

# Antibiotic resistance in fish-borne pathogens of public health significance: An emerging food safety issue

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## ABSTRACT

Fish are an essential part of the human diet worldwide. Human pathogenic bacteria found associated with fish can cause human illnesses when consumed raw or undercooked. Pathogenic bacteria are either naturally found in the environment inhabited by fish or introduced post-harvest during handling, transportation, or storage. Anthropogenic contamination is responsible for introducing diarrheagenic bacterial pathogens such as *Escherichia coli* and *Salmonella enterica*, some of which could be highly resistant to multiple drugs due to their human or animal origin. The use of antimicrobials in fish farms is also responsible for developing antimicrobial resistance in fish-borne human pathogens. The incidence of multidrug-resistant human pathogenic bacteria in wild-caught and farmed shrimp offers a formidable challenge in ensuring food safety and preventing the global spread of antibiotic-resistant bacteria *via* food fish.

**KEYWORDS:** fish, aquaculture, antibiotic resistance, mechanisms, seafood, pathogen.

## 1. Introduction

Food fish play a vital role in fulfilling the protein requirement in growing populations worldwide by contributing to nearly 20% of animal protein

consumed by 7.5 billion global population [1]. With the wild stocks of fish declining, farmed fish has formed an essential part of the human diet. However, fish can also cause human illnesses due to pathogenic microorganisms, biological toxins, and parasites [2]. Pathogenic bacteria are either naturally acquired, such as those belonging to *Vibrio* spp. which are the natural inhabitants of coastal-marine waters, or introduced as secondary contaminants from human or animal sources. Bacterial pathogens gain entry post-harvest at various stages of handling during processing. Several inputs, such as organic fertilizers, feeds, and seeds can constitute sources of contamination with pathogenic bacteria. The use of antimicrobials, disinfectants, and probiotics has a bearing on the microbial composition of water, sediment, and fish. Human pathogenic bacteria compromise fish safety and acceptability and can lead to detentions and rejections, resulting in economic losses to the producer. Therefore, good manufacturing practices, and the identification and management of possible sources of contamination from farm to the consumer is necessary to ensure safety of fish for consumption. An overview of the key pathogens of human health significance associated with fish and shellfish is provided in the following section.

## 2. Human pathogenic bacteria in seafood

### 2.1. *Vibrio* spp.

The *Vibrio* group constitutes one of the most important food-borne pathogens of human health

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significance in seafood. Vibrios are naturally present in coastal-marine waters and, as a result, are found in variable numbers in all seafood. Their numbers can vary depending on several factors such as the season, physico-chemical parameters of water such as the temperature, salinity, and nutrient concentrations [3]. A large number of *Vibrio* species are widely distributed in coastal-marine waters of which *V. cholerae*, *V. parahaemolyticus*, and *V. vulnificus* are important human pathogens that can cause varieties of infections such as gastroenteritis, wound infections, and septicemia [4]. *V. cholerae*, the primary causative agent of cholera, is also found in association with chitinous organisms such as copepods, crustaceans, as well as microalgae, zooplankton, and fish [5]. *V. cholerae* are of two types, O1 and non-O1, based on their agglutination with O1 antiserum. O1 *V. cholerae* and one non-O1 *V. cholerae* (O139 Bengal) are responsible for cholera, while all other non-O1 and non-O139 *V. cholerae* do not generally infect humans, or only cause mild illnesses [6]. The virulence of *V. cholerae* O1 and O139 is primarily due to their ability to produce cholera toxin CTX. Infection by *V. cholerae* occurs when contaminated water or improperly cooked seafood is consumed. Therefore, the presence of toxigenic *V. cholerae* in fish and shellfish should be considered as a health risk. Studies have reported the occurrence of *V. cholerae* in aquacultured fish and shellfish. Importing countries such as the USA and Canada prescribe zero tolerance for *V. cholerae* in ready-to-eat foods. There have been instances of seafood rejection due to the presence of *V. cholerae* in the past. However, farmed shrimp has not been implicated in any case of human cholera. Though several studies have reported the occurrence of non-O1 *V. cholerae* in aquaculture pond water, sediments, and shrimp [7], the presence of O1 *V. cholerae* has rarely been found. Therefore, the perceived health risk from *V. cholerae* in farmed shrimp is very low. Nevertheless, routine surveillance of farmed fish and shrimp, environments and finished products is necessary to ensure that these are free from choleraogenic *V. cholerae*.

The halophilic pathogenic bacterium *V. parahaemolyticus* is widely distributed in coastal waters worldwide and is commonly found in

seafood, sometimes in numbers as high as  $10^3$ - $10^4$ /g in oysters and about  $10^2$ /g in tropical shrimp [7, 8]. Physical characteristics of water, such as the temperature and salinity, influence their abundance in temperate waters [9, 10]. Food-borne infections occur when *V. parahaemolyticus* are present in numbers exceeding  $10^5$  CFU/g. Farmed oysters, mussels, and scallops have been responsible for *V. parahaemolyticus* infections worldwide [2]. Severe watery diarrhea, abdominal cramps, nausea, vomiting, and fever are symptoms of *V. parahaemolyticus* infection. Gastroenteritis occurs when shellfish such as oysters and clams are consumed raw or minimally cooked. However, not all *V. parahaemolyticus* microorganisms are pathogenic, but those producing either a thermostable direct hemolysin (TDH) and/or a TDH-related hemolysin (TRH) are pathogenic [11, 12]. TDH and TRH are encoded by *tdh* and *trh* genes, with about 70% nucleotide sequence similarity [13]. Studies have shown that <1% of the seafood isolates of *V. parahaemolyticus* are *tdh*<sup>+</sup>, while the occurrence of *trh*<sup>+</sup> *V. parahaemolyticus* is much higher, with some studies reporting as high as 60% [8]. Since 1996, a pandemic clone of O3:K6, which was first detected in Calcutta, India [14], has been responsible for numerous outbreaks in Asia and the USA. These strains harbor *tdh* but not the *trh* gene. Several other genetically and closely related serotypes were later identified as belonging to this group. At present, 27 clonally related serotypes constitute the pandemic *V. parahaemolyticus* group [15]. *V. parahaemolyticus* can be found in farmed shrimp, and recently, the occurrence of early mortality syndrome (EMS) has been attributed to *V. parahaemolyticus* in *Litopenaeus vannamei* farms [16]. However, the levels of human pathogenic strains of *V. parahaemolyticus* in farmed shrimps are very low and so far, no significant outbreaks of gastroenteritis have been attributed to farmed shrimp. Studies from south east Asia have shown however that cultured shrimps, aquaculture pond water and sediments harbor *V. parahaemolyticus* and some studies have reported a high prevalence (7-15%) of *tdh*<sup>+</sup> *V. parahaemolyticus* in shrimp farms [17]. Since *V. parahaemolyticus* cannot grow at low temperatures, proper icing or chilling after harvesting farmed shrimp can prevent this pathogen's multiplication in fresh shrimp.

Notably, the contamination of ready-to-eat products should be strictly prevented to avoid such foods being vehicles of *V. parahaemolyticus*.

*V. vulnificus* is another significant human pathogenic *Vibrio* species associated with fish and shellfish. *V. vulnificus* is known to cause severe infections in people with compromised immunity, liver disease, and iron overloaded conditions, with a fatality rate as high as 50% [18]. *V. vulnificus* infections are associated with eating raw molluscan shellfish which accumulate this pathogen from surrounding waters. *V. vulnificus* has been reported to be responsible for more than 85% of deaths associated with consumption of seafood [19]. *V. vulnificus* can cause fatal wound infections, since the bacterium can enter into the circulatory system and cause septicemia. Low salinities (5 to 25 ppt) and warm temperatures (20 to 35 °C) are reported to be favorable for this organism [20]. Filter feeding oysters and mussels, which concentrate *V. vulnificus* to several-fold higher concentrations than the surrounding water, have been commonly responsible for infections in the past. However, this organism's occurrence in frozen processed shrimp suggests potential health hazards if such products are consumed raw.

## 2.2. *Listeria monocytogenes*

*L. monocytogenes* is commonly distributed in nature and is an important pathogen of humans, especially in those with underlying immunological debilitations whose immune response is compromised [21]. Of 13 serovars of *L. monocytogenes*, 1/2a, 1/2b, and 4b are more frequently involved in human listeriosis [22]. The infection occurs when foods containing high levels of *L. monocytogenes* are consumed. Most outbreaks occurred with ready-to-eat, minimally processed foods [22]. *L. monocytogenes* is not indigenous to the marine environment and hence is not found in wild-caught or farmed fish. However, post-harvest contamination can result in the introduction of this pathogen into fish. Contamination of the ready-to-eat products can occur after the preparation of the products, and when such products are held at temperatures suitable for the growth of *L. monocytogenes*, their numbers can increase rapidly to infectious levels [23]. *L. monocytogenes* can grow in refrigeration temperatures of up to 3.3 °C,

and, hence, for storage of RTE products, temperatures of <3 °C are recommended. The US FDA (United States Food and Drug Administration) has imposed a zero-tolerance limit for *L. monocytogenes* in ready-to-eat seafood products such as smoked fish or crab meat [24].

## 2.3. *Salmonella enterica*

*Salmonella* contamination of the marine environment has been recognized as a serious threat to human health worldwide. Seafood harvested from contaminated water act as an essential vehicle of salmonellosis transmission, and human infection is generally associated with the consumption of raw or undercooked bivalve mollusks [25]. Though *Salmonella* is not indigenous to the marine environment, the bacterium has been isolated from it and seafood worldwide. It is primarily attributed to the discharge of domestic sewage into the marine-estuarine environment and the land runoff [26, 27]. Filter feeding animals such as oysters and clams concentrate pathogenic bacteria present in the surrounding water in their tissues and thus act as reservoirs of *Salmonella*. Hence, most seafood-associated salmonellosis worldwide have been associated with shellfish consumption [28].

*Salmonella* involved in human infections via seafood generally belongs to non-typhoid serotypes [29]. The occurrence of *Salmonella* in fish and shellfish is solely due to fecal contamination of culture environments as well as the post-harvest contaminations through various routes such as ice prepared from non-potable water, contaminated water used for washing fish and shellfish, and fish handlers. The prevalence of *Salmonella* in aquaculture environments has been reported [30, 31]. The accumulation of organic matter in the sediment provides a conducive environment for pathogen survival and accumulation in aquaculture ponds [31, 32]. Studies reported by the US FDA showed that farmed seafood was more likely to contain *Salmonella* than wild-caught seafood [33].

*Salmonella* contamination is a significant reason for the detentions of imported shrimps by the US and EU. Many countries, including the USA, EU, New Zealand, and Australia, recommend the absence of *Salmonella* in 25 g of raw or RTE shrimps [34]. Specific serovars such as S. Weltevreden, commonly

reported from Asian shrimp culture systems, are also significant agents of non-typhoidal salmonellosis [35, 36]. Their presence in farmed shrimp is a definite human health risk, and there are possibilities of these gaining more virulence due to complex interactions in the aquatic environment, and the exposure to antibiotics used in aquaculture farms can lead to the development of resistance to these agents. *Salmonella* can enter into the shrimp culture environments through water, feed, manures, and even probiotics [34]. The use of organic manures increases the risk of introducing *Salmonella* into the culture environment.

### 3. Antimicrobial resistance in bacteria

Antimicrobial compounds, which include antibiotics and chemicals intended to kill or halt the proliferation of unwanted bacteria, have positively changed the perspective of treating human infectious diseases in the last century. However, the use of such chemicals in treating diseases and food preservation was in vogue since time immemorial, though techniques were not available to determine their sources or obtain them in pure form. With the advent of microbiology, biochemistry, and analytical techniques, numerous antibacterial compounds could be identified, purified, or even synthesized for human use. Over the years, antibiotics, apart from being used in treating human and animal infections, have also been utilized in animal husbandry and aquaculture for growth promotion, feed efficiency, prophylaxis, and the treatment of infections [37]. However, unlike in humans where antibiotics are prescribed for a specific duration following the diagnosis of bacterial infections, the antibiotic use in animals and aquaculture is arbitrary, often without isolating or identifying the causative agent, and many a time, unwarranted [38]. Due to the unregulated use of antibiotics for purposes other than treatment of infection, humans are exposed to antibiotic-resistant pathogens in food and the environment, resulting in treatment crises worldwide. The US FDA has mandated the risk assessment of new antibiotics to experimentally prove that the use of such antibiotics in livestock does not contribute to the development of drug resistance in bacteria of human health significance [39]. The use of antibiotics in livestock and aquaculture invariably leads to the development

of resistance among human pathogens, and human infections by such bacteria become untreatable, or the treatment becomes prolonged and complicated. The antibiotics are primarily for treating infectious diseases of humans and animals with a dosage scientifically determined to avoid the development of resistance so that in the absence of any new antibiotics, we can still successfully treat human infections. However, the overuse or irrational use of antibiotics leads the world to the 'post-antibiotic era', with some pathogenic bacteria becoming extremely resistant or resistant to all currently available antibiotics [40]. Due to various infectious diseases in densely populated, low-income countries, both the morbidity and mortality rates are increasing, and their treatment is critically affected by the development of antibiotic resistance among pathogenic bacteria [41]. The use of antibiotics for short-term gains can inflict a long-lasting negative impact on human health and complicate antibiotics' biomedical applications.

#### 3.1. How significant is bacterial antimicrobial resistance?

Antimicrobial agents are used for treating infectious diseases caused by pathogenic and opportunistic bacteria in humans. In some cases, the microbial infections are self-limiting, requiring no chemotherapeutic interventions. Hence, the judicious use of antibiotics is also crucial. Despite all the scientific measures taken to prevent antibiotic resistance, bacteria have developed multidrug resistance, which circumvents such drugs' therapeutic efficacy [42-44]. Antimicrobial-resistant bacteria confound the chemotherapeutic efforts, leading to treatment failures as often observed in recent times all over the world [45, 46]. The association of multidrug-resistant bacteria with infectious diseases has become a serious global health concern. Particular examples are the New Delhi Metallo  $\beta$ -lactamase (NDM) producing *Klebsiella pneumoniae*, the methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant *Enterococcus faecium* (VRE), and extremely drug-resistant *Mycobacterium tuberculosis* (XDR) [40]. The global epidemiological data has identified multidrug-resistant bacteria belonging to the ESKAPE group consisting of *Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and

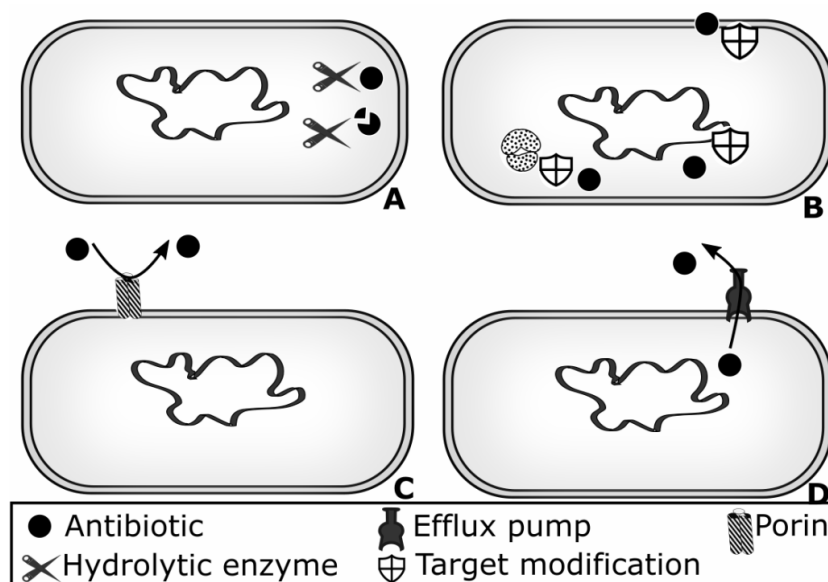
*Enterobacter* spp. as presenting an enormous treatment challenge and responsible for significant mortalities worldwide [47, 48].

### 3.2. Overview of bacterial antimicrobial resistance mechanisms

After exposure to antimicrobial agents, bacteria may develop drug resistance mechanisms or, in some cases, be intrinsically resistant to certain antimicrobials [49]. These resistance determinants reduce the antimicrobial activities of the drugs. There are four primary molecular mechanisms of bacterial drug resistance (Figure 1). (i) The metabolic degradation of the antimicrobial agent involves some microbial enzymes, which convert the drug into an ineffective compound incapable of killing or inhibiting the bacterium's growth [50]. (ii) Alteration of cellular drug target through various modifications so that the drug no longer effectively binds to its target and can be ineffective against the bacterium. Such targets in a bacterial cell include the ribosomal protein subunits, nucleic acids, metabolic enzymes, and bacterial cell wall components [51, 52]. (iii) Reduced drug permeability involves the drug's inability to gain entry into the cytoplasm of the bacterial cell. Such a drug-resistant bacterium might have opted to reduce the

expression of a putative drug transport system in the membrane, thus reducing cellular drug entry [53]. (iv) Antimicrobial agent efflux pump systems operate by extruding drugs from the bacterial cell, reducing the sufficient cellular drug concentrations [54]. The drug efflux pumps are embedded in the biological membrane and energized passively or actively [42, 55]. Table 1 gives an overview of bacterial mechanisms of antibiotic resistance against various classes of clinically relevant antibiotics.

Bacteria may be single-drug or multidrug-resistant (MDR). Multiple mechanisms are associated with the MDR phenotype, and these are genetically encoded on some mobile genetic elements such as the transposons and plasmids [56]. Bacteria become MDR when they acquire multiple genetic factors from different sources through various conjugation mechanisms, transduction, or transformation [57]. Apart from the acquired resistance, bacteria have chromosomally encoded intrinsic resistance mechanisms such as the *marRAB* locus of *Escherichia coli*, which confers intrinsic resistance to many antibiotics such as tetracyclines, chloramphenicol, cephalosporins, penicillins, nalidixic acid, fluoroquinolones, rifampin, organic solvents, and others [58, 59]. The antibiotic resistance mediated by the *mar* locus is due to the efflux mechanism,



**Figure 1.** Antibiotic resistance mechanisms are mediated by the enzymatic degradation or modification of antibiotics, reduced uptake of antibiotics, and the active extrusion of antibiotics from the cellular environments.

**Table 1.** Mechanisms of bacterial resistance to antibiotics.

Mechanism	Antibiotic class	Antibiotics	Resistance protein
Enzymatic hydrolysis	$\beta$ -Lactams	Penicillins Cephalosporins Carbapenems Monobactams	$\beta$ -lactamases
	Macrolides	Erythromycin	Macrolide esterase
	Epoxide	Fosfomicin	Epoxidase
Redox process	Tetracycline	Tetracycline	TetX monooxygenase
Group transfer/ enzymatic modification	Aminoglycosides	Gentamicin, Tobramycin, Amikacin	Aminoglycoside modifying enzymes (AME) • N-acetyltransferases (AAC) • O-phosphotransferases (ANT) • O-adenylyltransferases (ANT) • 16S rRNA methyltransferases
	Phenicol Streptogramins	Chloramphenicol Streptogramin A/B	Acetyltransferases
	Rifamycin	Rifampicin	Rifampicin monooxygenase (RIFMO)
Target modification	$\beta$ -Lactams	Penicillin Methicillin Cephalosporins	Modified penicillin-binding proteins
		Vancomycin	Modified cell wall precursor peptides
		Streptomycin	Modified ribosomal subunit
		Rifamycin	Modified RNA polymerase
	Oxazolidinone	Linezolid	Mutation in 23SrRNA
	Quinolones/ Fluoroquinolones	Ciprofloxacin Norfloxacin Moxifloxacin	Modified topoisomerase
	Macrolides	Erythromycin Clindamycin	Methylation of ribosomal RNA
Tetracycline		Ribosomal protection	
Reduced uptake	$\beta$ -Lactams Quinolones Tetracyclines Trimethoprim	Penicillins/cephalosporines Fluoroquinolones Tetracycline	Reduced porin expression
Active efflux	Diverse antibiotics		Efflux pumps

which involves the AcrAB efflux pump encoded on the *mar* locus [59, 60]. In *E. coli*, *Salmonella enterica*, and *K. pneumoniae*, the *mar* locus is constitutively expressed, making these bacteria resistant to several antibiotics and organic solvents [61]. Several *mar* transcription modulators have been identified, including benzoate, sodium salicylate, paraquat, dinitrophenol, menadione, and plumbagin, among others [59, 60].

#### 4. Antimicrobial-resistant bacteria in fish and fishery products

The use of antibiotics in food-growing facilities is responsible for resistant bacteria in the food chain. Many food animals and birds are the natural reservoirs of human pathogenic bacteria such as *Salmonella enterica*, Shiga toxin-producing *Escherichia coli* (STEC), and *Campylobacter* [62]. The use of antimicrobials in food animals naturally leads to the development of resistance in these bacteria, which get easily transmitted to humans *via* the food [37]. Also, since resistant bacteria reside in these food animals, their excreta can contaminate the environment with such bacteria, reaching humans *via* water or food [63, 64]. Further, humans can acquire such bacteria through touch or contact with the pet or livestock animals carrying resistant bacteria such as MRSA. When the resistant mechanisms are associated with plasmids and the transposons, they spread quickly among related and unrelated bacteria [42]. The magnitude of human health risk due to antibiotic-resistant bacteria has been highlighted by a study in which ready-to-eat shrimps from four countries were found to harbor multidrug-resistant bacteria [65]. Forty-two percent of the isolates from shrimp were resistant to antimicrobial agents, including important human pathogens such as *E. coli*, *Enterococcus* spp., *Salmonella* spp., *Shigella flexneri*, *Staphylococcus* spp. and *Vibrio* spp. The presence of antibiotic-resistant bacteria in ready-to-eat products constitutes a significant human health hazard since the consumers are directly exposed to the risk of infection by these pathogens upon consumption [66]. In a study in the United States, 105 *Salmonella* strains isolated from imported seafood during 2000-2005 were tested for antibiotic resistance and two isolates belonging to serovars Oslo and Bareily

resistant to trimethoprim/sulfamethoxazole, sulfisoxazole, ampicillin, tetracycline and chloramphenicol were found, which were isolated from seafood imported from India and Vietnam [67]. Both these isolates harbored Class I integrons suggesting that the resistance genes could be easily transferred to other pathogenic bacteria making them drug resistant. The growing concerns on the presence of multiple antibiotic resistant bacteria in seafood from Asian countries together with the unregulated use of antibiotics in aquaculture need to be addressed scientifically to develop strategies to prevent the development and spread of antibiotic-resistant bacteria in food fish.

##### 4.1. Antibiotic use in aquaculture and the development of resistance

The traditional, extensive types of aquaculture practices were environmentally friendly and sustenance oriented, but their output in terms of productivity was far too little. Commercial aquaculture, which has replaced traditional aquaculture, is intensive in nature, both in terms of capital input and in farming practices. Intensive or high density stocking, use of commercial feeds, growth-promoting products, and chemicals to control microbial and parasitic diseases are all integral to intensive aquaculture practices. Microbial diseases, both viral and bacterial, together with parasitic infestation and changes in physico-chemical conditions adversely affect the health of cultured animals leading to large scale mortalities and consequent economic losses to the farmer. These concerns force the fish farmers to use antibiotics, antimicrobials, pesticides, bioremediators (biological and chemical), and probiotics for prophylaxis and controlling the outbreaks of diseases [68]. When antibiotics are used, the residues in water find their way into the natural environments through the pond effluents. Sublethal amounts of such antibiotics in aquaculture ponds or the environment can lead to resistance development, both in pathogenic and non-pathogenic bacteria [69, 70]. Examples include *Aeromonas hydrophila*, *Edwardsiella tarda*, *Yersinia ruckeri*, *Citrobacter freundii*, *Lactococcus garviae*, *Photobacterium damsela*, *Vibrio anguillarum*, *Vibrio salmonicida*, *V. harveyi*, *Photobacterium psychrophilum*, and *Pseudomonas fluorescens* [71-73]. Bacteria become resistant to

multiple drugs when they acquire resistance mechanisms (genes) through horizontal transfer and mutations or increased expression of the efflux pumps [74]. More importantly, the use of one antibiotic can lead to the development of resistance to multiple other unrelated drugs, as reported by Fernández-Alarcón [75], who found multidrug resistance among bacteria isolated from a Salmon farm in Chile using only florfenicol. Scientific evidence suggests that some of the resistant forms of human pathogens acquired their genetic determinants from the aquaculture environment [75-77].

At present, antibiotics are used in aquaculture by farmers without proper scientific guidance. In many cases, antibiotic use can be avoided if good farming practices are followed in aquaculture, such as the maintenance of pond water quality, low to medium stocking density, water exchange and treatment, and proper diagnosis of the problem. Many fish and shrimp diseases are viral or parasitic in nature, and the knee-jerk reaction to mortalities occurring in the pond is to employ antibiotics to control infections, which serves little purpose. The use of antibiotics in aquaculture should be regulated and monitored, and the farmers should be extensively educated about the negative impact of using antibiotics in their farms.

#### 4.2. Legislation and control of the use of antibiotics

The World Health Organization (WHO) has classified some antibiotics as critically important for treating human infections and thus, has advocated against their use in veterinary or aquaculture [78]. In some countries, antibiotics cannot be purchased unless prescribed by a qualified veterinarian [68]. In the US, a few antibiotics such as tetracycline, florfenicol, sulfadimethoxine-ormetoprim are approved for administration in aquaculture settings by the Food and Drug Administration (FDA) [79, 80]. In developing countries, where most of the world's aquafarming occurs, there are no records of antibiotics sold or used in aquaculture. A Joint FAO/OIE/WHO expert consultation on antimicrobial use in aquaculture and antimicrobial resistance in 2006 calls for prudent use of antibiotics in aquaculture, proper monitoring, and management following the WHO global principles, the

recommendations of OIE (International office of epizootics), and the Codex code of practice for fish and fishery products.

#### CONFLICT OF INTEREST STATEMENT

The authors of this review article declare no conflicts of interest.

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