

Review

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Posted Date: 13 June 2024

doi: 10.20944/preprints202406.0874.v1

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Review

Antimicrobial Resistance in Aquaculture: Risk Mitigation within One Health Context

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Abstract: The application of antimicrobials in aquaculture primarily aims to prevent and treat bacterial infections in fish. Inappropriate use of antimicrobials in fish farming may result in the emergence of zoonotic antibiotic-resistant bacteria and subsequent transmission of resistant strains to humans via food consumption. From recently, AMR emerged as a significant public health concern in the aquaculture ecosystem and fisheries. The aquatic environment serves as a potential reservoir for resistant bacteria, with aquaculture practices providing an ideal breeding ground for AMR due to the excessive use of antimicrobials to prevent and treat diseases. The mutual inter-connection of intensive fish farming systems with terrestrial environments, food processing industry and human population creates pathways for the transmission of resistant bacteria, exacerbating the problem further. One Health concept, which recognizes the interconnectedness of human, animal and environmental health, enables a holistic approach to address the challenges posed by AMR. By understanding the evolution of such approach, the future of aquaculture, being the important source of a global animal protein supply, can be safeguarded. Risk mitigation strategies for AMR should be based on One Health concept to contribute to sustainable aquaculture practices that protect human and animal health, ensure food safety and protection of environment.

Keywords: antimicrobial resistance; aquaculture; fisheries; risk mitigation; One Health

1. Introduction

Over decades, human activities in agri-food chain have significantly affected the environment, resulting in depletion of natural resources and biodiversity. All forms of agricultural production represents the ideal platforms for development and expansion of antimicrobial resistance (AMR) through diverse ecosystems [1]. AMR occurs when bacteria, viruses, fungi and parasites no longer respond in vivo to antimicrobials in prescribed dosage and as a result of drug resistance, infections become difficult or impossible to treat, increasing the risk of disease spread, severe illness, disability and death [2].

In essence, antimicrobial resistance represents a microorganism's capacity to withstand the growth-inhibitory or lethal activity of an antimicrobial, beyond the typical susceptibility of the specific bacterial species [3]. It is the result of interactions between the microbial cell, its environment and the antimicrobial agent [1]. When people or animals come into contact with resistant pathogens, they may be subjected by infections that are no longer responsive to available antimicrobial (antibiotic) treatment. This not only results in the worsening of patients' health and potential fatalities but also leads to an increase in healthcare expenses due to prolonged hospitalization, disabilities and the ongoing spread of diseases. Therefore, an indiscriminate use of antimicrobials across veterinary, agricultural, and medical sectors is a significant contributing factor to development of AMR [1]. The World Health Organization (WHO) raised concerns about the alarming levels AMR in various parts of the world [2]. In a study on global burden of antimicrobial resistance using different bacterial pathogen-drug combinations it was observed that 4.95 AMR-associated million deaths occurred in 2019 and the six leading pathogens for deaths associated with AMR were identified, e.g. *Escherichia coli*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Streptococcus pneumoniae*, *Acinetobacter baumannii*, and

Pseudomonas aeruginosa [4]. Further, the AMR is responsible for an annual toll of 25,000 deaths in the European Union (EU) [5] of which 72.4% has been attributed to the health care system-nosocomial infections [6]. It is estimated that by 2050, bacterial resistance to antimicrobials could increase the annual rate to 10 million deaths and costs and losses to 100 trillion dollars [1,7–9]. The resistance to fluoroquinolones and β -lactam antibiotics (i.e., carbapenems, cephalosporins, and penicillins) was observed to be the most important since it was related to more than 70% of AMR-associated deaths [4]. Beyond its substantial public health impact, AMR inflicts significant economic losses, stemming from heightened treatment expenses and reduced productivity due to disease outbreaks. In the EU and European Economic Area (EEA), AMR-associated diseases are responsible for the average disability-adjusted life years (DALYs) at the level of 290.0 per 100 000 population [6]. Annual losses due to treatment and decreased productivity reach to 1.5 billion euros with prediction that by the middle of the 21st century, the economic losses caused by AMR will be on par with the recession experienced in 2008 [10].

Parallel to that finding, the study on AMR in aquaculture analyzing existence, design and implementation of National Action Plans (NAP) revealed that *Vibrio* spp. (resistance genes *hflk* and *chiA*), [11] *Aeromonas* spp. (resistant genes *tetE* and *tetA*), [12] *Streptococcus* (resistance genes *tet(O)*, *tet(M)*, *erm(B)*), [13] and *Edwardsiella* (resistance genes *blaTEM*, *sul1*, *tetA*, *blaCTX-M*, *aadA1*, *qnrS*, and *qnrA*) [14] were the most present resistant bacteria in fish farming sector. The most represented classes of antibiotics associated with AMR in aquaculture were β -lactam antibiotics, tetracyclines, sulfonamides, macrolides, fluoroquinolones [15,16].

The use of antimicrobials, whether warranted or not, has the potential for the development of AMR among microorganisms. It is essential to acknowledge that antimicrobials are frequently employed worldwide for prophylactic purposes and as growth promoters in animal husbandry and agriculture [17]. People and animals regularly exchange strains of antibiotic-resistant bacteria within the ecosystems in which they coexist. The EU introduced a ban on antibiotics as growth promoters in animal feed as of January 1, 2006 [18]. In addition, as of January 2022, the routine use of antimicrobials (antibiotics), either for prophylaxis or treatment of animals is banned in the EU and it only allows the preventative use of antimicrobials restricted only to individual animals required exceptional treatment. Therefore, antimicrobials can also no longer be applied as an alternative and/or to compensate the poor practices in livestock farming, such as hygiene and biosecurity [19]. However, outside of Europe many countries still continue to use antibiotic growth promoters to this day [20].

While the consumption of antimicrobials in the human health sector of developed countries is steadily declining, [21] their utilization in the agricultural sector, particularly in global pig and poultry farming, remains high [22,23] and less developed countries are witnessing an increase in antibiotic consumption for both medical purposes and animal husbandry. However, the precise data to support this statement are limited due to non-existent systems for monitoring antimicrobial usage in less developed countries [24].

The AMR associated with aquaculture is of global significance since fish, shellfish and other aquatic foods present important source of animal protein in many regions of the world, either being either developed (EU, USA, Japan) or developing countries (including China, Southeast Asia, India, Africa). One of the less commonly discussed avenues for AMR transmission is through the consumption of fish meat, especially non-heat treated Ready-To-Eat fish meat products, such as sushi and cold-smoked salmon. In order to suppress AMR, it is very important to establish a monitoring system for antimicrobial resistance genes (ARG) in microorganisms isolated from such fish products.

This review has a goal to provide a comprehensive overview of the current state of knowledge concerning AMR in aquaculture environments. It highlights the most relevant aspects of antimicrobials' use in aquaculture, development and transfer of AMR, detection methods, alternative solutions and practices to antimicrobials, and most effective risk mitigation strategies to combat AMR within the One Health concept. Ultimately, this review aims to deepen our understanding of the complexities surrounding AMR in aquaculture, as frequently neglected in comparison with other

food value chains, and support the development of evidence-based strategies to address this critical global challenge.

2. Materials and Methods

A literature review was performed by identifying and analyzing published scientific articles (research and review papers, technical reports by international organizations) and databases, published in domains of public health, zoonotic food borne pathogens and AMR originated from the scientific databases such as Web of Science, Scopus, Academic Search Complete, IEEE Xplore, PubMed, EBSCO and CAB Abstracts and the international guidelines. The official web sites of selected national AMR monitoring and surveillance schemes were analyzed, including the European Surveillance of Veterinary Antimicrobial Consumption (ESVAC). The relevant keywords and phrases related to the topic have been identified for the search. A search strategy based on defined keywords was based on Boolean operators (AND, OR, NOT) to combine keywords and narrow down results. These included terms like "AMR AND aquaculture", "AMR and public health", "AMR AND fish", "AMR AND fisheries", "AMR AND food borne disease", "AMR AND veterinary medicine", "AMR AND risk mitigation", "AMR AND One Health", "AMR AND monitoring", "AMR and drivers", "AMR and socioeconomic". The search was done for the years between 2000 and 2023. Each source of information was checked by reading through the titles and abstracts of the search results to assess its relevance and eligibility for the given topic. Once a list of relevant articles has been selected, a "snowballing" technique was used to discover more comprehensive and relevant literature, by including the review of the reference lists for additional sources that might not have appeared in the initial search. The selection criteria to identify the relevant articles within the scope of this review were as follows: 1) focus on antimicrobial use (AMU) in aquaculture and associated AMR, within One Health context; 2) focus on the potential for improvement of inter-sectoral cooperation between environment, veterinary and health authorities to prevent/reduce the occurrence of AMR in aquaculture. The data, as well as monitoring and surveillance programs on AMR of the major zoonotic foodborne pathogens associated with aquaculture of public health importance (*Vibrio spp.*, *Aeromonas spp.*, *Streptococcus*, *Edwardsiella*) and indicator/commensal bacteria, carriers of resistance genes (e.g. *E. coli*, *Enterococcus spp.*) were reviewed presenting the status in the EU/EEA countries, but also a global importance of AMR in aquaculture.

3. Tackling AMR in aquaculture

Aquaculture is one of the fastest growing sectors of global agricultural production. This agricultural sector produces more than half of the world's seafood and production has grown globally at 6% per year since 2001 [8]. Currently, aquaculture provides 10% of the protein consumed globally, but that number is expected to grow to 50% by 2030 [10,22]. Aquaculture production is very complex and under the influence of a large number of ecological, biological, cultural and socioeconomic factors. To reach the expected growth rate, the global aquaculture will need to be subjected to a high degree of intensification, which frequently entails the use of antimicrobials (included antibiotics) for the treatment and prevention of diseases, increasing productivity and compensating for poor biosecurity measures implemented in fish farms [25–27]. The majority of aquaculture production occurs in low and middle-income countries (LMICs) where the control of the use of antimicrobials and their quality is at a very low level, and where there are many opportunities for people, animals and microorganisms from the environment to come into close contact [22]. Antimicrobial use (AMU) can be minimized in aquaculture when farms implement more effective biosecurity measures, provide comprehensive training to workers in husbandry and management practices, and employ various disease prevention measures such as vaccination and improved water quality [8]. Additionally, the use of alternative treatments (e.g. probiotics, immunostimulants, peptides phage therapy and other natural products), can further reduce the reliance on antimicrobials. Understanding the problem of antimicrobial resistance in aquaculture and defining possible solutions requires a multidisciplinary and holistic approach to address AMR from multiple aspects within One Health concept reflecting environment-animal-human interconnection.

4. Antimicrobials Use in Aquaculture

In previous decades, AMU (including antibiotics) in animals has exceeded the amount used in humans, at the global level [28–31]. On the other hand, in the EU a decrease in AMU in food animals has been reported to the extent that it is actually lower than in humans [32]. This is in contrast with other regions worldwide where the AMU is still extensive and its quantity in food producing animals still exceed the use in humans [33].

Aquaculture stands as one of the fastest-growing food sectors on a global scale, and its rapid expansion entails heightening the risk of disease outbreaks due to lack of standardized approach and regulatory framework to disease prevention and treatment practices [34]. Intensive aquaculture, marked by densely stocked fish pools, sub-optimal hygienic conditions, physical stressors (e.g. overcrowding, handling and transportation, aquatic predators, lighting conditions, noise pollution, etc.), and water quality issues (e.g. water pH levels, temperature fluctuations, water flow and aeration) presents a range of challenges [35]. Such intensification, coming alongside with more frequent occurrence of aquatic pathogens, led to an increased reliance on antimicrobials, including antibiotics. Consequently, AMR has become a pressing concern, directly affecting a wide array of aquatic species that are farmed, such as catfish, trout, salmon, tilapia, shrimps [36–39]. The misuse and/or overuse of antibiotics can also be attributed to misdiagnoses often made by the breeders themselves. In many cases breeders, without veterinary consultation, resorted to antibiotic use in aquaculture to avert widespread mortality due to poor biosecurity and hygienic conditions and mitigate substantial economic losses [25]. From recently, there are promising data showing that fish farmers across the world are not only accepting limited antimicrobial/antibiotic usage but also embracing the new reality of industry rules and standards [34]. While antibiotic-free organic fish farming is a foreseeable future, the reality is that the water quality, fish farm waste and associated diseases are still ongoing challenges that sometimes require antimicrobial use [22,40]. Therefore, it is imperative that effective, safe and cost-competitive solutions are at disposal as the best alternatives available in place to replace and/or reduce antimicrobial (antibiotic) treatments. The overview on research regarding AMR in aquaculture, including classes of antimicrobials that are most frequently used is given in Table 1.

Table 1. Overview of research on AMR in aquaculture.

Author(s)	Year	Study location	Microorganism(s)	Type of samples	Antimicrobial agent(s)	Phase in aquaculture production chain
Raza et al.[41]	2022	South Korea	/	Water samples	SulfonamidesVCIA, HIA, TetracyclinesVCIA, HIA, QuinolonesVCIA,CIA, Beta lactamsVCIA,CIA	Pre-harvest
Osman et al.[42]	2021	Egypt	<i>Pseudomonas spp.</i>	Nile tilapia (<i>Oreochromis niloticus</i>)	Sulphamethoxazole/TrimethoprimVCIA, HIA, AmikacinVCIA,CIA, A,	Post-harvest

					ImipenemVCIA,CI A, TetracyclinesVCIA, HIA,, AmpicillinVCIA,CI A, Nalidixic acidVCIA,CIA, Chloramphenicol, GentamicinVCIA,C IA, CiprofloxacinVCIA, CIA, Aztreonam, Ampicillin/Sulbacta mVCIA,CIA, CefepimeVCIA,CI A, CeftriaxoneVCIA,C IA, CephalotinVCIA,CI A, CefotaximeVCIA,C IA, CeftazidimeVCIA,C IA	
Algammal et al.[14]	2022	Egypt	Edwardsiella tarda	Nile tilapia (Oreochromis niloticus), African catfish (Clarias gariepinus)	AmoxicillinVCIA,C IA, AmpicillinVCIA,CI A, CefotaximeVCIA,C IA, ErythromycinVCIA, CIA, StreptomycinVCIA, CIA, GentamycinVCIA,C IA, EnrofloxacinVCIA, CIA, CiprofloxacinVCIA, CIA, Colistin-sulfate,	Pre-harvest

					TetracyclineVCIA, HIA, Trimethoprim- SulfamethoxazoleV CIA,HIA	
Brunton et al.[25]	2019	Vietnam	/	Striped catfish (<i>Pangasianodon hypophthalmus</i>), White-leg shrimp (<i>Penaeus vannamei</i>)	N/A	Pre-harvest
Girijan et al.[43]	2020	India	<i>Escherichia coli</i>	Sediment, water, fish/shrimp/ clams	CiprofloxacinVCIA, CIA, Levofloxacin, MoxifloxacinVCIA, CIA, OfloxacinVCIA, CIA, NorfloxacinVCIA, CIA, Nalidixic acidVCIA, CIA, GentamicinVCIA, CIA, AmikacinVCIA, CIA, CefotaximeVCIA, CIA, Cefotetan, CeftazidimeVCIA, CIA, ImipenemVCIA, CIA, ColistinVCIA, CIA	Pre-harvest
Yang et al.[44]	2023	China	<i>Proteobacteria</i> , Firmicutes, <i>Enterococcus spp.</i> , <i>Escherichia</i>	Farm worker feces, water, sediment, fish guts,	AminoglycosidesVCIA, CIA, CIA, PhenicolesVCIA, HIA, TetracyclinesVCIA,	Pre-harvest

			<i>spp.</i> , <i>Streptococcus</i> <i>spp.</i> , <i>Klebsiella</i> <i>spp.</i> , <i>Acinetobacter</i> <i>spp.</i> , <i>Bacteroidetes</i> , <i>Cyanobacteri</i> <i>a</i> , <i>Klebsiella</i> <i>pneumoniae</i> ,	duck manure	HIA, SulfonamidesVCIA, HIA	
Garza et al.[34]	2022	Global/ review paper	General	General	General	N/A
Jones et al.[45]	2023	57 countries	<i>Aeromonas</i> <i>spp.</i>	Human, wastewater, drinking water, surface water, agriculture	AminoglycosidesV CIA,CIA, CarbapenemsVCIA, CIA, CephalosporinsVCI A,CIA, FluoroquinolonesV CIA,CIA, MacrolidesVCIA,CI A, Monobactams, PenicillinsVCIA,CI A, PhenicolVCIA,HI A, Polypeptides, SulfonamidesVCIA, HIA, TetracyclinesVCIA, HIA	N/A
Reddy et al.[46]	2022	Global/ review paper	General	General	General	N/A
Bell et al.[47]	2023	Bangladesh	/	Water samples	AminoglycosidesV CIA,CIA, SulphonamidesVCI A,HIA, CarbapenemsVCIA, CIA, CephalosporinsVCI A,CIA,	N/A

					CephameycinsVCIA, CIA, PenamsVCIA,CIA, FluoroquinolonesV CIA,CIA, Diaminopyrimidine sVCIA,CIA, PhenicolsVCIA,HI A	
Chowdhury et al.[35]	2022	Bangladesh	/	Various fish species	OxytetracyclineVCI A,HIA, CiprofloxacinVCIA, CIA, AmoxicillinVCIA,C IA, LevofloxacinVCIA, CIA, ErythromycinVCIA, CIA, SulfadiazineVCIA, HIA, TrimethoprimVCIA ,HIA	N/A
Kampouris et al.[48]	2022	/	<i>Flavobacteriu m,</i> <i>Pseudomonas,</i> <i>Lactococcus,</i> <i>Sphingomona s</i>	Water samples, biofilm from plastic mechanical filters from ponds with African catfish (<i>Clarias gariepinus</i>)	SulfonamidesVCIA, HIA, Beta lactamsVCIA,CIA, QuinolonesVCIA,C IA, MacrolidesVCIA,CI A	Pre-harvest
Alhaji[9]	2021	Nigeria	/	African catfish (<i>Clarias gariepinus,</i> <i>Clarias lazera</i>)	TetracyclinesVCIA, HIA, PenicillinVCIA,CIA , SulfonamidesVCIA, HIA, StreptomycinVCIA, CIA,	Pre-harvest

					NeomycinVCIA,CI A, AmpicillinVCIA,CI A, ColistinVCIA,CIA, ErythromycinVCIA, CIA, EnrofloxacinVCIA, CIA, ChloramphenicolV CIA,CIA	
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VCIA – Veterinary Critically Important Antimicrobial Agents; CIA - Critically Important Antimicrobials for Human Medicine; HIA – Highly Important Antimicrobials for Human Medicine; Pre-harvest: Aquaculture farming system; Post-Harvest: Aquatic animals after the completion of the rearing and growth cycle.

In aquaculture, antimicrobials are most often administered orally to all fish living in the same pool or cage [49]. The most common route for the delivery of antimicrobials to fish involves blending the antibiotics with specially formulated feed (i.e. medicated feed). Nonetheless, fish are not fully efficient in metabolizing antibiotics and other medications from their feed resulting in a significant portion passing through their bodies and being released into the aquatic environment, often in an unaltered form. It has been estimated that approximately 75% of the antibiotics administered to fish are excreted unchanged into the water and being still microbiologically active [49].

A wide range of antimicrobials (including antibiotics) is in use in aquaculture today. It is the frequent situation that the same classes of antibiotics are used both for medical purposes and in aquaculture production [39]. The type of antimicrobials, the method of their use, the frequency and the amount applied depend on a number of factors, such as: (i) the species grown in aquaculture, (ii) type of production (e.g. semi-intensive, intensive, pools, cages), (iii) environment, (iv) availability of drugs, (v) the role of veterinary services, (vi) legal frameworks and (vii) market control [26]. For example, the quinolones are very often used in aquaculture [39]. Nowadays, several classes of antibiotics banned in the EU are in regular use in aquaculture production in Asia (e.g. China and Vietnam), including chloramphenicol, ciprofloxacin, florfenicol, nitrofurans and enrofloxacin [50,51]. In addition, Carrizo et al. [52] observed the occurrence of amoxicillin, enrofloxacin, moxifloxacin, penicillin G, ciprofloxacin, oxolinic acid, sulfamethoxazole, trimethoprim, penicillin V, doxycycline, flumequine, oxacillin and pipemidic acid in samples of wild salmon in Chile. The presence of erythromycin, azithromycin, roxithromycin, sulfabenzamide, sulfamethazine, sulfapyridine, cephalexin and sulfaguanidine in samples of farmed salmon has been also found in the same study [52].

It is important to note that the afore mentioned classes of antibiotics belong to both, Veterinary Critically Important Antibiotics (VCIA) according to World Organization for Animal Health (WOAH) classification, [53] and Critically Important Antibiotics (CIA) in accordance with WHO [54]. This is related to the clear evidence of adverse human health consequences that led to increased episodes of treatment failures (including deaths) and increased severity and duration of infections that may occur due to resistant organisms resulted from excessive non-human usage of antimicrobials. To date, there is a scarcity of information and lack of research related to development and transfer of AMR in fisheries, connecting the AMU in aquaculture and human health.

Several antibiotics that are most commonly used in aquaculture are quinolones (27%), tetracyclines (20%), amphenicols (18%) and sulfonamides (14%) (Figure 1) [39]. These antibiotics are often used to treat or prevent bacterial infections in fish and other aquatic animals, and also belong to the VCIA, emphasizing their importance for public health [53,54].

Quinolones. Quinolones are a class of synthetic broad-spectrum antibiotics that are widely used to treat a variety of bacterial infections [39]. The most common quinolones include ciprofloxacin, levofloxacin, moxifloxacin, and ofloxacin. These group of antibiotics are considered as CIA by the

World Health Organization (WHO) and other health authorities [53,54]. This classification is due to their effectiveness in treating serious and life-threatening infections where few or no alternatives exist. Their broad-spectrum activity makes them essential for managing a wide range of bacterial diseases, particularly in hospital settings. However, the rise in antimicrobial resistance poses a significant public health challenge. As we indicated earlier, resistance to fluoroquinolones is associated with over 70% of deaths linked to AMR [4]. Balancing their benefits against the risks of resistance is essential to maintaining their status as CIA.

Oxytetracycline. It is a broad-spectrum antibiotic commonly employed in aquaculture to treat bacterial infections in fish, especially those caused by gram-negative bacteria. Oxytetracycline is poorly absorbed in fish intestines through passive diffusion, necessitating high doses for administration. Pharmacokinetics of oxytetracycline depends on various factors, such as fish species, age, size and health condition, oxytetracycline dosage, water temperature and salinity and the antibiotic's formulation [55,56]. This results in the slow release of significant antibiotic quantities, increasing selective pressure and potentially fostering the development of oxytetracycline-resistant bacterial strains within the fish's intestines [49,57]. In fish, oxytetracycline is primarily distributed to the liver, kidneys, and muscle tissues, where it can persist for several days after treatment. Most of the applied oxytetracycline is excreted in feces (70-80%). Although it is easily degraded in seawater, it remains in the sediments for a long time [49]. For example, some studies have shown that the concentration of oxytetracycline in sediments on salmon farms is up to 11 µg/g, while its half-life is from 9 to 415 days [49,58]. This is the likely reason why salmon farmed marine ecosystems have oxytetracycline-resistant bacteria as high as 25%, compared to less than 5% in non-salmon farmed locations [49]. Therefore, it is crucial to investigate and regularly monitor the persistence of various antibiotics in both water and sediments.

Sulfadiazine. It is a sulfonamide antibiotic frequently employed in aquaculture for its ability to inhibit bacterial growth and replication. This group is among the most used antibiotics in the European countries with contributions between 11 and 24% [59]. When bacteria come into contact with sulfadiazine, those possessing resistance genes or mutations that enable them to survive and reproduce will thrive, passing on their resistant traits to subsequent generations. Research indicates that the use of sulfadiazine in aquaculture can indeed contribute to the emergence of sulfonamide-resistant bacteria, affecting both fish and the surrounding aquatic environment. Such resistant bacteria have been detected in fish, shellfish, as well as in sediment and water samples from aquaculture facilities [60].

Florfenicol. It is a relatively new antibiotic, belonging to the class of phenicols, gaining popularity in aquaculture due to its effectiveness against a broad spectrum of bacteria and a lower risk of resistance development. It is frequently used to combat bacterial infections in fish, particularly those caused by gram-negative bacteria [61]. The impact of florfenicol on AMR is multifaceted and can hinge on various factors, including treatment dosage and duration, frequency of use, and the specific bacteria involved. Several studies have indicated that employing florfenicol in aquaculture can indeed foster the development of antibiotic-resistant bacterial strains [60,62]. To minimize the development of antimicrobial resistance linked to the use of florfenicol in aquaculture, it is imperative to exercise prudent antibiotic administration and adhere to good farming management practices. This involves utilizing appropriate treatment dosages and durations, reducing the frequency of usage, and enhancing hygiene and disease management protocols.

Amoxicillin. It is a broad-spectrum antibiotic widely used in human medicine to combat bacterial infections, has also found use in aquaculture for the treatment of bacterial infections in fish and aquatic animals [35]. However, its application in aquaculture has sparked controversy due to concerns about antibiotic resistance and potential environmental and food chain residues. While amoxicillin is approved for aquaculture use in certain countries, stringent regulations are in place to ensure its safe and responsible application, such as in the EU, USA and Japan [63].

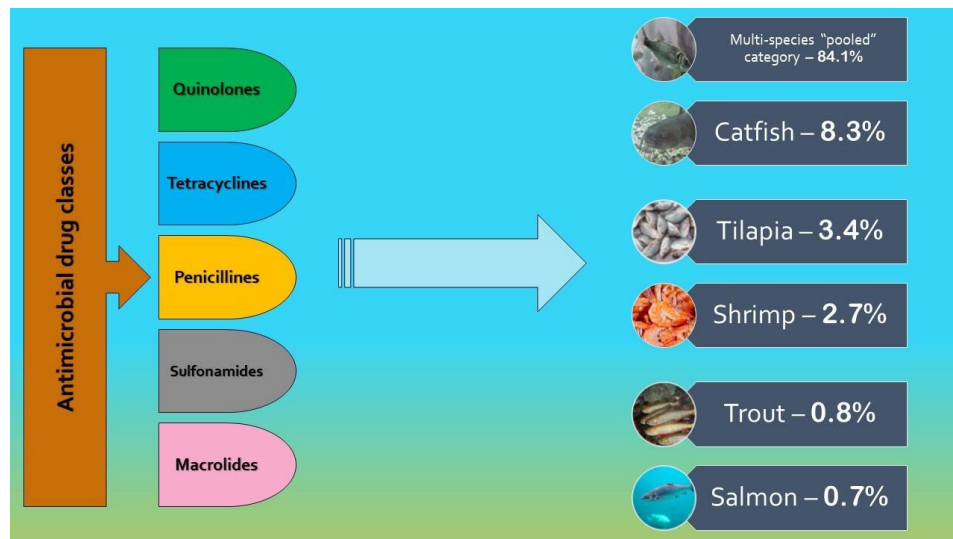


Figure 1. List of antimicrobial drug classes most commonly used in aquaculture and AMU trends (%) by species (adapted from Schar et al. [39]).

Regardless of the method or purpose of use, antibiotic residues can accumulate in fish tissues before being completely metabolized and eliminated from their bodies [64]. This happens, in particular, when they are given outside the indicated doses or manufacturer's recommendations. In addition, fish and aquatic organisms found in open waters are not spared from the influence of antimicrobial drugs due to discharges from farms and other agricultural environments [52,65]. Determining the levels of antimicrobial residues in tissues of aquatic animals can be used to track the source of contamination to which they were exposed [52,66]. In comparison with terrestrial animals, antimicrobial use in aquaculture has a greater potential for environmental dissemination, thus exerting a more significant impact on ecosystem and public health [39,51,67].

5. AMR Emergence, Transfer and Dissemination in Aquaculture

Antimicrobial-resistant bacteria are widespread in the interconnected ecosystem domains of environment, animals and humans. A profound understanding of the evolution of AMR and the dynamics of ARG dissemination throughout this triad is crucial for anticipating emerging pathogens and managing the spread of AMR. [68] The emergence of AMR can occur through vertical means, involving point mutations, [69] or horizontally through diffusion of antibiotic-resistance genes and mobile resistance genes enabling the acquisition of mobile genetic elements (MGE) like plasmids and transposons further spreading genetic determinants.[60]

AMR can be categorized into two primary forms: natural (intrinsic) resistance and acquired (transmissible) resistance. [70,71] Natural resistance is an inherent characteristic of a specific bacterial species or genus. It persists as it is transmitted from the parent cell to its progeny, unless subsequent mutations make them vulnerable. [72] It allows microorganisms to withstand the presence of antimicrobial agents due to their innate characteristics. [73] Such inherent resistance is consistent and it continuously exists within the bacterial species.

In contrast, acquired resistance becomes evident only after exposure to antimicrobial agents. This type of resistance, often referred to as induced resistance, is not an inherent trait of microorganisms but arises as a result of their response to external factors. [74] Bacteria can acquire resistance through various mechanisms, such as mutation within chromosomal DNA, [70] horizontal gene transfer, [75,76] or acquiring resistance genes from other bacteria. This property is not consistent or hereditary.

There are several ways that AMR can be transferred in aquaculture. For example, it can rapidly disseminate in aquatic bacterial populations through horizontal gene transfer (HGT). [48,77] Bacteria resistant to antibiotics harbor intracellular ARGs, which often have the potential to be transmitted to different bacteria via HGT, leading to the emergence of novel antibiotic-resistant bacteria. [28] This

can occur through a variety of mechanisms, including conjugation, transduction, and transformation (Figure 2).

Conjugation. This is the transfer of genetic material between bacteria through a physical connection. In this process, a plasmid carrying genes for the transfer process (F factor) is transferred from a donor bacterium to a recipient bacterium through a conjugation bridge. The F factor can integrate into the recipient bacterium's genome and transfer additional genetic material. The conjugation stands out as the primary mechanism responsible for the swift spread of ARGs. [78,79]

Transduction. It relates to the transfer of genetic material between bacteria through a bacteriophage. During the process of viral replication, the phage can accidentally package bacterial DNA instead of its own DNA, and then transfer this bacterial DNA to another bacterium. This is an efficient pathway for the transfer of ARGs, particularly among bacterial hosts of the same species. [80] Phage-mediated mechanism has been established as a significant driver for the transmission of ARGs related to tetracycline and β -lactam antibiotics in *Staphylococcus aureus*, as documented by some studies. [17,81] Further, transduction is also associated with emergence of multi-drug resistance to variety of antibiotics as observed among strains of *Streptococcus pyogenes*, *Enterococci*, *Escherichia coli*, *Salmonella*, and methicillin-resistant *Staphylococcus aureus* (MRSA). [82]

Transformation. It is based on uptake of DNA from the environment by a bacterium. Bacteria can take up DNA fragments released by other bacteria that have lysed, or they can take up DNA from the environment. [79] The DNA is then incorporated into the recipient bacterium's genome through recombination. This is the least efficient HGT mechanism, primarily because this process exposes DNA to the external environment outside the cell. [79]

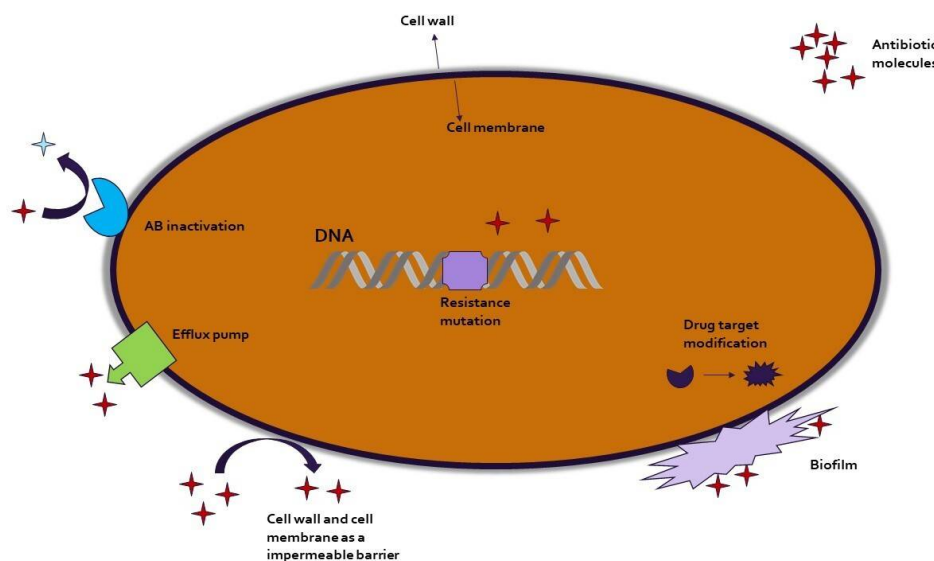


Figure 2. Principal mechanisms on development of AMR.

Evidently, HGT is an important mechanism for bacterial evolution as it allows bacteria to rapidly acquire new traits such as antibiotic resistance. A number of studies have investigated the possibility that aquaculture systems may represent a reservoir of AMR [83,84].

Further, wastewater treatment plants can be a significant source of antibiotic-resistant bacteria and genes, and effluents from these plants can discharge into aquatic environments. Similarly, agricultural runoff or discharges from livestock facilities can also introduce resistant bacteria and genes into aquatic environments [1]. In all, industrial waste from pharmaceutical companies and hospitals and runoff from livestock farms and agri-food sector, foster complex interactions between microbiota and human population that may have great influence on the presence of ARGs in aquatic environments [48,85].

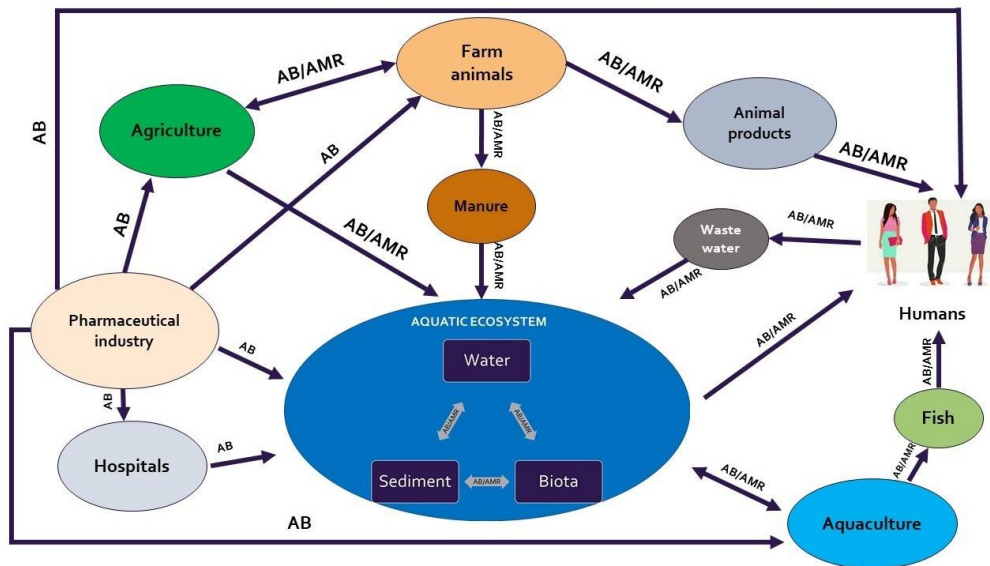


Figure 3. Transmissions routes of antimicrobial agents (AB) and AMR in environment.

Contaminated feed. It is another way in which AMR can be disseminated in the aquatic environment. Aquatic animals, such as fish and shrimp, are often fed with feed that contains antibiotics or other antimicrobial agents to promote growth or prevent infections. Therefore, use of antibiotics in animal feed can influence the development of resistant bacteria in the intestines of aquatic animals which become carriers of resistant bacteria and genes [86]. These bacteria can then be released into the environment through the animals' feces, which can contribute to the dissemination of AMR in the aquatic environment [49]. Further, the existence of antimicrobial-resistant bacteria within wild fish populations can occur via transmission of resistant organisms through water exchange between fish farms and adjacent natural habitats [9,35].

The disposal of unused or expired feed, such as dumping it in water or using it as fertilizer in aquaculture ponds, can also contribute to the dissemination of AMR in the aquatic environment [87]. To reduce the spread of AMR, it is important to promote responsible use of antibiotics in animal feed, improve feed quality and safety, and implement proper disposal practices for unused or expired feed.

Antibiotic residues in water and sediment. The important concern is that antibiotic residues can remain in the water or sediment of aquaculture systems even after the antibiotics have been removed. These residues can further contribute to the development of AMR. When antibiotics are used in aquaculture or agriculture, they can leave residues in water bodies through runoff, leaching or discharge of treated effluent. These residues can have negative impacts on the environment, including the selection and spread of antibiotic-resistant bacteria and genes [88]. Further, exposure to antibiotic residues can also induce changes in the microbial community composition and diversity in aquatic environments. This can alter the ecological balance of the microbial community, which can lead to the proliferation of opportunistic pathogens and the suppression of beneficial microorganisms [89].

Biofilms. These are other potential hotspots for AMR in aquaculture systems. Consequently, the excessive use of antibiotics in aquaculture can contribute to the formation of biofilms. Biofilms can form on various surfaces in aquaculture systems, including tanks, nets, and pipes, and can create environments that facilitate the growth and spread of antibiotic-resistant bacteria [42,90–92]. The extracellular polymeric substances in the biofilm matrix can form a physical barrier that prevents antibiotics from reaching the bacteria, making them less susceptible to antimicrobial treatment. Additionally, bacteria in biofilms can exchange genetic material more efficiently, which can increase the likelihood of horizontal transfer of resistance genes [93]. Antibiotics can disrupt the natural microbial communities in aquaculture systems, which can lead to the proliferation of opportunistic pathogens that can form biofilms. Once established, these biofilms can act as a reservoir for antibiotic-resistant bacteria and genes which can persist in the aquaculture system and potentially spread to other bacterial populations. Biofilms can also be difficult to remove, which can contribute to the long-term persistence of antibiotic-resistant bacteria in aquaculture systems, since the use of harsh

chemicals to remove biofilms can have negative impacts on the environment and can also promote the selection of antibiotic-resistant bacteria [94–97].

Climate change. The special attention should be given to the climate change impact on emergence of AMR in aquaculture systems. The rising temperatures may lead to the change in cell physiology of bacteria thus causing emergence of AMR. Some aquatoriums worldwide are more susceptible to the climate change, such as Mediterranean Sea [60]. The rising temperatures are mechanistic modulator that facilitate the transmission of AMR in bacteria, including within aquatic systems [98]. In a study carried over five years' period of time in Europe, it was observed that countries with 10°C warmer ambient experienced more rapid resistance increases across all antibiotic classes compared to other countries. The reported increase in AMR ranged from 0.33% - 1.2% / per year even taking into consideration recognized resistance drivers such as antibiotic consumption and population density. The trends of increased temperature may foster further global spread of AMR making risk mitigation strategies more complicated [98].

6. Clinical Breakpoints and Epidemiological Cut-Off Values

The data on clinical breakpoints (concentration of antibiotic used to define whether an infection by particular bacterial strain is likely to be treatable in patient) for those resistant bacteria in commercial fisheries are generally scarce. For example, the Minimum Inhibitory Concentrations/MICs (the lowest concentration of antimicrobial that could prevent visible bacterial growth) and Epidemiological Cut-Off Values/ECVs (highest MIC value of isolates that are not known to have resistance and are therefore considered representative of wild type bacterial isolates) for *Aeromonas hydrophila* and *Aeromonas veronii* were examined in a study by Woo et al. [12] against several selected antibiotics; the MICs for these *Aeromonas* spp. isolates ranged from 0.25–64 µg/mL for doxycycline, 0.03–32 µg/mL for enrofloxacin, and 0.03–64 µg/mL for erythromycin and florfenicol, while oxytetracycline had the highest MICs at >256 µg/mL. The MICs for *Vibrio* spp. were examined in bivalve shellfish by Mancini et al. [99] and it was observed that high resistance percentages to sulfonamide/sulfisoxazole (57.1%; 72/126) (MIC > 256 µg/mL), ampicillin (85.7%; 108/126), and cephalosporins/cefazolin (56.3%; 71/126) were found among all *Vibrio* species. In a study by de Oliveira et al. [100] MIC assay based on the protocol by Clinical and Laboratory Standards Institute (CLSI, 2014) was used and provisional ECV for *Streptococcus (agalactiae)* in tilapia farming, evaluating the profile of florfenicol resistance, was established observing that ECV was 8 µg/mL for 94% of the tested strains classified as a wild-type; ECV was calculated using two methodologies: the normalized resistance interpretation (NIR) [101] and ECOFFinder MS (<https://clsi.org/meetings/susceptibility-testing-subcommittees/ecoffinder/>). The MICs for *Edwardsiella* in aquatic culture was conducted using disk-diffusion test and provisional ECVs that were established were 8 µg/mL for erythromycin, 10 µg/mL for neomycin, 18 µg/mL for sulfamethoxazole-trimethoprim, 23 µg/mL for amoxicillin, 25 µg/mL for oxytetracycline, 26 µg/mL for norfloxacin and 27 µg/mL for florfenicol [102]. Further investigation is needed to establish more precisely ECVs for afore mentioned four most frequent bacteria associated with AMR in aquaculture.

7. Detection Methods for AMR

Detecting and monitoring AMR in aquaculture is crucial for gaining a comprehensive overview of potential threats to both animal and human health. The direct correlation between aquatic ecosystems and various human needs, such as water consumption, crop irrigation, and the direct consumption of fish and other aquaculture products, highlights the importance of these activities [82,103]. Additionally, it is essential to monitor the use of antimicrobial agents in human and veterinary medicine within One Health context encompassing interface between environment, animals and humans [32].

The determination of AMR profiles in bacterial isolates from aquatic animals often involves various *in vitro* procedures. These methods go beyond assessing sensitivity to antimicrobial agents and play a crucial role in monitoring the emergence and spread of resistant microorganisms within populations [104]. Ongoing enhancements and applicability of these monitoring techniques are ensured through continuous updates to guidelines and recommendations by relevant organizations, such as the Clinical and Laboratory Standards Institute/CLSI (USA) and WOHAI [105].

Standard classical methods for the detection of bacterial resistance primarily rely on the cultivation of microorganisms under specific conditions. The most often applied methods include paper diffusion, the disk diffusion method, the Epsilon (E)-test based on antibiotic diffusion, [106] broth dilution, agar dilution methods for determining the minimum inhibitory concentration (MIC), [107] and other classical approaches [108]. Although these methods are simple and easy to perform, they have certain limitations that make them less useful. One reason is that certain microorganisms isolated from nature may not be easily cultivable, or their multiplication in the natural environment may take a long time [109]. Additionally, they only provide phenotypic information about bacterial resistance and cannot detect resistance genes [108,110].

Beyond traditional methods, automated and semi-automated devices based on microdilution susceptibility testing have been widely used in recent decades, providing significantly faster results, such as VITEK System (bioMérieux, France), Phoenix System (BD Diagnostic Systems, USA), MicroScan Systems (USA) stand out as prominent examples [111,112].

Further, modern molecular methods, such as real-time PCR, DNA-Microarrays or Whole Genome Sequencing (WGS), facilitate the direct determination of genetic determinants responsible for AMR expression. In particular, WGS stands as a powerful tool for comprehending the interconnections between the health of aquatic animals, their ecosystems, and human health. The importance of WGS testing is emphasized with regard to the occurrence of HGT between bacteria in these environments and human pathogens, carrying significant public health implications [50,113–115]. WGS enables the detection of all the genes responsible for AMR, and thus the possibility to establish a comprehensive database of all resistance factors within a single species [112]. WOA guidelines [116] advocate for the integration of genotypic methods with phenotypic analysis to enhance specificity and sensitivity. This is crucial because the mere presence of AMR genes does not universally translate to phenotypic resistance.

WGS also facilitates understanding of how AMR genes move between different ecosystems, providing invaluable insights within One Health context for combating AMR effectively. Further, WGS also allows predictions of significant features important for public health, such as resistance to antimicrobials, serotyping, virulence factors, and pathogenicity of isolated strains. It also serves as a guide for conducting monitoring, surveillance and investigating outbreaks, [117] since WGS allows for the tracking of disease transmission pathways among aquatic organisms, helping to identify sources and routes of infection. However, molecular methods (including WGS) for testing AMR include certain drawbacks, such as limitations in detecting only previously recognized resistances, which can lead to false-negative results. Additionally, the inability to define MIC presents an additional challenge. These methods require validation against phenotypic data and extensive resistance databases, employing innovative bioinformatic approaches. Nevertheless, molecular methods for testing antimicrobial resistance prove to be a safe, efficient, and reliable tool in clinical settings. As experience with these tests grows, along with the collection of data on their effectiveness and clinical impact, they are likely to become more widely accepted [112,118]. Lastly, the routine use of WGS in food safety management was also recommended by FAO to facilitate One Health approach. It is expected that it should improve the current knowledge regarding microbiological diversity and genetic information and its application for identification and tracking of microorganisms (including AMR) in food production, in food control, clinical microbiology and epidemiology [119].

8. Alternatives to Antimicrobial Treatment in Aquaculture

There are several alternatives to the use of antimicrobials in aquaculture. The most important are prebiotics, probiotics, immunostimulants, vaccines, essential oils, peptides and phage therapy.

Prebiotics. These are non-digestible food ingredients that can stimulate the growth of beneficial bacteria in the gut. The novel functional foods for fish nutrition involves bioactive compounds, including prebiotics, which beneficial immune responses [120,121]. Non-digestible oligosaccharides promote the growth of intestinal microbiota, enhancing nutrient absorption and bolstering the immune system of fish [122]. There are several prebiotics that are commonly used in aquaculture and can help to stimulate the growth of beneficial bacteria in the intestines and improve the immune system of fish and other aquatic animals, as follows: Mannan oligosaccharides (MOS) derived from the cell walls of yeast [123]; Fructo-oligosaccharides (FOS) derived from fruits and vegetables [124];

Galacto-oligosaccharides (GOS) derived from milk [125]; Inulin derived from plants; Chitin and chitosan derived from the shells of crustaceans [126].

Probiotics. These refer to live microorganisms that confer health benefits when consumed in adequate amounts (*Lactic acid bacteria*, *Phaeobacter spp.*, *Bacillus spp.*). These microorganisms, predominantly bacteria and yeast, are known for their positive influence on the host organism, typically the human and animal body [121]. Probiotics contribute to the maintenance of a balanced microbial environment, particularly in the digestive system by promoting a healthy balance of gut bacteria, supporting digestion, and contributing to the modulation of the immune system. The difference in the effect of the use of probiotics in aquaculture compared to the breeding of mammals is that probiotics used in aquaculture can interact with the surrounding environment in a way that leads to improve its quality and prevent the growth and development of pathogens that could negatively affect the health status of fish [121,127]. The tests on probiotics in aquaculture were mostly directed towards lactic acid bacteria [49,128–130]. *Bacillus*, *Vibrio*, *Pseudomonas* and *Aeromonas* were also the subject of research [49,131,132] for its ecology relates to the competitive action of probiotic bacteria.

Immunostimulants are substances that can boost the immune system of fish and other aquatic animals. They can help to prevent infections and reduce the need for antibiotics [133]. For example, β -Glucans are complex polysaccharides derived from the cell walls of yeast, bacteria, and fungi [134]. Zinc and selenium are trace minerals that is essential for the proper functioning of the immune system [135]. Vitamin C is an antioxidant that can help to protect fish and other aquatic animals from oxidative stress [136]. They all can help to improve the growth and survival of fish in stressful conditions activating the immune system of fish and other aquatic animals leading to an increased production of white blood cells and antibodies. The dosage and administration of immunostimulants may vary depending on the species of fish, the age of the animals, and the specific health conditions present in the aquaculture system.

Vaccines. They can be used to prevent bacterial infections in fish and other aquatic animals. At present, bacterial vaccines such as those targeting *A. salmonicida*, *V. anguillarum*, and *Y. ruckeri* are being utilized, with preparations underway for the vaccination against viral diseases [137]. They can be administered through injection or through the feed.

Essential oils. Herbs and their extracts are emerging as increasingly promising supplements and alternatives due to their effectiveness, safety, environmental friendliness, and reduced drug resistance. Herbal medicines in preventing and controlling viral, bacterial, parasitic and fungal fish diseases are due to their robust immune enhancement, antioxidation, or direct anti-pathogenic effects of their active components (e.g. polyphenols, polysaccharides, saponins, flavonoids, alkaloids) [138]. A number of studies, mainly on poultry, have proven that the addition of essential oils (EOs) to food leads to a reduction of certain pathogenic microorganisms in the intestines [139,140]. The usage of EOs of *Ocimum gratissimum* and *Hesperozygis ringens* for its antimicrobial and antiparasitic properties, respectively, against different fish pathogens was studied and both EOs showed moderate activity against the bacteria *Aeromonas hydrophila* and *Aeromonas veronii* (MIC 400–800 $\mu\text{g/mL}$) and weak activity against *Citrobacter freundii* and *Raolteella ornithinolytica* [141].

Peptides. Antimicrobial peptides (AMPs) are short, gene-encoded peptides present in living organisms such as bacteria, insects, plants, vertebrates, as well as humans [142]. AMPs have an important role in maintenance of microbial ecology and the innate immunity of most organisms [142]. The antimicrobial activity of D-Caerin (synthetic all-D-amino acid peptide) was tested against four *Vibrio* species (*V. aestuarianus*, *V. anguillarum*, *V. harveyi*, *V. tapetis*) taking into consideration that vibriosis is one of the most usual infection diseases in bivalve mollusks (in particular, affecting seed and larvae) and can have a devastating effect in shellfish hatcheries. This is aggravated due to increased resistance of *Vibrio* spp. against traditional antibiotics [143]. It was confirmed that the D-Caerin had much higher antimicrobial action and was significantly more effective than its corresponding natural L-counterpart [143].

Phage therapy. By definition, phages (or bacteriophages) are viruses that can infect and kill bacteria [144]. Phages are made of protein shell composed of proteins and nucleic acids [24]. Due to intensification of aquaculture operations worldwide and increased use of antimicrobials (antibiotics) in prophylactic and therapeutical purposes thus provoking emergence of AMR, the phage therapy

can represent an alternative approach enabling effective and sustainable approach to control pathogenic bacteria in aquaculture production chain [144].

By using alternatives to antimicrobials, aquaculture producers can help to reduce the risk of AMR and promote the health of fish and other aquatic animals. It is recommended to plan strategies for AMR prevention and control in accordance with internationally recognized frameworks and locally available solutions [137] and use these alternatives to prevent the occurrence of aquatic animals' illnesses and reduce the use of antimicrobials.

9. Risk Mitigation Strategies for AMR in Aquaculture

Strategies to mitigate the spread of AMR in aquatic environments include, in general, reducing the use of antimicrobials (antibiotics) by improving genetics, biosecurity and applying alternative treatments (prebiotics, probiotics, immunostimulants, peptides, phage therapy), monitoring water quality, improving wastewater treatment processes, as well as promoting responsible and prudent use of antibiotics in both animal and human healthcare. Further to this, other synergistic actions should be use in attempt to enable integrated approach to combat the AMR in aquaculture.

Innovative Genetic Tools in Aquaculture. The genetics of farmed fish, by selectively breeding fish for disease resistance, is an alternative solution for disease prevention and reducing the reliance on antibiotics [145]. Genomic selection (GS) employs genetic markers spanning the entire genome to compute genomic estimated breeding values for selection candidates [146]. By selecting fish with these markers, farmers can breed for disease-resistant strains. For example, bacterial cold water disease (BCWD) provoked by *Flavobacterium psychrophilum*, stands as one of the most destructive afflictions in rainbow trout (*Oncorhynchus mykiss*) aquaculture. The utilization of licensed antibiotics for BCWD treatment amplifies production expenses, with the potential emergence of antibiotic-resistant pathogens. The selective breeding of resistance to BCWD emerges as a viable strategy to address this issue aimed at economic losses, enhancing animal welfare and allows production of Specific Pathogen Free (SPF) fish (raised in a controlled environment free from specific pathogens) [147].

Farm hygiene and sanitation management. Implementation of good agricultural and good sanitation practices is a key aspect of AMR prevention and control in aquaculture. For example, the low level of hygienic conditions in the pond environment leads to the bioaccumulation of residues in the form of sediments at the bottom of the pond, which consequently increases the risk of survival of pathogens resistant to antimicrobial drugs [148,149]. When implemented regularly and correctly hygienic practices can, in turn, reduce the need for antibiotics [150]. This encompass the application of adequate hygienic conditions in all stages of aquaculture production, from cultivation to processing (e.g. implementation and control of access to farms/pools, disinfection of equipment, facilities, pools and vehicles, control of waste and control of fish health with separation of sick animals).

Diet. A proper and balanced diet applied in aquaculture, using a high-quality feed in appropriate quantities, contributes to the prevention of diseases, and indirectly, to reduction of the need for antibiotics [151]. In conditions of excessive feeding and the use of low-quality food, stress and animal diseases occur as a result, which directly affects the increase in the consumption of antimicrobials.

Disease Prevention and Fish Health Monitoring. A disease prevention plan can help identify potential disease risks and establish preventive measures, such as vaccination, to reduce the need for antibiotics [27]. Certain vaccines can be used to prevent bacterial infections in fish and other aquatic animals, such as nucleic acid vaccines against *Nocardia seriolae* infection in orange-spotted grouper *Epinephelus coioides* [152] or nano immersion vaccine providing cross-immunoprotection against *Streptococcus agalactiae* and *Streptococcus iniae* infection in tilapia [153].

Water quality. Water quality parameters such as temperature, dissolved oxygen, pH, salinity, ammonia, nitrite, and nitrate can affect the health status of the fish in the pond [154]. Namely, poor water quality can increase the risk of disease and stress in aquaculture animals, which opens up the need for the use of antibiotics. Therefore, effective water quality management is essential to ensure optimal production and profitability of aquaculture operations. For assessment of pond water quality, the following information should be taken into consideration: pond type, pond age, water sources, feed type, pond fertilization, stocking density, and disease incidences [155].

AMU and AMR monitoring. Monitoring of antibiotics use in aquaculture will help to understand the current practices and its associated factors leading to emergence of AMR [35]. Usually, the misuse/overuse of antimicrobials in fish farming in ponds and/or freshwater systems is due to lack of farmers' training, poor farmers knowledge on the purposes of antibiotics and shorter farming experiences [35]. In 2017, global antimicrobial consumption in aquaculture was estimated at 10,259 tons. Projections indicate a 33% increase from this baseline, reaching 13,600 tons by the year 2030. The Asia-Pacific region dominates global consumption, accounting for an overwhelming 93.8%. This proportion is expected to remain constant from 2017 to 2030. Africa (2.3%) and Europe (1.8%) ranked as the second and third highest consuming regions in 2017. Europe's share is anticipated to decline to 1.7% by 2030, while Africa's is predicted to rise by 13%, reaching 2.6%. Remarkably, Africa and Latin America demonstrate the most substantial relative increases in consumption, with growth rates of 50.9% and 50.6% (Figure 4), respectively, between 2017 and 2030 [39]. AMU monitoring is related to collection of data on the antimicrobials taken by animals and humans [156], while AMR monitoring encompass the prevalence of antibiotic-resistant bacteria in aquaculture. The monitoring of the quantities and usage patterns of antimicrobials in aquatic animals is also recommended in WOAHA Aquatic Animal Health Code [157]. Elements for data collecting are kilograms of the active ingredient of the antimicrobial agent(s) used per year, divided into antimicrobial class/subclass [157]. The data on AMU can be collected from different sources, such as custom service, import-export, end-use sources (animal business operators), direct sources (wholesalers and feed manufacturers, feed stores, retailers), pharmaceutical industry and veterinary associations [118,157].

Tracking the AMR in aquaculture should be conducted within national AMR surveillance and monitoring programs. These programs should be mutually harmonized at regional and/or international level to provide the same level of public health protection and facilitate global trade. The important component of the national AMR monitoring program is to establish baseline data on the prevalence of antimicrobial resistant microorganisms and determinants, collect information on AMR trends in relevant microorganisms, explore the potential relationship between AMR in aquatic animal microorganisms and the use of antimicrobial agents, which are the elements to serve for risk analyses relevant to aquatic animal and human health [157].

It is of particular importance to establish antimicrobial (antibiotic) usage thresholds and imposing penalties for non-compliance as integral components of the legislation. The EU Farm to Fork Strategy aims to decrease the overall usage of antibiotics in farmed animals and aquaculture across 27 EU Member States from 118.3 mg/PCU in 2018 to 59.2 mg/PCU by 2030, representing a 50% reduction [158].

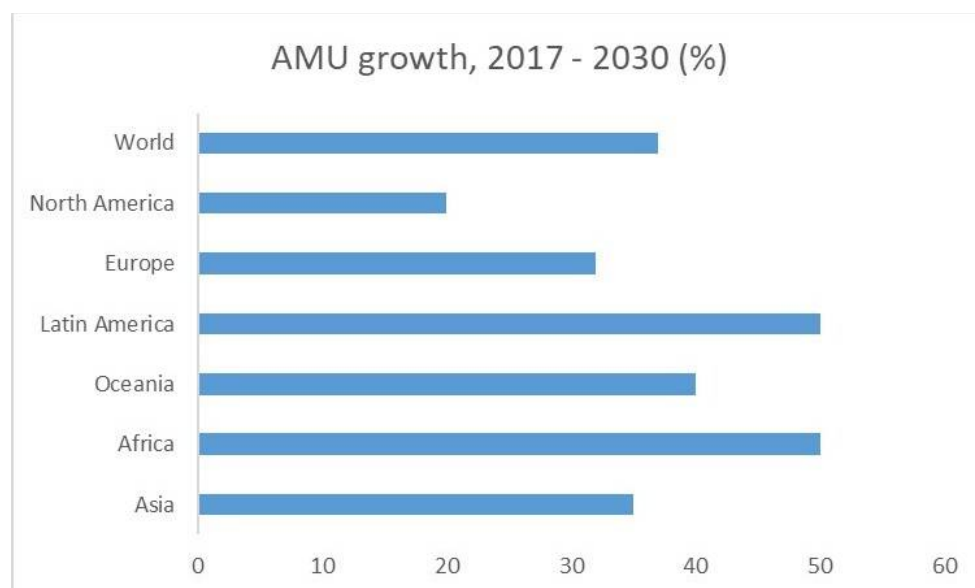


Figure 4. Estimated growth (shown as percentage) in AMU in aquaculture globally, between 2017 and 2030 (adapted from Schar et al. [39]).

Overall, a combination of afore mentioned alternative treatments and preventive measures in a synergistic manner is the best and most effective way to reduce the need for antimicrobials in aquaculture. Regular monitoring of health status of aquatic animals can help identify potential disease outbreaks in the early stage, enabling timely intervention. This includes using antibiotics only when necessary, using the right antibiotic for the specific disease or infection, and using antibiotics at the correct dose and duration [159]. Accurate diagnostic testing can help identify the specific pathogens causing disease in fish, which can lead to more targeted and effective use of antibiotics, thus reducing their use. It is important to work with a veterinarian or other qualified professional to develop a diagnostic testing plan that is appropriate for the farm. For example, such plan should be based on international approaches, such as FAO and the European Inland Fisheries Advisory Commission/EIFAC (<https://www.fao.org/fishery/en/organization/eifaac>) that have set forth guidelines for monitoring fish health and disease. It includes Specific Pathogen Free (SPF) aquaculture, used when transferring aquatic animals. Information on sample preparation, suitable diagnostic tests, import procedures, and physical site specifications are given in these guidelines [160,161]. Regular monitoring and record-keeping of antibiotic use (class of antibiotic, dose, duration of treatment) can help identify trends and patterns in use to develop more effective antibiotic stewardship programs.

10. One Health to Tackle AMR in Aquaculture

The roots of the One Health concept can be traced back to ancient civilizations that recognized the link between human health and the environment [162]. The formal recognition and promotion of this holistic approach occurred in the mid-20th century. The concept found its early expression in the fight against zoonotic diseases, such as rabies and brucellosis, where it became evident that addressing health issues in isolation failed to provide comprehensive solutions. As a holistic strategy that recognizes the interconnectedness of environmental, animal and human health, One Health approach became increasingly important in addressing the issue of AMR, particularly in the context of aquaculture (Figure 5). One Health interventions in aquaculture, to reduce the need for antimicrobials (antibiotics), include as follows: (i) Alternative disease management strategies, such as use of prebiotics, probiotics, vaccines, peptides and phage therapy; (ii) Biosecurity measures to prevent the spread of disease; (iii) Surveillance systems to monitor the use of antibiotics and the emergence of resistance in aquatic environments; (iv) Educating farmers and consumers about AMR risks and the importance of responsible antibiotic use in aquaculture [163].



Figure 5. One Health framework: environment-animal-human interface.

Food and Agriculture Organization (FAO) has undertaken efforts to implement risk analysis within One Health framework as a vital decision-making tool. Such approach was especially relevant

for responsible movement of live aquatic animals [164] and has been integrated into the framework of the National Strategy on Aquatic Animal Health [165].

In 2006, a significant milestone in the fight against AMR occurred during the Joint FAO/ WOA/WHO Expert Meeting on AMU and AMR in Aquaculture recognizing the critical hazards posed by antimicrobial residues and AMR in aquaculture [8]. Subsequent expert workshop held in 2008 identified seven major risk sectors related to aquaculture production [8,165], as follows: (i) pathogen risks, (ii) food safety and public health risks, (iii) ecological (pests) risks, (iv) genetic risks, (v) environmental risks, (vi) financial risks, and (vii) social risks.

The Global Action Plan on AMR, with contributions from FAO and WOA/WHO, was officially adopted during the 68th World Health Assembly in May 2015 [166]. The World Assembly of WOA/WHO adopted the AMR strategy in May 2015 [167]. In June 2015, FAO adopted a Resolution stating that AMR is an increasingly serious threat to public health and sustainable food production, and that an effective response should involve all sectors of government and society [168].

A critical moment in the fight against AMR came in September 2016, when a high-level meeting on AMR at the 71st United Nations General Assembly (UNGA) resulted in a political declaration [8]. UNGA called upon the Tripartite action, consisting of FAO as the global leader for food and agriculture, the WOA/WHO as the global leader for animal health and welfare, and WHO as the global leader for human health, to collaborate with other intergovernmental organizations in supporting the development and implementation of national action plans and AMR activities under the One Health platform.

The FAO Action Plan on AMR 2016–2020 was subsequently introduced to support the implementation of Resolution 4/2015. It focuses on four key areas: 1. Raising Awareness (Enhancing awareness regarding AMR and its associated threats); 2. Generating Evidence (Developing capacity for surveillance and monitoring of AMR and AMU in food and agriculture); 3. Strengthening Governance (Enhancing governance related to AMU and AMR in food and agriculture); 4. Promoting Best Practices (Encouraging the adoption of good practices in food and agricultural systems and promoting prudent AMU) [169].

From recently, at the High-Level Ministerial Conference on Antimicrobial Resistance in Muscat, Oman, the UN supported a Quadripartite action to accelerate the combat against AMR within One Health context, by establishing cooperation between the key four international agencies: FAO, WHO, WOA/WHO and UN Environment Programme (UNEP) [170,171].

11. Conclusions

The widespread use of antimicrobials in aquaculture, while aiming to prevent and treat bacterial infections in fish, poses a significant public health risk. This concern is particularly important in the rapidly expanding sector of global aquaculture production, generating more than half of the world's seafood. Risk mitigation strategies to tackle the emergence of AMR in aquaculture include implementing stringent antimicrobial (antibiotic) use guidelines, promoting disease control methods like enhanced biosecurity measures and vaccinations, alternatives to antibiotics (prebiotics, probiotics, immunostimulants, essential oils, peptides and phage therapy), feeding practices, genetics, monitoring water quality and improving wastewater treatment to curtail the release of antibiotic-resistant bacteria into the environment, as well as continuous monitoring and surveillance. Government should play a crucial role in combat against AMR in aquaculture. This should be achieved through strengthening regulations and enforcement mechanisms in monitoring antibiotic usage in aquaculture and providing recommendations on human health and aquatic animal health policies and programs, guidelines for prudent use of antimicrobials in aquaculture/aquatic animals. It is of particular importance to establish antimicrobial (antibiotic) usage thresholds and imposing penalties for non-compliance as integral components of the legislation. The national AMR monitoring plans should be regularly implemented and ideally harmonized at regional/international level to enable equivalency of public health protection and facilitate food trade on a global scale. This should include the information on AMR patterns from pre-harvest (intensive aquaculture/farming) and post-harvest level (e.g., non-heat treated Ready-To-Eat products such as sushi, cold-smoked salmon). One Health concept, connecting environmental, animal and human health, emerges as a vital holistic approach to combat the challenges of AMR in aquaculture. The quadripartite approach to tackle AMR

composed of environmental, animal health, food and agriculture, and public health agencies (UNEP, WOAAH, FAO, WHO, respectively) enables more effective actions to underscore the AMR threat, including in aquaculture. By embracing this interdisciplinary perspective, a sustainable future for global aquaculture can be envisioned that safeguards both human and animal health, along with environmental well-being.

List of Abbreviations

Abbreviation	Definition
AB	Antibiotics
AMU	Antimicrobial Use
AMPs	Antimicrobial Peptides
AMR	Antimicrobial Resistance
ARG	Antimicrobial Resistance Gene
BCWD	Bacterial Cold Water Disease
CIA	Critically Important Antibiotic
CLSI	Clinical and Laboratory Standards Institute
DALYs	Disability-Adjusted Life Years
ECV	Epidemiological Cut-Off Values
EEA	European Economic Area
EOs	Essential Oils
ESVAC	European Surveillance of Veterinary Antimicrobial Consumption
EU	European Union
FAO	Food and Agriculture Organization
FOS	Fructo-Oligosaccharides
GOS	Galacto-Oligosaccharides
GS	Genomic Selection
HGT	Horizontal Gene Transfer
LMIC	Low and Middle-Income Country
MGE	Mobile Genetic Element
MIC	Minimum Inhibitory Concentration
MOS	Mannan Oligosaccharides
MRSA	Methicillin-resistant Staphylococcus aureus
NAP	National Action Plan
SPF	Specific Pathogen Free
VCIA	Veterinary Critically Important Antibiotic
WGS	Whole Genome Sequencing
WHO	World Health Organization
WOAH	World Organisation for Animal Health

Author Contributions: Conceptualization, M.M. and I.N.; methodology, I.N.; validation, M.M., S.V.M. and I.N.; formal analysis, J.P.; investigation, M.M. and J.B.M.; resources, J.P., I.N.; data curation, J.B.M., S.V.M. and I.N.; writing—original draft preparation, M.M.; writing—review and editing, I.N. and M.M.; visualization, I.N.; supervision, I.N; All authors have read and agreed to the published version of the manuscript.

Funding: “This research was funded by the Ministry of Science, Technological Development and Innovations of Republic of Serbia, with reference to the Contract on realization and financing scientific and research work of Scientific Research Organization in 2024, number: 451-03-66/2024-03/200050 from February 5th 2024”.

Data Availability Statement: The data presented in this study are available on request from the corresponding author.

Conflicts of Interest: The authors declare no conflicts of interest.

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