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Review Article

Abuse of Antibiotics in Aquaculture and it's Effects on Human, Aquatic Animal and Environment

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Abstract: The faster growth of aquaculture has ensued in a series of developments harmful to the human and aquatic animal health as well as environment. People are using various prophylactic antibiotics indiscriminately in aquaculture mainly developing countries to prevent different bacterial infections resulting from sanitary shortcomings in fish and shellfish rearing. Also, the abuse of wide variety human non-biodegradable antibiotics remains in aquatic environment. It directly causes development of antibiotic-resistance bacteria and transferable resistance genes can be transferred to disease-causing bacteria, resulting in antibiotic-resistant infections for humans, fish and other aquatic animal. The greater the volume of antibiotics used, the greater the risks that antibiotic-resistant populations of bacteria will prevail in the contest for survival of the fittest at the bacterial level. The use of large amounts of antibiotics that have to be mixed with fish food also forms problems for industrial health and rises the opportunities for the presence of residual antibiotics in fish meat as well as fishery products. So, to overcome this global problem; strict measures, legislations and regulations for the use of antimicrobials in aquaculture should be developed and implemented especially in developing countries, to avoid such negative impacts in human, fish, animals and environment.
Keywords: Aquaculture, Antibiotic, Antibiotic Resistance and Environment.

INTRODUCTION

Aquaculture is growing rapidly in many regions of the world, and aquaculture products constitute an important food supply with increasing economic importance. World aquaculture production more than doubled during the period 1994–2004, and countries in Asia accounted for 80%–90% of the total production. In 2010, the world aquaculture production of food fish amounted to 79 million tons, of which 48.19 million tons were produced in China alone, whereas India, Vietnam, Thailand, Indonesia, and Bangladesh together accounted for 16.59 million tons [1]. The industry covers a wide range of species and methods, from simple traditional systems, in which fish or other aquatic animals are reared in small ponds for domestic consumption, to intensive industrial scale production systems. To control infectious diseases, similar strategies (eg. vaccination and use of antimicrobial agents) are employed in aquaculture as in other areas of animal production. Use of antimicrobial agents in aquaculture has resulted in the emergence of reservoirs of antimicrobial-resistant bacteria in fish and other aquatic animals, as well as in the aquatic environment [2-6]. Consequently, the use of antimicrobial agents in aquaculture results in a broad environmental application that impacts a wide variety of bacteria [7].

Intensive use of antimicrobial agents in aquaculture provides a selective pressure creating reservoirs of drug-resistant bacteria and transferable resistance genes in fish pathogens and other bacteria in the aquatic environment [3, 8]. From these reservoirs, resistance genes may disseminate by horizontal gene transfer and reach human pathogens, or drug-resistant pathogens from the aquatic environment may reach humans directly [9]. Horizontal gene transfer may occur in the aquaculture environment, in the food chain, or in the human intestinal tract. Among the antimicrobial agents commonly used in aquaculture, several are classified by the World Health Organization as critically important for use in humans. Occurrence of resistance to these antimicrobial agents in human pathogens severely limits the therapeutic options in human infections. Considering the rapid growth and importance of aquaculture industry in many regions of the world and the widespread, intensive, and often unregulated use of antimicrobial agents in this area of animal production, efforts are needed to prevent development and spread of antimicrobial resistance in aquaculture to reduce the risk to human health.

Antibiotic use in aquaculture

The natural or synthetic antibiotic should be safe to the host, permitting their use as chemotherapeutic agents for the treatment of infectious bacterial diseases. The efficiency of the fishes's immune system to clear up bacterial colonization and infection [10, 11, 12], it has become common to increase the use of prophylactic antibiotics. Fish are given antibiotics with their food, and sometimes in baths and injections [13, 8]. The unconsumed food, and fish faeces, containing antibiotics spread the sediment at the bottom of the raising pens; antibiotics are leached from the food and faeces and diffuse into the sediment and they can be washed by currents to distant sites [8, 14-21]. These residual antibiotics will remain in the sediment, exerting selective pressure, thereby changing the composition of the microflora of the sediment and selecting for antibiotic-resistant bacteria [8, 14, 22-30]. The determinants of antibiotic resistance that have emerged and selected in this aquatic environment have the potential of being transmitted by horizontal gene transfer to bacteria of the terrestrial environment, including human and animal pathogens [8, 22, 31-34]. In many aquaculture settings in developing countries, the possibilities of these exchanges have been intensified by the high level of contamination of seawater and freshwater with untreated sewage and agricultural and industrial wastewater containing normal intestinal flora and pathogens of animals and humans usually resistant to antibiotics [8, 11, 35]. This is also the case in settings in which aquaculture is integrated with agriculture [36], and such practices such as the use of manure and other agricultural residues as fish feed are widespread [36].

Resistance mechanisms and transference intensify

In aquaculture, antimicrobials are regularly added to the feed, which is then placed in the water where the fish are kept. In some cases, antimicrobials may be added directly to the water. These procedures result in a selective pressure in the exposed environments (usually water). The use of antimicrobials in aquaculture may involve a broad environmental application that affects a wide variety of bacteria. Several bacterial species may survive unfavorable conditions or environmental changes after selecting mutations that improve their fitness in the new conditions. Furthermore, bacteria take advantage of mobile genetic elements, such as plasmids and transposable elements. With these elements, bacteria can access a large pool of itinerant genes that move from one bacterial cell to another and can spread through bacterial populations.

Several mechanisms of antimicrobial resistance are readily spread to a variety of bacterial genera. The microorganism may acquire genes encoding enzymes, such as beta-lactamases, which destroy beta–lactams (penicillins). Other antibiotic-inactivating enzymatic reactions include phosphorylation, adenylation, and acetylation. Recently, Kumarasamy *et al*., [37] described the beta-lactamase NDM-1 as an example of how significant a single enzyme can be. Bacteria may acquire several genes for a metabolic pathway that ultimately produces an altered bacterial cell wall that no longer contains the binding site for the antimicrobial agent, or bacteria may acquire mutations that limit the access of antimicrobial agents to the intracellular target site via the down regulation of porin genes. Similarly, an important consequence of the large amounts of antibiotics used for farm animals and fish in aquaculture is the selection of pathogenic bacteria resistant to multiple drugs. Multidrug resistance in bacteria may be generated by one of two mechanisms. First, these bacteria may accumulate multiple genes, each coding for resistance to a single drug, within a single cell. This accumulation typically occurs on resistance plasmids. Second, multidrug resistance may also occur through the increased expression of genes that code for multidrug efflux pumps that excrete a wide range of drugs.

Aquaculture as a source of antibiotic resistance in human pathogens

Genes exchange for resistance to antibiotics between bacteria in the aquaculture environment and bacteria in the terrestrial environment, including bacteria of animals and human pathogens has recently been shown [8, 32, 33, 38-40]. For example, strong epidemiological and molecular indication exists showing that fish pathogens such as *Aeromonas* can transfer and share determinants for resistance to antibiotics with pathogens such as *Escherichia coli* isolated from humans [8, 19, 20, 32-34]. The antibiotic resistance determinants of *Salmonella typhimurium* DT104 are encoded by a transmissible genetic element in the chromosome that contains a resistance gene for florfenicol, an antibiotic extensively used in aquaculture in the Far East [41, 42]. Moreover, the DNA sequence of the transmissible element harboring these antibiotic resistance determinants has an important DNA sequence similarity to a plasmid of *Pasteurella piscicida*, which is also a fish pathogen [42-43]. This process demonstrates the potential role of carrying of antibiotic-resistant bacteria as an alternative mechanism responsible for the spread of antibiotic resistance determinants from the aquatic environment to the terrestrial environment [44, 45].

The existence of antibiotics in the aquatic environment can result in the appearance of resistance among human pathogens forming part of its microbiota [42]. The widespread transmission of antibiotic resistance determinants between bacteria of the aquatic and terrestrial environment has been recently revealed by the emergence of plasmid-mediated quinolone resistance among human Gram-negative pathogens [46-50]. Thus, the commonality of antibiotic resistance determinants and of genetic elements between aquatic bacteria, fish pathogens and bacteria from the terrestrial environment powerfully supports the idea that antibiotic usage in aquaculture will influence the appearance of resistance in bacteria of other places, including resistance in pathogens able to produce a variety of human and animal diseases [11, 32, 34, 42, 51-53].

Antibiotic effects on environmental bacteria

When an antibiotic treatment begins (usually via medicated feed) the gut micro flora and environmental bacteria can come in contact with the antibiotics present in fish farm and hatchery wastes. The use of oxytetracycline in fish farming has been demonstrated to match with an increased frequency of oxytetracycline-resistant microorganisms [54]. Miranda & Zemelman [24] also investigated the prevalence of oxytetracycline resistance in freshwater salmon farms, finding that the highest proportions of resistant bacteria were found in effluent samples (8-69%). Oxytetracycline, one of the most commonly used antibiotics in fish farms and hatcheries, is very poorly absorbed through the intestinal tract of fish. It has to be administered at a high dosage rate of 100–150 mg per kg fish per day for 10–15 days. This treatment consequently causes the slow excretion of large amounts of this antibiotic, thus increasing the selective pressure which might lead to the selection of oxytetracycline-resistant bacteria in the gut [55].

Therefore, most of the studies designate that increased levels of antibiotic resistance can be expected to occur for as long as antibiotics are used in aquaculture. A more comprehensive understanding of drug-resistance in aquaculture needs a clear-cut and quantitative analysis of resistance genes using a culture-independent method. Furthermore, the analysis of tetracyclines indicated that none of the samples contained therapeutic concentrations at any of the sampling times, suggesting that the prevalence of tetracycline- resistance genes may be caused by the perseverance of these genes in the absence of selection pressure. An increase in antibiotic-resistance genes in the absence of the antibiotic itself has also been recognized to co-selection with other antibiotics.

Molecular approaches and massive sequencing methods could be significant tools to clarify the diversity of antibiotic-resistance genes present in the environment. The resistome concept has been used to describe the diversity of antibiotic resistance that exists naturally in a particular environment [56]. However, the resistome of aquaculture environments has been poorly described. Recent studies have emphasized the observation that a single antibiotic has the potential to co-select for a diversity of resistances. To assess this potential risk, future studies should focus on the ability of different antibiotics used in aquatic environments to co-select for multiple resistances.

Antibiotic effects on host microbiota

The intestinal tracts of healthy fish harbor a microbiota that has been investigated by several authors due to its assumed importance in digestion; nutrition and disease control. It results the specificity of the host response, which depends on the bacterial species that colonize the digestive tract [57]. Possible modifications in gastrointestinal microbiota due to antibiotic treatment could change this presumably beneficial host-micro biota relationship.

However, very few studies have focused on determining the effects of antibiotic treatment on the microbial ecology of the fish gut. In general, published studies have mainly focused on describing the frequency of antibiotic resistance during and after the use of antibiotics [58], the susceptibility of fish pathogens isolated from fish and fish farms to antibiotics [58-60] and molecular determinants of antibiotic resistance [25, 61]. A special effort was prepared to describe the dominant bacterial components, especially the newly arising microbiota. Overall, this evidence suggests that antibiotic treatment may change the composition of the intestinal microbiota of farmed fish, causing a reduction in bacterial diversity. This evidence supports the current concern that antibiotic treatment can eradicate microorganisms of the normal microbiota, facilitating the proliferation of opportunistic bacteria by depleting competition.

Antibiotics and their effects on fish stress responses

The use of wide range of antibiotics in aquaculture has well-known positive effects on the control of bacterial infections; however, some side effects that affect both the fish and the environment are linked with excessive use. The effects of antibiotics on the environment are mainly due to the overuse of these drugs by the aquaculture industry and the presence of drug residues in fish products [62]. Unfortunately, there are only a few studies that analyze the side effects of antibiotic use on fish themselves. There is evidence that some antibiotics can persuade nephrotoxicity [63], but the most well recognized side effect is immunomodulation [64-67]. In the case of nephrotoxicity, a study conducted by the Bonventre group [63] determined that, as in rats and humans, gentamicin, an aminoglycoside antibiotic, induces acute renal failure in fish. Their results showed that gentamicin induced pericardial edema in a time- and dose-dependent manner, which resulted in the fish being unable to maintain fluid homeostasis.

Additional effects of the excessive use of antibiotics in aquaculture

The excessive use of antibiotics in industrial aquaculture is the presence of residual antibiotics in commercialized fish and shellfish products [8, 11, 68-72]. This problem has led to invisible consumption of antibiotics by consumers of fish with the added potential variation of their normal micro flora that enhances their susceptibility to bacterial infections and also selects for antibiotic-resistant bacteria [68, 69, 71, 73-76]. Besides, undetected consumption of antibiotics in food can create problems of allergy and toxicity, which are difficult to diagnose because of a lack of previous information on antibiotic ingestion [71, 73]. Allergy to antibiotics and problems of toxicity can also be generated for the unprotected workers in the aquaculture industry through the use of large amounts of antibiotics that come in contact with the skin, and intestinal and bronchial tracts as workers medicate the food in food mills, distributing it, and administer it to fish [68, 69, 77]. In aquaculture, the passage into and permanent existence of large amounts of antibiotics in the environment of water and sediments also have the potential to affect the presence of the normal flora and plankton in those niches, resulting in shifts in the diversity of the microbiota [8, 11, 35, 78, 79]. These moves can be amplified by the eutrophication produced in the aquaculture environment by the increased input of N, C and P generated by the non-ingested food and fish faeces [11, 70, 79]. As the microbiota carries important trophic and metabolic functions in aquatic and sediment niches, this heavy use antibiotic also has the potential to alter the ecological equilibria at those levels, thus creating situations that may impact fish and human health by promoting, for example, algal blooms and anoxic environments [79-82].

Antibiotics and public health

The use of antibiotics in aquaculture depends on the local regulations that vary widely between different countries. In some countries, regulations on the use of antibiotics are strict, and only a few antibiotics are licensed for use in aquaculture. However, a large proportion of global aquaculture production takes place in countries that have permissive regulations. The public health risk related with antimicrobial residues depends on the quantity of the antimicrobial encountered or consumed or the exposure. The presence of antibiotic-resistant bacteria in foods of animal origin is a potential health threat because resistance can be transferred among bacteria, and antibiotic-resistant pathogens may not respond to antibiotic treatments. In a microbiological study of market products, Duran & Marshall [83] examined several brands of ready-to-eat shrimp that were obtained from grocery stores. Several human pathogens were detected among the resistant isolates, including *Escherichia coli*, *Salmonella*, *Shigella* and *Vibrio* spp.

Antimicrobial-resistant bacteria in aquaculture represent a risk to public health. The appearance of acquired resistance in fish pathogens and other aquatic bacteria means that such resistant bacteria can work as a reservoir of resistance genes from which genes can be further distributed and may ultimately end up in human pathogens. It is important to consider that most antibiotics used for treating infections are produced by environmental microorganisms, meaning that the genes for antibiotic resistance must also have emerged in non-clinical/artificial habitats [84]. A better understanding of the ecological role of antibiotics and antibiotic resistance in natural environments may eventually help to predict and counteract the emergence and evolution of resistance.

Policies of antibiotic use in aquaculture

Antibiotic-resistant bacteria and antibiotic resistance determinants pass from the aquatic to the terrestrial environment has resulted in a drastic restriction of the use of antibiotics in aquaculture in many countries [8, 13, 71, 72, 77, 85]. Restrictions have included, increased control of the prescription of therapeutic antibiotics [8, 13, 68, 69, 77], almost total elimination of the use of antibiotic prophylaxis in this setting [8, 68, 69] and prohibition of the use of antibiotics in therapeutics that are still very useful in the therapy of human infections [13, 68-70, 77]. This increased control of antibiotic use, accompanied by sanitary measures that include the use of vaccines, have drastically reduced the use of antibiotics in the aquaculture industry of developed countries, therefore indicating that it is economically feasible to develop a productive aquaculture industry without excessive prophylactic use of antibiotics [8, 13, 68-70, 77]. However, the use of quinolones and many other antibiotics remains totally unrestricted in aquaculture in countries with growing aquaculture industries such as China and Chile [46, 71].

Alternative treatments: Probiotics, essential oils and phage therapy

The rise in bacterial antibiotic resistance and antibiotic residues has become global concerns, and there is a need to develop alternative therapies for bacterial pathogens in animal production, especially in aquaculture. Vaccination is an ideal method for preventing infectious diseases, but it is not a treatment for existing infections, and commercially available vaccines are still very limited in the aquaculture field. Several alternatives to the use of antibiotics have been used successfully in aquaculture. The use of innocuous microorganisms to avoid bacterial infection in aquatic organisms has been tested in aquaculture. Here, we include a brief review of the use of probiotics in aquaculture and discuss some of the suggested mechanisms by which they might control aquatic pathogens. Another source of alternative treatments is essential oils, which are natural components from plants that are generally recognized as safe substances (GRAS). Due to their antimicrobial properties, these oils may constitute alternative prophylactic and therapeutic agents in aquaculture. Furthermore, phage therapy has gained much attention for its advantages in preventing and controlling pathogen infections; since 1999, phages have been used successfully in aquaculture facilities.

CONCLUSION

Large quantities of antibiotics are used in aquaculture in some countries. They are used not only to treat diseases but also for prophylaxis. Consequently, many problems are associated with the use of antibacterials in aquaculture. More research is needed in order to determine the consequences of the application of large quantities of antibacterials. Considering the rapid growth and importance of the aquaculture industry in many regions of the world and the widespread, intensive, and often unregulated use of antibacterial agents for fish and shellfish production, additional efforts are required to prevent the development and spread of antibacterial resistance in aquaculture. Judicious use of antibiotics in animals is a requirement to delay the emergence of bacteria resistant to the still-working antibiotics. Furthermore, fish farmers must confirm that fish are kept in the best state of health and welfare. The invention of novel drugs or the use of alternatives to antibiotics should also be encouraged. Still, the increased awareness of the scientific community and the stakeholders in general is both alarming and promising at the same time.

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