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ECO-AQUACULTURE, SUSTAINABLE DEVELOPMENT AND PUBLIC HEALTH

Application of veterinary antibiotics in China's aquaculture industry and their potential human health risks

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Abstract China contributes to more than 60 % of the global aquaculture production, and its aquaculture industry has become one of the main players in food security. A large amount of antibiotics is believed to be used in fish cultivation for ensuring adequate production. The use of antibiotics as disease control agents and growth promoter in aquaculture in China has raised significant concerns recently because of the potential threats to human health. The extensive use of antibiotics in aquaculture may result in water and sediment contamination and the development of antibiotic resistance genes. In this review, the role of aquaculture in antibiotic contamination of the environment as well as the emerging concern of antibiotic resistance genes in China is discussed. Based on this review, it has been concluded that more information regarding the types and quantities of antibiotics used by Chinese fish farmers is required. Studies about the contribution of antibiotic usage in aquaculture to environmental levels in surface water, their potential risks on environment and human health, and the existence and spread of antibiotic resistance genes in aquaculture are needed.

Keywords Antibiotics · Antibiotic resistance genes · Aquaculture · China · Contamination

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Aquaculture industry of China

The latest available statistics indicated that in 2012, China as the largest aquaculture producer and exporter produced 61.7 % of the global aquaculture production (FAO 2014). The ratio of fishing and aquaculture production in 1978 was 74:26 and increased to 30:70 in 2008 (Fishery Bureau of Department of Agriculture 2009). In the future, it is expected that aquaculture production will account for a larger proportion of the total fish supply due to the fact that aquatic resources captured from the environment have leveled off in the past 20 years (FAO 2014). Protein from fish is an important protein source for coastal populations, and the production from the aquaculture industry will play a more important role in food security.

Aquacultural activities in China are concentrated in the coastal provinces, such as Jiangshu, Guangdong, Shangdong Fujian, Zhejiang, Guangxi, and Hainan, and inland provinces including Anhui, Jiangxi, Hubei and Hunan (Cao et al. 2007). The total area devoted to aquaculture production increased from 2.86 million hectares in 1979 to 5.63 million hectares in 2008 (Li et al. 2011). Fish ponds are the most popular type of culture system, while pen culture, rice paddy, cage cultures in lakes, and rivers are also used (Li et al. 2011). Moreover, integrated farming systems that co-culture crops together with fish and poultry are also popular in China (Su et al. 2011; Wong et al. 2004).

The top six finfish species currently farmed, by volume, include grass carp (*Ctenopharyngodon idella*), silver carp (*Hypophthalmichthysmolitrix*), common carp (*Cyprinus carpio*), bighead carp (*Hypophthalmichthys nobilis*), crucian carp (*Carassius carassius*), and tilapia (*Oreochromis sp.*). China produced over 17 million tonnes of carp and around 1.2 million tonnes of tilapia, accounting for about 90 % of the global carp production and about 36 % of the global tilapia production (FAO 2015). Waste-fed (feeding fish with agricultural waste or food waste, for cost-saving) and grass-fed culture (for culturing herbivorous fish) used to be popular in China (Prein 2002; Wong et al. 2004). However, in the study conducted by Chiu et al. (2013), more than 95 % of the fish farmers surveyed switched to manufactured feed.

Antibiotics in aquaculture industry in China

More and more fish farmers have adopted intensive culture in their fish farms, and the uneaten food and the waste excreted from fish contribute to the deterioration of water quality, which in turn create favorable conditions for the prevalence of disease. Increased fish stocking density, over-crowding, the lack of sanitary barriers between farming sites, and the failure to isolate fish farms with infected animals have led to the rapid spread of infection (Cabello 2006; Naylor et al. 2000; Naylor and Burke 2005). Pollution and crowding stress, for example, are well-known immunosuppressive factors resulting in higher disease susceptibility in fish (Köllner et al. 2002; Montero et al. 1999). Thus, the control of bacterial infections with antibiotics has become one of the solutions for fish farmers against bacterial pathogens. With the development of intensive culture, feeding antibiotics to animals has become necessary in aquacultural activities (Xu et al. 2006). It has been estimated that bacterial infections account for 15–20 % loss of annual total production, and more than 200 diseases have been identified in cultured aquatic species in China (Wei 2002). In order to reduce loss, antimicrobials, including various antibiotics, are heavily used in the aquaculture industry. Table 1 lists some of the commonly used antibiotics (therapeutic medicines) in aquaculture and their applications in fish diseases. *Aeromonas hydrophilia, Edwardsiella tarda, Vibrio anguillarum, V. harveyi, Streptococcus iniae*, and *Photobacterium damse*lae subsp. *piscicida* are some examples of bacterial pathogens found in China (Lan et al. 2008; Nielsen et al. 2001; Wang et al. 2002a, b, 2007a, b; Xiao et al. 1999; Zhou et al. 2008). The oral administration of antibiotics to animals by mixing the drugs with the fish feed using a fish feed mixer is the most common application method (Sarmah et al. 2006). Mixed medicated feed is also available commercially. The misuse or overuse of antibiotics in animal husbandry and aquaculture could be driven by financial incentives (by reducing monetary loss due to fish death), lack of knowledge about antibiotic safety, and poor management of antibiotic use (Currie et al. 2011; Zhao et al. 2010).

Some classes of antibiotics used for treating disease in fish include penicillins, macrolides, and quinolones and are classified as critically important for controlling diseases in humans (WHO 2012). In recent studies, various classes of antibiotics, including sulfonamides, fluoroquinolones (e.g., ciprofloxacin), tetracyclines (e.g., oxytetracycline), and macrolides (e.g., erythromycin), were detected in receiving waters or sediment near different types of aquaculture farms (Xu et al. 2013; Xue et al. 2013; Zheng et al. 2012; Zou et al. 2011). Sulfonamides, β-lactams, and macrolides are often used in animals for growth promotion and disease prevention in the form of feed additives or as veterinary drugs for the treatment of infections (Zhao et al. 2010).

Other than for the control of bacterial infections, antibiotics are also used as growth promoters. An antibiotic growth promoter refers to any medicine that destroys or inhibits bacteria and is administered at a low, sub-therapeutic dose (Hughes and Heritage 2004). It has also been hypothesized that cytokines produced from immune responses may also stimulate the release of catabolic hormones, which could in turn reduce muscle mass and thus result in reduced weight gain of animals (Thomke and Elwinger 1998). Moreover, energy in feed could be lost due to microbial fermentation in the gut rather than being efficiently utilized by the host (Jensen 1998). Thus, antibiotics are incorporated into feed in order to control bacterial infections as well as for manipulating bacterial populations (Hughes and Heritage 2004). It has been estimated that using antibiotic growth promoters could enhance growth and feed utilization by 4–8 and 2–5 %, respectively (Ewing and Cole 1994). In a more recent study, He et al. (2011) found that the dietary incorporation of florfenicol (0.02 g/kg) could significantly enhance growth and serum complement component concentrations of hybrid tilapia (*Oreochromis niloticus* female × *Oreochromis aureus* male), but the estimated bacterial count and bacterial diversity were found to be lower when compared to the control group. On the other hand, the effect of the use of antibiotic growth promoters has been questioned. Some authors pointed out that the withdrawal of antibiotic growth promoters in industrial production had no effect or resulted in a small loss in growth rate in poultry production (Emborg et al. 2001; Engster et al. 2002; Shane 2003).

In China, the annual production of antibiotics is about 210, 000 t, and 46 % of this is estimated to be used in livestock (Wang and Ma 2008), while other authors estimated the annual usage of raw antibiotic ingredients to be up to 180,000 t (Zheng et al. 2012). However, there is no government data which show the exact amount of antibiotics used in aquacultural activities. Depending on the usage, a wide range of concentrations of antibiotic is used. Chen et al. (2012) determined that the antibiotic concentrations in feeds for livestock production ranged from 0.2 to 46.8 μg/kg, while Wang et al. (2004) used a commercial medicated diet that contained 2000 mg/kg chloramphenicol, sulphamethoxazole, and oxytetracycline, respectively, to feed shrimp (*Penaeus chinensis*) in their study.

Antibiotics could also enter aquaculture farms via the application of fertilizers. The use of organic pond fertilizer is popular in Asian countries including China (Prein 2002). Swine and chicken dung are often used to maintain the fertility of fish ponds because they can enhance fish growth (Dhawan

Table 1 Occurrence of antibiotics in surface water of China and the methods of administering some commonly used antimicrobial compounds to fish and their target(s)

Antibiotic	Concentration (ng/L)	Location	Media	Application in aquaculture and method(s) of administration	References
Amoxicillin	nd-128	Victoria Harbour, Hong Kong	Sea water	Furunculosis, gill disease Oral: 60–80 mg/kg body weight of fish/ day/10 days	Austin and Austin 2007; Minh et al. 2009
Enrofloxacin	nd-21.5	Humen riverine outlet	River water	Furunculosis, streptococcicosis, vibriosis Oral: 5–10 mg/kg for 10 days (for *Streptococcus iniae* in hybrid striped bass); 10 mg/kg for 5 days (for vibriosis in rainbow trout) Bath: 2.5–5 mg/l of water, as a bath for 5 days	Dalsgaard and Bjeregaard 1991; Lewbart et al. 1997; Xu et al. 2013
Erythromycin (detected as erythromycin-H_2O)	9.5–486 988–1042 5.6–126 4.7–1974 0.13–6.7	Hong Kong Shenzhen river Humen riverine outlet Victoria Harbour, Hong Kong Bohai Sea and Yellow Sea	Coastal water River water River water Sea water Sea water	Bacterial kidney disease, streptococcicosis Oral: 25–100 mg/kg of fish/day for 4–21 days	Austin and Austin 2007; Gulkowska et al. 2007; Minh et al. 2009; Xu et al. 2013; Ye et al. 2007; Zhang et al. 2013
Florfenicol	0.45–89.5 6.85–46.63	Yangtze Estuary Huangpu River	River water River water	Furunculosis, vibriosis Oral: 10 mg/kg body weight of fish/day for 10 days	Austin and Austin 2007; Jiang et al. 2011; Yan et al. 2013
Oxytetracycline	nd-47.8 <LOQ-270	Victoria Harbour, Hong Kong Bohai Bay	Sea water Sea water	Acinetobacter disease, carp erythrodermatitis, coldwater disease, columnaris, edwardsiellosis, emphysematous putrefactive disease, enteric redmouth, enteric septicaemia, fin rot, furunculosis, gill disease, haemorrhagic septicaemia, redpest, salmonid blood spot, saltwater columnaris, Streptococcicosis, ulcer disease; Oral: 50–75 mg/kg of fish/day for 10 days	Austin and Austin 2007; Minh et al. 2009; Zou et al. 2011
Sulfadiazine	196–322 2.1–22.3 0.36–4.8	Shenzhen river Humen riverine outlet Bohai Sea and Yellow Sea	River water River water Sea water	Enteric redmouth, furunculosis haemorrhagic septicaemia, vibriosis Oral: 30 mg/kg of fish/day for 10 days (as potentiated sulfonamide) Note: Sulfonamides are usually used together with trimethoprim as potentiated sulfonamide	Austin and Austin 2007; Xu et al. 2013; Ye et al. 2007; Zhang et al. 2013
Sulfamethoxazole	645–907 6.2–28.6 < LOQ-56 nd-140 < LOQ-8.3	Shenzhen river Humen riverine outlet Yellow River Bohai Bay Bohai Sea and Yellow Sea	River water River water River water Sea water Sea water	Enteric redmouth, furunculosis haemorrhagic septicaemia, vibriosis Oral: 30 mg/kg of fish/day for 10 days (as potentiated sulfonamide) Note: Sulfonamides are usually used together with trimethoprim as potentiated sulfonamide	Austin and Austin 2007; Xu et al. 2013; Xu et al. 2009; Ye et al. 2007; Zhang et al. 2013; Zou et al. 2011
Sulfadimidine	1.2–12.1 nd-47.78 nd-0.16	Humen riverine outlet Victoria Harbour, Hong Kong Bohai Sea and Yellow Sea	River water Sea water Sea water	Enteric redmouth, furunculosis haemorrhagic septicaemia, vibriosis Oral: 30 mg/kg of fish/day for 10 days (as potentiated sulfonamide) Note: Sulfonamides are usually used together with trimethoprim as potentiated sulfonamide	Austin and Austin 2007; Minh et al. 2009; Xu et al. 2013; Zhang et al. 2013
Trimethoprim	<MDL-21.8 nd-149 <LOQ-120	Hong Kong Victoria Harbour, Hong Kong Bohai Bay	Coastal water Sea water Sea water	Enteric redmouth, furunculosis haemorrhagic septicaemia, vibriosis Oral: 30 mg/kg of fish/day for 10 days (as potentiated sulfonamide) Note: Sulfonamides are usually used together with trimethoprim as potentiated sulfonamide	Austin and Austin 2007; Gulkowska et al. 2007; Minh et al. 2009; Zou et al. 2011

nd not detected, *LOQ* limits of quantification, *MDL* method detection limit and Kaur 2002) as well as provide more food for filter feeders (Brunson et al. 1999). The use of animal manure in fish ponds is believed to be an ecological and economical farming practice (Su et al. 2011). However, a large proportion of antibiotics are also used in agriculture activities, and high concentrations of antibiotics have been detected in animal manure. For example, a study conducted by Zhao et al. (2010) found a maximum concentration of 99.43 mg/kg fleroxacin, 225.45 mg/kg norfloxacin, 45.59 mg/kg ciprofloxacin, and 1420.76 mg/kg enrofloxacin in chicken manure. Sarmah et al. (2006) pointed out that about 30 to 90 % of the antibiotics are excreted unchanged in feces and urine due to the poor absorption of antibiotics in animal gut. The excreta, which contain high concentrations of antibiotics, would in turn enter the aquaculture system through the application of manure used as pond fertilizer.

Occurrences of antibiotics and antibiotic resistance genes in environment associated with aquacultural activities and their potential health risk

It is a common practice to lower the water level of a fish pond by discharging the pond water directly to a nearby surface water body in order to facilitate the fish harvest process. Fish ponds are drained periodically, and pond sediment is removed. Pond sediment is considered as a good fertilizer source for vegetation. Moreover, the waste from fish farmed in cages is continuously discharged to the environment. These activities occur throughout the year. With a large area devoted to aquaculture, the area of land affected by antibiotics derived from aquaculture could be extensive. It has been reported that more than 20 antibiotic compounds can be found in various matrices in the Pearl River Delta, such as sewage water and sediment, vegetable and soil, river water, and in tap water and drinking water resources (Hu et al. 2010; Richardson et al. 2005; Xu et al. 2007; Zhang et al. 2012). Table 1 summarizes the occurrence of antibiotics in surface water in China that could be used for treating fish diseases.

Various antibiotics were also detected in fish and other aquaculture products. Chloramphenicol was detected in muscle of carp and chub from Guangzhou with concentrations higher than the minimum required performance limit (MRPL) (0.3 μg/kg) (Lu et al. 2009). Norfloxacin, ciprofloxacin, and enrofloxacin were detected in nine marine fish species, although the concentrations of these fluoroquinolones did not represent a risk to human health via the consumption of fish products (He et al. 2012). High concentrations of erythromycin-H_2O were detected in adult shrimp (*Fenneropenaeus penicillatus*) from Hailing Island, South China, and therefore consumption of shrimp could pose a human health risk (Chen et al. 2015). A recent publication estimated that 54,000 t of antibiotics were excreted by humans and animals in 2013, but no data was available regarding the contribution from aquaculture industry as the data was grouped together with other animals (Zhang et al. 2015). These antibiotics would eventually enter various water systems and contaminate other aquaculture products and wild fish. Various antibiotics were detected in different wild fish from different water systems such as the Baiyangdian Lake in north China (Li et al. 2012) and the Pearl River Delta in south China (Zhao et al. 2015). Quinolones were the major antibiotics detected in mollusks from coastal waters in the Bohai Sea (with concentrations of 0.71–1575.10 μg/kg) (Li et al. 2012).

The abuse of antibiotics for human and veterinary use in China is also widely reported by media ("Chinese government must tackle" 2015; Chen 2015; Foster 2010; Jiang 2012; Lau 2015; Luo 2015). Recently, the US FDA issued an import alert related to the presence of antibiotics or chemicals in farmed catfish, basa, shrimp, dace, and eel from China (FDA 2015).

Although there are no government data or reports showing the situation of antibiotics used in aquaculture, recent studies show clear evidence that antibiotics are used in fish and shrimp production. In the study near Haihe River conducted by Zou et al. (2011), it was determined that wastewater discharge from fish pond was dominated by oxytetracycline, while ofloxacin could be the main antibiotic used in the fish breeding farm and fish nursery plant, with concentrations up to 7900 and 5400 ng L^{-1} detected, respectively. Zheng et al. (2012) found erythromycin-H_2O detected in the sediment near shrimp farms in Baibu Bay, suggesting that erythromycin is the only antibiotic used in that area. Xu et al. (2013) pointed out that high concentrations of sulfonamides and ciprofloxacin (mainly used in livestock) detected in the Pearl River Estuary were related to their application in livestock production. Xue et al. (2013) suspected that the heavy use of sulfonamides in river cage culture was due to high concentrations of sulfonamides detected in river sediment in Nanning, Guangxi province. A recent study estimated that the annual mass loading of antibiotics from the Pearl River Delta to the Pearl River Estuary, and coast was 193 t (Xu et al. 2013). Antibiotics in the Pearl River Estuary partly originated from aquacultural activities (Liang et al. 2013). Prohibited antibiotics were frequently detected in exported fish products in the 2010s (CFS, 2006a; b; c; European Commission 2002). Recent studies showed that antibiotics are still detected in farmed or wild fish, although their concentrations are not likely to pose acute risk (He et al. 2012; Li et al. 2012).

Wang et al. (2015) observed that the presence of tetracyclines, quinolones, sulfonamides (both for human and veterinary use), and the antibiotics that are used exclusively for veterinary applications (chlortetracycline, enrofloxacin, and tylosin), in the urine samples of children, could be mainly due to the contaminated environment or food. Although sulfonamides are gradually being replaced by β-lactam, macrolides, and other antibiotics in China, they are still in use in poultry and aquaculture because of their lower cost (Zou et al. 2011). For example, depending on the region, the retail price of sulfadiazine ranges from RMB 70–100/kg while that of amoxicillin ranges from RMB 76–138/kg. The unintended consumption of antibiotics present in food or in water could lead to allergy and toxicity problems (Cabello 2006). Chronic exposure to tetracycline, for example, could lead to steatosis by altering genes related to lipid metabolism and transportation (Anthérieu et al. 2011). Exposure to chloramphenicol has been linked to an increased risk of aplastic anemia and leukemia in humans (Issaragrisil et al. 2005; Malkin et al. 1990). Liu and Wong (2013) reviewed human health risk studies and concluded that antibiotics would pose a low possibility of acute toxicity to humans. However, in extreme cases, the consumption of aquaculture products that are heavily contaminated with antibiotics could pose a human health risk. For example, Chen et al. (2015) revealed that the concentration of erythromycin in adult shrimp (*F. penicillatus*) from Hailing Island, south China ranged from 2498 to 15, 090 ng/g wet wt. and that the estimated daily intake (EDI) of erythromycin would therefore exceed the acceptable daily intake (ADI) value established by the Codex Alimentarius Commission.

Other than the various types of antibiotics detected in the environment, antibiotic resistance genes (ARGs) have also been widely detected in the aquatic environment of China (Gao et al. 2012; Hu et al. 2008; Luo et al. 2010; Su et al. 2011). Bacteria exposed to antibiotics in the environment may accelerate the persistence or emergence of ARGs which pose potential harm to both the ecosystem and to human health (Kemper 2008; Zhang et al. 2009). ARGs encoding resistance against a broad range of antibiotics, such as macrolides, sulfonamides, fluoroquinolones, and tetracyclines, occur ubiquitously in hospitals and livestock feeding effluents, municipal wastewater, surface water, as well as drinking water resources (Pruden et al. 2006; Zhang et al. 2009). The genes that code for resistance in bacteria are mainly located on mobile genetic elements such as plasmids, transposons, integrons, gene cassettes, and bacteriophages, indirectly transporting the ability for resistance from non-pathogens to pathogenic microorganisms (Kemper 2008). Selection of antibiotic resistant bacteria could occur even with very low concentrations of antibiotics (Gullberg et al. 2011).

The development of antibiotic resistance in bacteria in aquaculture environments could lead to bacterial antibiotic resistance among human populations. Recent studies indicated the roles of aquaculture in the development of antibiotic resistance. Studies in the Baltic Sea reported the persistence of tetracyclines, sulphonamide, and trimethoprim resistance genes in sediment samples in aquaculture farms, even though the concentrations of the antibiotics were very low (tetracyclines: below detection limit, limit of detection for tetracycline and oxytetracycline=25 and 66 ng/g sediment, respectively; sulphonamides and trimethoprim: <1 to 101 ng/g, limit of detection=1 ng/g sediment) (Muziasari et al. 2014; Tamminen et al. 2010).

In China, Su et al. (2011) reported that out of 203 *Enterobacteriaceae* isolates from four integrated fish farms in Zhongshan, south China, more than 50 % possessed tetracycline resistance genes (tet(A), tet(C)) and sulfonamide resistance genes (sul2), while the intI1 gene was found in 170 isolates (83.7 %). Gao et al. (2012) reported that sulfonamide resistance genes were prevalent in six studied aquaculture farms in Tianjin, and their concentrations were the highest detected among all studied ARGs, suggesting that the use of sulfonamides is more prevalent in the area. Furthermore, phylogentic analysis suggested genetic transmission between intestinal bacteria (e.g., *Enterococcus sp.*) and indigenous bacteria (e.g., *Bacillus sp.*). Xiong et al. (2015) indicated that fish ponds are reservoirs of ARGs and the presence of potential resistant and pathogen-associated taxonomic groups (*Acinetobacter*, *Arcobacter*, and *Clostridium*) in fish ponds might imply a potential risk to human health. The presence of multiple antibiotic resistant bacteria in aquaculture farms also exacerbates the situation (Gao et al. 2012; Su et al. 2011). The mechanisms of the development of antibiotic resistance have been described in detail elsewhere (Miranda et al. 2013).

Studies suggested that ARGs are likely localized in marine aquaculture farms, implying that the presence of ARGs at the farms is unlikely to cause serious effects to the environment around the farms (Muziasari et al. 2014; Tamminen et al. 2010). However, the wide distribution of inland aquaculture activities in China could lead to a different situation. It has been suggested that drinking water is a reservoir for antibiotic resistance bacteria (ARB) and ARGs (Armstrong et al. 1982; Schwartz et al. 2003). Recently, Shi et al. (2013) reported the highest abundance among the ARGs detected in drinking water in Nanjing, China. It is a difficult task to conduct risk assessment on antibiotic resistance in aquaculture because of the lack of data and the complex pathways of gene flow among various aquatic species and environmental compartments (Pruden et al. 2013). Figure 1 shows the flow of antibiotics and antibiotic resistance genes in fish ponds.

Alternatives to antibiotics in aquaculture

High occurrences of diseases in high-stocking density environments and polluted water are inevitable, and it is a difficult mission to ensure food security. It has been suggested that the use of antibiotics in China is necessary (Xu et al. 2006), and it would not be possible to stop the use of antibiotics in aquaculture. However, possible alternatives to antibiotics should be promoted and used.

Probiotic microorganism refers to a live microbial adjunct which confers beneficial effects on the host by modifying the host-associated or ambient microbial community, by ensuring

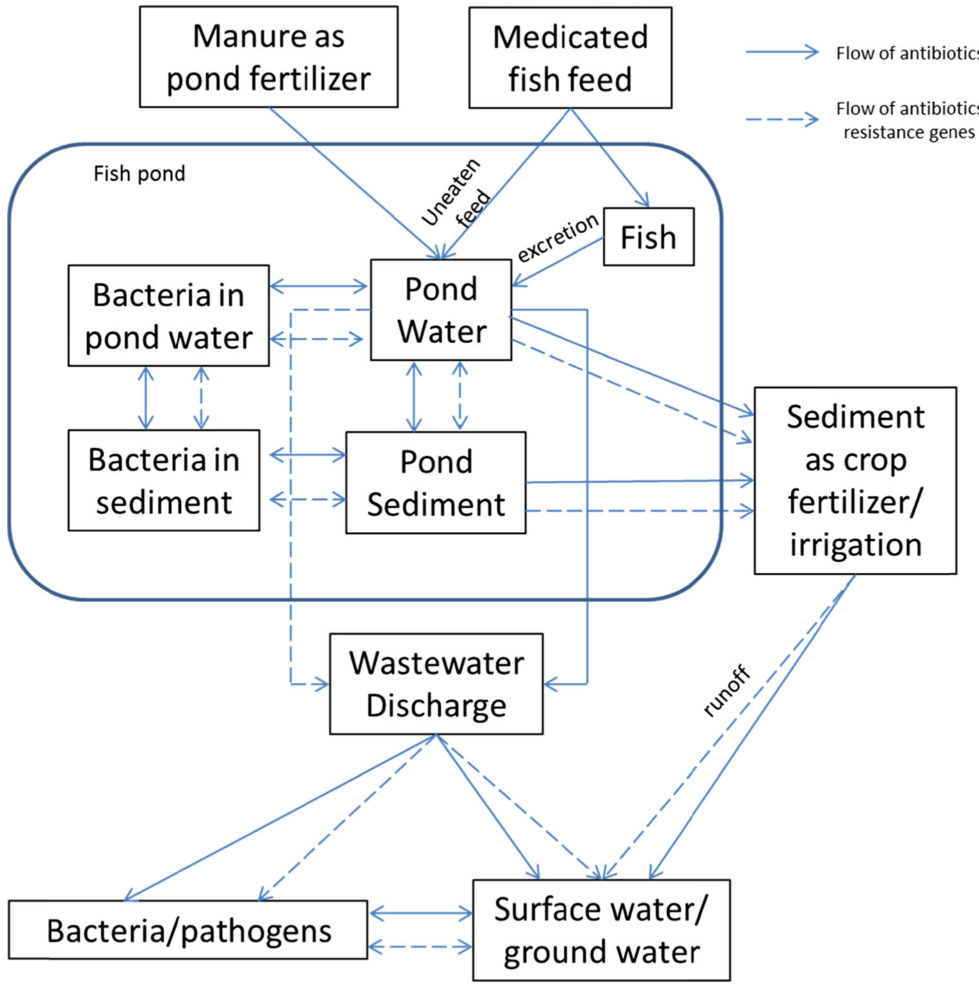

Fig. 1 Flow of antibiotics and antibiotic resistance genes in fish ponds

improved use of the feed or enhancing its nutritional value, by enhancing the host response towards disease, or by improving the quality of its ambient environment (Verschuere et al. 2000). It could be used in aquaculture for promoting growth and/or preventing diseases. Manipulating the gut bacteria in fish via the administration of antagonistic bacteria could be a feasible way for reducing the incidences of opportunistic pathogens (Balcazar et al. 2006). Some opportunistic pathogens, such as *Aeromonas hydrophila*, is commonly found in fish gut (Ray et al. 2012) and is capable of producing extracellular enzymes such as proteases and chitinase to assist food digestion (Pemberton et al. 1997; Sugita et al. 1999). Various isolates of *Bacillus subtilis*, for example, have been proven to be effective in controlling infections of *A. hydrophila* (Kumar et al. 2006, 2008; Newaj-Fyzul et al. 2007).

The use of lactic acid bacteria could enhance the growth of fish as well as fish immunity. *Lactobacillus plantarum* administered at 10^8 cfu/kg to *Epinephelus coioides* could promote growth, enhance innate immune responses as well as disease resistance against *Streptococcus* sp. (Son et al. 2009). Pollution is one of the factors that could suppress the immune system of fish (Köllne et al. 2002). Thus, improving water quality could partially remove the immunosupressive effect. Some bacteria that are capable of reducing nitrate and nitrite to nitrogen, such as *Microbacterium* and *Bacillus sp.*, were isolated from a shrimp pond, thus exhibiting the potential to reduce the amount of nitrogenous waste in fish ponds (Wang et al. 2007a, b).

Traditional Chinese medicines (TCM) could also serve as an alternative to antibiotic usage in the aquaculture industry. The effects of TCM on fish health, especially non-specific immunity and disease resistance, have been extensively studied in the last decade. For example, Ardó et al. (2008) reported that the inclusion of 0.1 % of *Astragalus membranaceus* (dried root of the plant used as medicine and a common herb used as an immuno-stimulant in humans) in the diet of Nile tilapia significantly enhanced phagocytic and respiratory burst activities of blood phagocytic cells. Significant reduction in mortalities attributed to bacterial infection in fish fed with TCM was noted in common carp (*Cyprinus carpio*) and grouper (*Epinephelus tauvina*)

(Punitha et al. 2008; Yin et al. 2009). Jian and Wu (2003) noted that feeding large yellow croaker (*Pseufosciaena crocea*) with 1.0 and 1.5 % astragalus root (*Astragalus propinquus*) and Chinese angelica root (*Angelica sinensis*) (mixed at a ratio of 5:1) increased the number of nitroblue tetrazolium (NBT) positive cells, and lysoyme activities enhanced the complement activities of the fish. Choi et al. (2013) also reported that grass carp juveniles fed with a diet containing 2 % mixture of *Radix scutellaria, Rhizoma coptidis, Herba andrographis*, and *Radix sophorae flavescentis* in a ratio of 1:1:2:3 resulted in significantly lower mortality after *A. hydrophila* challenge in both laboratory and field trial.

In addition to the effects on immunity, certain TCM also possesses antimicrobial properties. TCM such as *Angelica dahurica, Lycium barbarum, Scutellaria barbata*, and *Zingiber officinale* could inhibit a wide range of bacteria (Agarwal 2001; Jin et al. 1995; Sato et al. 2000; Yu et al. 2004). In fact, the use and dosage of several TCMs against bacterial infections, such as *Andrographis paniculata, Scutellaria baicalensis*, are also mentioned in the Safety Food—Criterion for usage of Fishery Drugs (MOA 2002b). Choi (2013) observed that multiple passages of bacteria pathogens to TCM extract at sub-inhibitory concentrations had no long-term effects on their minimal inhibitory concentration, probably due to the multiple actions of TCM extracts to inhibit bacteria (Meng et al. 2003), making TCM a more promising choice against bacterial diseases. Other than the antibacterial and immunostimulating effects, feeding fish with TCM could also enhance growth performance. For example, feeding grass carp with *Gynostemma pentaphyllum* and *Lycium barbarum* could enhance weight gain, feed conversion efficiency, and specific growth rate (Mo 2014; Wu et al. 1998). In general, herbal based medicines pose effects on growth, antimicrobials, disease resistance, stimulate appetite, and result in anti-stress (Choi 2013; Citarasu 2010).

Management approach

Regulations on the use of antibiotics are strict in some developed countries and only a few antibiotics are licensed for use in aquaculture. In Europe, the use of non-therapeutic antibiotics, including antibiotic growth promoters, in livestock production has been banned since 2006 (Cogliani et al. 2011). The sales and usage of veterinary antimicrobial across the European Union are monitored under the European Surveillance of Veterinary Antimicrobial Consumption (ESVAC) project (EMA 2015). In the USA, the FDA planned to phase out the indiscriminate use of antibiotics in cows, pigs, and chickens raised for meat in 2013 (FDA 2013), while the FDA also collects data regarding the amount of antimicrobial active ingredients in their drugs sold or distributed for use in food-producing animals (FDA 2015). Currently, the FDA has approved four antibiotics for the treatment of bacterial infections in food fish: oxytetracyclin, sulfadimethoxine (with ormetoprim), florfenicol, and sulfamerazine (Kelly 2013).

Table 2 shows some regulations and standards regarding aquaculture in China. A number of antibiotics, including chloramphenicol, nitrofuran, and metronidazole, have been banned in the production of food animals (MOA 2002a). Residue levels of veterinary drugs, their withdrawal period, and a list of banned antibiotics in fish product are also regulated and listed in Safety Food—Criterion for Usage of Fishery Drugs (MOA 2002b). The purchase of antibiotics for livestock requires a prescription from veterinarians, but farmers could buy antibiotics directly from chemical companies without a prescription (Wang et al. 2014). Moreover, fish farmers or feed producers could purchase a large variety of antibiotics, such as tetracyclines, quinolones, penicillins, macrolides, and the banned chloramphenicol and metronidazole, via the internet without a prescription. Wastewater discharge volume from pharmaceutical manufacturers producing chloramphenicol, sulfadiazine, furazolidone, amoxicillin, and

Table 2 Laws, regulations and standards for aquaculture in China

Laws, regulations, and standards	Year of issue	Last amendment	References
Marine Protection Law	1982	1999	NPC (1999)
Law on the Prevention and Control of Water Pollution	1984	2008	NPC (2008)
Water Law	1988	2002	NPC (2002)
Environmental Protection Law	1989	2014	NPC (2014)
Administrative Regulation of Feed and Feed Additives	1999	2001	State Council of China (2001)
Safety Food – Criterion for Usage of Fishery Drugs	2001	2002	MOA (2002a)
List of Banned Veterinary Drugs and Other Chemical Compounds for Food Animals	2002		MOA (2002b)
Administrative Regulation of Quality and Safety for Aquaculture	2003		MOA (2003)
Regulations on the Administration of Veterinary Drugs	2004		State Council of China (2004)
Requirement for Water Discharge from Freshwater Aquaculture Pond	2007		MOA (2007)
Food Safety Law	2009		NPC (2009)

cephradine is currently regulated (MEP 2008), and this is the only regulatory measure in place to control antibiotics in wastewater. The discharge of wastewater from fish ponds is regulated under the Ministry of Agriculture (MOA 2007); however, the regulations only focus on the concentrations of macronutrients, metals, and pesticides, while antibiotics or other veterinary drugs in discharge wastewater are not regulated. Since fish pen culture, rice paddy, and cages cultures in lakes and rivers are also used by fish farmers in China (Li et al. 2011), the discharge from these cultures should also be included in the regulations. It has been suggested that limiting the use and types of antibiotics in animal production is the most direct route for controlling antibiotics and probably the release of resistance genes into the environment (Pruden et al. 2013). Table 3 lists some common examples of banned antibiotics. For example, although ciprofloxacin has been banned since 2002, it was detected in some fish cultured in the Pearl River Delta (He et al. 2012), suggesting the possibility of illegal use in food fish. More stringent regulations on the application of antibiotics should also be established, and the illegal sale and use of antibiotics should be strictly controlled. Moreover, management strategies for the application of animal manure as pond fertilizer should be established for controlling and preventing the transmission of antibiotic resistance from animals to humans via aquaculture (Xiong et al. 2015).

No requirement currently exists to test for antibiotics in tap water (Huang et al. 2015). The estimated volume of wastewater discharged from shrimp culture in Guangdong province in 2001 was 2.21×10^9 m^3 (Li et al. 2004). However, there is no data available regarding the exact annual discharge of wastewater from all types of aquaculture activities. Due to the fact that some antibiotics could resist biodegradation (Capone et al. 1996; Lai et al. 2008; Marengo et al. 1997), they could be transported from upstream (source of drug application) to downstream and potentially contaminate drinking water sources. Management and supervision of drinking water sources need to be strengthened (Liu and Wong 2013).

In order to achieve sustainable aquaculture practices, there are several factors that have been suggested by The World Bank in a publication that reviewed disease outbreaks in the aquaculture industry in Chile, Vietnam, Mozambique and Madagascar. These include establishing biosecurity measures to prevent disease outbreak, setting limits on maximum production in farming areas so as not to exceed the carrying capacity of the biological system (such as the water body supporting aquaculture), developing programs to support effective regulations and enforcement, regular monitoring of key performance indicators (such as frequency of antibiotic treatments for bacterial diseases), reducing the use of drug treatments, and establishing effective communication channels between various stakeholders to ensure issues are tackled in a timely and efficient manner (Brummett et al. 2014). These measures could be considered and referred to by the Chinese Government for strengthening laws and regulations pertaining to aquaculture activities.

Currently, the supervision of laws and regulations of food safety falls within a number of government departments which hinders enforcement efficiency. Ni and Zeng (2009) pointed out that this would dampen the efficiency of the supervision efforts; thus, it would be necessary to harmonize all relevant government bodies to rectify the situation as well as enhance the Food Safety Law performance. There is an urgent need to gather information regarding the use and sale of antibiotics and revise the regulations in order to tackle the problems of the overuse and abuse of antibiotics in aquaculture.

Table 3 Permitted antibiotics and their maximium residue limit (MRL) and banned antibiotics in fishery product in China (adapted from MOA 2002a, b)

Antibiotics	MRL (μg/kg)
Permitted antibiotics	
Chlortetracycline	100
Oxytetracycline	100
Tetracycline	100
Sulfonamides (total 4: sulfadiazine, sulfamethoxazole, sulfadimidine, and sulfamethazine)	100
Oxilinic acid	300
Trimethoprim	50
Banned antibiotics	
Avoparcin	
Ciprofloxacin	
Chloramphenicol	
Dapsone	
Furazolidone	
Erythromycin	
All nitrofurans	
Tylosin	

Conclusion

Although the situation with respect to antibiotic overuse and abuse in aquaculture is severe, the aquaculture industry of China will continue to play an important role in food security in the future. The consumption of high levels of antibiotics and the rapid growth of antibiotic resistance in China derived from antibiotic abuse warrants urgent attention. Because of the lack of information regarding sales and usage of antibiotics, the amount of antibiotics applied in the aquaculture industry still remains largely unknown. Meanwhile, most studies available so far mainly focus on the occurrence of antibiotics in the environment. More studies are required for evaluating the contribution of antibiotics used in aquaculture and their concentrations in surface water. Acute and chronic toxicity studies for different antibiotics need to be conducted to assess their potential risks on the environment and human health. Further studies are needed to evaluate the importance and contribution of aquaculture in the development of antibiotic resistance genes and their transmission between various bacteria. More studies are also required to investigate the existence and spread of ARGs in aquaculture.

Although the Chinese government has numerous laws and regulations to monitor the performance and safety of the industry, they are not efficiently enforced and their current coverage is not sufficient. Various antibiotics, including those banned in livestock production, are readily available and can be purchased easily, and their discharges from aquaculture activities are not currently regulated. Last, but not the least, the use of alternatives to antibiotics have been extensively studied and some of them have been proven to be applicable, and thus, they should be encouraged and promoted in the aquaculture industry.

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