialized databases that facilitate identification and classification of resistance genes. Major databases commonly used in antimicrobial resistance studies include:

- Comprehensive Antibiotic Resistance Database (CARD)
- ResFinder
- ARG-ANNOT
- MEGARes
- NCBI AMRFinderPlus

These resources enable comparison of environmental DNA sequences with known resistance determinants and support large-scale resistome analyses. Advances in machine learning and computational biology are further enhancing the ability to predict novel resistance genes and assess their potential risks (Chaudhry et al., 2026). The integration of bioinformatics with metagenomic technologies has greatly improved understanding of resistance gene ecology within fish-associated microbial communities.

### **9.10 Future Directions in Resistome Surveillance**

Emerging technologies are expected to further transform resistance monitoring in aquaculture. Long-read sequencing platforms such as Oxford Nanopore and PacBio now allow more accurate reconstruction of plasmids and mobile genetic elements carrying ARGs. These approaches improve understanding of genetic contexts and transfer pathways that are often difficult to resolve using short-read sequencing alone. Additionally, environmental DNA (eDNA) monitoring, single-cell genomics, transcriptomics, and artificial intelligence-assisted bioinformatics are expanding the capacity to investigate resistance evolution in real time. Such technologies may eventually enable early detection of emerging resistance threats before they become established within aquaculture systems (Yang et al., 2024). From a surveillance perspective, integration of metagenomics with One Health monitoring frameworks represents a promising strategy for tracking resistance transmission among environmental, animal, and human reservoirs.

### **9.11 Significance of Molecular Surveillance in Aquaculture**

The transition from traditional microbiology to metagenomic and genomic approaches has fundamentally changed understanding of antibiotic resistance in fish microflora. Modern technologies reveal that resistance genes are far more diverse, abundant, and mobile than previously recognized. They also demonstrate that fish-associated microbial communities function as dynamic reservoirs interconnected with broader environmental resistomes (Meradji et al., 2025). Consequently, molecular surveillance has become an essential component of sustainable aquaculture management. By identifying emerging resistance determinants, tracking dissemination pathways, and evaluating intervention strategies, these approaches provide critical information for mitigating antimicrobial resistance and protecting both aquatic animal health and public health.



## **X. PUBLIC HEALTH AND FOOD SAFETY IMPLICATIONS OF ANTIBIOTIC RESISTANCE IN FISH MICROFLORA**

Antibiotic resistance in fish microflora is no longer regarded solely as an aquaculture issue. Increasing evidence indicates that aquatic environments serve as important reservoirs of antibiotic-resistant bacteria (ARB) and antibiotic resistance genes (ARGs) that may influence human, animal, and environmental health. This interconnectedness forms the foundation of the One Health concept, which recognizes that the health of humans, animals, and ecosystems is inseparably linked (WHO, 2023 and Al-Khalaifah et al., 2025). The expansion of global aquaculture production, international seafood trade, and widespread environmental contamination has heightened concerns regarding the potential transmission of resistant microorganisms from aquatic environments to humans. Fish-associated microbial communities therefore represent a critical interface between environmental resistomes and public health.

### **10.1 Aquaculture Within the One Health Framework**

The One Health approach has become central to contemporary antimicrobial resistance research because resistance genes move freely among environmental, animal, and human microbial communities. Aquaculture occupies a particularly important position within this framework because fish are continuously exposed to environmental microorganisms while simultaneously serving as food resources for human populations. Antibiotics used in aquaculture may select for resistant bacteria within fish microbiota, surrounding water, and sediments. These microorganisms can subsequently interact with wildlife, domestic animals, wastewater systems, and human populations through multiple transmission pathways. Consequently, resistance emerging within aquaculture environments may ultimately contribute to the broader global burden of antimicrobial resistance (Bondad-Reantaso et al., 2023).

Several studies have demonstrated that resistance genes commonly encountered in clinical settings, including *bla*CTX-M, *bla*TEM, *qnr*, *sul*, and *tet* genes, are also present in aquaculture ecosystems (Wojnarowski et al., 2025). The occurrence of shared resistance determinants across environmental and clinical compartments highlights the interconnected nature of contemporary resistomes.

### **10.2 Fish as Reservoirs of Antibiotic-Resistant Bacteria**

Fish-associated microbial communities contain numerous bacterial taxa capable of harboring antibiotic resistance determinants. These include both opportunistic fish pathogens and environmental microorganisms that persist as components of normal microflora. Resistant strains of *Aeromonas*, *Vibrio*, *Pseudomonas*, *Acinetobacter*, *Edwardsiella*, and Enterobacteriaceae have been isolated from cultured and wild fish worldwide. Many of these bacteria possess multidrug resistance profiles involving resistance to tetracyclines, sulfonamides, quinolones, aminoglycosides, and  $\beta$ -lactams (Debnath et al., 2025). Although not all resistant bacteria associated with fish are pathogenic to humans, their significance lies in their ability to function as reservoirs of transferable resistance genes. Through horizontal gene transfer, these determinants may eventually be acquired by clinically important human pathogens. Consequently, fish microflora should be viewed not only as indicators of antimicrobial exposure but also as dynamic genetic reservoirs contributing to resistance dissemination across ecological boundaries.

### **10.3 Seafood as a Potential Transmission Route**

Consumption of fish and seafood represents one of the most frequently discussed pathways through which resistant bacteria may enter human populations. Global fish consumption has increased substantially during the past several decades, making seafood an increasingly important component of human diets. Fish products may carry resistant bacteria originating from intestinal contents, skin surfaces, gills, processing environments, or contaminated water sources. If seafood is consumed raw, undercooked, or improperly handled, these microorganisms may reach consumers directly. Numerous investigations have reported the presence of multidrug-resistant bacteria in retail seafood products, including resistant strains of *Aeromonas*, *Vibrio*, *Escherichia coli*, and *Klebsiella* species (Xedzro et al., 2023). While the actual risk of transmission varies depending on processing, cooking practices, and bacterial survival, these findings highlight



the potential role of seafood in resistance dissemination. It is important to note that properly cooked fish products generally present substantially lower risks because most bacterial contaminants are eliminated during adequate thermal processing.

#### **10.4 Occupational Exposure and Human Contact**

Individuals working in aquaculture facilities, hatcheries, fish-processing plants, and seafood markets may experience greater exposure to resistant microorganisms than the general population. Direct contact with fish, water, sediments, and biofilms creates opportunities for bacterial transfer through skin wounds, mucosal surfaces, or accidental ingestion. Occupational exposure is particularly relevant for bacterial genera such as *Aeromonas* and *Vibrio*, which are capable of causing opportunistic infections in humans. Although severe infections remain relatively uncommon, the presence of antibiotic-resistant strains may complicate treatment when infections occur (Fernández-Bravo & Figueras, 2020). The increasing recognition of occupational exposure pathways further supports the need for comprehensive antimicrobial stewardship and biosafety measures within aquaculture industries.

#### **10.5 Environmental Dissemination and Human Health**

The public health implications of aquaculture-associated resistance extend beyond direct seafood consumption. Antibiotic-resistant bacteria and ARGs released into aquatic environments may spread through rivers, lakes, estuaries, coastal waters, and groundwater systems. Aquaculture effluents frequently contain resistant microorganisms, residual antibiotics, and mobile genetic elements. These contaminants may interact with environmental bacterial communities and contribute to resistance dissemination across broader ecological networks (Mushtaq et al., 2026).

Environmental dissemination is particularly concerning because aquatic ecosystems often connect with drinking-water sources, recreational waters, agricultural irrigation systems, and wastewater treatment infrastructures. Such connectivity increases opportunities for resistance determinants to circulate between environmental and human-associated microbial populations.

#### **10.6 Clinically Important Resistance Genes in Aquaculture**

One of the most significant concerns regarding aquaculture-associated resistance involves the occurrence of genes that are also important in clinical medicine. Resistance determinants conferring protection against critically important antibiotics have increasingly been detected in fish-associated microbiota (Milijasevic et al., 2024).

Examples include:

- *blaCTX-M*, *blaTEM*, and *blaSHV* (extended-spectrum  $\beta$ -lactamase genes)
- *qnrA*, *qnrB*, and *qnrS* (quinolone resistance genes)
- *mcr* genes (colistin resistance)
- *tetM* and *tetO* (tetracycline resistance)
- *sul1* and *sul2* (sulfonamide resistance)

The detection of such genes in aquaculture environments raises concerns regarding their potential transfer into human pathogens. Although direct transmission events are often difficult to demonstrate conclusively, genomic evidence increasingly supports ecological connectivity between environmental and clinical resistance reservoirs (Liu et al., 2016).

#### **10.7 Multidrug Resistance and Therapeutic Challenges**

The emergence of multidrug-resistant (MDR) bacteria within fish-associated microbial communities has important implications for both aquaculture and public health. MDR organisms are capable of resisting multiple classes of antibiotics simultaneously, reducing available treatment options. Several fish-associated bacterial genera now frequently exhibit multidrug resistance phenotypes. In some cases, isolates possess resistance determinants against tetracyclines, sulfonamides, quinolones, aminoglycosides,  $\beta$ -lactams, and phenicols concurrently. Such resistance profiles complicate disease management and increase the likelihood of therapeutic failure.



From a public health perspective, MDR bacteria represent particularly important reservoirs because they frequently carry mobile genetic elements capable of disseminating multiple resistance genes through a single transfer event (Hossain et al., 2026). The continued expansion of MDR populations within aquatic environments underscores the urgency of implementing effective resistance mitigation strategies.

#### ***10.8 Antibiotic Residues and Consumer Safety***

In addition to resistant bacteria, antibiotic residues in fish products represent another important food-safety concern. Antibiotic residues may persist in edible tissues when withdrawal periods are not properly observed or when antimicrobials are used inappropriately. International regulatory agencies have established maximum residue limits (MRLs) for various veterinary drugs to protect consumer health. Monitoring programs implemented by regulatory authorities routinely evaluate seafood products for compliance with residue standards. Although residue violations are relatively uncommon in well-regulated production systems, improper antibiotic usage may still contribute to residue-related concerns in certain regions. Continued surveillance remains essential for maintaining consumer confidence and ensuring food safety.

#### ***10.9 Global Trade and Transboundary Dissemination***

The globalization of seafood markets has transformed antimicrobial resistance into an international concern. Fish and seafood products are traded extensively across national borders, creating opportunities for resistant bacteria and resistance genes to move between regions.

A resistance determinant that emerges in one aquaculture-producing country may eventually appear in distant geographical locations through trade networks, environmental dispersal, or human movement. Consequently, antimicrobial resistance should be regarded as a transboundary challenge requiring coordinated international responses. Organizations such as the World Health Organization (WHO), Food and Agriculture Organization (FAO), and World Organisation for Animal Health (WOAH) have emphasized the importance of harmonized surveillance systems and responsible antimicrobial stewardship across all food production sectors (WHO, 2023).

#### ***10.10 Public Health Significance and Future Concerns***

The growing body of evidence linking fish-associated microbial communities with broader resistance dissemination highlights the public health significance of antibiotic resistance in aquaculture. Fish microflora function as reservoirs, amplifiers, and exchange platforms for resistance determinants that may ultimately affect human and animal health.

Although the precise magnitude of transmission from aquaculture to humans remains difficult to quantify, the widespread occurrence of clinically relevant resistance genes in fish-associated microbiota demonstrates the importance of continued surveillance and preventive action. The issue extends beyond individual bacterial species and reflects broader ecological processes occurring across interconnected environmental and biological systems. For these reasons, antibiotic resistance in fish microflora should be viewed as a One Health challenge requiring integrated solutions involving aquaculture management, environmental protection, public health surveillance, and international cooperation.

### **XI. CONCLUSION**

Antibiotic resistance has become one of the defining biological and environmental challenges of the twenty-first century. Within aquatic ecosystems, fish-associated microbial communities have emerged as important reservoirs, amplifiers, and disseminators of antibiotic resistance genes and resistant bacteria. The increasing prevalence of antimicrobial resistance in fish microflora reflects the cumulative influence of antibiotic exposure, environmental contamination, microbial adaptation, and horizontal gene transfer occurring across interconnected ecological systems.

This review has demonstrated that fish microflora comprise diverse microbial communities inhabiting the gastrointestinal tract, skin mucus, gills, and other host-associated niches. These microbial populations perform essential physiological



functions, including nutrient metabolism, immune modulation, and pathogen exclusion. However, they also serve as reservoirs of resistance determinants capable of responding rapidly to antimicrobial selective pressures.

The extensive use of antibiotics in aquaculture has played a major role in shaping contemporary aquatic resistomes. Antimicrobial compounds introduced through therapeutic, prophylactic, and historical production practices have selected for resistant bacterial populations and promoted the persistence of resistance genes within fish-associated microbiota. As a result, numerous bacterial genera commonly associated with fish, including *Aeromonas*, *Vibrio*, *Pseudomonas*, *Edwardsiella*, *Acinetobacter*, and members of the Enterobacteriaceae, now frequently exhibit resistance to multiple antibiotic classes.

At the molecular level, resistance is mediated through diverse mechanisms including enzymatic antibiotic degradation, target-site modification, active efflux systems, reduced membrane permeability, biofilm formation, and genetic mutation. These mechanisms are often encoded by mobile genetic elements such as plasmids, transposons, and integrons that facilitate rapid dissemination among bacterial populations.

The review further highlights the central role of antibiotic resistance genes in shaping resistance dynamics. Resistance determinants associated with tetracyclines, sulfonamides, quinolones,  $\beta$ -lactams, aminoglycosides, and phenicols have been widely documented in fish-associated microbial communities. Their occurrence within mobile genetic structures enables extensive horizontal gene transfer among environmental bacteria, commensal microorganisms, fish pathogens, and potentially human-associated bacterial populations.

Horizontal gene transfer represents one of the most important processes driving resistance dissemination within aquatic ecosystems. Through conjugation, transformation, and transduction, resistance genes move across taxonomic boundaries and contribute to the emergence of multidrug-resistant bacterial populations. Biofilms, sediments, and fish gastrointestinal tracts function as important ecological hotspots supporting these genetic exchanges.

The development of metagenomics and other advanced molecular technologies has transformed understanding of resistance ecology. Modern resistome analyses reveal that aquatic environments contain far greater diversity and abundance of resistance genes than previously recognized through culture-based approaches. These technologies have established fish microflora as integral components of broader environmental resistomes and have significantly enhanced the capacity to monitor emerging resistance threats.

The public health implications of antibiotic resistance in fish microflora are substantial. Fish-associated microbial communities may contribute to dissemination of clinically relevant resistance genes through food chains, occupational exposure, environmental pathways, and international trade networks. Although the precise magnitude of transmission from aquaculture to human populations remains difficult to quantify, the widespread occurrence of shared resistance determinants across environmental and clinical settings underscores the importance of adopting a One Health perspective. Environmental consequences are equally significant. Resistant bacteria and ARGs persist within aquatic ecosystems, accumulate in sediments, colonize biofilms, and interact with wildlife populations. Environmental factors such as heavy metal contamination, wastewater discharge, climate change, and hydrological connectivity further influence resistance persistence and dissemination. These findings demonstrate that antimicrobial resistance is not solely a clinical problem but also an ecological challenge affecting entire ecosystems.

Encouragingly, numerous mitigation strategies have shown promise for reducing antibiotic dependence and limiting resistance development. Improved biosecurity, vaccination programs, probiotics, microbiome management, phage therapy, immunostimulants, responsible antimicrobial stewardship, and strengthened regulatory frameworks collectively provide opportunities for sustainable disease management. The integration of these approaches within comprehensive One Health strategies offers the most effective pathway toward long-term resistance control.

Despite substantial scientific progress, significant challenges remain. Continued expansion of global aquaculture production, increasing environmental pressures, climate change, and evolving microbial populations will likely influence future resistance dynamics. Addressing these challenges will require interdisciplinary collaboration among microbiologists, aquaculture scientists, veterinarians, environmental researchers, public health professionals, policymakers, and industry stakeholders.



Ultimately, antibiotic resistance in fish microflora represents a complex ecological phenomenon arising from interactions among microorganisms, hosts, environments, and human activities. Sustainable management will depend upon balancing aquaculture productivity with responsible antimicrobial use, environmental protection, and public health priorities. Continued research, surveillance, innovation, and international cooperation will be essential for preserving antimicrobial effectiveness and ensuring the long-term sustainability of global aquaculture systems.

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