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Perspective

Antibiotics and Food Safety in Aquaculture

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ABSTRACT: Antibiotics are widely used in aquaculture. Intensive farming drives indiscriminate use of antibiotics, which results in residues of antibiotics in cultured aquatic products and bacterial resistance. This perspective attempts to present a brief update on usage, regulations, residues, and potential human health risk of antibiotics used in aquaculture. Through the comprehensive literature review, we provide a view that the safety of aquatic products still requires further attention and more rigorous risk assessment. Finally, we make a few suggestions for future research directions: reduce the use of antibiotics to bring down the speed of resistance development and monitor resistant pathogens and genes, strictly manage the environmental sanitation of aquaculture and pay attention to the quality of water bodies introduced into aquaculture, seek international cooperation to establish an information bank of antibiotic residues and antibiotic-resistant genes, and set up a quantitative model to assess the risk of antibiotic resistance associated with the antibiotic residues.

KEYWORDS: *antibiotics, food safety, aquaculture*

INTRODUCTION

Aquaculture is one of the sustainable modes of agricultural production that provides edible protein. Aquaculture has kept a lasting growth over the past 2 decades. Therein, Asia contributed to nearly 90%.[1] The rapid growth has benefited from contributions of a semi-intensive or intensive aquaculture method and the usage of antibiotics.[2] Previous studies reported that half the amount of antibiotics produced in China, the world's largest producer and user of antibiotics, ended up in animal feed in recent years, meaning that approximately 105 000 tonnes was used for animal consumption.[3] Antibiotics via oral administration, bath, pond sprinkle, and injection can prevent and treat aquaculture diseases, and some of them are even used as growth promoters.[2−4] However, antibiotics also cause stresses on the aquatic environment.[5] Numerous studies have investigated the residues of antibiotics in water bodies and sediments and confirmed the antibiotic contamination.[6−9]

Large-scale usage of antibiotics is bound to give rise to residues in cultured aquatic products.[10−12] Antibiotic residues in aquatic products are complex because of the regional differences and physicochemical properties of different antibiotics.[13] Up to date, there is no database about residues in cultured aquatic products around the world. The studies on antibiotic residues in aquatic products are relatively limited in comparison to the studies about the residues in the aquaculture environment or antibiotic resistance. Antibiotic residues in cultured aquatic products can pose potential risks to humans, including allergy, toxicity, and antibiotic resistance.[3,5] The impacts of antibiotic resistance on aquatic organisms and humans are difficult to eliminate. Thus, the collection of resistant genes in aquaculture is a significant topic around the world,[14] while the dietary risk is also a main concern.

In this perspective, the global usage and regulations of antibiotics in aquaculture are summarized. On account of the increasing concerns about potential human health risk from antibiotics via consumption of cultured aquatic products,[15] we reviewed the residues of antibiotics in cultured aquatic products and human health risk from antibiotics. This paper is not only a review but an attempt to understand the food safety status of aquaculture products and suggest research perspectives for a sustainable aquaculture. All papers cited are primarily those found in the Web of Science database. The search terms used were "aquaculture" and "antibiotics" for publications in the Web of Science database between 1990 and May 2020.

USAGE AND REGULATION OF ANTIBIOTICS IN AQUACULTURE

Usage. On the basis of the chemical structures, antibiotics in aquaculture are divided into aminoglycosides (AGs), quinolones (QNs), sulfonamides (SAs), tetracyclines (TCs), macrolides (MLs), chloramphenicols (CAPs), *β*-lactams, nitrofurans (NFs), lincosamides (LINs), polymyxins (PLs), and some others (Table 1). Natural antibiotics (e.g., erythromycin, oxytetracycline, tetracycline, and chloramphenicol) were widely used in the past and developed antibiotic resistance. However, later semi-synthetic antibiotics (e.g., mostly *β*-lactams), synthetic antibiotics (e.g., quinolones,

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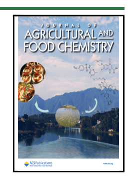

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Table 1. Important Classifications and Maximum Residue Limits (MRLs) of Antibiotics

antibiotics	rank in human medicine[17]	MRL (μg/kg)[31]	target disease/species[3,21,24]
			Aminoglycosides
neomycin	critically important	500	septicemia (2.5−5 mg/kg of BW)[3]
			β-Lactams
amoxicillin	critically important	50	infection of furunculosis (fish) (80−160 mg/kg for 10 days)[24]
ampicillin	critically important	50	NA[a]
			Quinolones
enrofloxacin	veterinary use only	100 (for ENR and CIP)	bacterial diseases (2500 mg/kg feed); shrimp (4.00 mg/kg of BW for 5−7 days)[3]
flumequine	critically important	600	infections of Gram-negative bacteria (such as *Piscirickettsia salmonis*, furunculosis, and *Vibrio* infections)[24]
oxolinic acid	critically important	100	red fin/skin (10−20 mg/kg of BW for 4−7 days);[3] infections of Gram-negative bacteria (such as *Piscirickettsia salmonis*, furunculosis, and *Vibrio* infections)[24]
			Sulfonamides
sulfamethoxazole	highly important	100 (sum of total SAs)	bacterial enteritis (100−200 mg/kg of BW for 5 days)[3]
sulfamethazine	highly important	100 (sum of total SAs)	NA
sulfadimethoxine	highly important	100 (sum of total SAs)	enteric septicemia of catfish (ESC) caused by *Edwardsiella ictaluri* strains[b,21]
sulfadiazine	highly important	100 (sum of total SAs)	infections of Gram-negative bacteria, such as furunculosis and *Vibrio* (salmon) (30−75 mg/kg for 5−10 days)[c,24]
			Macrolides
erythromycin	critically important	200	infections of Gram-positive and non-enteric Gram-negative bacteria responsible for causing bacterial kidney disease (50−100 mg/kg for 21 days)[24]
			Chloramphenicols
chloramphenicol	important	0	NA (for human only)[3]
florfenicol	veterinary use only	1000	infection of furunculosis (salmon) (10 mg/kg for 10 days);[24] infection of *E. ictaluri*-associated ESC (catfish) (10−15 mg/kg for 10 days)[21]
thiamphenicol	highly important	50	NA
			Tetracyclines
chlortetracycline	highly important	100 (sum of parent drug and its 4-epimer)	NA
oxytetracycline	highly important	100 (sum of parent drug and its 4-epimer)	infection of furunculosis and *Vibrio* (50−125 mg/kg for 4−10 days);[24] bacterial hemorrhagic septicemia caused by *Aeromonas liquefaciens* and *Pseudomonas* disease (catfish) (2.5−3.75 g/100 lbs)[21]
tetracycline	highly important	100 (sum of parent drug and its 4-epimer)	infection of furunculosis and *Vibrio* (salmon)
			Nitrofurans
furazolidone	important	1 (EC, 2003)[23]	fish (100−200 mg/kg of BW); shrimp (0.1−0.15% feed)[3]
			Polymyxins
colistin	critically important	150	NA
			Lincosamides
lincosamide	highly important	100	NA
			Others
trimethoprim	highly important	50	infections of Gram-negative bacteria, such as furunculosis and *Vibrio* (salmon) (30−75 mg/kg for 5−10 days)[c,24]

[a]NA = not available. [b]The combination of SM2 and ORM (NADA 125-933).[21] [c]Sulfadiazine/trimethoprin (5:1) (tribrissen).[24]

nitrofurans, and florfenicol), and other chemically modified derivatives (e.g., oritavancin, telavancin, and ivermectin) have gradually replaced the natural antibiotics.[3] SAs and *β*-lactams are prevalent in Asia, while the dominant antibiotics in the past were SAs, QNs, and TCs.[16] This might be associated with the poor treatment response as a result of the antibiotic resistance and the regulation updates. Some antibiotics are used for veterinary purposes (e.g., enrofloxacin and florfenicol), while some are for humans (e.g., chloramphenicol, erythromycin, and ciprofloxacin). Some antibiotics are for dual uses: aquaculture and human. However, regardless of the original purposes, many types of antibiotics were detected in aquacultural products and environments. The World Health Organization (WHO) has classified antibiotics according to their importance in human medicine:[17] "critically important" (e.g., amoxicillin, flumequine, and oxolinic acid), "highly important" (e.g., sulfadiazine, sulfamethoxazole, oxytetracycline, and tetracycline), and "important" (e.g., chloramphenicol

Table 2. Lists of Authorized Antibiotics or Antibiotics Which Have Been Used among the Major Aquaculture-Producing Countries and Organizations

country	authorized/used antibiotics
China[3,16]	13 antibiotics authorized: doxycycline, enrofloxacin, florfenicol, flumequine, neomycin, norfloxacin, oxolinic acid, sulphadiazine, sulphamethazine, sulphamethoxazole, sulphamonomethoxine, thiamphenicol, and trimethoprim (33 antibiotics used from 2008 to 2018)[15]
Vietnam[16,20]	30 antibiotics authorized: amoxicillin, benzylpencillin, ciprofloxacin, cloxacillin, colistin, chlortetracycline, cypermethrim, danofloxacin, dicloxacillin, difloxacin, emamecyin, erythromycin, flumequine, neomycin, oxolinic acid, ormetoprinm, oxytetracycline, oxacillin, paromomycin, sarafloxacin, sulfadimethoxine, sulfadiazine, sulfamonomethoxine, sulfamethoxazole, sulfamethazine, spectinomycin, tetracycline, tilmicosin, trimethoprim, and tylosin (39 antibiotics used from 2008 to 2018)[15]
U.K.[15]	5 antibiotics authorized: oxytetracycline, oxolinic acid, amoxicillin, sarafloxacin, and cotrimazine
U.S.A.[15]	4 antibiotics authorized: oxytetracycline, florfenicol, sulfadiazine/trimethoprim, and sulfadimethoxine/ormetoprim
Italy[15]	6 antibiotics authorized: tetracycline, oxytetracycline, amoxicillin, flumequine, and sulfadiazine/trimethoprim
Brazil[37]	2 antibiotics authorized: florfenicol and oxytetracycline
Thailand[16]	14 antibiotics used from 2008 to 2018: amoxicillin, enrofloxacin, norfloxacin, oxytetracycline, ormetoprim, penicillin, sulfadiazine, sulfadimethoxine, sulphamonomethoxine, sulfadimethoxine, sulphaguanidine, trimethoprim, tribrissen, and tetracycline
South Korea[16]	17 antibiotics used from 2008 to 2018: amoxicillin, ciprofloxacin, chlortetracycline, enrofloxacin, erythromycin, florfenicol, nalidixic acid, ormetoprim, oxolinic acid, oxytetracycline, sulfadiazine, sulphachloropyridazine, sulphamethoxazole, sulfadimethoxine, sulphamethazine, trimethoprim, and tetracycline
Chile[15]	19 antibiotics authorized: amoxicillin, chloramphenicol, doxycycline, enrofloxacin, erythromycin, florfenicol, flumequine, furazolidin, gentamyin, neomycin, norfloxacin, oxolinic acid, oxytetracycline, sulphadiazine, sulphamethazine, sulphamethoxazole, sulphamonomethoxine, thiamphenicol, and trimethoprim
Bangladesh[36,38]	12 antibiotics are reported in aquaculture: amoxicillin, chlortetracycline, doxycycline, erythromycin, oxytetracycline, penicillin G, sulfadiazine, sulfamethazine, sulfamethizole, sulfamethoxazole, trimethoprim, and tylosin
Japan[39]	amoxicillin, carbolic acid, doxycycline, erythromycin, fosfomycin, oxolinic acid, lincosamide, oxytetracycline, sulphamonomethoxine, sodium alkane sulfonate, and thiamphenicol
Australia[15]	NA[a]
South Africa[15]	NA
U.S. FDA[15]	4 antibiotics authorized: florfenicol, oxytetracycline, and sulfadimethoxine/ormetoprim
FAO 2005[15]	4 + SA antibiotics authorized: florfenicol, oxytetracycline, sarafloxacin, eythromycin, and sulfonamides

[a]NA = not available.

and furazolidone) (Table 1). Using these first-line antibiotics for human medicine in aquaculture may give rise to bacterial resistance on human clinic medication;[4] thus, these should be avoided as much as possible. Oral administration, for which antibiotics are mixed in feed,[11] is the main route of application in aquaculture. Bath treatment, pond sprinkle, and injection are the other three application routes.[3,4] Except injection, other routes directly affect not only aquatic organisms but also the aquatic environment.

The amount of antibiotics used in aquaculture worldwide is difficult to estimate.[18] This is subject to the differences of limits on antibiotics,[3,19−21] various farming modes, and diversity of aquaculture species. Nevertheless, we can summarize the universal laws on the basis of many previous studies. Two-thirds of antibiotics are broad-spectrum bacteriostatic agents, which are active against both Gram-positive and Gram-negative bacteria.[22] Antibiotics have their target diseases and species, which differ significantly (Table 1). For example, QNs are frequently administered in treatment of septicaemia or skin diseases in fish.[23] SAs and TCs are therapeutics and prophylactics for bacterial infections.[3] Oxytetracycline and tetracycline are widely used in salmon treatment, such as infections of furunculosis and *Vibrio*,[24] with prevalent usage of erythromycin in bacterial kidney disease (Table 1).[24] Drug products often contain multiple antibiotic active ingredients.[24] Tribrissen, for example, is a 5:1 mixture of sulfadiazine and trimethoprim, used to treat the vibriosis for flatfish, jacopever, and yellowtail.[25] Additionally, the adjustment of species is common in aquaculture as a result of the diversity of cultured species, which affects the usage of antibiotics.[26] The shift of the Thai shrimp industry from black tiger shrimp (*Penaeus monodon*) to white leg shrimp (*Litopenaeus vannamei*) had reduced the use of antibiotics from 78% in 2000 to 3% in 2011−2012.[27,28] Another example is that, over the past 20 years, the change of Pacific salmon into Atlantic salmon in Canada and the United States has resulted in a 10-fold reduction in the use of antibiotics (i.e., oxytetracycline) in salmon farming.[29] Bacterial kidney disease in Pacific salmon is harder to control than that in Atlantic salmon.[29] Climate is also a factor influencing antibiotic use. A previous study found that a hot and rainy climate was beneficial to the occurrence and spread of aquaculture diseases, causing greater use of antibiotics in places such as the coastal regions of Vietnam and South China.[30]

Regulation. The usage of antibiotics is characterized by "geographical heterogeneity", because of the different regulations on approved antibiotics in aquaculture among regions. Table 2 summarizes the regulations among the major aquaculture countries and organizations. The types and numbers of antibiotics vary considerably with regions. According to the study of Ronald et al.,[16] oxytetracycline, sulphadiazine and florfenicol were used in 11 of the 15 main producing countries, while amoxicillin, enrofloxacin, sulphadimethoxine and erythromycin were used by 8 main producing countries. On average, 15 antibiotics were applied by those countries.

China and Vietnam are the biggest consumers of antibiotics, which might be linked to the rampant prophylactic usage of antibiotics.[16] Of course, the Chinese and Vietnamese governments have taken measures to control the use of antibiotics and have updated lists of banned antibiotics.[3,32] In China, chloramphenicol, erythromycin, furazolidone, and ciprofloxacin were banned in 2002, while lomefloxacin, ofloxacin, and norfloxacin were banned in 2016.[3] Back in 2013, the usage of antibiotics in aquaculture was regulated and a prescription was required.[33] Nowadays, 13 common antibiotics are authorized by the Chinese government (Table 2).[3] The antibiotic application is also limited by the government authorities.

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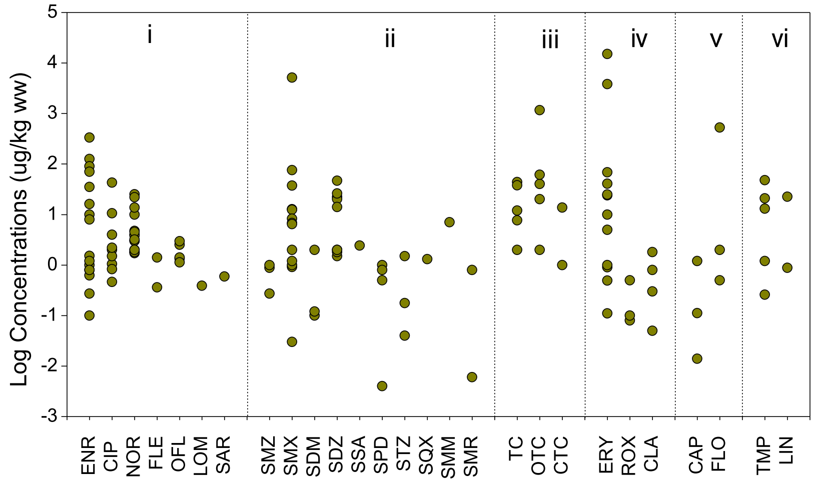


Figure 1. Concentrations of antibiotics detected in aquatic products. The concentrations were calculated on a basis of μg/kg of wet weight (ww) by assuming a moisture content in aquatic products at 80%. i, ii, iii, iv, v, and vi represent quinolones, sulfonamides, tetracyclines, macrolides, chloramphenicols, and others, respectively.[6,11,12,48,49,53−56] ENR, enrofloxacin; CIP, ciprofloxacin; NOR, norfloxacin; FLE, fleroxacin; OFL, ofloxacin; LOM, lomefloxacin; SAR, sarafloxacin; SMZ, sulfamethazine; SMX, sulfamethoxazole; SDM, sulfadimethoxine; SDZ, sulfadiazine; SSA, sulfisoxazole; SPD, sulfapyridine; STZ, sulfathiazole; SQX, sulfaquinoxaline; SMM, sulfamonomethoxine; SMR, sulfamerazine; TC, tetracycline; OTC, oxytetracycline; CTC, chlortetracycline; ERY, erythromycin; ROX, roxithromycin; CLA, clarithromycin; CAP, chloramphenicol; FLO, florfenicol; TMP, trimethoprim; and LIN, lincosamide.

However, antibiotics are still allowed for use in food-producing animals to promote growth and prevent diseases, resulting in great difficulties in enforcement and monitoring in China. Indiscriminate antibiotic usage exists in Vietnamese aquaculture.[16,34] Antibiotics often emerge in convenient stores and can be purchased without a prescription.[16,34] To tackle these problems in aquaculture, the Vietnamese government authority has issued strict regulations for the use of antibiotics in aquaculture since 2002.[16] Up to 2014, 30 antibiotics were authorized (Table 2).[20] In 2016, the list of banned antibiotics was updated again to include ciprofloxacin and the fluoroquinolones.[32] However, some antibiotics can still be detected in later studies, even though they have been banned. For example, enrofloxacin has been banned in Vietnam since 2009;[20] nowadays, it can still be frequently detected in aquacultural products in Vietnam.[34,35] In contrast to China and Vietnam, the number of antibiotics used in India, Thailand, South Korea, Bangladesh, and other Asian countries is fewer.[16,36,38,39] This can be explained by the fact that these countries have to meet the strict needs of importing countries, such as the United States, Japan, and the European Union (EU), while Vietnam and China have relatively huge domestic markets.[7,16]

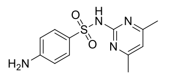
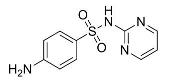
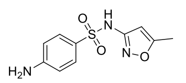
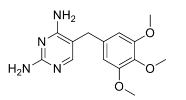
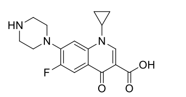
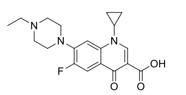
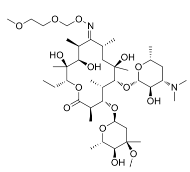
The United States, Japan, and the EU have strict regulations on the use of antibiotics and restrict minimum limits approved in aquaculture.[15] Antibiotics in the United States are regulated strictly by the Food and Drug Administration (FDA) and Environment Production Agency (EPA). Only four antibiotics are permitted for use (Table 2) and also limited in specific aquaculture species or specific diseases.[21] Oxytetracycline, for example, can be used only to treat certain diseases in catfish, salmon, and lobster (Table 1).[21] Antibiotics approved for the treatment will not be used as growth hormones to prevent diseases; thus, the harm to humans or the environment from aquaculture antibiotics is seldom reported in the United States. In the United Kingdom, only oxytetracycline, oxolinic acid, amoxicillin, sarafloxacin, and cotrimazine have been acknowledged (Table 2).[19] Furthermore, to ensure the food safety of cultured aquatic products, the usage of antibiotics as growth promoters has been prohibited[10] and maximum residue levels (MRLs) were established for antibiotics in fish by the European legislation (Table 1).[31] There are efforts to harmonize MRLs, but MRLs were set for the permitted veterinary origin. In contrast, no tolerance levels have yet been established for non-permitted substances. Therefore, the significant geographical differences always remain among the MRLs set by different agencies. Major players in Europe are the European Commission, FDA, European Medicines Agency, Norwegian Veterinary Institute, the Norwegian Food Safety Authority, Codex, and government ministries.[16] Most developing countries have not yet established their MRLs for antibiotics.[10]

RESIDUES OF ANTIBIOTICS IN CULTURED AQUATIC PRODUCTS

Types of Antibiotics. The residual levels of different types of antibiotics vary in cultured aquatic products. Figure 1 shows the common antibiotics in cultured aquatic products and their residual concentrations. This can be associated with their exposure levels as well as physicochemical properties (Table 3). Logarithm octanol−water partition coefficients (log K_{ow}) and water solubility are two important factors governing the bioaccumulation capacity of hydrophobic compounds. It is generally accepted that antibiotics with poor water solubility and log K_{ow} of >1 are accumulative in organisms.[40]

QNs (especially enrofloxacin, norfloxacin, and ofloxacin) are widely administered in aquaculture worldwide, particularly in Asia, leading to elevated exposure levels.[3,12,41] They also have relatively high stability in the aquatic environment[42] and are known as "pseudo-persistent" emerging contaminants.[43] QNs exhibit strong bioaccumulation capacity as a result of their poor water solubility and log K_{ow} of greater than 1 (Figure 2 and Table 3). For instance, a previous study reported that norfloxacin in bile of grass carp was up to 5600 μg/L.[44] However, Holten et al. considered that the adsorption of QNs to solid particles would bring down bioavailability for fish.[45]

Table 3. Physicochemical Properties of Selected Common Antibiotics

Antibiotics	Water solubility (20°C) g/L	Log K_{ow}	Chemical Formula[3]	Structural formula
1) SAs	NA	-0.1–1.7[3]		
SMZ	1.5[34]	0.89[34]	$C_{12}H_{14}N_4O_2S$	
SDZ	0.1[34]	-0.09[34]	$C_{10}H_{10}N_4O_2S$	
SMX	2.8[34]	0.66[34]	$C_{10}H_{11}N_3O_3S$	
2) TMP	1.0[34]	0.59[34]	$C_{14}H_{18}N_4O_3$	
3) FQs	NA	-1–2.31[3,34]		
CIP	0.5[34]	1.63[34]	$C_{17}H_{18}FN_3O_3$	
ENR	0.1[34]	1.1[6], 2.31[34]	$C_{16}H_{18}N_2O_4S$	
4) MLs	NA	1.5–3[3]		
ROX	NA	2.1–2.8[6]	$C_{16}H_{18}N_2O_4S$	

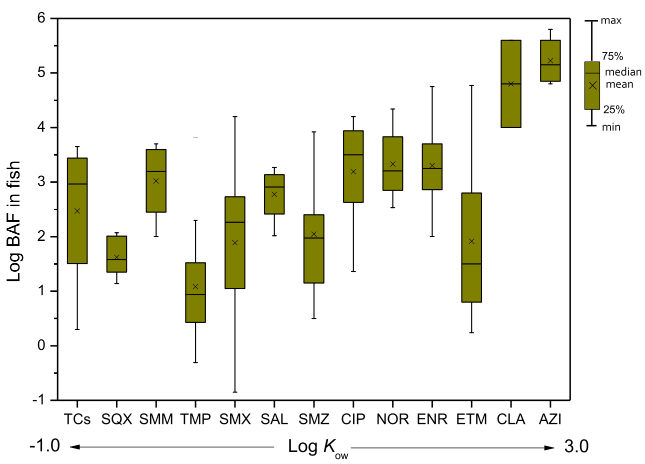


Figure 2. Log BAFs of different antibiotics in fish tissues.[44,50,53,57,58] AZI = azithromycin.

Sulfadiazine, sulfamethoxazole, and sulfadimidine are the three typical chemicals of SAs.[8] SAs are economical with a wide antibiotic spectrum and strong efficacy in treatment, causing prevalent usage in animal productions. In the EU, SAs are the most commonly used antibiotics after TCs.[46] SAs have strong hydrophilicity, weak degradation, high mobility, and persistence.[47] The high usage amount of SAs also makes them familiar in cultured products, although they have high water solubility.[48] Oxytetracycline is one of the few antibiotics authorized in most of the aquaculture countries. It is common that oxytetracycline residues occurred in aquatic products (Figure 3c).[21,49] Even in Brazil, where oxytetracycline is one of the two antibiotics allowed,[37] the mean concentration of oxytetracycline was approximately up to its MRL. MLs have a strong bioaccumulation capacity as a result of their higher log K_{ow} (1.5−3 for MLs) compared to other antibiotics (from −1 to 1.6 and from −0.1 to 1.7 for QNs and SAs, respectively) (Table 3).[34] MLs have a great lipophilicity;[50] therefore, they are inclined to remain in the liver, which is full of phospholipids compared to other tissues. For example, erythromycin, a typical antibiotic of MLs, has different accumulation ability in the liver and muscle. This explains why the log bioaccumulation factor (BAF) of erythromycin has a large span of values (Figure 2). In addition, the acid dissociation constant (pK_a) value plays a role in the accumulation of antibiotics in aquatic organisms. Antibiotics that have diverse pK_a can exist as zwitterion in natural environments with neutral pH values.[51] The pK_a values of enrofloxacin were 3.85, 6.19, 7.59, and 9.86, which probably explains why enrofloxacin has a great ability of bioaccumulation.[44] Chloramphenicol is the most representative CAP, with serious toxic effects. It has been widely prohibited in animal production in many countries, including China, the European Union, etc. but can still be detected frequently in certain areas.[3,52] Thiamphenicol and florfenicol have been used extensively as alternatives to chloramphenicol, and in particular, florfenicol is authorized in many farming countries.[16,52] It is worth noting that aminoglycosides and β-lactams are also commonly used in aquaculture but less commonly found in aquatic products.

Species and Tissues. Fish (e.g., grass carp, silver carp, and Nile tilapia), crustaceans (e.g., white leg shrimp), and bivalves (e.g., oysters and mussels) are the major aquaculture objects. The residual concentrations and detection rates of antibiotics in cultured aquatic products vary among species and tissues.[41] The accumulation of antibiotics in aquaculture organisms is mainly through absorption via skin, gills from water, and ingestion of contaminated food.[48] Consequently, species-specific bioaccumulation of antibiotics is related to trophic levels, habitats, and feeding habits.[59] Concentrations of antibiotics in carnivorous fish (e.g., eel, bass, and snakehead fish) are generally higher than in the herbivorous fish (e.g., grass carp) or omnivorous fish (e.g., red drum and tilapia).[48] Carnivorous fish is at the top of the aquatic food chain and has amplification to the environmental pollutants.[60] Carnivorous fish is often fed with the iced fresh fish feed and fish feed pellets, which may have been contaminated, and then causes antibiotic accumulation via secondary exposure.[8,48,61] As an example, the concentrations of SAs in the predatory fish bass from the Pearl River Delta (140.5 ± 12.5 μg/kg) were significantly higher than those in the herbivorous fish grass carp (82.0 μg/kg) and the omnivorous fish tilapia (38.0 μg/kg).[48] Other than that, the antibiotic residue in the sediment is probably another route leading to bioaccumulation in the cultured fish. Benthic fish (e.g., eel) is susceptible to residual antibiotics in the sediment, resulting in high antibiotic concentrations.[8] Furthermore, the content of antibiotic residues in oysters or mussels is usually less than that of the fish and shrimps. Oysters and mussels need to ingest natural

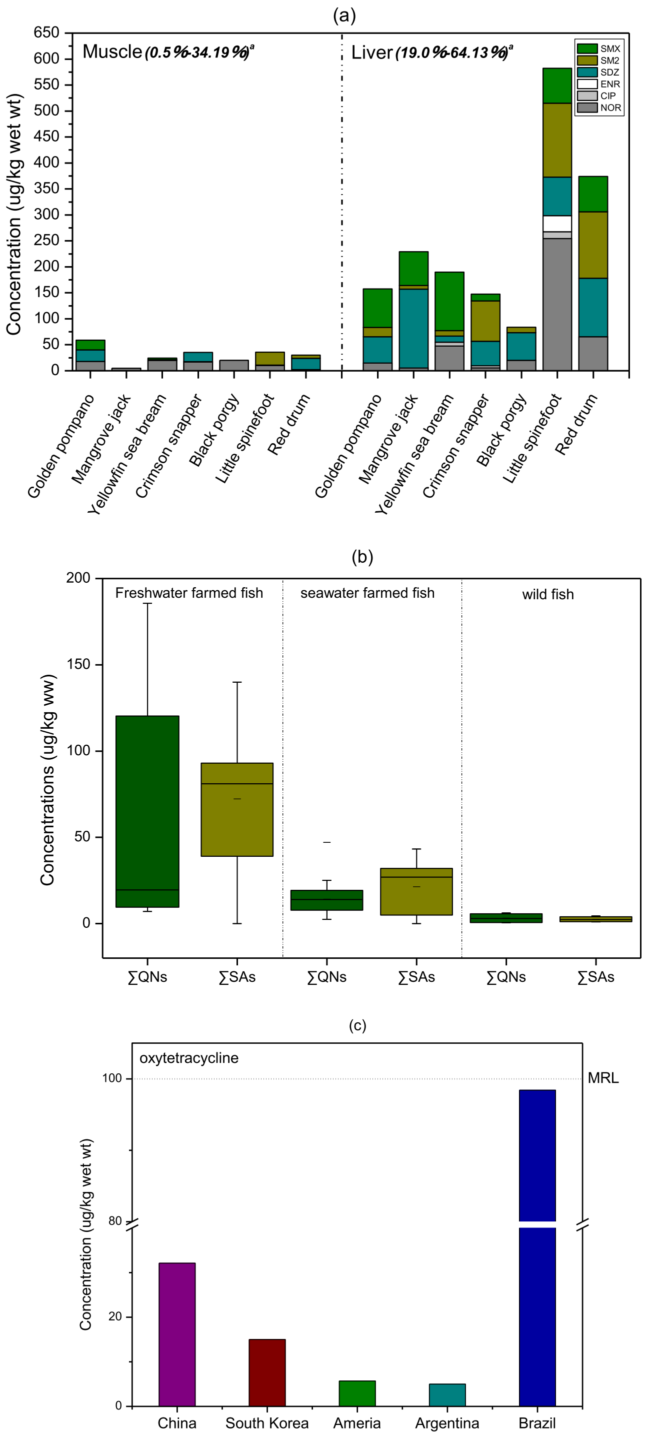


Figure 3. (a) Residues of QNs and SAs in muscle and liver of marine aquaculture products of Guangdong Province, South China.[8,61] Lipid content of muscle and liver (because each individual has a different lipid content, here are just *Takifugu flavidus* and *Pampus cinereus* as examples).[91,92] (b) Comparison of ∑QNs (enrofloxacin, norfloxacin, and ciprofloxacin) and SAs (sulfadiazine, sulfamethoxazole, sulfamethazine, and sulfadimidine).[5,6,8,44,48,50,53,57,58,61,68] (c) Mean concentration of oxytetracycline (top antibiotic in aquaculture) from China, South Korea, U.S.A., Argentina, and Brazil.[53,93−96]

phytoplankton and are usually cultured in open estuaries or shallow seas, while the fish and shrimps live on the artificial feed and are more often raised in relatively closed ponds.[11,53] Besides, a study had shown that the blue mussel had low bioconcentration potential (their log K_{ow} of <2) for most antibiotics, such as β-lactams, sulfonamides, and tetracyclines.[62]

The residual levels are also associated with the growth stage and the body size of cultured organisms; for example, the fish size influenced the depletion rate of TCs.[53,61] In addition, a study found that the lighter and younger fish had higher drug metabolism than the bigger and older fish, showing that the time of accumulation is another significant factor (e.g., older fish).[63] To date, no evidence has shown a significant difference between males and females in the uptake of the given drugs.[64]

The lipid-rich tissues, such as liver, kidney, and bile, are inclined to accumulate lipophilic antibiotics with high K_{ow} values, such as MLs and enrofloxacin, compared to muscles.[44,50,61] In particular, the liver, an important detoxification tissue and digestive tissue with rich phospholipids, has the capability to accumulate antibiotics in the metabolic processes of fish.[11,44] High levels of antibiotics were also found in fish gills.[13,50] This is probably due to the reabsorption of the antibiotics excreted in the feces into the fish via gills.[13] The same is true for the other farmed organisms. For example, the antibiotic concentration in crab roe was higher than that in the leg muscle and pereion muscle, because the crab roe has high lipids and its one component hepatopancreas is a crucial digestive tissue in crab.[11] The residual levels of antibiotics in the head of prawn were also generally higher than those in the muscle, because the hepatopancreas in the head can remain with higher residues over a long period of time in comparison to the muscle.[64] Trimethoprim and SAs tend to accumulate in muscle that has a low accumulative potential.[44,50,53] Therefore, the different accumulative potential plus large lipid content make the residual concentrations of livers generally higher than those of muscles (Figure 3a).[8,50,61] Besides, the liver is often used as off-cuts in the feed production, leading to the risk of secondary exposure, even if it is rarely consumed directly by humans.[8] However, the time of administration, dosage, and external conditions can explain the few cases that the muscle concentrations are greater than the liver, bile, and other tissues.[8]

Farming Models. The types of aquaculture farming models can lead to different levels of antibiotic residues in aquatic products. In general, freshwater organisms are supposed to be more polluted than the seawater organisms as a result of the self-purification capacity of the aquatic environment (Figure 3b).[7,48] Marine aquaculture is a relatively open system, which is conducive to the dilution and diffusion of antibiotics. In contrast, freshwater aquaculture has poor water exchanges and high stocking density.[48] In addition, the cultured aquatic organisms exporting to the developed countries have lower residual concentrations than those for local markets,[7,12,65] because the exports are subject to stricter regulations.[28] In the case of Vietnam, 555 and 5555 g/ton of antibiotics of fish and lobster, respectively, were used in the domestic markets,[65] while 93 g/ton for *Pangasius* and 1.4 g/ton for shrimp were intended for export from Vietnam.[28] Moreover, stereoscopic culture models (e.g., swine−chicken−geese−fish model) provide possible antibiotic pollution from non-aquaculture sources, because they are easily affected by the antibiotic cross-contamination. Stereoscopic culture is considered as an economic tillage method, and animal manure (e.g., pig and chicken) is used to promote the growth of fish and maintain the fertility in the fish ponds.[66] However, Sarmah et al. discovered that 30−90% of antibiotics were unalterably

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excreted in the feces and urine of animals.[67] A study of typical cultures in Guangdong Province, China, calculated the daily antibiotic excretion of nearby cultured animals: 0.76, 35, and 151 μg/day for chicken, geese, and pigs, respectively.[44]

It is noteworthy that an aquatic environment is easily polluted by the antibiotics from various sources. Therefore, antibiotic residues were even detected in some wild aquatic organisms (Figure 3b).[6,50,57] The antibiotic pollution primarily came from the aquaculture, livestock manure, sewage discharge, and pharmaceutical effluent.[11,6,57] At present, there are relatively fewer studies on antibiotic contamination in the wild fish than those in cultured fish. It implies that more attention should be paid to the wild fish products in the future.

HUMAN HEALTH RISK OF ANTIBIOTICS VIA CONSUMPTION OF CULTURED AQUATIC PRODUCTS

There are some guidelines issued by different countries and national organizations to evaluate the risk in cultured aquatic products of human health. In general, the consumption assessment based on the estimated daily intake (EDI) is popularly used. EDI is expressed as the percentages of acceptable daily intake (ADI) values that are recommended by the Food and Agriculture Organization of the United Nations and World Health Organization (FAO/WHO). Also, the body weight of the consumer used to calculate EDI is usually assumed to be 60 kg by the Codex Alimentarius Commission and WHO.[53,70] Established according to ADI, MRL is also applied to evaluate the chronic effects.[61] Most studies indicated that the antibiotic residue levels were legal in cultured products and wildlife, and these products pose no or low risk to human health.[8,11,44,48,61] Nevertheless, extreme cases still existed: products were not suitable for consumption in certain areas or species. For examples, concentrations of erythromycin in adult *Fenneropenaeus penicillatus* ranged from 2498 to 15 090 μg/kg on Hailing Island in South China.[53] The levels of oxytetracycline in muscles of Brazilian tilapia ranged from 15.6 to 1231.8 μg/kg.[49] The residue of sulfamethoxazole in a common eel from Korea was up to 5140 μg/kg.[55] An extreme case was that antibiotic residues of 17.3% fish samples exceeded MRLs in Turkey.[69] The reason why the highly contaminated products emerged frequently is likely the overspend of antibiotics and non-compliance with withdrawal periods. The withdrawal period is a time when the residue levels fall below the MRLs,[10] and 3 weeks or longer is generally considered the appropriate time span to prevent the short-term toxicological risk of antibiotics.[70]

To our best knowledge, most studies assessed the human health risk associated with the consumption of antibiotic-contaminated aquatic products by comparing the concentrations of antibiotics in the products to MRLs. However, only a few studies have thus far estimated the local daily intakes of antibiotics through the consumption of aquatic products to compare to ADIs. This phenomenon may detract from the value of studies, because, in different countries or regions, different groups of people on the consumption of aquatic products vary significantly. Additionally, there is a study that has suggested chronic exposure to legal aquaculture doses and low environment concentrations of antibiotics can also provoke health risk.[71] Moreover, the risk of antibiotic resistance is not fully considered in human health risk assessment of aquaculture food when exposed at sub-inhibitory concentrations.[3] Therefore, the safety of aquatic products still requires further attention and more rigorous risk assessment.

Allergy and Toxicity. Excessive use of antibiotics can lead to antibiotic residues in the fish and shellfish, which can cause allergies and toxicity for consumers through consumption.[10] In addition, antibiotics can come into direct contact with the skin, respiratory tracts, and intestines of those who apply antibiotics or feed containing antibiotics without precautions as well as those who transport and sell the aquatic products, causing allergies and poisoning. Most antibiotics (e.g., TCs, SAs, and penicillin G) are antigenic. The consumption of these contaminated products may contribute to the allergic symptoms.[72] Another adverse impact is the toxicity, including carcinogenicity, mutagenicity, teratogenicity, bone marrow suppression, and destruction of normal intestinal flora.[3,10] For example, exposure to chloramphenicol may increase the incidence of aplastic anemia and agranulocytosis in humans.[73] Long-term exposure to antibiotics can bring about steatosis by altering genes related to lipid metabolism and transportation.[74] FQs are a special class of antibiotics that can inhibit DNA gyrase, a key enzyme in DNA replication.[75] SAs that enter human bodies through consumption would destroy the human hematopoietic system and, consequentially, cause hemolytic anemia.[8] Some SAs, such as sulfadimidine, were carcinogenic.[8] Maternal use and use by children of cephalosporins, sulphonamides and trimethoprim, macrolides, and amoxicillin may increase the risk of asthma in children, and the class of cephalosporins has the strongest association with asthma.[76]

Antibiotic Resistance. The occurrence of antibiotic-resistant bacteria (ARB) and antibiotic-resistant genes (ARGs) is another way that antibiotics create a public health hazard, because antibiotic resistance can transfer from the aquatic organisms to humans. The fish pathogens and other aquatic bacteria exposure to antibiotics can develop ARGs, through mechanisms including efflux pump mechanism, target modifications, production of enzymes, and changes in cell permeability.[77] The development of different classes of antibiotic resistance is presented by Preena et al.[15] Recently, Brunton et al. also applied a system-thinking approach to identify the two hotspots for emergence and selection of antibiotic resistance and thought that the ARB and AGRs may have selected and enriched in the early and grow-out phases of aquatic production.[78]

Antibiotic resistance is transferred to humans in two ways: indirect and direct.[4] It was thought that ARGs indirectly disseminate to human pathogens. There are three significant gene transfer systems: the bacterial conjugative plasmids (the extrachromosomal mobile genetic element comprises of various genes that can confer resistance[79]), the transposable elements, and the integron systems.[15] For example, multi-drug-resistant plasmids are transferable to *Escherichia coli* from *Aeromonas salmonicida*, *Aeromonas hydrophila*, *Edwardsiella tarda*, *Citrobacter freundii*, *Photobacterium damselae*, *Vibrio anguillarum*, and *Vibrio salmonicida*.[80] Antibiotic-resistant pathogens can be directly transmitted to the human bodies. The zoonotic pathogen plays an important role in this transmission. Gram-negative bacteria, such as *Aeromonas*, *Vibrio*, *Edwardsiella*, *Salmonella*, and *Mycobacterium*, and Gram-positive bacteria, such as *Streptococcus* and *Straphylococcus*, are zoonotically significant fish pathogens.[15] Among them, *Vibrio* has the biggest percentage distribution of fish pathogens exhibiting antibiotic resistance and accounts for 23%, followed by *Aeromonas* and Enterobacteriaceae (20

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and 10%, respectively). The multi-drug resistance of these pathogens is widely reported.[15]

The consequences for humans of transferring antibiotic resistance from aquaculture have been reviewed by Kruse et al.[81] One consequence is the increased number of infections: antibiotic agents may interfere with the microflora of the human intestinal tract, and thus, individuals taking an antibiotic agent are at increased infectious risks as a result of the fact that pathogens are resistant to the antibiotic agents. Another is the increased frequency of treatment failure and the increased severity of infection because antibiotic resistance probably causes prolonged duration of illness, increased frequency of bloodstream infection, increased hospitalization, or increased mortality.

■ FUTURE PERSPECTIVES

The main reason why the problem of antibiotics in aquaculture has become an imminent booming public health crisis is that the occurrence of antibiotic resistance has caused serious burden to the human medical service and human health security. Therefore, as far as we can tell, alleviating the problem of antibiotic resistance is still the focus and hotspot in the near future.

Andersson and Hughes suggested that, under the condition of reducing the selective pressure of antibiotics, susceptible bacteria theoretically could outcompete resistant bacteria but the rate of this reversibility is too low or even non-existant.[82] Because the reversibility is difficult to achieve, reducing the likelihood of antibiotic resistance in the future is a significant task. Decreasing the usage of antibiotic agents is the most direct and effective way to bring down the development of ARGs: (1) Fish vaccines[83] and alternatives to antibiotics ought to be more explored and used on a large scale in the future. Nowadays, there are many studies that have found alternatives to antibiotics, e.g., functional feed additives,[84] probiotic bacteria,[83,84] phages, etc.[85] (2) A complete ban on antibiotics as the growth-promoting or prophylactic agents is strongly recommended, and antibiotics that are widely used in human medicine should also be avoided in aquaculture.[4] Using as growth promoters and prophylactic agents is a typical manifestation of antibiotic abuse; however, it is still permitted in many developing countries.

Antibiotic resistance is a big threat to the world,; therefore, a proper multi-stage monitoring of antibiotic resistant pathogens and the collection of ARGs are inevitable. A recently developed multi-residue analysis method, called "QuEChERSER", i.e., more than QuEChERS, would be useful for antibiotic monitoring.[86] In addition, there is an urgent need to take new therapeutic strategies in the future, e.g., chemical inhibitors of antibiotic resistance mechanisms.[77] Chemical inhibitors may include enzymatic inhibitors, efflux pump inhibitors, inhibitors of bacterial biofilm formation, and other molecules targeting multi-drug resistance mechanisms. Such inhibitors may serve as add-on treatments for antibiotic-resistant infections and hold promise for the treatment of resistant infections and even multi-drug-resistant infections.

Besides, the aquaculture environment deserves our attention. Sanitary shortcomings in fish rearing are at the root of the misuse of antibiotics, contributing to the high incidence of fish diseases.[87] Providing good management and environmental hygiene in aquaculture is the way to tackle the root cause. Particularly, the culture of tropical fish is more severe on the sanitary conditions of the aquaculture environment, needing to change the water in time to ensure water quality.[30,87] In addition, sewage treatment of aquaculture can effectively reduce the abundance of ARGs and avoid the transmission to other environments.[88] However, water bodies entering the aquaculture environment have no specific treatment measures. A study on sources of antibiotics in the aquatic environment showed that hospital sewage accounted for a large proportion.[89] If such a water body is introduced into the aquaculture environment, it will aggravate antibiotic pollution to aquaculture. Thus, greater treatments about water introduced to aquaculture are expected.

As for risks of antibiotic residues, there are two major research needs in the future: (1) Because the regulations and enforcement about antibiotics vary from country to country, the number and residues of antibiotics used are difficult to come by, even the maximum residue limits and acceptable daily intake are not uniform. The lack of information is a problem that cannot wait to be solved. Seeking international cooperation and jointly establishing an information bank of antibiotic residues and antibiotic-resistant genes are a way of obtaining twice the result with half of the effort. (2) There is an urgent need to derive the equation between antibiotic residue levels and pathogenic antibiotic resistance development in different settings and establish a quantitative model to appropriately assess the risk of antibiotic resistance associated with antibiotic residues.[90]

What makes antibiotics special compared to other compounds is that they are so important in the human medical treatment. The abuse of antibiotics in aquaculture will unconsciously accelerate the emergence and spread of antibiotic resistance, which is one of the great obstacles to human health care in the future. However, antibiotics also face the same problems as other compounds that need to be addressed. Recently, studies focused more on ecological effects and environmental toxicity, while further studies on human health risk are in high demand.[71] The human risk assessment standards are relatively few, because of the lack of independent standards for different age groups and sensitive groups, such as children, the elderly, and pregnant women. These populations differ from the adult male in the sensitivity to antibiotics through consumption of cultured aquatic products. It is known, for instance, that children are vulnerable to toxic burdens from xenobiotics.[71] The ADI should not only be limited to individual antibiotics but also to multiple antibiotics, particularly those which antibiotics have the same mode of action. Some antibiotics have synergistic effects, and the co-existence of multiple antibiotics may pose a greater risk. The background information on antibiotic drugs in the consumption of cultured aquatic products, therefore, requires establishing a detailed and strict risk assessment framework.

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Notes

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REFERENCES

(1) Food and Agriculture Organization of the United Nations (FAO). *The State of World Fisheries and Aquaculture 2018—Meeting the Sustainable Development Goals*; FAO: Rome, Italy, 2018; ISBN: 978-92-5-130562-1.

(2) Santos, L.; Soares, B.; Rosa, J.; Freitas, A.; Leston, S.; Barbosa, J.; Ramos, F. Detection and quantification of 41 antibiotic residues' in Gilthead sea bream (*Sparus aurata*) from aquaculture origin, using a multiclass and multi-residue UHPLC-MS/MS method. *Toxicol. Lett.* **2015**, *238*, S85.

(3) Liu, X.; Steele, J.; Meng, X. Usage, residue, and human health risk of antibiotics in Chinese aquaculture: A review. *Environ. Pollut.* **2017**, *223*, 161−169.

(4) Heuer, O. E.; Kruse, H.; Grave, K.; Collignon, P.; Karunasagar, I.; Angulo, F. J. Human health consequences of use of antimicrobial agents in aquaculture. *Clin. Infect. Dis.* **2009**, *49*, 1248−1253.

(5) Song, C.; Zhang, C.; Kamira, B.; Qiu, L.; Fan, L.; Wu, W.; Meng, S.; Hu, G.; Chen, J. Occurrence and human dietary assessment of fluoroquinolones antibiotics in cultured fish around Tai Lake, China. *Environ. Toxicol. Chem.* **2017**, *36*, 2899−2905.

(6) Li, W.; Shi, Y.; Gao, L.; Liu, J.; Cai, Y. Occurrence of antibiotics in water, sediments, aquatic plants, and animals from Baiyangdian Lake in north China. *Chemosphere* **2012**, *89*, 1307−1315.

(7) Hossain, A.; Nakamichi, S.; Habibullah-Al-Mamun, M.; Tani, K.; Masunaga, S.; Matsuda, H. Occurrence, distribution, ecological and resistance risks of antibiotics in surface water of finfish and shellfish aquaculture in Bangladesh. *Chemosphere* **2017**, *188*, 329−336.

(8) He, X.; Wang, Q.; Nie, X.; Yang, Y.; Cheng, Z. Residues and health risk assessment of sulfonamides in sediment and fish from typical marine aquaculture regions of Guangdong Province, China. *Environ. Sci.* **2014**, *35*, 2728−2735 (in Chinese).

(9) Zou, S.; Xu, W.; Zhang, R.; Tang, J.; Chen, Y.; Zhang, G. Occurrence and distribution of antibiotics in coastal water of the Bohai Bay, China: Impacts of river discharge and aquaculture activities. *Environ. Pollut.* **2011**, *159*, 2913−2920.

(10) Okocha, R. C.; Olatoye, I. O.; Adedeji, O. B. Food safety impacts of antimicrobial use and their residues in aquaculture. *Public. Health. Rev.* **2018**, *39*, 21.

(11) Zhang, R.; Pei, J.; Zhang, R.; Wang, S.; Zeng, W.; Huang, D.; Wang, Y.; Zhang, Y.; Wang, Y.; Yu, K. Occurrence and distribution of antibiotics in mariculture farms, estuaries and the coast of the Beibu Gulf, China: Bioconcentration and diet safety of seafood. *Ecotoxicol. Environ. Saf.* **2018**, *154*, 27−35.

(12) Uchida, K.; Konishi, Y.; Harada, K.; Okihashi, M.; Yamaguchi, T.; Do, M. H. N.; Thi Bui, L.; Duc Nguyen, T.; Do Nguyen, P.; Thi Khong, D.; Thi Tran, H.; Nam Nguyen, T.; Viet Le, H.; Van Chau, V.; Thi Van Dao, K.; Thi Ngoc Nguyen, H.; Kajimura, K.; Kumeda, Y.; Tran Pham, K.; Ngoc Pham, K.; Trong Bui, C.; Quang Vien, M.; Hoang Le, N.; Van Dang, C.; Hirata, K.; Yamamoto, Y. Monitoring of Antibiotic Residues in Aquatic Products in Urban and Rural Areas of Vietnam. *J. Agric. Food Chem.* **2016**, *64*, 6133−6138.

(13) Xu, N.; Li, M.; Fu, Y.; Zhang, X.; Ai, X.; Lin, Z. Tissue residue depletion kinetics and withdrawal time estimation of doxycycline in grass carp, Ctenopharyngodon idella, following multiple oral administrations. *Food Chem. Toxicol.* **2019**, *131*, 110592.

(14) Zhang, H.; Zhang, J.; Zhu, Y. Identification of Microcystins in waters used for Daiky life by people who live on tai lake during a serious cyanobacteria dominated bloom with risk analysis to human health. *Environ. Toxicol.* **2009**, *24*, 82−86.

(15) Preena, P. G.; Swaminathan, T. R.; Kumar, V. J. R.; Singh, I. S. B. Antimicrobial resistance in aquaculture: A crisis for concern. *Biologia* **2020**, *75*, 1497−1517.

(16) Lulijwa, R.; Rupia, E. J.; Alfaro, A. C. Antibiotic use in aquaculture, policies and regulation, health and environmental risks: A review of the top 15 major producers. *Rev. Aquacult.* **2020**, *12*, 640−663.

(17) Collignon, P. C.; Conly, J. M.; Andremont, A.; McEwen, S. A.; Aidara-Kane, A. World Health Organization Ranking of Antimicrobials According to Their Importance in Human Medicine: A Critical Step for Developing Risk Management Strategies to Control Antimicrobial Resistance From Food Animal Production. *Clin. Infect. Dis.* **2016**, *63*, 1087−1093.

(18) Cabello, F. C.; Godfrey, H. P.; Tomova, A.; Ivanova, L.; Dölz, H.; Millanao, A.; Buschmann, A. H. Antimicrobial use in aquaculture re-examined: Its relevance to antimicrobial resistance and to animal and human health. *Environ. Microbiol.* **2013**, *15*, 1917−1942.

(19) Adams, A.; Aoki, T.; Berthe, F. C. J.; Grisez, L.; Karunasagar, I. Recent technological advancements on aquatic animal health and their contributions towards reducing disease risks—A review. *Proceedings of the Sixth Symposium on Diseases in Asian Aquaculture*; Fish Health Section, Asian Fisheries Society: Manila, Philippines, 2005; pp 71−88.

(20) Ministry of Agriculture and Rural Development (MARD). *List of Drugs, Chemicals and Antibiotics of Banned or Limited Use for Aquaculture and Veterinary Purposes*; MARD: Hanoi, Vietnam, 2014.

(21) Chuah, L. O.; Effarizah, M. E.; Goni, A. M.; Rusul, G. Antibiotic Application and Emergence of Multiple Antibiotic Resistance (MAR) in Global Catfish Aquaculture. *Curr. Environ. Health Rep.* **2016**, 3, 118−127.

(22) Zhang, Z. P. Antibiotics and antimicrobials phylogeny. *Cap. Med.* **2004**, 2, 14−16 (in Chinese).

(23) Samanidou, V. F.; Evaggelopoulou, E. N. Analytical strategies to determine antibiotic residues in fish. *J. Sep. Sci.* **2007**, *30*, 2549−2569.

(24) Burridge, L.; Weis, J. S.; Cabello, F.; Pizarro, J.; Bostick, K. Chemical use in salmon aquaculture: A review of current practices and possible environmental effects. *Aquaculture* **2010**, *306*, 7−23.

(25) Chung, J. K. *The Research of the Antibiotics Reduction in Aquarium and the Environmental Influence of Parasiticide*; Ministry of Food, Agriculture, Forestry and Fisheries & Pukyong National University Press: Busan, South Korea, 2008.

(26) Henriksson, P. J. G.; Rico, A.; Troell, M.; Klinger, D. H.; Buschmann, A. H.; Saksida, S.; Chadag, M. V.; Zhang, W. Unpacking factors influencing antimicrobial use in global aquaculture and their

implication for management: A review from a systems perspective. *Sustainability Sci.* **2018**, *13*, 1105−1120.
(27) Holmström, K.; Gräslund, S.; Wahlström, A.; Poungshompoo, S.; Bengtsson, B. E.; Kautsky, N. Antibiotic use in shrimp farming and implications for environmental impacts and human health. *Int. J. Food Sci. Technol.* **2003**, *38*, 255−266.
(28) Rico, A.; Phu, T. M.; Satapornvanit, K.; Min, J.; Shahabuddin, A. M.; Henriksson, P. J. G.; Murray, F. J.; Little, D. C.; Dalsgaard, A.; van den Brink, P. J. Use of veterinary medicines, feed additives and probiotics in four major internationally traded aquaculture species farmed in Asia. *Aquaculture* **2013**, *412*, 231−243.
(29) Morrison, D.; Saksida, S. Trends in antimicrobial use in Marine Harvest Canada farmed salmon production in British Columbia (2003−2011). *Can. Vet. J.* **2013**, *54*, 1160−1163.
(30) Nguyen Dang Giang, C.; Sebesvari, Z.; Renaud, F.; Rosendahl, I.; Hoang Minh, Q.; Amelung, W. Occurrence and Dissipation of the Antibiotics Sulfamethoxazole, Sulfadiazine, Trimethoprim, and Enrofloxacin in the Mekong Delta, Vietnam. *PLoS One* **2015**, *10*, e0131855.
(31) European Commission (EC).. Council Regulation 2377/90/EC. *Off. J. Eur. Union* **1990**, *33*, 1−8 (consolidated version of the Annexes I to IV updated up to 20.01.2008 obtained from www.emea.eu.int).
(32) Minister of Agriculture and Rural Development (MARD). *List of Veterinary Drugs Banned from Use (Promulgated Together with Circular No. 2016/TT-BNN Dated 2016 of the Minister of Agriculture and Rural Development) (Department of Animal Health MoAaRD)*; MARD: Hanoi, Vietnam, 2016.
(33) Ministry of Agriculture of the People's Republic of China (MAPRC). *Catalogue of Veterinary Prescription Drug Varieties (First Batch)*; MAPRC: Beijing, China, 2013; Bulletin 1997.
(34) Braun, G.; Braun, M.; Kruse, J.; Amelung, W.; Renaud, F. G.; Khoi, C. M.; Duong, M. V.; Sebesvari, Z. Pesticides and antibiotics in permanent rice, alternating rice-shrimp and permanent shrimp systems of the coastal Mekong Delta, Vietnam. *Environ. Int.* **2019**, *127*, 442−451.
(35) Strom, G. H.; Bjorklund, H.; Barnes, A. C.; Da, C. T.; Nhi, N. H. Y.; Lan, T. T.; Magnusson, U.; Norman Halden, A.; Boqvist, S. Antibiotic use by small-scale farmers in freshwater aquaculture in the upper Mekong Delta, Vietnam. *J. Aquat. Anim. Health* **2019**, *31*, 290−298.
(36) Hossain, A.; Nakamichi, S.; Habibullah-Al-Mamun, M.; Tani, K.; Masunaga, S.; Matsuda, H. Occurrence, distribution, ecological and resistance risks of antibiotics in surface water of finfish and shellfish aquaculture in Bangladesh. *Chemosphere* **2017**, *188*, 329−336.
(37) Santos, L.; Ramos, F. Analytical strategies for the detection and quantification of antibiotic residues in aquaculture fishes: A review. *Trends Food Sci. Technol.* **2016**, *52*, 16−30.
(38) Ali, H.; Rico, A.; Murshed-e-Jahan, K.; Belton, B. An assessment of chemical and biological product use in aquaculture in Bangladesh. *Aquaculture* **2016**, *454*, 199−20.
(39) *Pharmaceuticals in Aquaculture Practices*; https://www.maff.go.jp/j/syouan/suisan/suisan_yobo/attach/pdf/fishmed-3.pdf.
(40) Kümmerer, K. Antibiotics in the aquatic environment—a review—Part I. *Chemosphere* **2009**, *75*, 417−434.
(41) Chen, J.; Ying, G. G.; Deng, W. J. Antibiotic Residues in Food: Extraction, Analysis, and Human Health Concerns. *J. Agric. Food Chem.* **2019**, *67*, 7569−7586.
(42) Binh, V. N.; Dang, N.; Anh, N. T. K.; Ky, L. X.; Thai, P. K. Antibiotics in the aquatic environment of Vietnam: Sources, concentrations, risk and control strategy. *Chemosphere* **2018**, *197*, 438−450.
(43) Tang, Y. J.; Xu, J. Q.; Chen, L.; Qiu, J. L.; Liu, Y.; Ouyang, G.-F. Rapid in vivo determination of fluoroquinolones in cultured puffer fish (*Takifugu obscurus*) muscle by solid-phase microextraction coupled with liquid chromatography-tandem mass spectrometry. *Talanta* **2017**, *175*, 550−556.
(44) Chen, H.; Liu, S.; Xu, X. R.; Diao, Z. H.; Sun, K. F.; Hao, Q. W.; Liu, S. S.; Ying, G. G. Tissue distribution, bioaccumulation characteristics and health risk of antibiotics in cultured fish from a typical aquaculture area. *J. Hazard. Mater.* **2018**, *343*, 140−148.
(45) Holten Lutzhøft, H.-C.; Vaes, W. H. J.; Freidig, A. P.; Halling-Sørensen, B.; Hermens, J. L. M. Influence of pH and other modifying factors on the distribution behavior of 4-quinolones to solid phases and humic acids studied by "negligible-depletion" SPME-HPLC. *Environ. Sci. Technol.* **2000**, *34*, 4989−4994.
(46) Nunes, K. S. D.; Vallim, J. H.; Assalin, M. R.; Queiroz, S. C. N.; Paraíba, L. C.; Jonsson, C. M.; Reyes, F. G. R. Depletion study, withdrawal period calculation and bioaccumulation of sulfamethazine in tilapia (*Oreochromis niloticus*) treated with medicated feed. *Chemosphere* **2018**, *197*, 89−95.
(47) Liu, X.; Lv, K.; Deng, C.; Yu, Z.; Shi, J.; Johnson, A. C. Persistence and migration of tetracycline, sulfonamide, fluoroquinolone, and macrolide antibiotics in streams using a simulated hydrodynamic system. *Environ. Pollut.* **2019**, *252*, 1532−1538.
(48) He, X.; Deng, M.; Wang, Q.; Yang, Y.; Yang, Y.; Nie, X. Residues and health risk assessment of quinolones and sulfonamides in cultured fish from Pearl River Delta, China. *Aquaculture* **2016**, *458*, 38−46.
(49) Monteiro, S. H.; Francisco, J. G.; Campion, T. F.; Pimpinato, R. F.; Moura Andrade, G. C. R.; Garcia, F.; Tornisielo, V. L. Multiresidue antimicrobial determination in Nile tilapia (*Oreochromis niloticus*) cage farming by liquid chromatography tandem mass spectrometry. *Aquaculture* **2015**, *447*, 37−43.
(50) Liu, S.; Bekele, T. G.; Zhao, H.; Cai, X.; Chen, J. Bioaccumulation and tissue distribution of antibiotics in wild marine fish from Laizhou Bay, North China. *Sci. Total Environ.* **2018**, *631−632*, 1398−1405.
(51) Erickson, R. J.; McKim, J. M.; Lien, G. J.; Hoffman, A. D.; Batterman, S. L. Uptake and elimination of ionizable organic chemicals at fish gills: II. Observed and predicted effects of pH, alkalinity, and chemical properties. *Environ. Toxicol. Chem.* **2006**, *25*, 1522−1532.
(52) Cañada-Cañada, F.; Muñoz de la Peña, A.; Espinosa-Mansilla, A. Analysis of antibiotics in fish samples. *Anal. Bioanal. Chem.* **2009**, *395*, 987−1008.
(53) Chen, H.; Liu, S.; Xu, X. R.; Liu, S. S.; Zhou, G. J.; Sun, K. Y.; Zhao, J. L.; Ying, G. G. Antibiotics in typical marine aquaculture farms surrounding Hailing Island, South China: Occurrence, bioaccumulation and human dietary exposure. *Mar. Pollut. Bull.* **2015**, *90*, 181−187.
(54) Song, C.; Li, L.; Zhang, C.; Qiu, L.; Fan, L.; Wu, W.; Meng, S. L.; Hu, G. D.; Chen, J. Z.; Liu, Y.; Mao, A. M. Dietary risk ranking for residual antibiotics in cultured aquatic products around Tai Lake, China. *Ecotoxicol. Environ. Saf.* **2017**, *144*, 252−257.
(55) Won, S. Y.; Lee, C. H.; Chang, H. S.; Kim, S. O.; Lee, S. H.; Kim, D. S. Monitoring of 14 sulfonamide antibiotic residues in marine products using HPLC-PDA and LC-MS/MS. *Food Control* **2011**, *22*, 1101−1107.
(56) Swapna, K. M.; Rajesh, R.; Lakshmanan, P. T. Incidence of antibiotic residues in farmed shrimps from the southern states of India. *Indian J. Mar Sci.* **2012**, *41*, 344−347.
(57) Zhao, J. L.; Liu, Y. S.; Liu, W. R.; Jiang, Y. X.; Su, H. C.; Zhang, Q. Q.; Chen, X. W.; Yang, Y. Y.; Chen, J.; Liu, S. S.; Pan, C. G.; Huang, G. Y.; Ying, G. G. Tissue−specific bioaccumulation of human and veterinary antibiotics in bile, plasma, liver and muscle tissues of wild fish from a highly urbanized region. *Environ. Pollut.* **2015**, *198*, 15−24.
(58) Na, G. S.; Fang, X. D.; Cai, Y. Q.; Ge, L. K.; Zong, H. M.; Yuan, X. T.; Yao, Z. W.; Zhang, Z. F. Occurrence, distribution, and bioaccumulation of antibiotics in coastal environment of Dalian, China. *Mar. Pollut. Bull.* **2013**, *69*, 233−237.
(59) Fisher, S. W. Mechanisms of Bioaccumulation in Aquatic Systems. *Rev. Environ. Contam. Toxicol.* **1995**, *142*, 87−117.

(60) Boonsaner, M.; Hawker, D. W. Evaluation of food chain transfer of the antibiotic oxytetracycline and human risk assessment. *Chemosphere* **2013**, *93*, 1009−1014.
(61) He, X.; Wang, Z.; Nie, X.; Yang, Y.; Pan, D.; Leung, A. O. W.; Cheng, Z.; Yang, Y. T.; Li, K. B.; Chen, K. Residues of fluoroquinolones in marine aquaculture environment of the Pearl River Delta, South China. *Environ. Geochem. Health* **2012**, *34*, 323−335.
(62) Le Bris, H.; Pouliquen, H. Experimental study on the bioaccumulation of oxytetracycline and oxolinic acid by the blue mussel (*Mytilus edulis*). An evaluation of its ability to bio-monitor antibiotics in the marine environment. *Mar. Pollut. Bull.* **2004**, *48*, 434−440.
(63) Paschoal, J. A.; Bicudo, A. J.; Cyrino, J. E.; Reyes, F. G.; Rath, S. Depletion study and estimation of the withdrawal period for oxytetracycline in tilapia cultured in Brazil. *J. Vet. Pharmacol. Ther.* **2012**, *35*, 90−96.
(64) Xu, N.; Li, M.; Fu, Y.; Zhang, X.; Ai, X.; Lin, Z. Tissue residue depletion kinetics and withdrawal time estimation of doxycycline in grass carp, *Ctenopharyngodon idella*, following multiple oral administrations. *Food Chem. Toxicol.* **2019**, *131*, 110592.
(65) Hedberg, N.; Stenson, I.; Nitz Pettersson, M.; Warshan, D.; Nguyen-Kim, H.; Tedengren, M.; Kautsky, N. Antibiotic use in Vietnamese fish and lobster sea cage farms; implications for coral reefs and human health. *Aquaculture* **2018**, *495*, 366−375.
(66) Dhawan, A.; Kaur, S. Pig dung as pond manure: Effect on water quality, pond productivity and growth of carps in polyculture system. *Naga ICLARM Q.* **2002**, *25*, 11−14.
(67) Sarmah, A. K.; Meyer, M. T.; Boxall, A. B. A. A global perspective on the use, sales, exposure pathways, occurrence, fate and effects of veterinary antibiotics (VAs) in the environment. *Chemosphere* **2006**, *65*, 725−759.
(68) García-Galán, M. J.; Díaz-Cruz, M. S.; Barceló, D. Occurrence of sulfonamide residues along the Ebro river basin: Removal in wastewater treatment plants and environmental impact assessment. *Environ. Int.* **2011**, *37*, 462−473.
(69) Yipel, M.; Kürekci, C.; Tekeli, İ. O.; Metli, M.; Sakin, F. Determination of selected antibiotics in farmed fish species using LC-MS/MS. *Aquacult. Res.* **2017**, *48*, 3829−3836.
(70) Sun, M.; Chang, Z.; Van den Brink, P. J.; Li, J.; Zhao, F.; Rico, A. Environmental and human health risks of antimicrobials used in *Fenneropenaeus chinensis* aquaculture production in China. *Environ. Sci. Pollut. Res.* **2016**, *23*, 15689−15702.
(71) Limbu, S. M.; Zhou, L.; Sun, S. X.; Zhang, M. L.; Du, Z. Y. Chronic exposure to low environmental concentrations and legal aquaculture doses of antibiotics cause systemic adverse effects in Nile tilapia and provoke differential human health risk. *Environ. Int.* **2018**, *115*, 205−219.
(72) Li, Z. J. Advantages and disadvantages and strategies of antibiotic application in aquaculture. *Guizhou Anim. Sci. Vet. Med.* **2008**, *32*, 23−24 (in Chinese).
(73) Malkin, D.; Koren, G.; Saunders, E. F. Drug-induced aplastic anemia: Pathogenesis and clinical aspects. *J. Pediatr. Hematol./Oncol.* **1990**, *12*, 402−410.
(74) Anthérieu, S.; Rogue, A.; Fromenty, B.; Guillouzo, A.; Robin, M. A. Induction of vesicular steatosis by amiodarone and tetracycline is associated with up-regulation of lipogenic genes in heparg cells. *Hepatology* **2011**, *53*, 1895−1905.
(75) Bogialli, S.; D'Ascenzo, G.; Di Corcia, A.; Laganà, A.; Nicolardi, S. A simple and rapid assay based on hot water extraction and liquid chromatography-tandem mass spectrometry for monitoring quinolone residues in bovine milk. *Food Chem.* **2008**, *108*, 354−360.
(76) Metsälä, J.; Lundqvist, A.; Virta, L. J.; Kaila, M.; Gissler, M.; Virtanen, S. M. Prenatal and post-natal exposure to antibiotics and risk of asthma in childhood. *Clin. Exp. Allergy* **2015**, *45*, 137−145.
(77) Schillaci, D.; Spanò, V.; Parrino, B.; Carbone, A.; Montalbano, A.; Barraja, P.; Diana, P.; Cirrincione, G.; Cascioferro, S. Pharmaceutical Approaches to Target Antibiotic Resistance Mechanisms. *J. Med. Chem.* **2017**, *60*, 8268−8297.
(78) Brunton, L. A.; Desbois, A. P.; Garza, M.; Wieland, B.; Mohan, C. V.; Hasler, B.; Tam, C. C.; Le, P. N. T.; Phuong, N. T.; Van, P. T.; Nguyen-Viet, H.; Eltholth, M. M.; Pham, D. K.; Duc, P. P.; Linh, N. T.; Rich, K. M.; Mateus, A. L.P.; Hoque, M. A.; Ahad, A.; Khan, M. N. A.; Adams, A.; Guitian, J. Identifying hotspots for antibiotic resistance emergence and selection, and elucidating pathways to human exposure: Application of a systems-thinking approach to aquaculture systems. *Sci. Total Environ.* **2019**, *687*, 1344−1356.
(79) Partridge, S. R.; Tsafnat, G.; Coiera, E.; Iredell, J. R. Gene cassettes and cassette arrays in mobile resistance integrons. *FEMS Microbiol Rev.* **2009**, *33*, 757−784.
(80) Sørum, H. Antimicrobial drug resistance in fish pathogens. In *Antimicrobial Resistance in Bacteria of Animal Origin*; Aarestrup, F, Ed.; ASM Press: Washington, D.C., 2006.
(81) Kruse, H.; Sørum, H. Transfer of multiresistance plasmids between bacteria of diverse origin in natural micro-environments. *Appl. Environ. Microbiol.* **1994**, *60*, 4015−4021.
(82) Andersson, D. I.; Hughes, D. Antibiotic resistance and its cost: Is it possible to reverse resistance? *Nat. Rev. Microbiol.* **2010**, *8*, 260−271.
(83) Newaj-Fyzul, A.; Austin, B. Probiotics, immunostimulants, plant products and oral vaccines, and their role as feed supplements in the control of bacterial fish diseases. *J. Fish Dis.* **2015**, *38*, 937−955.
(84) Dawood, M. A. O.; Koshio, S.; Esteban, M. Á. Beneficial roles of feed additives as immunostimulants in aquaculture: A review. *Rev. Aquacult.* **2018**, *10*, 950−974.
(85) Defoirdt, T.; Sorgeloos, P.; Bossier, P. Alternatives to antibiotics for the control of bacterial disease in aquaculture. *Curr. Opin. Microbiol.* **2011**, *14*, 251−258.
(86) Ninga, E.; Sapozhnikova, Y.; Lehotay, S. J.; Lightfield, A.; Monteiro, S. H. High-throughput mega-method for the analysis of pesticides, veterinary drugs, and environmental contaminants by UHPLC-MS/MS and robotic Mini-SPE Cleanup + LPGC-MS/MS, Part 2: Catfish. *J. Agric. Food Chem.* **2020**, DOI: 10.1021/acs.jafc.0c00995.
(87) Cabello, F. C. Heavy use of prophylactic antibiotics in aquaculture: A growing problem for human and animal health and for the environment. *Environ. Microbiol.* **2006**, *8*, 1137−1144.
(88) He, Y.; Yuan, Q.; Mathieu, J.; Stadler, L.; Senehi, N.; Sun, R.; Alvarez, P. J. J. Antibiotic resistance genes from livestock waste: Occurrence, dissemination, and treatment. *npj Clean Water* **2020**, *3*, 4.
(89) Binh, V. N.; Dang, N.; Anh, N. T. K.; Ky, L. X.; Thai, P. K. Antibiotics in the aquatic environment of Vietnam: Sources, concentrations, risk and control strategy. *Chemosphere* **2018**, *197*, 438−450.
(90) Ben, Y.; Fu, C.; Hu, M.; Liu, L.; Wong, M. H.; Zheng, C. Human health risk assessment of antibiotic resistance associated with antibiotic residues in the environment: A review. *Environ. Res.* **2019**, *169*, 483−493.
(91) Xu, J.; Shi, Y.; Zhang, G.; Liu, Y.; Zhang, H.; Lu, G.; Deng, P.; Zhang, Z. Analysis of Lipid and Fatty Acid Composition in Different Tissues of Adult Female and Male *Takifugu flavidus*. *Food Sci.* **2014**, *35*, 133−137.
(92) Shi, Z.; Huang, X.; Li, W.; Hu, P.; Luo, H.; Fu, R. Analysis of lipid and fatty acid compositions in different tissues of the wild-caught *Pampus cinereus* broodstocks. *J. Fish. China* **2008**, *32*, 309−314.
(93) Griboff, J.; Carrizo, J. C.; Bonansea, R. I.; Valdés, M. E.; Wunderlin, D. A.; Amé, M. V. Multiantibiotic residues in commercial fish from Argentina. The presence of mixtures of antibiotics in edible fish, a challenge to health risk assessment. *Food Chem.* **2020**, *332*, 127380.
(94) Done, H. Y.; Halden, R. U. Reconnaissance of 47 antibiotics and associated microbial risks in seafood sold in the United States. *J. Hazard. Mater.* **2015**, *282*, 10−17.
(95) Kang, H.-S.; Lee, S.-B.; Shin, D.; Jeong, J.; Hong, J.-H.; Rhee, G.-S. Occurrence of veterinary drug residues in farmed fishery products in South Korea. *Food Control* **2018**, *85*, 57−65.
(96) Guidi, L. R.; Santos, F. A.; Ribeiro, A. C. S. R.; Fernandes, C.; Silva, L. H. M.; Gloria, M. B. A. Quinolones and tetracyclines in

aquaculture fish by a simple and rapid LC-MS/MS method. *Food Chem.* **2018**, *245*, 1232−1238.