Antimicrobial Resistance Assessment in Aquacultures

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Aquaculture is a new food production sector that has been increasing dramatically over the last two decades due to the high demand for a healthy protein source. The aquaculture production figures indicate a substantial increase in the relative contribution of aquaculture to total fish consumption from 5% in 1962 to 49% in 2002. The use of antimicrobials in aquaculture, even at subinhibitory concentrations, has been promoting antimicrobial resistance in bacteria from the aquatic environment. New omics tools and approaches pave the way for the assessment of antimicrobial resistance in these environments.

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1. Introduction

The need to provide adequate and safe food to a growing global population of 9.8 billion people by 2050 [1], has intensified the importance of the aquaculture industry. The reduction in wild fishery stocks, a rising human population, a continuing demand for seafood and international trade has driven a great expansion of aquaculture during the last decades in terms of production and economic value. Aquaculture production provides almost half of the fish that is consumed worldwide, which has led producers to move towards intensive and semi-intensive production systems [2]. Nine of the top-ten ranked countries for aquaculture species diversity are in Asia, with China leading by a wide margin.

The other side of this reality is that the use of intensive and semi-intensive practices for fish production leads to a higher concentration of animals in small spaces, substantially increasing the risk of contagious diseases [3]. Therefore, the prophylactic and therapeutic use of antimicrobials is currently employed to control disease outbreaks, and the substances widely used in aquaculture are the same as those licensed for therapy and the prophylaxis of infectious diseases in humans and livestock. Quinolones (i.e., oxalinic acid, flumequine, and enrofloxacin), tetracyclines (i.e., oxytetracycline), and phenicols (i.e., florfenicol) are the most widely antibiotics in aquaculture to control the bacterial fish disease [4] and are administered mostly in food.

The use of antimicrobials in aquaculture, even in sub-inhibitory concentrations, may favour the emergence of durable and stable antimicrobial resistance (AMR) [5] and promote horizontal gene transfer (HGT) and mutagenesis in bacteria of the aquatic environment [6][7][8]. Due to the connections of epidemiological pathways between humans, animals, and the environment, the identification of the factors influencing AMR emergence and spread in animal production, such as aquacultures, will contribute to the ability to control resistance in the areas of food production, the environment, and public health [9].

Antibiotics are molecules, widespread in nature, which are naturally produced by bacteria and other microorganisms, as part of their natural life within a microbial community. In parallel, bacteria from microbial communities develop natural antibiotic resistance genes (ARG). Both antibiotics and ARG are as old as bacteria are [10][11][12]. For example, ancient DNA found in 30,000-year-old permafrost sediments has revealed a highly diverse collection of genes encoding antimicrobial resistance mechanisms to beta-lactam, tetracycline, and glycopeptide groups [11][13] that are associated with modern bacterial pathogens, highlighting the ancient origins of antibiotic resistance.

Antibiotic production and release into the environment can be a strategy that allows antibiotic-producing microorganisms to compete and communicate with each other within a microbiome (the collection of all the microorganisms and their genomes of that microbial community). Cooperation, competition, and inhibition among organisms within a microbiome are different aspects of the interplay between these different counterparts. Environmental antimicrobials are ubiquitous and naturally present in low concentrations, playing a very important ecological role in microbiome dynamics.

The resistome, as a collection of all the ARGs and their precursors in a microbial community, is a peculiarity of both pathogenic and non-pathogenic microorganisms since it is required for the survival and evolution of the bacteria in a dynamic environment [14][15]. The complex networks and interactions occurring between microbial species from diverse environments facilitate the gene flow, expanding the AMR between humans, animals, and the environment, resulting in a widespread issue.

Bacterial resistance to antibiotics relies on three main factors: the impermeability of the bacterial cell to the antibiotic molecule (e.g., the physicochemical properties of the molecules, the presence of efflux pumps etc.), the lack of the target molecules in the cell or the inactivation of the antibiotic compound by means of degrading enzymes [15]. Schmieder and Edwards reported at least four well-known mechanisms that contribute to antibiotic resistance in bacteria: “(i) the inactivation or modification of the antibiotic; (ii) the modification of metabolic pathways to circumvent the antibiotic effect; and (iv)

Upon environmental changes, bacterial evolution relies on their genomic flexibility to adapt to the surrounding environment including, among others, the ability to protect themselves from toxic substances [16]. Moreover, the genetically determined resistance set up by given bacteria is efficiently transmitted to its clonal expansion and/or other bacterial species through mobile genetic elements, such as plasmids, transposons, and integrons [17]. The concerted activity of both genetic/heritable elements and phenotypic traits are involved in a wide array of metabolic functions, which have been considered essential for the onset and diffusion of antimicrobial resistance.

2. Microbial Communities and Antimicrobial Resistance in Aquacultures

In December 2017, the United Nations Environment Programme (UNEP) identified the environmental resistance to antibiotics as the major concern out of six emerging issues and referred to the fact that up to 75% of antibiotics used in aquaculture may be lost into the surrounding environment [18]. A strong positive correlation between flumequine and florfenicol consumption in aquafarms and the detection of their residues in surface water and sediment samples were identified [19], proving that the antibiotics used in aquaculture can reach the near aquatic environment. In Europe, Japan, and North America, the use of antibiotics in aquaculture is strictly limited to therapeutic applications where only a limited number of antibiotics are approved [20]. For example, between 1987 and 2013, the salmon production system in Norway reduced antibiotic use by 99%, and the use of vaccines, hygienic measures, and scientific research has been crucial for such an improvement [20].

Rearing animals in small tanks increases the stress of the animals and the incidence of infectious diseases, resulting in whole stock losses associated with economically important damages [3]. This leads to the use of antibiotics for both prophylactic and therapeutic purposes, promoting the selection and dissemination of antibiotic resistance genes by various routes (food, feed, and environment) [2][21][22]. Fish farming has been suggested as a reservoir of ARGs [23][24] and a significant correlation between the occurrence of ARGs and the concentration of antibiotics in aquaculture sites has been reported [25].

Thus, antibiotics intended for human and veterinary medicine sectors are also improperly used in the aquaculture which contributes to an exacerbation of the impact of AMR onset and dissemination [2][26]. Six antibiotic classes, listed by the World Health Organization (WHO), critically important in human medicine (aminoglycosides, macrolides, penicillins, quinolones, sulphonamides, and tetracyclines), have been widely used in both terrestrial and aquaculture husbandries, thus compromising their effective use in the treatment of infectious diseases in humans [2][27][28]. Therefore, antibiotic residues remain for long periods in fish meat, which promotes their entry into the food chain. Additionally, it is estimated that approximately 70 to 80% of antibiotic residues still active are eliminated in faeces, contaminating wastewater and affecting the ecosystem [27].

Most of the aquaculture open farms use antibiotics and despite the need for a quarantine period, some antibiotic remnants may be discarded into the open sea [29]. Antibiotics are usually provided in feedstuffs [30] but not all the medicated feed is consumed by fish or crustaceans, and, after lixiviation, small amounts of antibiotics are released into the sediment or water and make contact with environmental bacteria that can develop resistance. Antibiotic resistance genes can be incorporated into aquaculture systems through manure, commonly used as natural fertilizer, containing bacteria with antibiotic resistance [31][32].

Currently, China has the world’s largest areas for marine farming production [33]; the industry of the Chinese mitten crab Eriocheir sinensis, one of the main freshwater aquaculture industries in that country. However, the real situation of antibiotic resistance in aquaculture of E. sinensis is not yet adequately known [34]. Much attention has been paid to the distribution of antibiotics, antibiotic resistance genes (ARGs), and the bacterial composition of the community in this environment [35]. These include tetracycline and sulfonamides resistance genes, some of the emerging plasmid-mediated quinolone resistance (PMQR) genes, the new determinant of fosfomycin resistance, the widely disseminated emergingfloRgene of human pathogens, and the chloramphenicol catII, catB9 and catB2 from aquatic Photobacterium sp.

The use of antibiotics in aquaculture environments, combined with anthropogenic disturbances resulting from environmental contamination with antibiotics, not only affects bacteria in the microbiota of fish and shellfish but also affects the aquaculture sediments. Marti and colleagues have compared the bacterial composition and the content of antibiotic-resistant genes blaTEM, ermB, qnrS and sulI in two different ecosystems with high and low anthropogenic disturbance, respectively, and detected significant differences in bacterial community composition between the fish species, suggesting that anthropogenic activities promote the emergence and spread of antibiotic resistance in aquatic organisms [36].

The sediments below fish farms can be also enriched with ARG, as they are constantly receiving fish feces, such as those encoding resistance to sulfonamide (sul1), trimethoprim (dfrA1), tetracycline (tet(32), tetM, tetO, tetW), aminoglycoside (aadA1, aadA2), chloramphenicol (catA1), and efflux-pumps resistance genes (emrB,matA, mefA, msrA), together with class 1 integron-associated genes (intI1,qacED1), and transposases (tnpA) [37].

Oxytetracycline is a tetracycline broad-spectrum antibiotic that is widely used in aquaculture both for therapeutics and as a prophylactic agent. However, it has also been linked to the emergence of antibiotic-resistant bacteria in aquaculture environments, and to the contamination of fish meat and products with antibiotic residues and with human bacterial pathogens [38].

Many fish pathogens and aquatic bacteria, such as Aeromonas salmonicida ,A. hydrophila, Citrobacter freundii, Edwardsiella tarda, Yersinia ruckeri, Lactococcus garviae, Photobacterium damselae subsp. piscicida,Vibrio anguillarum, V. salmonicida, Photobacterium psychrophilum, and Pseudomonas fluorescent [38], have been reported as having developed resistance to antibiotics as a consequence of antimicrobial exposure. Antibiotic resistance can be transferred from plasmids-encoding resistance genes from fish pathogens into other bacteria within the same genus but also to E. coli. These observations reinforce the idea that aquaculture environments can act as reservoirs of antibiotic resistance genes.

Vibrio parahaemolyticusandVibrio vulnificus is the leading foodborne bacterial pathogens that cause seafood associated infections and death in the US. They are reported as being usually susceptible to most antimicrobials of veterinary and human significance; however, Elmahdi and colleagues [39] have performed a comparative study across many different countries worldwide and have concluded that of the antibiotic resistance profiles involved, regardless of the country, ampicillin, penicillin, and tetracycline were frequently observed in aquacultures. The presence of multiple-antibiotic resistant bacteria in seafood and aquatic environments is a major concern in fish and shellfish farming and human health.

In a comprehensive metagenomic study on the structure of bacterial communities, and the abundance and diversity of ARGs, as well as MGEs in the three Chinese mitten crab aquaculture ponds in Jiangsu Province, China contained in [34] it was revealed that resistance to bacitracin was very prevalent in the water, while sediments were enriched in multidrug resistance traits. There was also a significant correlation between MGEs (particularly plasmids) and ARGs which may cause a potential risk to human health [34].

Another work using quantitative PCR and bacterial culture-dependent methods to evaluate ARGs and antibiotic-resistant bacteria in marine fish farming areas indicated that the sul and yet family genes were widely distributed in Hainan, China; specifically,sul1andtetBwere the most abundant genes detected [40]. It was found that salinity also has an important effect on the abundance of ARGs and antibiotic-resistant bacteria in the marine fish farming area [40][41], and tetS were the prominent ARGs with high detection frequencies ranging from 30.9 to 51.1% in total samples. This study also showed that ARGs are more abundant in freshwater aquatic animals than in marine animals, reflecting a discrepancy in the cultivation patterns between freshwater and marine aquacultures [40].

In the Drwęca river in Poland, fish farming drove an increase in the diversity of tetracycline-resistance genes [42]. Resistance to tetracyclines (tetracycline, oxytetracycline or chlortetracycline) are among the most frequently detected in aquacultures across the world The Polish Drwęca River study showed that fish farming influenced the quality of the water directly by increasing the diversity of tetracycline-resistance genes by HGT induced between Aeromonas sp. Therefore, we can conclude that the anthropogenic activities and the pollution of aquatic environments, particularly in relation to antibiotic residuals, can trigger the emergence and spread of antibiotic resistance [36].

Aquaculture is a new food production sector that has been increasing dramatically over the last two decades due to the high demand for a healthy protein source [43]. The aquaculture production figures indicate a substantial increase in the relative contribution of aquaculture to total fish consumption from 5% in 1962 to 49% in 2002 [44]. In 2014, food fish produced via aquaculture systems reached 70.5 million tons [45], and in 2018 seafood provided almost 20% of all animal protein in diets, globally [46].

Most aquaculture production units (>90%) are located in Southeast Asian countries, where fish farming is practised in oceanic networks or contained in lagoons or reservoirs, and often integrates fish farming practices that use the waste from animal husbandry potentially contaminated with antibiotic residues [47]. Overcrowding, unhygienic measures and other manipulations may promote the spreading of bacterial infection and concomitantly an increase in antimicrobial use particularly in the shrimp and salmon industries [48].

Although disease control in fish farming can be vaccine-based, antimicrobial treatment in medicated feed or bathing with fluoroquinolones, florfenicol, tetracycline, sulfonamides, and amoxicillin are often used. Antimicrobial substances are thus used in the fish farming industry for therapeutic and prophylactic purposes and in some countries, albeit not in European ones, they are also added to animal feeds as growth promoters [24]. In fact, the antimicrobial agents that are most widely used in aquaculture are the same as those authorized for therapy and the prophylaxis of infectious diseases in humans and animals [49][50]. The OIE has conveyed that tetracyclines were the most commonly reported antimicrobial class among the 116 countries providing quantitative data, accounting for 34.5% of those used from 2015 to 2017 [51].

The widespread application of antimicrobials to fish will lead to the release of uneaten feed and faecal particles dispersed in water, which may contain residues that persist in the surrounding environment. In addition, as fish do not metabolize antibiotics effectively, 70% to 80% of the active substance can pass into the environment through the faeces or remain in the fish tissues for long periods of time [2]. It is known that bacterial populations exposed to low antimicrobial levels concentrations are selected for AMR [43][48][52]. In aquaculture, AMR can develop in the gut microbiota of fish and on other bacteria in the aquatic environment as a result of antimicrobial pressure.

In a bibliographic analysis to review the use of antibiotics in the top 15 aquaculture producing countries between 2008 and 2018, it was pointed out that 67 antibiotic compounds were used in 11 of the 15 countries, including oxytetracycline, sulphadiazine, and florfenicol, and that on average, countries used 15 antibiotics; Vietnam, China and Bangladesh were the top users [53].

The emergence of β-lactamase- and carbapenemase-producing Enterobacteriaceae at integrated fish farms is particularly worrisome [54]; carbapenemase-producing E. coli from oysters, aquatic shrimps, and lakes have been reported in Brazil [52]. In addition, the detection of carbapenemase in non-fermentative Gram-negative organisms, such as Stenotrophomonas, Myroides, and Pseudomonas spp. isolates, recovered from frozen seafood imported from Asian countries, suggests that non-pathogenic bacteria excluded from antimicrobial surveillance programs may act as reservoirs of the carbapenemases genes in the food supply [55]. Recently, researchers are questioning whether fish commonly used in raw preparations such as sushi and sashimi constitute a public health problem [56].

Resistance to more than one antibiotic has also been described in Vibrio parahaemolyticus, Clostridium difficile, and some Enterobacteriaceae, among others, isolated from bivalve molluscs from coastal areas [57][58][59]. These food products pose a potential risk to humans as they are consumed without adequate heat treatment or even raw, such as flat oysters (Ostrea edulis) and large scallops (Pecten maximus). Bivalve plays an important role in resistance transfer determinants, as they are suspension feeders, actively filtering, retaining, and concentrating particles, including free-living or particulate-bound bacteria, from the surrounding water [57]. Thus, the presence of antibiotic-resistant bacteria in fish, seafood, and molluscs may represent a threat to human health, and could also result in the transfer of resistant determinants to other clinically important bacteria [24].

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