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Problems of antibiotic resistance associated with oxytetracycline use in aquaculture: A review

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Abstract
Aquaculture is one of the fastest-growing industries in the world which has led to the intensification of fish farming methods to meet the global demands of fish production. To prevent and treat diseases that occur in aquaculture, antibiotics are being widely applied especially in developing countries, to forestall bacterial infections resulting from sanitary shortcomings in fish rearing. Antibiotics are mixed with feed and feed ingredients and are fed to fish to enhance their growth and ultimately boost up production. Oxytetracycline (OTC) is a tetracycline broad-spectrum antibiotic being widely used in aquaculture as a therapeutic and prophylactic agent ever since it was first approved by USFDA for use in finfish aquaculture. The indiscriminate use of oxytetracycline has led to a lot of problems such as the emergence of antibiotic-resistant bacteria in aquaculture environments which in turn transfer these resistance factors to bacteria of terrestrial animals and human pathogens. Moreover, it can also create problems for industrial health as antibiotic residues can get accumulated in fish meat and fish products. Residues of antibiotics also result in lowering the marketing and export value of aquaculture products. This review paper provides information on the rising problems of antibiotic resistance due to the indiscriminate use of oxytetracycline in aquaculture systems.

Keywords: Antibiotic resistance, aquaculture, antibiotics, oxytetracycline

Introduction
Fish aquaculture is one of the most rapidly growing industries worldwide. This has led to a concomitant increase in aquaculture intensity methods, which increases the susceptibility to disease outbreaks and the necessary use of a medical diet especially antibiotics. Antibiotics are one of the most frequent groups being used as feed additives in the form of growth promoters. Several antibiotics have been in use as growth promoters in fish farms ever since. Aquafarm owners are using various preventive measures to boost up production. Antibiotics are mixed with feed and feed ingredients with a sub-therapeutic dose to maintain good water quality and appropriate dietary management. One of the most used antibiotics in fish farms is oxytetracycline (OTC) [1]. OTC is a broad-spectrum tetracycline based antibiotic with bacteriostatic action produced by *Streptomyces* spp. fungi used to treat general bacterial infections of fish [2]. Oxytetracycline (OTC) has been widely used in aquaculture as a therapeutic and prophylactic agent because of its broad-spectrum activity. OTC was the first antibacterial approved by USFDA for use in finfish aquaculture. Antibiotic resistance bacteria (ARB) have become a global concern due to the massive use or misuse of antibiotics to prevent possible diseases and overcome major production problems, as confirmed by several research reports [3]. Most frequently, Antibiotic resistance has been reported against oxytetracycline [4]. Moreover, indiscriminate use of antibiotics could lead to undesirable deposition of their residues in edible tissues of fish when they are fed with fish feeds mixed with antibiotics and could hamper public health to some extent. Antibiotic residues deposited in the fish meat could be transferred to humans consuming this food and can alter the intestinal ecology thereby favoring the emergence of resistant microflora [5]. Residues of antimicrobials also result in lowering the marketing and export value of aquaculture products [6]. Several classes of antibiotics are commonly used in large quantities in the fish industry, especially in developing countries where their uses are not regulated. Some of these antibiotics are often non-biodegradable and persist in the aquatic environment as residues.

Antibiotic use in aquaculture

An antibiotic is a kind of antimicrobial substances that can kill or inhibit the growth of bacteria and is the most significant kind of antibacterial agent for fighting bacterial infections. Antibiotics are one of the most frequent groups used as feed additives in the form of growth promoters. Aquafarm owners are using various preventive measures to boost up production. Antibiotics are mixed with feed and feed ingredients with a sub-therapeutic dose to maintain good water quality and appropriate dietary management. It has been proved that using antibiotics at low dose mixed with feed remain active for a long period rather than direct application to a fish pond [7]. Antibiotics are still used as a form of growth promoters in most of the aquafarms in the world [8]. Several studies have shown the effects of extended time antibiotic use on fish which can cause nephrotoxicity and liver damage or malfunctions [9]. Moreover, the use of a large number of antibiotics may cause the presence of antibiotic residues in fish tissue and products [10].

Antibiotic resistance in aquaculture

The occurrence and distribution of antibiotic resistance (AR) phenomena in areas designed for fish farming have exponentially increased in the last decades. Antibiotic resistance bacteria (ARB) have become a global concern due to the massive use or misuse of antibiotics to prevent possible diseases and overcome major production problems, as confirmed by several research reports [3]. As consequences of the selective pressure exerted by antibiotics, ARB (Antibiotic resistance bacteria) has been developed and multi-drug resistant bacteria (MDR) have become difficult to be controlled and eradicated. In salmonid farming in Europe and America, an effective range of vaccines has been developed, which have allowed decreasing the use of antimicrobial agents for most of the bacterial diseases [11]. Most frequently, AR has been reported against oxytetracycline [4], ampicillin [12], florfenicol [13].

The mechanisms of AR, consist of two main pathways [14] first inherent or intrinsic resistance, which occurs when a bacterial species is not normally susceptible to an antibacterial agent, due to the inability of this agent to reach its target site inside the cell, or a lack of affinity between the antibacterial and its target site and second is acquired resistance when the bacterial species is normally susceptible to a particular drug, but some strains are resistant and proliferate under the selective pressure induced by the use of that agent. AR genes can be transferred between bacteria by transformation; transduction or conjugation processes that involve lateral DNA transfer [14].

Antibiotic-resistant bacteria that persist in sediments and farm environments can act as sources of antibiotic-resistance genes for fish pathogens in the vicinity of the farms. In 2011, a 5-year action plan was launched to address the growing risks posed by AR based on a holistic approach; prudent use of antimicrobial in veterinary medicine was recommended. To comply with this action, in 2015 specific guidelines have been issued (2015/C 299/04, commission notice-guidelines for the prudent use of antimicrobials in veterinary medicine); to limit AR bacteria originated from livestock animals. Among the actions recommended to prevent and reduce the use of antimicrobials in aquaculture, it has been underlined to encourage the use of vaccines, when possible; to implement specific biosecurity measures and to develop specific disease surveillance programs to prevent or reduce possible disease outbreaks; to develop production systems optimal concerning water quality, oxygen levels and able to warrant the welfare of the reared animals. Particularly, the use of probiotics to promote health maintenance and disease prevention has recently gained increasing interest as an alternative to antibiotics [15].

About the treatment of aquaculture effluents, antibiotic residues should be removed before being released to the environment. In aquaculture systems to remove antibiotics has to be applied physical, chemical and biological methods including adsorption, biodegradation, disinfection, membrane separation, hydrolysis, photolysis and volatilization [16]. Oxytetracyclines are removed mostly by adsorption onto the biomass flocs, beta-lactams antibiotics by hydrolysis reactions driven by bacteria or physical-chemical processes, while removal of ciprofloxacin and erythromycin by biodegradation is difficult. Advanced oxidation methods are cost-effective mechanisms for the reduction and removal of flumequine from aqueous systems, as conventional processes in wastewater treatment plants fail to remove this antibiotic [17].

Based on the experimental evidence proving the spread of AR, the best way to solve this problem is to avoid abuse antibiotic use in aquaculture. A holistic approach to the use of antibiotics in fish farming is suggested, which relies on reducing the need for antibiotics through prevention (i.e. vaccines), nutrition and better management of rearing sites.

Antibiotic resistance genes

Fish pathogens and aquatic bacteria can develop resistance as a consequence of antimicrobial exposure [18]. Examples include *Aeromonas salmonicida*, *A. hydrophila*, *Citrobacter freundii*, *Edwardsiella tarda*, *Yersinia ruckeri*, *Lactococcus garviae*, *Photobacterium damselae* subsp. *piscicida*, *Vibrio anguillarum*, *V. salmonicida*, *Photobacterium psychrophilum* and *Pseudomonas fluorescens*. For example, *A. salmonicida*, a bacteria which causes disease in fish of temperate and colder areas, easily develop resistance. Acquired sulfonamide resistance in *A. salmonicida* was reported already in 1955 in the USA and the 1960s, multiresistant strains were observed in Japan. Later on, multiresistant *A. salmonicida* has been described from many countries in various parts of the world and transferable resistance plasmids are commonly detected in these strains [18]. Typically transferable resistance determinants are those conferring resistance to sulphonamide, tetracycline, trimethoprim and streptomycin. The use of quinolones in the control of bacterial infections from the 1980s resulted in the development of quinolone resistance in strains of *A. salmonicida*. This resistance in *Aeromonas* was mainly mediated by a mutation in the gyrase A gene, gyrA, and has so far not been shown to be transferable [18]. Antibiotic resistance in *V. harveyi*, a shrimp pathogens due to exposure to antimicrobials has been reported [19]. Numerous studies have confirmed that plasmids harboring resistance factors often are transferable from fish pathogens and aquatic bacteria not only to other bacteria within the same genus but also to *E. coli*. For example, multi-resistance plasmids are transferable to *E. coli* from *A. salmonicida*, *A. hydrophila*, *Edwardsiella tarda*, *Citrobacterfreundii*, *Photobacterium damselae* subsp. *piscicida*, *V. anguillarum* and *V. salmonicida* [18]. Genes coding for tetracycline resistance in fish farm bacteria and clinical isolates in Japan showed high similarity suggesting that they may be derived from the same source [20]. Bacteria from aquaculture sites could be a reservoir of the genes tet(M) and tet(S) encoding ribosomal protection proteins responsible for resistance to tetracyclines in several clinical isolates [21]. *Vibrio spp*, *L. garvieae*, *P. damselae* subsp. *Piscicida* harbored tet(D) gene, while *Vibrio* spp. and *L. garvieae* harbored tet(S) gene. Molecular characterization shows that some of the antimicrobial resistance factors in multiresistant *Salmonella typhimurium* DT104, such as tet(G) causing tetracycline resistance and flo-like gene that confers resistance to both florfenicol and chloramphenicol, are also present in some fish pathogenic bacteria [22].

Several studies have indicated that 70-80% of the drug used in aquaculture systems ends up in the environment [23]. Such environmental contamination with antimicrobials could result in the selection of resistance in environmental microflora and resistance genes could further disseminate and eventually reach human pathogens. Furthermore, aquatic bacteria and fish pathogens can develop antimicrobial resistance as a response to antimicrobial exposure and such resistant bacteria can act as a reservoir of resistance genes from which the genes can be further disseminated and ultimately end up in human pathogens.

Discovery and development of the oxytetracycline

Oxytetracycline and Chlortetracycline, both were discovered in the late 1940s, and are the first members of the tetracycline group to be described. These antimicrobial substances were products of *Streptomyces aureofaciens* and *S. rimosus*, respectively. Other tetracycline groups were identified later, either as naturally occurring molecules, e.g., demethylchlortetracycline from *S. aureofaciens* and tetracycline from *S. aureofaciens*, *S. rimosus* and *S. viridofaciens*, or as products of semisynthetic methods, e.g., doxycycline, methacycline and minocycline. Despite the success of the early tetracyclines, analogs were sought with improved water solubility either to allow parenteral administration or to enhance oral absorption. These approaches resulted in the development of the semi-synthetic compounds rolitetracycline and lymecycline.

The most recently discovered tetracyclines are the semisynthetic group referred to as glycylcyclines [24] e.g., 9-(N,N-dimethylglycylamido)-6-dimethyl-6-deoxytetracycline, 9-(N,N-dimethylglycylamido)-minocycline and 9-t-(butylglycylamido)-minocycline. These compounds possess a 9-glycylamido substituent.

Oxytetracycline as growth promoters

Fish aquaculture constitutes a rapidly growing industry worldwide. This has led to a concomitant increase in aquaculture intensity methods, which increases the susceptibility to disease outbreaks and the necessary use of a medical diet. Especially, antibiotics are still used until now as growth promoters in fish farms. The most used antibiotics in fish farms are oxytetracycline (OTC) and florfenicol (FLO) [1]. OTC is a kind of tetracycline based broad-spectrum antibiotic with bacteriostatic action produced by *Streptomyces spp.* fungi used for treating systemic bacterial infections in fish [2]. FLO is a structural analog of chloramphenicol similar to thiamphenicol but is more active against some bacteria than chloramphenicol [25].

Antibiotics are one of the most common groups used as feed additives in the frame of growth promoters. Several antibiotics have been in use as growth promoters in fish farms ever since. Channel cat fish (*Ictalurus punctatus*) supplemented with feed containing oxytetracycline showed a significant increase in weight gain suggesting a growth promotion action of this antibiotic agent [26]

Previous studies stated in the literature showed the negative effects of antibiotics on the immune system of fish [27]. OTC interferes with humoral innate immune parameters and increases the cellular parameters in gilthead seabream [27]. Few studies elucidated the side effects of long term antibiotic use on fish which can induce nephrotoxicity and liver damage [9]. Moreover, the use of the number of antibiotics leads to the presence of residual antibiotics in fish tissue and fish products [10]

Withdrawal period and residue for oxytetracycline in fish

The current trend in fish production has created a lot of challenges for fish health conditions. Infectious and non-infectious diseases constitute major constraints to aquaculture productivity. Antibiotic regimens are being employed prophylactically and therapeutically to combat these challenges as well as for growth promotion. To reduce disease outbreak in aquaculture, a wide range of chemotherapeutics has been used. Oxytetracycline (OTC) has been widely used in aquaculture as a therapeutic and prophylactic agent because of its broad-spectrum activity. OTC was the first antibacterial approved by USFDA for use in finfish aquaculture.

OTC is currently approved in the United States for treating diseases caused by *Aeromonas, Pseudomonas* and *Haemophilus* bacteria in salmonids and channel catfish. In Brazil, (National Plan for the Control of Residues in Livestock Products), instituted by the Ministry of Agriculture in 1995, contemplates a residue control program of antimicrobial substances in fish. Currently, this program includes an inspection for residues of tetracycline and OTC, both with a maximum residue limit (MRL) of 100 ng⁄g in fish flesh. Indiscriminate use of antibiotics could lead to undesirable deposition of their residues in edible tissues which could hamper public health to some extent. Transfer of antibiotic residues to humans through food can also alter the intestinal microflora thereby favoring the emergence of resistant microflora [5]. Residues of antimicrobials also result in lowering the marketing and export value of aquaculture products [6]. Several classes of antibiotics are commonly used in large quantities in the fish industry, especially in developing countries where their uses are not regulated. Some of these antibiotics are often non-biodegradable and persist in the aquatic environment as residues. When antibiotics are mixed with fish feeds, residues may be deposited in the meat, and consumers are inadvertently consuming their residues which may lead to changes in their microbial flora.

Another important aspect that must be considered in the establishment of pharmacokinetic parameters is related to the maximum limit permitted. The Codex Alimentarius does not have an MRL established for OTC in fish. The U.S. Food and Drug Administration (USFDA) has established a tolerance level of 2.0 mg/g of OTC in muscle and skin of fish, which is twenty-fold higher than the MRL established in Brazil (MAPA, Ministerio da Agricultura e do Abastecimento do Brasil, 1999). These discrepancies must be taken into account when the withdrawal period is established. Even when dealing with the same fish species, the same antimicrobial and the same climatic condition of cultivation, the withdrawal period established for a country with higher maximum limit permitted for the veterinary drug will provide a shorter withdrawal period compared with a country with a lower permitted maximum limit, considering the same treatment dose and the same treatment period.

Immune-related gene expression effects of oxytetracycline

Immuno related gene expression effects of the tetracycline in fish have been described in several publications [28]. Regarding fish, the fish species, temperature, antibiotics, route of administration are also important in this respect because of the influence of drug absorption and withdrawal [29]. Antibiotics are lipophilic and persistent in water, soil and organisms because they are produced to induce a biological effect [30]. The OTC is an antibiotic that has low bioavailability when administered orally [31], although it persists in tissues of fish. In several fish species, levels of OTC were always higher in the liver, blood, muscle tissue and kidney [32]. OTC is held or retained in bone tissues, scales and also in the pronephros. The pronephros is a vital lymphoid organ of fish, and for this reason, the OTC might interact with the immune cells and have an influence on immune responses [33]. The penetration of OTC in immune cells could enhance or, conversely, impair the cell functions [34]. Most of the studies focus on the effects of OTC on fish immune systems that have been carried out *in vitro*. It has been described that OTC has immunosuppressive effects on fish [35], which may cause oxidative stress [36] and has a high incidence of bacterial resistance [37]. OTC was administered at 75 mg kg^{-1} for 10 days and increased nitroblue tetrazolium (+) cells and total erythrocyte and leucocyte numbers. The effects of OTC on the immune system lasted around 21 days after ceasing the administration and the parameters evaluated then returned to normal levels [38].

Exposure to elevated levels of 17<beta>-estradiol (E2), the most potent natural estrogen [39], could alter components of the immune system, inducing immunosuppressive effects and increased disease susceptibility in individuals and may have significant implications at the population level of fish. E2 may also have immunostimulatory effects during the reproductive season, modulating leukocyte functions at the end of gametogenesis, spawning and post-spawning stages. Besides, E2 appears to promote the filtration of acidophilic granulocytes and cause a resumption of gonad cell proliferation. The 17a-ethynylestradiol, a potent synthetic estrogenic compound even more than E2, seems to have the capacity to alter fish immune function and decrease pathogen resistance, which may have important implications for fish survival and population growth. Since estrogen receptors (ERs) are known to exist in the immune system organs, their actions are executed by the classical genomic pathway, through nuclear translocation of estrogen-ER complexes and estrogen response elements (EREs)-mediated regulation of transcription and by a rapid non-genomic pathway, which involves phosphorylation cascade through G protein-coupled receptors.

A group of small non-coding RNAs has been increasingly implicated in the modulation of the innate and acquired immune response in vertebrates. MiRNAs are associated with the development of hematopoietic cell lineages, regulation of host-pathogen interactions and regulation of signal transduction in immune cells. Both ER<alpha> and ER<beta> can inhibit the activity of the Drosha complex, thereby preventing the conversion of pri-miRNAs to pre-miRNAs.

Mechanism of action of oxytetracycline

Oxytetracycline inhibits cell growth by inhibiting translation. It prevents the amino-acyl tRNA from binding to the A site of the ribosome by binding to the 30s ribosomal subunit. The binding is reversible. Oxytetracycline is lipophilic and can easily pass through the cell membrane or passively diffuses through the cell membrane or passively diffuses through porin channels in the bacterial membrane.

It is well established that tetracyclines inhibit bacterial protein synthesis by preventing the association of aminoacyl tRNA with the bacterial ribosome [40]. Hence, to interact with their targets, these molecules need to traverse one or more membrane systems depending on whether the susceptible organism is gram-positive or gram-negative.

Oxytetracycline passes through the outer membrane of gram-negative enteric bacteria through the OmpC and OmpF porin channels, as positively charged cation (probably magnesium) tetracycline coordination complexes [41]. Uptake of tetracyclines across the cytoplasmic membrane is energy-dependent and driven by the DPH component of the proton motive force [42]. Tetracycline molecules are likely to become chelated within the cytoplasm since the internal pH and divalent metal ion concentrations are higher than those outside the cell. Indeed, it is probable that the active drug species which binds to the ribosome is a magnesium tetracycline complex [43]. Association of tetracyclines with the ribosome is reversible, explaining the bacteriostatic effects of these antibiotics. Several studies have indicated a single, high-affinity binding site for tetracyclines in the ribosomal 30S subunit, with indications through photo affinity labeling and chemical footprinting studies that protein S7 and 16S rRNA bases G693, A892, U1052, C1054, G1300 and G1338 contribute to the binding pocket [44]. However, naturally occurring oxytetracycline-resistant bacteria such as *propionibacteria* contain a cytosine-to-guanine point mutation at position 1058 in 16S rRNA, which does at least suggest that the neighboring bases U1052 and C1054 identified by chemical footprinting may have functional significance for the binding of tetracyclines to the 30S subunit.

Tetracyclines inhibit protein syntheses in mitochondria [45] due to the presence of 70S ribosomes in these organelles. It has been known for some time that the spectrum of activity of tetracyclines includes various protozoan parasites such as *Entamoeba histolytica, P. falciparum, Leishmania major*, *Giardia lamblia, Trichomonas vaginalis* and *Toxoplasma gondii* [46].

Legal use of antibiotic

Many international and regional codes of practice, agreements and technical guidelines exist for aquatic animals [47]. The drugs available for use and their treatment protocols are tightly regulated. The consumers of fish and particularly in the world’s richer economies are increasingly demanding that retailers guarantee that the fish which they offer are not only of high quality and safe to eat, but also that they derive from sustainable fisheries [48]. The prescription scheme could be discussed and improved and non-approved and even banned antibacterials are purchased "over-the-counter" (without the need for a prescription) or their use is undeclared in fish feed formulations. The usage of banned antimicrobials in aquaculture is a violation of regulations [49]. Drugs are assessed for the definition of their maximum residue limits (MRLs) and their environmental impact and efficacy (Table 1) [50]. MRLs are generated by several bodies, such as the EU and more globally within the framework of the FAO/WHO Codex Alimentarius Commission, which is advised scientifically by the JECFA (Joint FAO/WHO Expert Committee on Food Additives). The use of antibacterial agents in food animal species, including fish, is controlled by particularly in Europe and the USA.

Table 1: Main antibacterial compounds having fixed Maximum Residue Limits (MRLs) [51]

Antibacterial	MRL(pg/kg)
Amoxicillin	50
Ampicillin	50
Benzylpenicillin	50
Chlortetracycline	100
Cloxacillin	300
Colistine	150
Danofloxacin	100
Dicloxacillin	300
Difloxacin	300
Enrofloxacin	100
Erythromycin	200
Florfenicol (Fish)	1000
Flumequine	600
Lincomycin	100
Neomycin (Incl. Framycetin)	500
Oxacillin	300
Oxolinic Acid	100
Oxytetracycline	100
Paromomycin	500
Sarafloxacin (Fish& Poultry)	30
Spectinomycin	300
Sulphonamides (All)	100
Tetracycline	100
Thiamphenicol	50
Tilmicosine	50
Trimethoprim	50
Tylosin	100

Problems associated with antibiotic use in aquaculture

Any hazards associated with feed medication whether in feed mills or on farms must be considered, as must any hazards to the final user (the fish farm staff) [52]. The majority of the fish farmers are in developing countries and mainly in Asia which has experienced the largest increases over recent decades, reflecting the rapid expansion of aquaculture activities [53]. Fish diseases are usually associated with cultured fish and regarded as a result of aquaculture [54]. As a consequence, probably, the majority of antibacterial use in world aquaculture is not associated with any classification of the target bacterium or of its susceptibility to the range of available antibacterials [53]. There is a need for assurance that the usage of antimicrobials will not harm animals or humans [55]. The volume of fisheries and aquaculture products consumed is expected to rise due to an increase in consumers' recognition of the health benefits associated with seafood consumption [56]. There is little doubt that aquaculture production will continue to grow [57]. The world food supply will probably have to double in quantity and increase in quality over the next 30–50 years as populations and incomes rise. The global demand for fish as food is expected to be double and could even more than double [58]. Consequently, an increase in the number of problems associated with aquaculture production may be expected.

Environmental impact of antibiotic use in aquaculture

Aquaculture is so integrally linked to the surrounding environment that if sustainable practices are not employed, the degradation of the surrounding environment will ultimately lead to the degradation of the industry itself [54]. The wellbeing of the environment in cases of disease and treatment is related to two aspects of biota conservation; the transmission of microbial pathogens to wild populations and the pollution from chemotherapeutics [59].

The input of resistant bacteria into the environment from different sources seems to be the most important basis of resistance in the environment. The possible impact of resistant bacteria on the environment is not yet known and the health risks of active pharmaceutical ingredients remain poorly understood [60]. The physicochemical fate and environmental concentrations of antibacterials in soil have been the subject of several recent studies. During recent years, significant attention has been paid to the occurrence of drugs in the environment. Several classes of antibacterials have been detected in field soils and their sorption behavior and degradation have been studied to a large extent [61, 62]. Fish production can generate considerable amounts of dissolved effluents, which potentially affect water quality in the vicinity of the farms and, due to rapid dilution, also at larger scales (km-scale) [63]. High antibacterial load in sediments and in concentrations potent enough to inhibit the growth of bacteria have been reported for aquaculture [64].

Tetracycline has low bioavailability in fish (< 10%), due to binding with sea-water borne divalent cations such as Mg^{+2} and Ca^{+2}. It is noteworthy that non-bioavailable tetracyclines contaminate the environment [1]. However, it has been shown that residues of oxytetracycline in marine sediments were very stable over months [65]. In one study [66] conducted in China (Jiangsu Province) the biggest aquaculture producer [48] contamination with antibiotics indicated that ten veterinary antibiotics around farms were found in animal wastewaters, eight antibiotics were detected in pond waters and animal farm- effluents and river water samples were contaminated by nine antibacterials. The most commonly detected antibiotics were sulfamethazine (75%), oxytetracycline (64%), tetracycline (60%), sulfadiazine (55%) and sulphamethoxazole (51%). Antibacterials may be detected in effluent entering receiving waters and be detectable 500m from the source [63]. There is very little information about the chronic toxicity or the bioaccumulation potential of pharmaceuticals in biota and food chains [67]. For example, the exposure of eels to pollution during their development is inducing changes in the biomarkers involved in physiological functions that are determinants for the survival and performance of the eels, namely biotransformation enzymes and antioxidative stress defenses, and these alterations may have negative effects on sexual development. Also, the mechanisms used to face chemical stress need energy which is probably allocated from other functions, such as tissue repair, growth and weight increase, and which are determinants for a successful migration into the reproduction area [68].

Antibacterial usage suggestions in aquaculture

When it is apparent that treatment is necessary, the following checklists may be useful before treating [69]:

1. Accurately determine the water-volume, flow-rate and temperature.
2. Accurately determine the number and total weight of fish in the rearing unit.
3. Accurately determine the number and total weight of fish in the rearing unit.
4. Confirm the identity, expiration date and active ingredient concentration of the regulated product to be applied.

5. Double-check treatment calculations. Beware of confusion from mixing standard units and metric units.
6. Have aeration devices ready for use if needed.
7. If the treated water is to be discharged, make sure all appropriate permits are in place and regulatory authorities have been notified.

Conclusion

Oxytetracycline (OTC), which is a broad-spectrum antibiotic has been widely used in aquaculture as a therapeutic and prophylactic agent ever since its approval by USFDA for use as an antimicrobial in aquaculture. It is being continuously used at subtherapeutic levels in fish feeds for growth promotion, improvement of feed efficiency, and disease prophylaxis. Although few developed countries monitor and regulate the use of antibiotics in aquaculture. However, aquaculture activities carried out in developing countries lack regulations and enforcement on the use of antibiotics, leading to high variability in antibiotic use. The indiscriminate use of antibiotics especially OTC in aquaculture has led to the rise of antibiotic resistance bacteria (ARB) which have become a global concern. Most frequently, antibiotic resistance has been reported against oxytetracycline (OTC) [4]. Moreover, its frequent use can also create problems for industrial health as antibiotic residues can get accumulated in fish meat and fish products.

The use of antibiotics for prophylaxis and as growth promoters can be prohibited and regulated in the developing countries [70], alternative strategies can be used to lessen pathogen activity such as vaccination; immune stimulation using nutritional factors derived from bacterial, algal, or animal (including hormones and cytokines) sources; use of probiotics; phage therapy; and quorum sensing disruption (affecting virulence). Also, the disinfection of system water may be managed with UV application or, as is often the case for intensive systems, via ozone treatment [70]. These alternative strategies may provide solutions to improve aquaculture health and function while reducing the use of antibiotics in aquaculture and ultimately reducing the problems associated with antibiotic use.

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