Methods for containment of antimicrobial resistance in farmed fish intended for food

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Introduction:

An estimated three million deaths/year occur around the world, in developed and developing countries, from food and water-borne diseases, with millions more becoming sick as reported by the Food and Agriculture Organisation. Occurrence of food poisoning outbreaks can adversely impact national economies and livelihoods leading to closure of export markets, and to high cost of addressing both the effects of the threat on public health and commerce. Furthermore, according to the Centres for Disease Control and Prevention (USA), which lead several programmes to monitor antimicrobial resistance of bacteria, resistant strains of three major human pathogens – *Salmonella* spp., *Campylobacter* spp. and *Escherichia coli* – are linked to the use of antibiotics in animal husbandry. Literature has reported that the major route of transmission of resistant microorganisms from animals to humans is through the food chain [1, 2]. Considering the trade globalisation, hazards related to antibiotic use are not limited to producing countries, but concern importing countries as well. The World Health Organisation has then targeted antibiotic resistance as one of the major emerging public health concern that need a global strategy for its containment.

Aquaculture is currently the fastest growing food-producing sector in the world. It now accounts for nearly half of the world’s food fish. Developing countries account for 50 percent of the world’s traded fish and seafood by value and 61 percent by volume (FAO, 2010). Some 50 percent by value of the fisheries exports from developing countries are destined to developed country markets (EU, USA) which have stringent requirements regarding food safety, quality standards, certification as well as the social and environmental awareness of the consumers of those markets. Asean countries account for about 90% of the world's fish farming production. The aquaculture sector in Vietnam has greatly developed in the last two decades. Aquaculture production has increased from a total of 1,202,500 tonnes in 2004 to 2,430,944 tonnes in 2008. Shrimp and catfish are considered as two of the major aquaculture products for Vietnam which are mostly produced in the Mekong River Delta. Penaeid shrimp (*Penaeus monodon*) production has increased from 281,800 tonnes in 2004 to 381,728 tonnes in 2008. Catfish culture (*Pangasius hypophthalmus* and *Pangasius bocourti*) has increased from 315,000 tonnes in 2004 to over one million tonnes in 2008, equal to about 55% of the total fish aquaculture production of Vietnam. In value terms, shrimp is the principal seafood export from Vietnam.

Among the main hazards threatening aquaculture industry, infectious diseases may cause serious economic and stock losses. So antibiotics have been used over the world for prophylactic and therapeutic purposes. However, it is now recognized that the intensive use of antibiotics in aquaculture has been associated with the increase of antibiotic residues in food products; and of bacterial resistance in products and the exposed microbial environment (water and sediment bodies) as well. The literature has shown that (i) once acquired, resistance genes could be maintained even in the absence of the corresponding antibiotic, (ii) farming practices impact extends beyond the individual farm environment, (iii) in response to the antibiotic pressure, bacteria optimises its resistance system towards multiple drugs to survive which leads to multiresistance patterns. Consequently, the contamination of the environment with bacterial pathogens resistant to antimicrobial agents is a real threat not only as a source of disease but also as a source from which resistance genes can easily spread to others pathogens of diverse origins. This phenomenon has severe implications on both animal and human health. From this point of view, control of diseases with veterinary drugs and antimicrobials should be carried out only on the basis of an accurate diagnosis and knowledge that the drug is effective for control or treatment of a specific disease. Prophylactic use of veterinary medicinal products, particularly antimicrobial agents, should be avoided. Furthermore, an increase in water temperatures due to climate change is expected to promote the growth of organisms such as opportunistic *Vibrio* spp. leading to an increased human health risk from handling or consuming fish grown in these waters. For these reasons, international organisations (FAO, WHO, OIE) recommend that antibiotics should be restricted to therapeutic purposes only, and that preventive approaches for disease management should be foster through improvement in animal husbandry and production practices to reduce infections spread.

To this end, it is important to emphasize that good aquaculture management practices are essential to maintain a healthy environment for farmed finfish and crustaceans. As recommended by the European Union Conference of the Microbial Threat [1], priority should be given to good hygiene with proper handling practices and other preventive measures in containment of resistant infections. Among guidelines specified by FAO [3], aquaculture facilities and operations should maintain good culture and hygienic conditions, including:

- Good Hygiene Practices in the farm surroundings aiming at minimizing contamination of water bodies, particularly from waste materials or faecal contaminations from animals or humans, because rearing water is the main source for entry of pathogens in the host.
- Good Aquaculture Practices to ensure good hygienic culture conditions and safety and quality of aquaculture products
- Farms should institute a pest control program, so that rodents, birds and other wild and domesticated animals are controlled, especially around feed storage areas

Workers should be trained to good hygienic practices to ensure they are aware of their roles and responsibilities for protecting aquaculture products from contamination. Evidence from the literature suggests that beyond traditional approaches based on training, food analysis and official inspections, there is a need to ensure a “food safety culture” to improve food safety performance. As defined by Yiannas [4], food safety culture is the way in which an organisation or a group approaches food safety in thought and in behaviour. It means that operators: (i) know the risk associated with the food they produce; (ii) know how it should be managed and effectively manage it; (iii) promote a value system that focuses on preventing illness [5].

Traceability of products and record-keeping of farming activities and inputs related to food safety should be ensured by documenting*:*

- The source of inputs such as feed, seed, veterinary drugs and antibacterials, additives, chemicals

- Type, concentration, dosage, method of administration and withdrawal times of chemicals, veterinary drugs and antibacterials and the rationale for their use

To cope with a sustainable development of aquaculture over the world and to mitigate adverse effects of antibiotic resistance towards animal and human health, applied research, education and training activities are required to address these new challenges.

What is antibiotic resistance of bacteria?

A bacterial strain or a species is termed “resistant” if it has the ability to function, survive, or persist in the presence of higher concentrations of an antimicrobial agent than the members of the parental population from which it emerged, or than other species respectively. Cross-resistance refers to the fact that resistance to one antimicrobial compound within a class of antimicrobials often confers resistance to other members of the same class.

Resistance to a variety of antibiotics has been largely reported in bacteria isolated from aquaculture environments such as the water body, sediments and fish. In many studies, the bacterial resistance levels were correlated to the pattern of antimicrobial use in the farms [6-8]. Antibiotic resistance among *Vibrio* and *Aeromonas* strains has been found higher in the shrimp hatcheries than in the *Penaeus monodon* culture ponds, suggesting use of antibiotics in the hatcheries rather than in the farms [9]. The analysis of resistance susceptibility of bacterial isolates from water, sediment and different fish farms (catfish, tilapia, common carp and gouramy) in the Mekong river showed high levels of resistance to tetracycline, ampicillin, chloramphenicol, nitrofurantoin and trimethoprim-sulfamethoxazole [10, 11]

A bacterium that is naturally susceptible to an antibiotic can acquire resistance in two distinct ways: through mutation in the relevant gene or through uptake of copy of a resistance gene present in other bacteria. Resistance acquired through mutation will be confined to the mutant clone and emergence and spread will depend on the clone's ability to multiply and infect new hosts (vertical transmission). More common is uptake of resistance genes (horizontal gene transfer). These may spread from one bacterial cell to another. Several resistance determinants in fish bacteria have been reported to be carried by transferable genetic elements such as plasmids, transposons or integrons [12-15]. Mobile DNA elements-encoded resistance determinants were often found in fish or water-associated bacteria as for tetracyclines, chloramphenicol, sulphonamide, trimethoprime or ß-lactams resistance [14, 16-19].

What alternatives to antibiotic?

Vibrio spp. such as *V. cholerae, V. parahaemolyticus, V. vulnificus* and *V. alginolyticus* are human pathogens that are often isolated from fish or their immediate environment. Vibriosis are a major constraint on the intensive production of shrimps as Vibrionaceae family is one of the most important groups in marine environments and the major pathogenic bacteria for penaeid crustacean larvae [20, 21]. Since these bacteria are common in the marine environment, the culture pond serves then as a constant source of exposure for the shrimp. Thus massive mortalities of shrimp larvae associated with luminescent strains of *Vibrio* spp. have been reported in hatcheries from several countries [22, 23].

Therefore, strategies to prevent and to control infectious diseases need to be developed in order to make the aquaculture industry more sustainable and efficient [24-26]. Vaccination of fish for instance (not applied to shrimps culture) has allowed significant reduction of antibiotics in salmon culture [27]. Commercial probiotics including *Lactobacillus sp., Bacillus sp.* and other bacteria from different genera, but also yeast are increasingly used for shrimp farming (Farfanzar, 2006; Ravi *et al.*, 2007). A probiotic is a mono or mixed culture of live microorganisms that affects beneficially the host by improving the properties of the endogenous microflora (Havenaar *et al*., 1992). Though the modes of action of probiotics are not fully understood, it is assumed that they may display multiple effects involving bacterial antagonism, competition with harmful bacteria, production of inhibitory compounds (e.g. bacteriocines) or stimulation of the host immune system. However, high doses of probiotics might be needed, as they are not able to maintain themselves in natural environment. This variability in response to probiotics and the lack of reliable data hinder the use of this practice in routine.

Among alternatives to antibiotics in aquaculture, use of plant extracts as immunostimulants for enhancement of nonspecific host defence mechanisms or as antimicrobials for bacterial growth inhibition has been reported as a relevant strategy [28-32]. Plants are generally considered as rich sources of safe and economical active compounds [33]. Use of plant extracts to replace chemotherapeutics in aquaculture, and in organic farming in particular, has been generally achieved through oral administration (bioencapsulation) or direct mixture with feed ingredients. As the plant extracts are non-palatable to shrimps, they could be first fed to *Artemia* which acts as a biological carrier [34]. Similar mortalities have been found between *Streptococcus iniae*-infected tilapia which were treated with *Rosmarinus officinalis* extract or leaf powder and those treated with oxytetracycline [35]. Juveniles of *Penaeus indicus* fed with the enriched diets (from terrestrial plants: *Ricinus communis*, *Phyllanthus niruri*, *Leucus aspera*, *Manihot esculenta*, and sea weeds: *Ulva lactuca* and *Sargassum wightii*) had better survival and growth in addition to inhibit bacterial load of *V. parahaemolyticus* [34]. Similarly, the hot-water extracts from leaves and twigs of *Camphor kanekirae* have also shown better immunity of *Litopenaeus vannamei* shrimp and disease resistance to *V. alginolyticus* [29]. The supplementation of enriched *Artemia* with plant extracts of *Withania somnifera* and *Mucuna pruriens* showed higher larval quality indices including the cumulative larval survival of *P. monodon* [36]. The protection elicited by *Dendrilla nigra*, a marine sponge, has been reported to be due to its antibiotic effect against *V. harveyi* and *V. alginolyticus* rather than its influence on the host defense system of shrimps [37]. Several essential oil components have been reported as efficient antibacterial against fish and human pathogens [38]. Their inhibitory activity results from a complex interaction between their different constituents, which may produce additive, synergistic or antagonistic effects, even for those present at low concentrations [39]. Use of essential oils of *Cinnamosma fragrans* (an endemic tree to Madagascar) in water tank have significantly reduced the bacterial load of *Vibrio* spp. population in *in vivo* conditions of *Penaeus monodon* larval culture; and it has enhanced the survival rate of *Penaeus monodon* larvae as well [40, 41]. Due to their mode of action affecting several targets, no particular resistance or adaptation to essential oils has been described so far in the literature [42].

Conclusion:

Different techniques have been investigated to reduce the use of antibiotics in aquaculture but each one has its advantages and its limitations. Further research is needed to adapt these techniques to the system of production and the type of culture. Efficiency might be reached through the combination of different methodologies to avoid bacterial resistance and to look for synergies among the selected methodologies. It would be interesting to explore antimicrobial essential oils as they are natural substances. In this regards, research is needed (i) to characterize active essential oils from the rich biodiversity that exist in many Asean producing countries; (ii) to test their implementation in different aquaculture systems against fish pathogens and opportunistic strains; and (iii) to determine their limitations and how they could be complementary to other measures.

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