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Review Article

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Antibiotic Residues and Resistant Bacteria in Aquaculture

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Abstract Fish are treated routinely with antibiotics to prevent, treat, or control diseases. One consequence of the use of antibiotics in aquaculture is the presence of residues of the drug in the edible tissues of the treated fish, as well as environmental hazards. Another potential consequence is the exposure of the human consumer to resistant bacteria. Regulatory frameworks have concerns about the use of veterinary drugs, including antibiotics in aquaculture, and one of these concerns is that it does not lead to residues in fish. However, effective analytical methods are required to rapidly and accurately detect, quantify, and confirm antibiotic residues in food aiming to verify that regulatory standards have been fulfilled and have avoided the appearance of antibiotic resistant bacteria in aquaculture environments, in the decrease of antibiotic resistance in fish pathogens, and in the avoid the transfer of these resistance determinants to bacteria of other animals and to human pathogens. This review summarizes the current knowledge and concerns about antibiotics residues and resistant bacteria in aquaculture, including the main techniques of analysis and what has been studied around the world. Researchers, policy makers, governments, and aquaculture industries must invest and collaborate in exchanging critical information and developing targeted policies that are practical, effective, and enforceable in order to adequately understand and prevent the impacts of the antibiotics use in aquaculture.

Keyword: Antibiotic Residues, Resistant Bacteria, Aquaculture

Introduction

Fisheries and aquaculture make crucial contributions to the world's wellbeing and prosperity. In the last five decades, world fish food supply has outpaced global population growth, and today fish constitute an important source of nutritious food and animal protein for much of the world's population [1]. In addition, the sector provides livelihood and income, both directly and indirectly, for a significant share of the world's population. Fish and fishery products are among the most traded food commodities worldwide with trade volumes and values reaching new highs and expected to carry on rising, and developing countries continue to account for the bulk of world exports [2]. While capture fisheries production remains stable, aquaculture production keeps on expanding. Aquaculture is set to remain one of the fastest-growing animal food-producing sectors and, in the next decade, total production from both capture and aquaculture will exceed that of beef, pork or poultry [3-4].

This widespread growth of aquaculture has been accompanied by an increased use of a wide range of chemicals including antibiotics. The results of the therapeutic uses are healthy animals that contribute to a healthful and plentiful food supply [5]. However, one consequence of the use of antibiotics in aquaculture is the presence of residues of the drug in the edible tissues of the treated fish. The residues of the antibiotic could be systemically toxic to the consumer, residues of the antibiotic in consumed food could have direct adverse effects on the complex microflora that inhabits the human gastrointestinal system with potentially disastrous consequences for the human consumer. Another potential consequence is the exposure of the human consumer to bacteria, which have been exposed to the drug through the treated fish and have survived the exposure, are less susceptible to that antibiotic. People who develop a human disease resulting from exposure to these bacteria may find that the causative organisms are resistant to antibacterial used in human medicine and the disease refractory to standard treatments [6].
In order to avoid, or at least, to be prepared to control the incidence of antibiotics contamination in aquaculture production and the spread of resistant bacteria, protect the production, and also prevent the transfer of these to humans, we need to have knowledge and be updated on existing methodologies for the identification and quantification of antibiotic residues in fish. Surveys of contamination that have been risen around the world to date, procedures for the identification of resistant bacteria and also what and where they were already identified must be considered so that we can makes policies in order to control this resistant bacteria contamination and follow with an increase in animal protein production through the production of fish. For this, this review has this purpose.

Maximum Residue Limits

Permission for the use of veterinary drugs is conditioned on several studies, including toxicity evaluated by determining the acceptable daily intake (ADI) and maximum residue limit (MRL). ADI is expressed in $mg.kg^{-1}$ body mass, established by long toxicological evaluation in experimental animals, defined as "the amount of a chemical that can be ingested daily by humans over a lifetime without appreciable risk to your health, in the light of the knowledge available at the time of evaluation". MRL is defined as the maximum concentration of residue resulting from the use of a veterinary drug (expressed in $mg.kg^{-1}$ on a fresh weight basis) to be legally permitted or recognized as acceptable in or on food [7].
MRLs are also related to ADI. However, they are not derived from ADI and are not a direct part of the ADI. In fact, they are reflective of the residue concentrations found under the controlled conditions of use determined by validated analytical methods. Since the MRLs reflect only the conditions in which they were established, they may not be fully representative and therefore need to be evaluated constantly as new uses of veterinary medicinal products are made and filed. Inherent in relating MRLs back to the ADI is the assumption that all animal-derived edible products will be eaten at their maximum consumption values every day (i.e., no partitioning of the ADI), and quantifying human exposure to drug residues regulated through MRLs necessitates the assignment of MRLs for all appropriate edible products. Further, relating overall food safety regulated with MRLs according to the ADI is often achieved using a theoretical maximum daily intake (TMDI) calculation: (tissue-specific MRL) × (marker : total ratio) × (tissue-specific consumption value) = tissue residue contribution to TMDI. The TMDI is compared to the ADI. The TMDI must not result in exposures more than the ADI. In the example above, the TMDI represents 95% of the ADI. While a specific study and its associated analytical method may provide the data used to establish the marker : total ratio, there is no unbreakable link between the MRLs and any specific analytical method for residue monitoring [7].
There are notable differences among MRLs or tolerances set by different agencies. For instance, only EU regulation permits the use of fluoroquinolones in fish. The Codex Alimentarius Commission and Chile Health Department have established a MRL for flumequine in trouts at 500 μg kg^{-1}. On the other hand, the agencies have set different residue levels for the same drug. The MRL, for the sum of residues of tetracycline in fish, has been set at 100 μg kg^{-1} in the EU countries, at 200 μg kg^{-1} in Canada, Brazil and Australia, and at 2,000 μg kg^{-1} in the US [6].
Several countries and regulatory authorities list their official MRLs online. The Table 1 provides the URLs for a selection of sites providing MRLs/Tolerances established by national regulatory authorities.

Sample Preparation Procedures

The detection of antibiotic residues in the aquaculture environment provides critical information on their potential to cause undesirable effects to the ecosystem function and to animal and human health. Increasing concern over the presence of antibiotics in fish mainly from aquaculture industries has required fast development and efficient methods for detecting these contaminants. Some of the challenges include need for multi-component quantitation, preference for simple sample preparation, and desire for an automated, online method to make quality control screening cost-effective.

Appointing which antibiotics should be included in the analytical methods for detection is a difficult task and usually the methods have focused on heavily used antibiotics, especially those frequently used in treating or preventing infections or in promoting growth in animals' production. Generally, methods used for monitoring antibiotic residues can be classified in two groups: screening and confirmatory.

Immunoassay, microbiological assay or biosensor technique are quite used for fast screening of antibiotic residues and among the benefits are short analysis time, high sensitivity, selectivity for some immunoassays, simplicity, and automation. However, cross-reactivity may occur with related substances, such as metabolites, and the methods are effective within a particular concentration range, nevertheless when low levels are expected, only more sensitive HPLC method is suitable [8].

For confirmatory methods, the sample pretreatment technique plays a crucial role in analyzing veterinary drug residues. The extraction and clean-up of drugs from the complex matrix are one of the most difficult steps in antibiotic analysis.

Various extraction methods have been proposed for sample preparation in antibiotics analysis, such as liquid–liquid extraction – LLE [9-10], pressurized liquid extraction – PLE [11], solid-phase microextraction – SPM [12], dispersive liquid–liquid microextraction – DLLM [13], and quick, easy, cheap, effective, rugged, and safe – QuEChERS [14] and for detecting the methods are typically based on liquid chromatography coupled to mass spectrometry (LC/MS).

But independently of the technique used for the extraction, a clean-up procedure is often required prior to chromatographic analysis in order to reduce the amount of co-extracted compounds, and consequently minimizing the matrix effects. The most common technique for sample clean-up is the use of sorbents in SPE and several authors have reported the use of octadecyl-C18 bonded silica [15-17], hydroplilic-lipophilic balanced polymeric sorbents (Oasis HLB) [18-21], and anion exchangers SPE materials [22] from a variety of matrices. The advantage of this technique is the possibility of concentration of analytes, by the injection of a large volume of sample and elution with small volume. However, this technique presents high cost, since the cartridges are used only once [15].

Liquid chromatography tandem mass spectrometry (LC-MS/MS) is popular for antibiotics analysis because it is sensitive and specific for the unequivocal identification of compounds and to avoid situations of false-positive and false-negative results. Retention time, ion transitions, and transition ratios are used for identification and quantification. By monitoring ion transitions, tandem mass spectrometry increases selectivity by filtering specific ions. This selectivity removes noise, resulting in a large increase in the signal-to-noise ratio, thereby enhancing sensitivity [23].

Residues of the antibiotics frequently administered, such as tetracyclines and sulfonamides, are typically detected at $\mu g.kg^{-1}$ to $mg.kg^{-1}$ levels in aquaculture system [24] and fish farms [25], and other classes most frequently administrated in samples originated in aquaculture are quinolones, amphenicols, penicillins, macrolides, and aminoglycosides that should be considered as target compounds in analytical methods. Hence, several examples of antibiotics analyses have been described in the literature, as exemplified in Table 2, which presents an overview of the analytical methodologies for determination of antibiotic residues in fish samples from 2006 to the present. The fish is a very complex matrix and the compounds are difficult to be extracted and analyzed. Compounds prohibited in the EU, the USA and other countries are also listed (metronidazole, chloramphenicol).

In most studies reported in Table 2, the following procedures have been considered: the fishes were collected from the aquaculture sites and transported alive to the laboratory in Ziploc polyethylene bags or wrapped in an aluminum

foil and then were eviscerated and peeled and their tissues such as muscle, liver and bile were separated, mixed and homogenized using a blender; then, the samples were kept in a freezer at −20°C until the moment of extraction.

Actually, cost-effectiveness of analytical procedures is becoming a major issue for all laboratories involved in residue analysis, as the reagents and equipment are very expensive and therefore increasingly the need to develop simpler, faster, and still, very sensitive and selective techniques for residues monitoring and control.

Thus, considering the different chemical structures of different antibiotics, as well as their different physicochemical properties, implies that significant improvements are still needed in the sample pre-treatment step.

Antibiotics Residues around the World

The current definition of aquaculture according to FAO is "the farming of aquatic organisms including fish, mollusks, crustaceans and aquatic plants". Farming implies some sort of intervention in the rearing process to enhance production, such as regular stocking, feeding, and protection from predators. In aquaculture, antibiotics have been used mainly for therapeutic purposes and as prophylactic agents, although they promote the development of antibiotic-resistant bacteria [26]. The excessive use of antibiotics in animal rearing has the potential of backfiring and negatively affecting all the aspects of the industry, including its economic health. In addition, the horizontal gene transfer between antibiotic-resistant aquaculture bacteria and human pathogens may result in more frequent hospitalizations and significantly higher medical costs [27].

We conducted a search at the scientific literature regarding the results of antibiotics analysis in fish. Most of the papers found only depict methods for the antibiotics determination in fish and sometimes prove their efficiency applying their methods in real samples, but monitoring studies representing real situation in fish and food that we eat are very scarce in these papers. The Table 3 presents the results and just looking at these results we can be satisfied in relation to fish contamination consumed around the world, few samples showed antibiotic residues above the MRLs, which leads us to believe that we are consuming fish free, or at least, within acceptable antibiotics residue.

Although the current antibiotic residue in fish appears to be relatively safe, this survey results suggest the possibility of drug misuse and incomplete observation of prescribed withdrawal times by producers. Consequently, antibiotic residues are exposed to consumers. Therefore, we need to monitor these antibiotic residues in aquaculture products continuously. It is necessary to clarify that the results obtained cannot be considered representative of the total number of fresh fish commercialized in the world, because the number of samples analyzed is relatively low compared with the production.

Better representative results that we could have about antibiotics residues in fish could be seen by monitoring programs realized by governments' agencies. Table 4 provides a selection of sites with information about these programs in countries around the world.

The use of antibiotics in aquaculture is widespread and largely unregulated and undocumented all over the world and this could be resulting in consumer's exposure to residues and contributing to selection of resistant bacteria. We can find many studies that provide an assessment of antibiotics residues detected in aquaculture, and in fact we have evaluated a small part of the production and consumption of the fish around the world for the last 5 years, but our intention is provide baseline data in view of a larger and more exhaustive study. Surveys of larger samples of fish are needed to achieve more reliable consumer risk assessments. Consumer protection policies aimed at reducing drug residues in aquaculture products must therefore involve the entire supply chain through wider and more frequent monitoring.

Antibiotic Resistance in Fish Bacteria

Worldwide concern about antibiotic resistance is great, especially the increase in morbidity and mortality rates attributed to failures in the treatment of bacterial infections, mainly caused by the transfer of antibiotic-resistant bacteria to humans through the food chain, which the aquaculture has contributed to this situation due to the therapeutic and prophylactic use of antibiotics, many of them used in human medicine (Ahmed et al., 2015). Centers for Disease Control and Prevention reported that in 2013 two million people were infected with bacteria resistant to at least one antibiotic agent commonly used in the United States and 23.000 people died as a result of antibiotic-

resistant infections. In Europe, 400.000 people were infected with multidrug-resistant bacteria, which caused about 25.000 deaths in 2007 [28].

The constant use of antibiotics in aquaculture causes selective pressure for antibiotic resistance in bacteria, in addition these compounds remain for long periods in the sediment or the water column. The chemicals (or heavy metals disinfectants) used in aquaculture may also enhance the antibiotic resistance in the environment [29]. It is known that the use of antibiotics in aquaculture is increasing, however, the pressure for more sustainable products, which do not impact the environment and has as a result a quality product to the consumer, is great. At present, it is known that to produce 1 ton of salmon in Chile, 279 g of antibiotics is used, whereas only 4.8 g of antibiotics is used to produce the same amount of salmon in Norway. This difference is probably reflected in animal health and also in the quality of the final product [29]. By contrast, in China, according to the Ministry of Agriculture data, the gross value of veterinary products produced in the country increased from 20 to 30 billion Chinese Yuan in the period 2009-2011, with three-quarters of this amount being designated by chemical drugs. It is also estimated that the antibiotic dose used in farm animals, including fish in China has risen from 50 to 703 mg/kg of biomass between 2001-2007 [30]. Thus, many studies have been conducted throughout the world on the possible transfer resistance of fish bacteria to humans.

Schmidt et al. [31] evaluated the occurrence of antibiotic resistance in bacteria present in trout, water and sediment from four fish farms in Denmark. Oxolinic acid and sulfadiazine-trimethoprim, amoxicillin, oxytetracycline and florfenicol were studied. Three hundred thirteen *Aeromonas* were isolated (110 from water, 140 from sediment and 63 from gills and mucus from healthy fish). Two hundred sixteen *Aeromonas* isolates (69%) were resistant to oxytetracycline, 135 (43%) were resistant to sulfadiazine-trimethoprim, 63 (20%) were resistant to oxolinic acid and only one isolate was resistant to florfenicol. All isolates and type strains were amoxicillin resistant. Regarding *Flavobacterium psychrophilum*, all isolates proved to be resistant to oxolinic acid, 63 isolates (71%) were oxytetracycline resistant, 44 isolates (50%) were amoxicillin resistant, and florfenicol resistance was not found. Resistance levels were not significantly different in water and fish isolates. *Yersinia ruckeri* isolated did not detect antibiotic resistance among the isolates. The study demonstrates the significant impact of trout farming in the environmental bacteria and fish. The antibiotic resistance levels were higher in ponds or outlet samples than in samples from the inlets and thus, preventive measures in freshwater aquaculture should be improved to minimize the usage of antibiotic agents as well as their release into the effluent water.

In the Czech Republic, researchers evaluated the antibiotic resistance and genetic determinants (tet(A-E) genes, integrase genes and gene cassettes) in motile *aeromonads* isolated from Koi carp (*Cyprinus carpio koi*) and common carp (*Cyrpinus carpio*). The results showed that the 72 isolates from koi carp, 36 (50%) was resistant to oxytetracycline, 18 (25%) to ciprofloxacin, 5 (7%) to chloramphenicol, 5 (7%) to florfenicol, and 11 (15%) to trimethoprim. From carp, 49 isolates of motile aeromonads were isolated, 20 (41%) was resistant to oxytetracycline, 3 (6%) to chloramphenicol, and 3 (6%) to florfenicol. About the genetic determinants, the Tet genes were detected in 40% (48/121) of isolates, with tet(E) being the most dominant, showing a significant difference in the incidence of resistant isolates collected from koi carp and common carp. The potential risk for resistant bacteria to spread and transmit infection to humans should be considered in cases of technological crossover between the two types of fish farms [32].

A study reported by Ahmed et al. [33] in seafood sold in supermarkets in Japan searched mobile genetic elements resistance (*integrons*) and resistance genes in gram negative bacteria present in the products. In total, 215 gram negative bacteria were isolated and among them, six isolates (three *Aeromonas hydrophila*, one *Citrobacter freundii*, one *Enterobacter cloacae*, and one *Klebsiella oxytoca*) showed class 1 integrons containing gene cassettes encoding resistance to trimethoprim (dfrA12 and dfrA17), aminoglycosides (aadA2), and β-lactams (blaPSE-1), and seven isolates (four *C. freundii* and three *E. cloacae*) had four different β-lactamase-encoding (penicillin) genes (including blaTEM-1, blaCMY-2, blaCMY-13, and blaCMY-39). Plasmid-mediated quinolone resistance genes were identified in ten isolates (six *C. freundii*, two *E. cloacae*, one *C. koseri*, and one *Pantoea spp.*). Therefore, the authors concluded that the seafood is a possible route of transmission of resistant bacteria to humans.

In China researchers studied the prevalence of antibiotic resistance of non-typhoidal *Salmonella serovars* in retail aquaculture products (fish, shellfish, bullfrog, clam, shrimp, and others) and they observed high resistance to sulfonamides (56.5%), tetracycline (34.1%), streptomycin (28.6%), ampicillin (23.5%), and nalidixic acid (21.2%). A total of 43.3% of the Salmonella isolates were multidrug-resistant and 44 different resistance patterns were found. The authors suggest urgency in monitoring programs for microbiologic safety in such projects and for more prudent drug use in aquaculture production in order to reduce the risk of development and spread of antibiotic resistance [34].

Another state assessed screened for the presence of GyrA and ParC substitutions, efflux activity and the prevalence of plasmid-mediated quinolone resistance genes, qnr and aac-6'-Ib-cr from *Aeromonas* strains (*Aeromonas veronii biovar sobria/veronii, A. allosaccharophila*, one *A. caviae*, one *A. jandaei*, one *A. salmonicida*, and five *A. hydrophila*) obtained in three fish farms producing tilapia, *Oreochromis mossambicus*; trout, *Oncorhynchus mykiss*; and koi, *Cyprinus carpio* from South Africa. This study is very important, since the diversity of resistance loci present in *Aeromonas* is able to move among bacteria in the aquatic environment and be transmitted to other fish pathogens and humans through consumption of fish or their products prepared improperly or by cross contamination of other foods by fish intestinal bacteria. The results revealed that 44% of isolates were resistant to nalidixic acid, the majority was susceptible to ciprofloxacin and ofloxacin, qnrB and qnrS were detected for 41% and 24% of isolates, respectively. Quinolone resistance in these fish associated *Aeromonas* isolates was related to mutations in the quinolone resistance determining regions of GyrA and ParC and presence of *qnrB* and *qnrS*. The presence of *qnr* alleles in *Aeromonas spp*. isolates may facilitate high-level fluoroquinolone resistance and potentially serve as reservoirs for the dissemination of *qnr* genes to other aquatic pathogens [35].

In India, a mortality surge in goldfish from commercial farms caused by *virus-2 herpes cyprinid (CyHV-2)* in association with multidrug-resistant *Aeromonas hydrophila* was reported. The isolate was resistant to more than 10 classes of antibiotic groups*viz*., Carbapenems (imipenem), folate pathway inhibitors (sulphatriad) anti β-lactams staphyloccal (oxacillin), Glycopeptides (vancomycin), Lincosamides (lincomycin), penicillins (ampicillin, penicillin G, cloxacillin, methicillin), penicillin and β-lactamase inhibitors (amoxyclav) monobactams (aztreonam), extended spectrum cephalosporins (3rd and 4th generation cephalosporins like ceftazidime, cefotaxime) and non-extended spectrum cephalosporins (3rd and 4th generation cephalosporins like cefuroxime) [36]. This can be a serious problem, especially from the therapeutic point of view and public health, since multidrug-resistant *Aeromonas hydrophila* can convey resistance genes to other pathogens of aquatic medium and reach the human.

The Relationship between Aquaculture and Antibiotic Resistance

Antibiotic resistance has become a great world problem in disease control, causing prolongation of disease and increasing risk of death of patients. Moreover, resistance is stopped being of a particular antibiotic and come to be a multiple and extreme resistance to severe drugs [37-38].

Susceptible bacteria can become resistant to antibiotics by many mechanisms: exclusion of the antibiotic by cell membrane, intracellular change or deactivation of the antibiotic, sensitivity reduction of cell target, cell extrusion and intracellular sequestration [39]. These mechanisms can involve genetic code mutation and selection and the genetic code resistance can be acquired from other bacteria.

The concept of antibiotic resistance has been used a lot in public health and limited as a phenomenon of natural environments. It is because antibiotic concentrations detected in environmental setting are very low [40]. However, recent studies has demonstrated that selection of antibiotic resistant bacteria occurs in environments with extremely low concentrations, like that one detected in aquatic environments and soil [41-42].

Initial hypothesis that antibiotics are degraded and diluted in aquatic environment were proved to be incorrect because many antibiotics continue active and able to select resistance in the environment, mainly in sites where they are often used [43]. Another declined hypothesis is that terrestrial bacteria populations are genetically different of aquatic bacteria populations. Aquatic environment contamination around the world by terrestrial and human pathogens allowed the bidirectional horizontal gens transfer [44].

Many aquatic environments shelter a great amount of mobile genetic elements (plasmids, integrons, transposons and conjugative integrative elements) that can be recombined to generate bacteria more adaptable to an environment which contains variable amount of antibiotics, both of natural and anthropic occurrences [45]. The intensification of aquaculture has caused an industrialization process of this activity that leadsto an increase use of antibiotics, both as prophylaxis and therapy, spreading their residues in the aquatic environment.

The use of antibiotic in aquaculture differs of the use in terrestrial animal production by some aspects, as for example, the amount of studies of its effect. In aquaculture, their use is incipient. There is little knowledge about how the great amount of fish excretain aquaculture, containing non digested antibiotics, is able to stimulate the genetic variability and horizontal gene transfer in the sediment around aquaculture [45-46].

Studies show that both antibiotics used in aquaculture and antibiotics of anthropic source can be retained in sediments, which are an interface for a complex and dynamic community of microorganisms, favoring the transference, maintenance and dissemination of mobile genetic elements [47], because as in terrestrial environment the great concentration of organic matter in the aquatic sediment favors the horizontal genes transfer [48-49].

The anthropogenic action has caused an increase of antibiotic resistance by two manners. The presence of antibiotic in the environment makes a pressure of bacteria selection, selecting resistant populations and increasing the amount of ARGs (antibiotic resistance genes) and these genes originating from humans and animals, for example, are transported by outflow to aquatic environments [50].

Bacteria from aquatic environment that contain ARGs share these genetic elements with fish, shellfish and human pathogens [21, 51-52], besides colonizing zooplankton and phytoplankton [39]. Aquatic animals concentrate antibiotics in their tissues and organs, where they interact with their natural microbiota [44]. Biofilms also act as mediators of horizontal gene transfer and they are present both in live aquatic organisms and in inanimate ones [53].

In the aquatic environment high concentrations of bacteriophages and other agents of genes transfer are also present and they are able to mediate and stimulate the horizontal gene transfer [54]. These characteristics convert the intestinal tract and tegument of fish in dynamic hotspots to generate new arrangements of ARGs and new bacteria resistant to antibiotics [44-45].

Even as food supply sourced from terrestrial animals, the food supply sourced from aquaculture can also have antibiotic resistant bacteria, including human pathogens from aquatic source (*Vibrios and Aeromonas*) and terrestrial pathogens (*Salmonella, E. coli*) [55-56]. Experts alert for the necessity of studies that evaluate the potential of antibiotic resistant bacteria that cause disease in fish and are human opportunistic pathogens, which may change the normal microbiota of aquaculture workers exposed to antibiotics [57-59].

Occurrence of new ARGs and antibiotic resistant bacteria globalization in environments around aquaculture facilities show that the prevention of these should be made firstly per region, because there is a variable number of molecules used around the world. In salmon industry, for example, the amount of antibiotics used to produce one ton of this fish ranges from 0.0008 kg to 1.4 kg [45], according to the producer country.

It is necessary to increase fish farmers and veterinarians' consciousness level to improve fish health reducing the antibiotics use in countries where aquaculture is being developed [60], mainly in geographic regions with high water temperature (30.8°C), because recent studies [61] have demonstrated that genes transfer is more frequent in higher temperature water than in water with lower temperature (25.8°C).

An interesting example is Norway that has recently reached a technical production level in which the antibiotic amount used is almost nil. This country had this advance because of the improvement in disease diagnosis, including tests of susceptibility and the use of vaccines and prebiotics. Moreover, the salmon production systems use collectors under the cages that catch not consumed diet by fish, monitoring the feeding to avoid wastes, reducing then, the exposition of bacteria from sediment to antibiotics and their metabolites [62-63].

Conclusion and Future Priorities

Studies that determine and quantify the contribution of aquaculture and aquatic environments on the appearance of infections by antibiotic resistant bacteria are essential, because without this advance, development of new antibiotic

molecules will not be sufficient to prevent the greater crisis of bacterial infections treatment both in human and animal populations.

Many aquacultures aspects must be carefully treated by all society segments, producers, government, civil society, academic, and industry. Some of these points are: More monitoring programs of antibiotics in environments and fish, further, it is worthwhile to formulate internationally acceptable standard protocols about the use of antibiotics in animal husbandry and about surveillance programs to monitor global emergence of resistance bacteria; More studies about bacteria resistance to know how the use of antibiotics are impacting in the environment and what's the impact to the population heath and the hazards to aquaculture production; Improve the technology production practices of fish, it can be done by the uses of existing vaccines and optimal use of prebiotics to minimize the use of antibiotics must be a viable alternative, therefore, new methods to manage infectious diseases in animal husbandry are required, improving the hygiene, using enzymes, probiotics, prebiotics, and acids to improve health, and utilizing bacteriocins, antimicrobial peptides, and bacteriophages as substitutes for antibiotics might be good methods to promote growth in food animals and decrease infectious diseases in them; Producers' education, manly to the smaller to show the problems of incorrect use of them, promoting the prudent use of antibiotics and the alternatives methods to minimize the infectious diseases; Public policies about fish production get by the government in addition to the fish production and antibiotics uses inspection are fundamental to protect the fish production and the population heath, avoiding this away a serious problem in relation to the antibiotics use and bacteria resistance.

Table 1: Online Sources[a] of National/Regulatory Authority MRL/Tolerance Information

Country / Regulatory Authority	Agency	URL for MRL/Tolerance Information
Australia	Dept. Agriculture, Fisheries, and Forestry (veterinary and pesticide MRLs)	https://www.legislation.gov.au/Details/F2016C00256
Canada	Health Canada (veterinary MRLs)	http://www.hc-sc.gc.ca/dhp-mps/vet/mrl-lmr/mrl-lmr_versus_new-nouveau-eng.php
The European Union	European Medicines Agency	http://www.ema.europa.eu/ema/index.jsp?curl=pages%2Fmedicines%2Flanding%2Fvet_mrl_search.jsp&mid=WC0b01ac058008d7ad&docType=epmar&searchkwByEnter=false&alreadyLoaded=true&isNewQuery=true&keyword=fish
New Zealand	NZ Food Safety Authority (veterinary and pesticide MRLs)	http://www.foodsafety.govt.nz/index.htm
The United Nations	Codex Alimentarius (veterinary MRLs)	http://www.fao.org/fao-who-codexalimentarius/codex-texts/maximum-residue-limits/en/
The United States of America	US Food and Drug Administration	http://www.fas.usda.gov/maximum-residue-limits-mrl-database
International Database	Global MRL	https://www.globalmrl.com/home/index.html (not include fish)

[a]URLs accessed on July 16, 2018.

Table 2: Selection of methods for the analysis of antibiotics in fish

Compounds	Samples	Sample treatment	Chromatographic conditions	Detection	Analysis time	LOQ's	Reference
enrofloxacin	fish from markets	extraction with 2% acetic acid in ACN twice clean-up using SPE with h-MIPs ($K_2Ti_4O_9$)	C18 column (250 × 4.6 mm, 5 μm) column gradient elution (A) ACN (B) 0. 5 mol L−1 phosphoric acid (15:85, v/v) in water 1 mL min^{-1}	fluorescence detector with excitation at 280 nm and emission at 450 nm	10 min	0.8 μg kg^{-1}	[64]
screening of 40 antibiotics (sulfonamides, trimethoprim, tetracyclines, macrolides, quinolones, and penicillins)	samples of milk, muscle, liver, and fish	extraction with ACN and 0.1 M EDTA defatting with n-hexane clean-up using SPE Oasis HLB cartridges	Acquity HSS T3 column (2.1×100 mm, 1.8 μm) gradient elution: (A) 0.1 % acid formic in water (B) ACN 0.45 mL min^{-1}	triple quadrupolemassanalyzer (ESI+) MRM acquisition mode	12 min	-	[65]
crystal violet, chloramphenicol, gentamicin, enrofloxacin, malachite green, furaltadone, and furazolidone	feed and fish from aquaculture production	extraction with buffer of ELISA kits from Bio Scientific	-	spectrophotometer analyser	-	CCβ 0.075 - 5.0 μg kg^{-1}	[66]
14 sulfonamides	fish from markets	extraction with ACN formic acid 1% clean-up with C18 dispersive-SPE	ZORBAX SB-C18 column (4.6 × 150 mm, 5 μm) gradient elution (A) 5 mM ammonium acetate and formic acid in water (pH 3.5) (B) MeOH 0.5 mL min^{-1}	triple quadrupolemassanalyzer (ESI+) (ESI-) MRM acquisition mode	20 min	1.27–3.71 μg kg^{-1}	[67]
143 veterinary drugs and pharmaceuticals (quinolones, tetracyclines, sulfonamides, and amphenicols)	milk and fish from markets and aquaculture production	extraction with 0.1% formic acid/ 0.1% EDTA/ MeOH/ ACN and ultrasonic bath at 60°C overnight −20°C defatting with hexane	ACQUITY BEH C18 column (2.1 × 50 mm, 1.7 μm) gradient elution (A) 0.01 % acid formic in water (B) MeOH 0.1 mL min^{-1}	Q-TOF MS (ESI+) MRM acquisition	17 min	20 - 200 μg kg^{-1}	[68]
51 pharmaceuticals drugs	macroalgaes, bivalves, and fish from coastal areas	extraction withpressurised liquid extraction using MeOH clean-up using gel permeation chromatography	Acquity HSS T3 column (50 × 2.1 mm , 1.8 μm) gradient elution (A) 10 mM formic acid/ ammonium formate (pH 3.2) in water (B) MeOH 0.5 mL min^{-1}	triple quadrupole-linear ion trap mass spectrometer mass analyzer (ESI+) MRM acquisition mode	40 min	0.10 - 1.66 μg kg^{-1}	[69]
fleroxacin, ofloxacin, norfloxacin, ciprofloxacin, and enrofloxacin	fish, chicken, pork and beef from markets	extraction with phase separation method based on ultrasound-assisted, salt-induced, liquid–liquid microextraction	Zorbax Eclipse XDB-C18 column (150 × 4.6 mm, 5 μm) (A) MeOH/ ACN/ water (15:5:80, v/v) 0.8 mL min^{-1}	fluorescence detector with excitation at 290 nm and emission at 455 nm	10 min	0.28 - 1.57 μg kg^{-1}	[70]
12 antibiotics (amphenicols, quinolone, sulfonamides, tetracyclines and	fish from aquaculture production	extraction with 0.1 M Na_2EDTA of ACN: water (0.1% formic acid; 70:30) clean-up by Captiva ND cartridges	Zorbax Eclipse Plus C18 column (100 × 3 mm; 3.5 μm) gradient elution (A) 0.1% formic acid in water (B) 0.1% formic acid in ACN 0.4 mL min^{-1}	triple quadrupolemassanalyzer (ESI+) (ESI-) MRM acquisition mode	15 min	0.9 - 4.30 μg kg^{-1}	[26]
metronidazole)	water, sediment and fish from aquaculture production	extraction with 1% acetic acid in ACN, drying with MgSO4 and clean-up using SPE with Supelco NH2 cartridge	Gemini-NX C18 column (150 × 4.6 mm, 5 μm) gradient elution: A) 1 mM ammonium acetate in water : ACN (90:10,v/v), pH = 3.57 (B) ACN 0.5 mL min^{-1}	ion trap mass spectrometer analyzer (ESI+) MRM acquisition mode	15 min	1.0 μg kg^{-1}	[71]
41 antibiotics (sulfonamides, trimethoprim,	fish from aquaculture production	comparison of 14 different extraction procedures	Acquity HSS T3 column (2.1×100 mm, 1.8 μm) gradient elution:	triple quadrupolemassanalyzer (ESI+) (ESI-)	10 min	CCβ 0.2 -413.6 μg kg^{-1}	[65]

tetracyclines, macrolides, quinolones, penicillins and chloramphenicol)			A) formic acid 0.1 % in water (B) ACN 0.45 mL min^{-1}	MRM acquisition mode			
erythromycin, josamycin, tilmicosin, tylosin, spiramycin and neospiramycin	fish from markets	extraction with ethanol twice and defatted with hexane	XTerraC18 (150 × 2.1 mm, 5 µm) gradient elution: (A) 1 % acid acetic in water (B) 1 % acid acetic in MeOH 0.3 mL min^{-1}	Q-TOF MS (ESI+) full scan acquisition	16 min	17 - 82 µg kg^{-1}	[72]
fleroxacin, ofloxacin, norfloxacin, pefloxacin, ciprofloxacin, lomefloxacin, enrofloxacin and gatifloxacin	fish from markets	extraction with MSPD using DMIP with dimethylsulfoxide−ACN (1:1.8, v/v)	Zorbax SB-C18 column (250 × 4.6 mm, 5 µm) gradient elution (A) 0.1 % acid formic in water (B) MeOH (C) ACN 1 mL min^{-1}	fluorescence detector with excitation at 290 nm and emission at 480 nm	36 min	0.2 - 0.7 µg kg^{-1}	[73]
dimetridazole, ipronidazole, metronidazole, ornidazole, ronidazole and metabolites	prawn and finfish from markets	extraction with acetonitrile, water, $MgSO_4$ and NaCl and defatted with hexane pre-saturated with acetonitrile	Acquity BEH C18 column (100 mm × 2.1, 1.7 µm) gradient elution (A) 0.01 % acid formic in water (B) 0.01 % acid formic in MeOH 0.45 mL min^{-1}	triple quadrupolemassanalyzer (ESI+) MRM acquisition mode	8 min	CCβ 0.14 - 1.2 µg kg^{-1}	[74]
nitrofurans residues (3-amino-2-oxazolidinone and 3-amino-5-morpholinomethyl-2-oxazolidinone)	fish from aquaculture production	acidification with HCl solutions and addition of derivatizing agent (2-NBA), neutralisation with K_2HPO_4, defatting with hexane, and partitioning with ethyl acetate.	Inertsil ODS-3 (150 × 2.1 mm, 5 µm) column gradient elution (A) 0.1 % acid formic in water (B) 0.1 % acid formic in ACN 1 mL min^{-1}	triple quadrupolemassanalyzer (ESI+) MRM acquisition mode	37 min	0.17 and 0.7 µg kg^{-1}	[75]
34 antibiotics (aminoglycosides, β-lactams, fluoroquinolones, macrolides, sulfonamides, trimethoprim and tetracyclines)	fish from aquaculture production	double extraction with m-phosphoric acid and heptafluorobutyric acid as an ion-pair agent and ACN clean-up using SPE Strata X-CW	Luna C18 column (50 × 4.6 mm, 3 µm) gradient elution A) 0.025% HFBA in water (B) ACN 0.25 mL min^{-1}	triple quadrupolemassanalyzer (ESI+) MRM acquisition mode	18 min	CCβ 59.5 - 1141 µg kg^{-1}	[22]
oxytetracycline, doxycycline, tetracycline and chlortetracycline	fish from aquaculture production	comparison of 4 different extraction procedures, better extraction with McIlvaine solution, containing 0.1 mol L^{-1} EDTA, pH 4.00 defatting with trichloroacetic acid clean-up using SPE comparing phenyl, C18 and Oasis HLB cartridge	ACE C18 column (250 × 4.6 mm, 5 µm) gradient elution (A) sodium acetate (0.0375 mol L^{-1}), calcium chloride (0.0175 mol L^{-1}) and EDTA (0.0125 mol L^{-1}), in water, pH 7.00 (B) MeOH: ACN (1:1) 1 mL min^{-1}	fluorescence detector with excitation at 327/280 nm and emission at 367/450 nm	17 min	8.25 - 46.2 µg kg^{-1}	[15]
norfloxacin, danofloxacin, enrofloxacin and ciprofloxacin	fish from markets	extraction with solution A (1% acetic acid in MeOH), 150 mg of PSA, solution B (1% acetic acid in ACN) defatting with n-hexane	XTerra RP18 column (150 × 2.1 mm, 5 µm) gradient elution: (A) 0.1 % acid formic in water (B) ACN 0.2 mL min^{-1}	Q-TOF MS (ESI+)	8 min	25 µg kg^{-1}	[76]
9 antibiotics (penicillin and amphenicol)	fish from markets	extraction with water: acetone (50:50) twice clean-up using SPE Oasis HLB cartridge	Inertsil C_8column (250 × 4 mm), 5 µm gradient elution: (A) 0.05 M ammonium acetate (B) ACN 1.0 mL min^{-1}	Diode Array Detector at 225, 240 and 278 nm	20 min	33.2 - 61.7 µg kg^{-1}	[77]
ciprofloxacin, danofloxacin, enrofloxacin,	fish from markets	extraction with citrate buffer solution (pH = 4.7) twice	Perfectsil ODS-2 120 column (250 × 4 mm, 5 µm) gradient elution:	Diode Array Detector at 225, and 275 nm	25 min	5.7 - 35.0 µg kg^{-1}	[78]

sarafloxacin, oxolinicacid, nalidixicacid and flumequine		clean-up using SPE Oasis HLB cartridge	(A) 0.1% TFA (B) ACN (C) MeOH 1.0 mL min^{-1}				
13 aminoglycosides	samples of pork muscle, fish, and veal liver and kidney	extraction with trichloroacetic acid and EDTA solution clean-up using SPE OASIS MCX cartridge	Kinetex C18 column, (150 × 2.1mm, 2.6 µm) gradient elution: (A) (97.4: 2.5: 0.07 v/v/v) water: ACN: heptafluorobutyric acid (B) (97.4: 2.5: 0.07 v/v/v) ACN: water: heptafluorobutyric acid 0.3 mL min^{-1}	triple quadrupolemassanalyzer (ESI+) MRM mode	8 min	1 - 30 µg kg^{-1}	[79]
3 sulfonamides and 3 tetracyclines	feed and fish from markets and aquaculture production	comparison of immunoassays (ELISA and TR-FIA) and LC-MS/MS	Mediterranean sea18 column (150 × 2.1 mm, 5 µm) gradient elution (A) 0.1% formic acid in water (B) 0.1% formic acid in ACN 0.3 mL min^{-1}	photometric and time-resolved fluorometric detection/ triple quadrupole mass analyzer (ESI+) MRM acquisitionmode	35 min	0.3 - 360 µg kg^{-1}	[80]
17 sulfonamides and 5 tetracyclines	fish, porcine and poultry tissue from aquaculture production and markets respectively	extraction with MeOH:ACN 50:50 (v/v) acidified with 0.05% formic acid	Zorbax Eclipse plus C18column (50 × 2.1 mm, 1.8 µm) gradient elution: (A) 0.1 % acid formic in water (B) ACN 0.1 mL min^{-1}	triple quadrupolemassanalyzer (ESI+) data dependent scan mode	21 min	17.1 - 78.1 µg kg^{-1}	[81]
furazolidone, furaltadone, nitrofurazone, nitrofurantoin and metabolites	samples of fish	samples were acid-hydrolyzed, treated with 2-nitrobenzaldehyde and extracted with ethyl acetate	ZORBAX SB-C18 column (150 × 4.6 mm, 5 µm) gradient elution: (A) 0.5 mM ammonium acetate in water (B) MeOH 0.7 mL min^{-1}	triple quadrupolemassanalyzer (ESI+) MRM acquisition mode	9 min	1 - 10 µg kg^{-1}	[82]
11 quinolones	fish from markets	extraction with McIlvaine solution, containing 0.2 mol/L disodium hydrogen phosphate, 0.1 mol/L citric acid, pH 4.0 twice clean-up using SPE Varian Bond elutplexa cartridge	comparison of 4 different columns, better Zorbax Eclipse XDB-C18 column (20 × 3.0 mm, 1.8 µm) gradient elution (A) 0.2% formic acid in water (B) 0.2% formic acid in ACN 0.5 mL min^{-1}	fluorescence detector with excitation at 270/ 320 nm and emission at 450/ 366nm	13 min	5.3 - 142.9 µg kg^{-1}	[83]
flumequine, oxolinicacid, sarafloxacin, danofloxacin, enrofloxacin, and ciprofloxacin	fish from aquaculture production	extraction with 10% trichloroacetic acid and MeOH (80:20) twice clean-up using SPE Oasis HLB cartridge	XTerra C18hybridsilicacolumn (250 × 4.6 mm, 5 µm) gradient elution: (A) 0.010 mol L−1 oxalic acid in water, pH 4 (B) ACN 1.0 mL min^{-1}	fluorescence detector with excitation at 385 nm and emission at 528 nm and confirmations by LC-MS/MS-TOF	30 min	5 - 27 µg kg^{-1}	[84]
ciprofloxacin, danofloxacin, enrofloxacin, and sarafloxacin	fishs and shrimp from aquaculture production	extraction with an acidic acetonitrile solution and diluted with dichloromethane clean-up using STRATA SPE cartridge	HSS T3 C18 column (50 × 2.1 mm, 1.8 µm) gradient elution (A) 4 mM NH4OH/50 mM FA buffer in 10% MeCN (B) 4 mM NH_4OH/50 mM FA buffer in 90% MeCN. 0.4 mL min^{-1}	Quattro Premier XE tandem quadrapolemassanalyzer (ESI+) MRM acquisition mode	8 min	0.06 - 0.56 µg kg^{-1}	[85]
7 macrolides	raw meat and fishfrom markets	extraction with pressurized liquid extraction	Kromasil 100 C18 column (250 × 4.6 mm, 5 µm) gradient elution (A) 0.1 % acid formic in water (B) ACN 1 mL min^{-1}	single quadrupolemassanalyzer (ESI+) SIM acquisition mode	25 min	25.0 - 50.0 µg kg^{-1}	[86]
quinolones, fluoroquinolones, malachite green and metabolites, tetracyclines, sulfonamides,	shrimp, marine fish, freshwater fish, and canned fish form	a different extraction for each class of antibiotic	-	triple quadrupolemassanalyzer (ESI+) MRM acquisition mode	-	0.3 - 990 µg kg^{-1}	[87]

avermectins, nitrofuran metabolites and amphenicols	markets						
14 sulfonamide	fish and shrimp from markets	extraction with 0.2% acetic acid/ MeOH/ ACN (85:10:5) and methylene chloride clean-up using SPE with SCX cartridges	Symmetry C18 column (150 mm × 4.6 mm, 3.5 µm) gradient elution (A) 2% acetic acid: MeOH: ACN (85:10:5 v/v/v) in water (B) 2% acetic acid: MeOH: ACN (85:10:5 v/v/v) in water derivatized postcolumn 1 mL min^{-1}	fluorescence detector with excitation at 400 nm and emission at 495 nm	25 min	1 µg kg^{-1}	[88]

MeOH: methanol; ACN: acetonitrile

Table 3: Results of antibiotics residues analyses in fish samples around the world

Sample	Country	Antibiotic	Results (µg kg^{-1})	Reference
15 Gilthead	Italy	Chroamphenicol	0.57	[66]
15 Seabass		Gentamicin	< LOQ	
		Enrofloxacin	0.14	
		Malachite green	0.48	
		Crystal violet	2.05	
		Furaltadone	0.29	
		Furazolidone	0.09	
		AMOZ	< LOQ	
		AOZ	< LOQ	
Gilthead Seabream (Quantity not informed)	Spain	Albendazole	< LOQ	[89]
		Enrofloxacin	< LOQ	
		Fenbendazole	< LOQ	
		Mebendazole	< LOQ	
		Oxfendazole	< LOQ	
		Oxolinic acid	< LOQ	
		Thiabendazole	< LOQ	
3 Gilthead Seabrem 2 Seabass	Greece	143 Antibiotics	< LOQ	[90]
22 Fish (not identified)	Greece	115 Antibiotics	1 (4.6) with Flumequine 1 (4.8) with Enrofloxacine	[68]
Gilthead Seabrem (Quantity not informed)	Greece	Ampicillin	< LOQ	[77]
		Penicillin	< LOQ	
		Penicillin G	< LOQ	
		Oxacillin V	< LOQ	
		Cloxocillin	< LOQ	
		Dicloxacillin	< LOQ	
		Thiamphenicol	< LOQ	
		Florfenicol	< LOQ	
		Chloramphenicol	< LOQ	
Salmon solar L. (Quantity not informed)	Greece	Ciprofloxacin	< LOQ	[78]
		Danofloxacin	< LOQ	
		Enrofloxacin	< LOQ	

		Sarafloxacin	< LOQ	
		Oxolinic Acid	< LOQ	
		Nalidixic Acid	< LOQ	
		Flumequine	< LOQ	
20 Tilapia	Taiwan	Sulfadiazine	< LOQ	[67]
		Sulfathiazole	< LOQ	
		Sulfamethoxazole	< LOQ	
		Sulfadoxine	< LOQ	
		Sulfaphenazole	1 (2.7)	
		Sulfachloropyridazine	< LOQ	
		Sulfamethazine	< LOQ	
		Sulfadimethoxine	< LOQ	
		Sulfacetamide	< LOQ	
		Sulfaquinoxaline	< LOQ	
		Sulfamonomethoxine	< LOQ	
		Sulfamerazine	< LOQ	
		Sulfamethizole	< LOQ	
		Sulfamethoxypyridazine	< LOQ	
18 Tilapia	Brazil	Ciprofloxacin	< LOQ	[76]
13 Pacu		Danofloxacin	< LOQ	
		Enrofloxacin	< LOQ	
		Norfloxacin	< LOQ	
		Ofloxacin	< LOQ	
36 Tilapia	Brazil	Oxytetracycline	9 (15.6 – 528.0)	[25]
		Tetracycline	1 (7.7)	
		Chlortetracycline	< LOQ	
		Ciprofloxacin	< LOQ	
		Enrofloxacin	< LOQ	
		Sarafloxacin	< LOQ	
		Norfloxacin	< LOQ	
		Sulfathiazole	< LOQ	
		Sulfadimethoxine	< LOQ	
		Sulfamethazine	3 (521.2 – 528.0)	
		Florfenicol	< LOQ	
		Chloramphenicol	< LOQ	
Rainbow Trout (Quantity not informed)	Poland	MNZ	< LOQ	[71]
		Metronidazole	1 (1.5)	
33 Flatfish	South	Sulfamethoxazole	1 Eel (5104)	[91]
42 Jacopever	Korea	Sulfamethoxypyridazine	< LOQ	
53 Seabream		Sulfachloropyridazine	< LOQ	
43 Eel		Fulfadimethoxine	< LOQ	
34 Blue Crab		Sulfamonomethoxine	< LOQ	
57 Shrimp		Sulfaphenazole	< LOQ	
47 Abalone		Sulfadoxine	< LOQ	
		Sulfasoxazole	< LOQ	
		Sulfachloropyrazine	< LOQ	
		Sulfamethazine	< LOQ	

		Sulfathiazole	< LOQ	
		Sulfaquonoxaline	< LOQ	
		Sulfadiazine	1 Flat fish (14)	
			1 Jacopever (26)	
		Sulfamerazine	< LOQ	
67 Eel	South Kore	Malachitegreen	1 shrimp (2.6)	[92]
41 Salmon		Crystal violet	1 Eel (168.4)	
78 Shrimp				
67 Fish cakes				
33 Fish	China	Sulfacetamide	< LOQ	[93]
		Sulfathiazole	3 (2.7 – 6.1)	
		Trimethoprim	< LOQ	
		Norfloxacin	4 (2.8 – 38,5)	
		Ofloxacin	3 (1.9 – 4.5)	
		Oxytetracycline	2 (2.8 and 1.5)	
		Ciprofloxacin	3 (1.7 – 3.9)	
		Tetracycline	2 (3.7 and 4.2)	
		Sulfamethiazole	< LOQ	
		Chlortetracycline	< LOQ	
		Sulfaclororpyridazine	< LOQ	
		Sulfamethoxazole	4 (0.9 – 2.3)	
		Sulfaphisoxazole	< LOQ	
		Sulfadimethoxine	2 (0.4 and 5.3)	
		Enrofloxacin	< LOQ	
		Erythromycin	1 (0.7)	
		Roxithromycin	1 (0.4)	
73 Frehwater Fish	China	Norfloxacin	3.1 – 100.5	[4]
		Ciprofloxacin	< LOQ – 33.3	
		Enrofloxacin	< LOQ – 51,9	
		Sulfadiazine	11.1 – 85.2	
		Sulfadimidine	< LOQ – 84.3	
		Sulfamethoxazole	< LOQ – 26.9	
21 Marine Fish	China	Norfloxacin	2.0 – 43.5	[4]
		Ciprofloxacin	< LOQ – 2.2	
		Enrofloxacin	< LOQ – 1.0	
		Sulfadiazine	< LOQ – 37.5	
		Sulfadimidine	< LOQ – 27.8	
		Sulfamethoxazole	< LOQ – 20.0	
20 Sea Bream	Spain	Tetracycline	3 (11.1 – 13.1)	[94]
		Oxytetracycline	4 (49 – 59)	
		Chlortetracycline	4 (20 – 23.2)	
		Sulfadiazine	< LOQ	
		Sulfadimethoxine	< LOQ	
		Sulfamerazine	< LOQ	
9 Fish	Spain	Tetracycline	< LOQ	[94]
		Oxytetracycline	4 (< LOQ - 60)	
		Chlortetracycline	< LOQ	
		Sulfadiazine	< LOQ	

		Sulfadimethoxine	< LOQ	
		Sulfamerazine	< LOQ	
100 Rainbow	Iran	Tetracycline	30 (2.09 – 22.12)	[95]
Trout		Chloramphenicol	7 (0.09 – 0.25)	
		Sulfonamide	19 (2.01 – 7.06)	

Table 4: Sites that provide results about veterinary drugs programs

Country/Regul. Authority	URL for MRL/Tolerance Information
The United Kington / Department for Environment, Food & Rural Affairs and Health and Safety Executive	https://www.gov.uk/government/collections/residues-statutory-and-non-statutory-surveillance-results
The United States of America / Food and Drug Administration (FDA)	https://www.fda.gov/Food/FoodScienceResearch/default.htm#Monitoring
European community / European Food Safety Authority	http://www.efsa.europa.eu/en/press/news/datex100419
Norway / The National Institute of Nutrition and Seafood Research	https://sjomatdata.nifes.no/#search/
Australia / Australian Pesticide and Veterinary Medicines Authority	https://apvma.gov.au/node/32
Canada / Canadian Food Inspection Agency (CFIA)	http://www.inspection.gc.ca/food/chemical-residues-microbiology/chemical-residues/eng/1324258929171/1324264923941
Brazil / Secretary of Animal and Plant Healthy and Inspection of the Ministry of Agriculture, Livestock and Food Supply	http://www.agricultura.gov.br/assuntos/inspecao/produtos-animal/plano-de-nacional-de-controle-de-residuos-e-contaminantes

accessed on July 16, 2018.

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