Minimising antimicrobial use in aquaculture and improving food safety

Iddya Karunasagar
Fisheries and Aquaculture Department
Food and Agriculture Organization
Rome, Italy

SUMMARY

Aquaculture is contributing to about 50 percent of global fish production and in most parts of the world fish capture production has stagnated for over a decade because maximum sustainable yields have been reached. Therefore the increasing demand for fish has to be met by increasing fish production by aquaculture. This has been a challenge because disease outbreaks have been causing serious losses in both finfish as well as shellfish aquaculture. Detection of residues, in some countries, of certain banned antibiotics in aquaculture products has led to consumer concerns about the safety of these products. There is also growing concern about the emergence and spread of bacteria resistant to antimicrobial agents and transfer of resistance determinants to human pathogens that may be associated with the aquatic environment. In this context, there is a need to look for alternatives to antimicrobial agents for health management in aquaculture.

Most often, disease problems in aquaculture are because of a shift in the delicate balance between the host, the pathogen and the environment. Therefore, disease problems can be significantly reduced by adopting good management practices. In the aquaculture of salmonids, the use of antimicrobials could be minimised substantially by vaccinating fish against some of the common bacterial and viral diseases. However, currently, no vaccines are available for parasitic diseases. Further, global aquaculture is dominated by Asian cyprinids and currently, there are no commercial vaccines available for these species. Crustaceans have a poorly developed immune system and there are no commercial vaccines for this sector of aquaculture. However, the innate immune response of fish and crustaceans can be stimulated by certain microbial molecules like glucans that can act as immunostimulants. Currently, immunostimulants are widely used for health management both in finfish as well as crustacean aquaculture.

Probiotics have become useful tools for health management and the term "probiotics" has been more broadly used in aquaculture to refer to microbial agents that have beneficial effects on cultured animals in a number of ways. Most of the aquaculture probiotics are thought to act by modifying the microbial community around the animals in favour of beneficial microorganisms that may improve the water or sediment quality, suppress pathogenic bacteria, stimulate the immune system of the host or improve host digestion. The technology of bacteriophage therapy is attracting the attention of medical professionals because of the increasing incidence of human infections with multi-drug resistant bacteria. Scientific studies show that even in bacterial diseases of fish and shrimp, bacteriophage therapy could be effective. Commercial products based on bacteriophages for pathogen control in agriculture, aquaculture and food processing are available in some countries. Thus, there are

number of alternative technologies for health management in aquaculture and these have potential to contribute to minimising antimicrobial use in this sector.

PUBLIC HEALTH AND TRADE IMPACT OF THE USE OF ANTIMICROBIALS IN AQUACULTURE

The contribution of aquaculture to world fish production is increasing rapidly. In 2006, 47 percent of the 110 million tonnes of world food fish supply came from aquaculture (FAO, 2009). The annual growth rate in world aquaculture production during 2004 to 2006 was 6.1 percent in volume terms and 11 percent in value terms. The Asia Pacific Region accounts for 89 percent of production in terms of quantity and 77 percent in terms of value. As increasing quantities of aquaculture product are reaching markets, there is also considerable concern on safety issues and the detection of residues of antibiotics has been one the major issues. It is very difficult to obtain data on the usage of antimicrobials in aquaculture. The World Organisation for Animal Health (OIE) prepared a list of antimicrobials of veterinary importance (Table 1). This follows the recommendation of the FAO/OIE/WHO Expert Workshop on non-human antimicrobial usage and antimicrobial resistance that OIE and WHO should develop a list of critically important antimicrobials in veterinary medicine and human medicine respectively (FAO/OIE/WHO, 2003).

The OIE list was prepared based on response to a questionnaire sent to member countries. Two criteria were used to assess the importance of antimicrobials in veterinary medicine:

a) Response rate to the questionnaire regarding veterinary critically important antimicrobials. This criterion was met when more than 50 percent of the respondents identified the importance of the antimicrobial.

b) Treatment of serious animal disease and availability of alternative antimicrobials.

Antimicrobials meeting both criteria were designated "veterinary critically important antimicrobials". Those meeting one of the criteria were designated "veterinary highly important antimicrobials". Those meeting none of the criteria were designated "veterinary important antimicrobials". Table 1 shows all antimicrobials used in aquaculture appearing in the OIE list and antimicrobials licensed for use in aquaculture in the United States of America and in the European Union.

There is no reliable data on licensing or national usage from the Asia Pacific region, but available evidence suggests that considerable quantities are used in some countries, often without professional consultation or supervision. Insufficient regulations and limited enforcement in many countries where aquaculture is an important industry are major problems that need to be addressed.

At the international level, it is being recognized that while antimicrobial agents are important for animal health protection, the negative impacts of their use in food producing animals should be minimized. The Food and Agriculture Organization of the United Nations (FAO), the World Health Organization (WHO) and the World Organisation for Animal Health (OIE) have organized several expert consultations and technical meetings to review the global situation and develop recommendations.

TABLE 1
Antimicrobials licensed/used in aquaculture

Antimicrobials appearing in OIE list[1]	Antimicrobials approved by US FDA[2]	Antimicrobials approved in EU[3]
Spectinomycin	Oxytetracycline	Amoxycillin
Streptomycin	Florfenicol	Florfenicol
Kanamycin	Sulfadimethoxine/ ormetoprim	Oxolonic acid
Bicozamycin		Oxytetracycline
Fosfomycin		Flumequine
Lincomycin		Sarafloxacin
Erythromycin		Sulphadiazine + trimethoprim
Josamycin		
Spiramycin		
Novobiocin		
Amoxycillin		
Ampicillin		
Tobicillin		
Florphenicol		
Thiamphenicol		
Flumequin		
Miloxacin		
Oxalonic acid		
Enrofloxacin		
Sulphadimethoxine		
Sulphafurazole		
Sulphamethoxine		
Sulphamonomethoxine		
Trimethoprim + sulphonamide		
Doxycycline, Oxytetracycline, Tetracycline		

[1] www.oie.int/downld/Antimicrobials/OIE_list_antimicrobials.pdf.
[2] www.fda.gov/AnimalVeterinary/DevelopmentApprovalProcess/Aquaculture/ucm132954.htm.
[3] Rodgers and Furones, 2009 accessed at ressources.ciheam.org/om/pdf/a86/00801061.pdf.

The public health hazards related to antimicrobial use in aquaculture include the development and spread of antimicrobial resistant bacteria and resistance genes and the occurrence of antimicrobial residues in products of aquaculture (FAO/OIE/WHO, 2006). Bacterial resistance to antimicrobial agents is a significant public health concern. The widespread use of antibiotics in different sectors such as animal husbandry, agriculture and human medicine has contributed to selection and spread of antibiotic-resistant bacteria in the environment. Antibiotic resistance genes can spread among unrelated bacteria without any phylogenetic, ecological or geographical barriers. The Joint FAO/OIE/WHO Expert Consultation on Antimicrobial Use in Aquaculture and Antimicrobial Resistance held in 2006 identified two types of hazards with respect of antimicrobial resistance:

1. Development of acquired resistance in bacteria in aquatic environments that can infect humans. This can be regarded as a direct spread of resistance from aquatic environments to humans; and
2. Development of acquired resistance in bacteria in aquatic environments whereby such resistant bacteria can act as a reservoir of resistance genes from which the genes can be further disseminated and ultimately end up in human pathogens. This can be viewed as an indirect spread of resistance from aquatic environments to humans caused by horizontal gene transfer.

The consequences of antimicrobial resistance in bacteria causing human infections could include increased severity of infection and increased frequency of treatment failures (FAO/OIE/WHO, 2006). However, there are no recorded cases of human infections caused by antibiotic-resistant bacteria from aquaculture products.

While the issue of selection and spread of antibiotic-resistant bacteria in aquaculture has been deliberated upon for quite some time, the issue of antimicrobial residues in aquaculture products came to the fore in 2001 following marked improvements in laboratory methods to detect residues. This was followed by disruptions of trade in aquaculture products. According to the World Trade Organization's (WTO) Sanitary and Phytosanitary (SPS) Agreement, countries have the right to establish measures to protect the life and health of their population and also to determine the level of protection that is appropriate for the country; however, available scientific evidence should be used when establishing control measures, and these measures should not be taken only to favour the domestic industry. Measures adopted by countries with respect to antibiotic residues and antibiotic-resistant bacteria would be within the framework of the SPS agreement.

At the international level, the responsibility of providing advice on risk management concerning veterinary drug residues lies with the Codex Alimentarius Commission (CAC) and its subsidiary body, the Codex Committee on Residues of Veterinary Drugs in Foods (CCRVDF). The primary responsibility for risk assessment lies with the Joint FAO/WHO Expert Committee on Food Additives (JECFA). CCRVDF determines the priorities for consideration of residues of veterinary drugs and JECFA provides independent scientific advice by evaluating the available data on veterinary drugs prioritized by CCRVDF. The Risk Assessment Policy for Setting of MRLs in Food established by the CAC defines the responsibilities of CCRVDF and JECFA and their interactions. For the establishment of a priority list, CCRVDF identifies, with the assistance of Members, the veterinary drugs that may pose a consumer safety problem and/or have a potential adverse impact on international trade. Veterinary drugs meeting some or all of the following criteria could appear on the priority list:

- A Member has proposed the compound for evaluation;
- A Member has established good veterinary practices with regard to the compound;
- The compound has the potential to cause public health and/or trade problems;
- It is available as a commercial product; and
- There is commitment that a dossier will be made available (CAC, 2010).

JECFA uses a risk assessment process to establish acceptable daily intake (ADI) and maximum residue limits (MRLs). Veterinary drugs that are toxic or have carcinogenic potential are not evaluated by JECFA and therefore no ADI or MRL is established. Chloramphenicol and nitrofurans, the main compounds that caused disruptions in trade in aquaculture products, belong to this category and are banned for use in food-producing animals in most countries. Presently, there is a Codex MRL only for chlortetracycline/oxytetracycline/tetracycline in fish and shrimp (CAC, 2009). However, there are national/regional MRLs for several other antimicrobial agents. In the European Union (EU), Commission Regulation (EC) No. 1181/2002 establishes MRLs for veterinary drugs in foods of animal origin, including aquaculture products. A lack of Codex MRLs for veterinary drugs could be a problem for many developing countries that adopt Codex MRLs as national MRLs. This situation led FAO/WHO (2004) to recommend that for veterinary drugs that have been evaluated by national governments and are legally used in many countries, a comprehensive approach needs to be adopted to expedite harmonization. A JECFA evaluation of substances may be constrained by a lack of sponsors. FAO/WHO (2004) recommended that with the assistance of JECFA and based on national/regional MRLs, an initial list of temporary/operative MRLs could be adopted by CCRVDF. This list could be made permanent by CAC, if the national/regional risk assessments are not questioned or if JECFA could establish an ADI using the data used by the country/region to propose an MRL. Substances that do not fulfil these requirements could then be moved to the list of compounds not to be used in food animals.

For veterinary drugs without an ADI or MRL, regulatory authorities generally adopt a zero tolerance approach. In this situation, as the analytical capability improves, levels that were not detectable by earlier technologies become detectable and hence reportable. Therefore, independent of any toxicological risk posed by the food product, the residues would attract regulatory action. The countries taking a zero tolerance approach argue that the products are not acceptable because they have evidence of the use of a banned drug in animal production and therefore it represents a violation of regulations.

Table 2 shows the rapid alerts that appeared in the European Union market resulting from residues of antimicrobials being found in fish and fishery products. The major veterinary drugs involved are chloramphenicol, nitrofuran metabolites and malachite green.

TABLE 2

Rapid alerts from detection of residues of veterinary drugs in the European Union

Veterinary drug	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010	Total
Chloramphenicol	44	102	9	8	1	1	4	2	3	4	178
Malachite green	0	2	11	18	50	17	9	2	5	4	118
Nitrofuran (including all metabolites)	0	89	51	27	30	41	31	48	89	10	416

Following the trade disruptions caused by the detection of residues, a Joint FAO/WHO Technical Workshop on Residues of Veterinary Drugs without ADI/MRL was held in 2004. This technical meeting recommended that for residues of drugs without an ADI/MRL, CCRVDF should request JECFA to perform and report, if possible, an estimate of the risks associated with the exposure to residues, because such risk estimates would be useful in risk management and that CAC should include consideration of cost–benefit and risk comparisons in their risk analysis process (FAO, 2004). Use of alternate risk management approaches that reflect the toxicological risk of the residue for regulatory analytical methods such as Recommended Performance Level (RPL) or a control strategy chosen by the competent authority were also recommended (FAO/WHO, 2004). They further emphasized that the illegal use of veterinary drugs cannot be condoned. The current lack of epidemiological data on the perceived public health risks and the cost of implementing regulatory measures based on analytical capability emphasize the need for more innovative approaches to manage this problem.

ALTERNATIVES TO ANTIMICROBIALS IN HEALTH MANAGEMENT IN AQUACULTURE

One of the major constraints faced by aquaculture is the serious loss because of disease outbreaks. Some examples are indicated in Table 3. Both shrimp and finfish farmers have lost millions of dollars because of outbreaks of diseases. Therefore, a health management strategy is very important for the success of commercial aquaculture. Most often, pathogens causing diseases are present in the environment in which fish are grown but disease outbreaks occur when the conditions are unfavourable for the fish e.g. overcrowding, environmental stress like drops in temperature, salinity. In the case of shrimp, for example, the presence of multiple viruses has been detected by sensitive diagnostic tests, like polymerase chain reaction, in shrimp farms showing normal growth (Umesha *et al.*, 2006). This suggests that for an infection (presence of a pathogen in a host system) to lead to a disease (alteration of the normal physiology of the animal), environmental factors are very important. Thus effective health management strategies should consider the interaction between the host, the pathogen and the environment. The goal of the strategies should be to prevent disease outbreaks occurring, thus minimizing the need for use of any antimicrobials.

TABLE 3
Some examples of economic losses because of diseases in aquaculture

Disease	Country	Economic impact	Reference
White spot disease of shrimp	Bangladesh	US$80.14 million during late 1990's	Alam *et al.*, 2007
White spot disease of shrimp	India	US$120 million during late 1990's	Karunasagar and Karunasagar, 1999
Yellow head disease and white spot disease of shrimp	Thailand	US$650 million in 1994	Chanratchakool *et al.*, 2001.
Shrimp viral diseases	Viet Nam	US$100 million in 1993	Khoa *et al.*, 2001
White spot disease of shrimp	Ecuador	US$280 million in 1999	Alday de Graindorge and Griffith, 2001
Bacterial diseases of finfish	China	Over US$120 million annually during 1990–1992	Wei, 2002
Infectious salmon anaemia (ISA) virus disease	United States of America United Kingdom	US$20 million in 2001 US$32 million in 1998–1999	Valderhaug, 2008

However, it should be pointed out that global aquaculture systems encompass diverse fish species and varied pond conditions. For example, carps in Asia are cultured in earthen ponds with fairly high organic matter content, while salmonids are cultured in rather clean waters in temperate environments. Aquaculture involves not only the vertebrate finfish, but also includes invertebrates like crustaceans and molluscs, which are at different evolutionary scales and have diverse physiological systems. A good understanding of the animal physiology, nutrition and immunological system would be essential to develop appropriate health management strategies. Some of the health management tools that have been successfully used in various aquaculture systems are detailed below.

Good aquaculture practices

Epidemiological studies have indicated that outbreaks of diseases in aquaculture systems are related to certain risk factors such as high stocking density, inadequate management of feed, fertilizers, water and sediment quality, the use of infected seeds, sudden changes in environmental conditions, such as temperature, salinity, dissolved oxygen, etc. Studies conducted in Asia in shrimp aquaculture have shown that, in some ponds, one can find animals infected with two or three viruses but growing normally (Umesha *et al.*, 2006). This shows that often pathogens are present in the environment (e.g. in water/sediment in the case of bacteria or in carrier animals in the case of viruses), but may not result in disease unless there are additional environmental factors affecting the host. Thus, for disease management, it is important to consider developing and implementing good aquaculture practices (GAPs). The general aspects to be looked into, such as site selection, water quality, source of fry and fingerlings, identification of hazards and defects, and production operations including feed and use of veterinary drugs, have been elaborated in the Codex Code of Practice for Fish and Fishery Products (CAC/RCP 52-2003). Depending on the species cultured and the surrounding environmental conditions, site-specific GAPs need to be worked out. One of the well known success stories is that of shrimp aquaculture in India. Following the outbreak of disease from whitespot syndrome in India, there were massive crop losses. The Marine Products Export Development Authority (MPEDA) of India, in collaboration with the Network of Aquaculture Centers in Asia and Pacific (NACA), initiated a programme to develop "Better Management Practices" (BMPs) in the State of Andhra Pradesh. The BMPs developed included a comprehensive set of measures such as good pond preparation, water quality management, pond bottom management, biosecurity and avoidance of animals carrying White Spot Syndrome Virus, good quality seed selection, feed management and waste management (Umesh *et al.*, 2010). Because most shrimp farmers in India operate small farms, often with a single pond, a cluster approach was used, so that farmers in an area joined together and followed the same practices. Over a period of 4 years, this approach led to a 31 percent reduction in disease prevalence compared with non-BMP ponds (Umesh *et al.*, 2010). This example illustrates that it is possible to achieve marked gains in production by following BMPs.

Vaccination

Vaccination has been successfully used for prevention of disease outbreaks in animal husbandry and some diseases have even been eradicated e.g. Bovine riderpest viral disease. Even in the aquaculture sector, there are success stories like the minimization of antimicrobial use in salmon culture in Norway. Bacterial vaccines were commercially used in aquaculture for the first time in the United States of America during the late 1970s against enteric red mouth disease (yersiniosis) and vibriosis (Evelyn, 1997). These early vaccines were based on inactivated whole bacterial cells administered by immersion. Application of industrially produced vaccines in aquaculture perhaps began in Norway, the major driving force being the huge losses to the salmon aquaculture industry because of vibriosis in the 1980s. In 1987, nearly 50 000 kg of antibiotics were used for production of about 5 000 tonnes of salmon, but the usage dropped following development of vaccines (Sommerset *et al.*, 2005). The antibiotics used in Norwegian salmon industry in 2003 were only 805 kg active ingredient for over 500 000 tonnes fish production (Burridge *et al.*, 2008). However, the use of antibiotics in salmon aquaculture varies depending upon the country. In Chile during 2003, 133 800 kg antibiotics were used for the production of 280 481 tonnes of salmon and in Canada, 30 373 kg antibiotics were used for production of 111 178 tonnes of salmon. This trend seems to be continuing with the salmon aquaculture industry in Chile using 385 600 kg antibiotics in 2007 and 325 600 kg antibiotics in 2008 to produce between 300 000 to 400 000 MT salmon (Burridge *et al.*, 2010). Thus, apart from availability of commercial vaccines, there are other factors like regulatory pressure that influence antimicrobial use in the aquaculture industry. Presently, vaccines are available for a large number of bacterial diseases and a few viral diseases (Tables 4 and 5). However, most of the vaccines available are for salmonids and there are very few vaccines for use in tropical aquaculture, one example being the streptococcosis vaccine for tilapia. According to FAO statistics (FAO, 2007), global aquaculture production in 2004 was dominated by carps and cyprinids (18.3 million tonnes) and shrimps and prawns (2.76 million tonnes) while salmon and trout production was only about 1.9 million tonnes. Thus, there are no commercial vaccines available for some of the major commercial fish species.

TABLE 4

Examples of multivalent/bivalent vaccines available for aquaculture in different regions

Diseases/Type of vaccines	Pathogen	Fish	Countries/Regions			
			North America	Europe	Chile	Japan/ Asia
Bivalent/Multivalent vaccines						
Furunculosis, Vibriosis	*Aeromonas salmonicida, Vibrio anguillarum, V. ordalli*	Salmonids	+	+		
Vibriosis, Yersiniosis	*Vibrio anguillarum, Yersinia ruckeri, V. ordalli*	Salmonids, cod	+	+		
Furunculosis, Vibriosis Coldwater vibriosis, Winter sore, Pancreas disease	*Aeromonas salmonicida, Vibrio anguillarum, V. salmonicida, Moritella viscosa,* Infectious pancreatic necrosis virus	Salmonids		+		
Furunculosis, Vibriosis, Infectious pancreatic necrosis, Salmonid Ricketsial Septicaemia (SRS), Infectious salmon anaemia (ISA)	*Aeromonas salmonicida, Vibrio anguillarum, Piscirickettsia salmonis*, Infectious pancreatic necrosis virus, ISA virus	Salmonids			+	
Furunculosis, Vibriosis, Infectious pancreatic necrosis, Salmonid Ricketsial Septicaemia (SRS)	*Aeromonas salmonicida, Vibrio anguillarum, Piscirickettsia salmonis*, Infectious pancreatic necrosis virus	Salmonids			+	
Infectious pancreatic necrosis, Salmonid Ricketsial Septicaemia (SRS)	*Piscirickettsia salmonis*, Infectious pancreatic necrosis virus	Salmonids			+	
Vibriosis, Infectious pancreatic necrosis	*Vibrio anguillarum*, Infectious pancreatic necrosis virus	Salmonids	+	+		
Furunculosis, Vibriosis, Infectious pancreatic necrosis	*Aeromonas salmonicida, Vibrio anguillarum,* Infectious pancreatic necrosis virus	Salmonids			+	
Vibriosis, Infectious pancreatic necrosis, Ricketsial Septicaemia (SRS)	*Vibrio anguillarum* Infectious pancreatic necrosis virus, *Piscirickettsia salmonis*	Salmonids			+	
Pasteurellosis, Vibriosis	*Photobacterium damselae, Vibrio anguillarum*	Salmonids		+		
Furunculosis, Infectious pancreatic necrosis (IPN)	*Aeromonas salmonicida,* Infectious pancreatic necrosis virus	Salmonids		+		
Pasteurellosis and Streptococcosis	*Photobacterium damselae, Lactococcus garvieae*	Yellowtail				+
Vibriosis, cold water vibriosis	*Vibrio anguillarum, V. ordalli, V. salmonicida*	Salmonids	+			
Vibriosis	*Vibrio anguillarum,* serotype O1, O2a, O2b	Salmonids, halibut, cod, seabass, seabream, Amberjack, yellowtail	+	+	+	+

TABLE 5

Examples of monovalent vaccines available for aquaculture in different regions

Diseases/Type of vaccines	Pathogen	Fish	Countries/Region			
			North America	Europe	Chile	Japan/ Asia
Vibriosis	*Vibrio anguillarum*, serotype O1	Yellowtails				+
Furunculosis	*Aeromonas salmonicida*	Salmonids	+	+		
Infectious salmon anaemia (ISA)	ISA virus	Salmonids			+	
Infectious pancreatic necrosis	IPN virus	Salmonids			+	
Enteric septicaemia	*Edwardsiella ictaluri*	Catfish	+			
Yersiniosis	*Yersinia ruckeri*	Salmonids	+	+	+	
Columnaris disease	*Flavobacterium columnare*	Catfish				+
Streptococcosis	*Streptococcus iniae*	Tilapia, seabass, grouper, flounder, halibut		+		+
Pasteurellosis	*Photobacterium damselae* subsp *piscicida*	Seabass and seabream	+	+		+
Lactococcosis	*Lactococcus garvieae*	Trout, Amberjack/ yellowtail		+		+
Cold water vibriosis	*V. salmonicida*	Salmonids	+	+		
Flavobacteriosis	*Flavobacterium psychrophilum*	Salmonids	+		+	
Bacterial kidney disease	*Renibacterium salmoninarum*	Salmonids	+		+	

While most available bacterial vaccines are based on inactivated bacterial cells (bacterins), there are a few examples of live attenuated vaccines. The efficacy of bacterins containing *Edwardsiella ictaluri* is low but, but a live attenuated vaccine has been found to be efficacious by immersion at 7 to 10 days post hatching (Shoemaker *et al.*, 1999). A live vaccine based on cross-reactive property of *Arthrobacter* spp. has been used in a vaccine licensed in North America and Chile against the intracellular bacterium *Renibacterium salmoninarum* causing bacterial kidney disease (Sommerset *et al.*, 2005). As shown in Tables 4 and 5, there are a few viral vaccines available and most of these are based on inactivated viruses or recombinant proteins. The efficacy of inactivated viral vaccines is low unless delivered by injection at relatively high doses (Sommerset *et al.*, 2005). There are safety concerns about use of live inactivated viruses as vaccines. As such, there is a need to demonstrate that they are non-pathogenic to non-target species of aquatic animals because they are likely to reach the aquatic environment, particularly if they are used for animals reared in open waters in cages. Generating such data would involve enormous cost and effort. DNA vaccines show promising results in experimental trails, but this involves introduction of a bacterial plasmid encoding antigen of interest. There are concerns that the plasmids may reach the environment and could reach other organisms with unforeseeable consequences (Magnadottir, 2010).

There are currently no vaccines available for parasites, though this group of pathogens can cause considerable economic losses. Celiate parasites like *Trichodina*, monogeneans like *Gyrodactylus* and *Dactylogyrus* and copepod parasites like *Lernea* are serious problems in warm water aquaculture. In salmon aquaculture in the northern hemisphere, the lice (*Lepeophtheirus salmonis*) alone are responsible for US$50–100 million annual losses through mortality, growth and quality reduction and pharmaceutical costs (Sommerset *et al.*, 2005). In Chile, the copepods belonging

Caligus spp. are a major problem. Parasites have complex cellular structure and the identification of a protective antigen would be important. More research efforts are needed to develop vaccines for parasites affecting aquacultured fish.

The two common methods of vaccine delivery to fish are immersion and injection. In the former, fish are immersed in a dilute vaccine suspension and this is a cheap, convenient method for a large number of fish, usually, at the fry stage. Vaccination by immersion has been found to be effective for several bacterial vaccines. On the other hand, vaccination by injection is labour-intensive and cannot be delivered to fish at the fry stage. In salmon aquaculture, use of a multiple component vaccine is common and in such multivalent vaccines, some components require delivery by injection with an oil adjuvant. In commercial salmon aquaculture, automated vaccine machines are commonly used. Because of the problems involved in delivering vaccines, the farmers prefer vaccination only once during the culture period and this has led to the development of polyvalent vaccines. Some of the commonly used vaccines for salmon contain six antigens (Table 4).

Being vertebrates, fish have a fairly developed specific immune system that has several similarities with the mammalian system. Fish produce antibodies, predominantly of the IgM type. On the other hand, the immune system of invertebrate shrimp is poorly understood. Though it is commonly believed that they do not have an adaptive immunity comparable to vertebrates, experimental studies indicate that it is possible to induce protection in shrimp through injection/oral administration of viral proteins (Witteveldt *et al.*, 2004a; 2004b), but the mechanism of protection is not known. There are no commercially available vaccines for shrimp aquaculture.

Immunostimulants

Though vertebrate finfish have a fairly developed specific immune response, the innate immune response plays an important role in preventing attack by pathogens. In the case of invertebrates like shrimp, there is no evidence of any specific immune response and the innate immunity is very important in the defence against pathogens. Even in finfish, development and maturation components involved in a specific immune response takes a few months after hatching (Zapata *et al.*, 2006) and therefore at this early stage of life, they are dependent on an innate immune response. Even after maturation, the specific immune response in fish has several constraints, such as limited classes of antibodies (IgG, IgA and IgE have not been found in fish), limited memory and relatively slow lymphocyte proliferation (Magnadottir, 2006).

Immunostimulants are naturally occurring compounds that enhance disease resistance in the host through modulation of the immune system (Bricknell and Dalmo, 2005). Studies done with various fish species show that the innate immune system can be upregulated with the help of various immunostimulants (Sakai, 1999). Many of the immunostimulants reported are molecules derived from microbial cell walls or outer membranes with characteristic patterns such as repeating units e.g. glucans, lipopolysaccharides, peptidoglycans, chitin and chitosan, and have been termed "pathogen associated molecular patterns" (PAMP). These recognise pattern recognition receptors (PRR) or pattern recognition proteins (PRP) of the innate immune system present in host cells. Stimulation of the innate immune response is indicated by parameters such as phagocytosis, activation of reactive oxygen and microbicidal activity in granulocytes, macrophage migration, complement activation and resistance to challenge by microbial pathogens (Sakai, 1999). There are numerous studies on immunostimulants and most of them report improved resistance to challenge by various bacterial pathogens, but some studies indicate no effect (Sakai, 1999).

Most commercial immunostimulants are derived from yeast and seaweeds containing β 1-3 and β 1-6 glucans in the case of former and alginates and polysaccharides in the case of latter. Delivery of immunostimulants is generally by bath immersion or through feed. Pulse feeding is commonly practiced. In shrimp aquaculture in India, the intervals of feeding range from 4 to 7 days (Karunasagar and Karunasagar, 1999) and in salmonid culture, it could range from 4 to 6 weeks (Bricknell and Dalmo, 2005). In salmonid aquaculture, feeding with diet supplemented with immunostimulants has been demonstrated to reduce sea lice settlement and provide better protection against furunculosis and vibriosis (Bricknell and Dalmo, 2005). Immunostimulants are reported to be widely used in seabass and sea bream aquaculture.

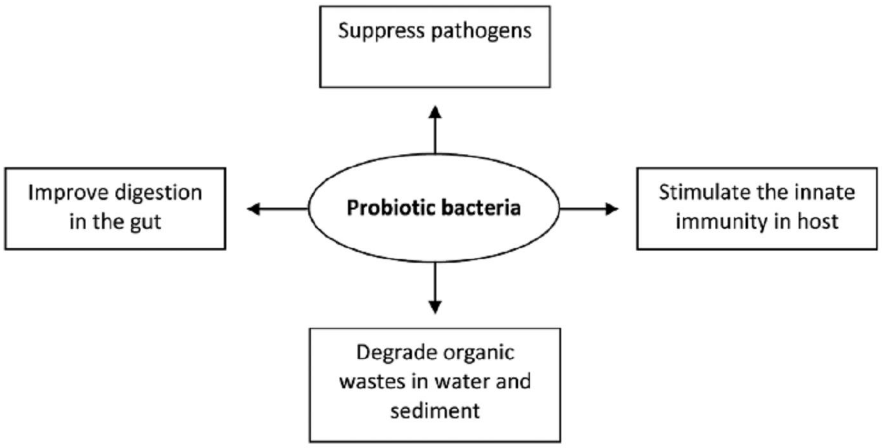
Probiotics

Probiotics have been in use in human and veterinary medicine for a long time and the term has been traditionally used to refer to live microbial feed supplements that beneficially affect the host by improving the intestinal microbial balance (Fuller, 1989). A Joint FAO/WHO Working group on drafting guidelines for the evaluation of probiotics in foods recommended the following definition: "Live microorganisms which when administered in adequate amounts confer a health benefit on the host" (FAO/WHO, 2002). In the aquatic environment, the animals are in intimate contact with the environment including the microflora therein and even gut flora of aquatic animals are greatly influenced by the microflora in the surrounding environment.

Considering this interaction between environmental microflora and fish health, Verschuere *et al.* (2000) suggested the following definition for probiotics in aquaculture: "A live microbial adjunct which has a beneficial effect on the host by modifying the host associated or ambient microbial community, by ensuring improved use of feed or enhancing its nutritional value, by enhancing the host response towards disease or by improving the quality of its ambient environment". Thus, probiotic bacteria could improve the animal health either by suppressing the pathogens present in the environment, by stimulating the immune response in the host, by improving the digestion in the gut or by improving water/sediment quality by degrading accumulated wastes (Figure 1).

FIGURE 1

Potential roles of probiotic bacteria in improvement of animal health



Probiotic bacteria may modify the host associated microbial community by competitive exclusion of pathogens. The competition could be for nutrients, iron or for adhesion sites and some are known to produce compounds inhibitory to the pathogens. In fact, a common technique used by several investigators looking for potential probiotic bacteria is to screen the cultures for the ability to suppress potential fish/shrimp pathogens (Vershuere *et al.*, 2000). Lactic acid bacteria, commonly used as probiotics in mammalian systems, are known to produce bacteriocins that inhibit, predominantly, gram positive bacteria. Most fish/shrimp pathogenic bacteria are gram negative and bacteria such as *Bacillus* spp. have been shown to produce inhibitory compounds against gram negative bacteria (Karunasagar *et al.*, 2005) and have been used as probiotics in shrimp aquaculture. When added to shrimp larval rearing water or when administered through diets, *Bacillus* spp. have been shown to improve survival and weight of larvae (Rengpipat *et al.*, 1998; Moriarty, 1998). There are also reports of *Bacillus*, *Carnobacterium* and *Vibrio* spp. that enhance survival of fish eggs, larvae, juveniles or adults when challenged with pathogens (Vershuere *et al.*, 2000). Though production of inhibitory compounds by probiotic bacteria that suppress pathogens has been demonstrated *in vitro*, this has not been demonstrated under *in vivo* conditions. However, enhanced survival, moulting rate and growth of black tiger shrimp, *P. monodon*, has been reported under farm conditions (Rengpipat *et al.*,1998). Addition of probiotic bacteria such as *Lactobacillus*, *Bacillus*, *Carnobacterium* or *Roseobacter* to larval rearing water has been found to improve survival of turbot larvae, salmonid fingerlings and channel catfish (Balcazar *et al.*, 2006). Feed supplementation has been preferred in grow-out ponds and has been found to be more effective than direct addition to rearing water (Hai *et al.*, 2009).

Improvement of water/sediment quality by improving oxidation of ammonia or by oxidizing sulphides by a consortium of probiotic bacteria that included *Bacillus* spp., *Nitrosomans* and *Nitobacter* has been demonstrated under laboratory conditions in microcosms simulating shrimp pond conditions (Karunasagar, 2011). However, some studies were unable to find this effect (Vershuere *et al.*, 2000). Photosynthetic purple non-sulphur bacteria are widely used as probiotics in shrimp farms in South East Asia and in fish and shrimp farms in China (Qi *et al.*, 2009). These bacteria are reported to be efficient degraders of organic wastes in aquaculture ponds.

It has been proposed that in the case of filter feeders or larval stages of crustaceans, probiotic bacteria may serve as a complementary food source and enhance digestion (Vershuere *et al.*, 2000). Protease producing bacteria such as *Bacillus* spp. have been shown to improve growth performance in shrimp, *Litopenaeus vannamei* (Liu *et al.*, 2009).

The immunomodulating activity of probiotics in various fish species has been reported in the literature (Nayak, 2010). Stimulation of both innate immune response as well as increase in immunoglobulin levels has been demonstrated in fish. Probiotic bacteria such as *Lactobacillus* spp., *Bacillus* spp., *Carnobacterium* spp., *Clostridium butyricum*, have been demonstrated to stimulate the immune system of several fish species like tilapia, seabream and trout. But some investigators reported variable results. The high degree of variability observed by some investigators may be related to the bacterial species used as a probiotic and their source. It is now common to use multi-species probiotics. Organisms belonging to different families like *Lactobacillus* and *Bacillus* spp. have been found to act synergistically in immunomodulation (Salinas *et al.*, 2005).

In China, the biggest aquaculture producer in the world, it has been reported that over one hundred companies are involved in producing about 50 000 tonnes of probiotics with a market value of 50 million Euros. Though probiotics for aquaculture had a booming market in 2008, there was about a 50 percent decline in the market because of a lack of confidence by farmers and an issue with quality control of the commercial products (Qi *et al.*, 2009). This seems to be the experience in many countries. Regulatory approval for the use of probiotics as feed supplements has been documented in some regions. European Union regulation EC/710/2009 permits the use of authorized probiotics for disease control in organic aquaculture.

Biocontrol agents

The use of microorganisms as biological control agents for insect pests has been practiced in various forms. Bacteriophages (bacteria eaters) are viruses that replicate by using bacteria as hosts. Recently, there has been a surge of interest in using bacteriophages as therapeutic agents, particularly in the context of widespread occurrence of antibiotic resistance in several pathogenic bacteria. Bacteriophages are abundant in nature and have been found in both terrestrial and aquatic environments and in association with plants and animals. In non-polluted waters, 2 x 10^8 bacteriophages per ml have been found (Bergh *et al.*, 1989). The life cycle of a bacteriophage may include a lytic stage and some bacteriophages have their genome inserted into the host chromosome and enter a lysogenic stage. Lysogenic bacteriophages are involved in gene transfer in bacteria and some of the virulence factors found in bacteria (e.g. the ability to produce cholera toxin by *Vibrio cholerae* O1) have been associated with bacteriophages inserted into the bacterial genome.

Soon after the discovery of bacteriophages in 1917, the potential to use them against bacteria was realized. However, the interest in bacteriophages declined after the discovery of antibiotics, the subsequent scaling up of antibiotic production to industrial levels and their effectiveness in treating infections in soldiers during the World War II. But the treatment failures because bacteria show resistance to multiple antibiotics has led to a renewed interest in bacteriophage therapy. Bacteriophages are host specific, hence they lyse only the target bacteria, unlike antibiotics that suppress most of the bacterial groups. Thus bacteriophage therapy would not suppress useful commensal flora that are required for the health of the animals. This would be a great advantage in aquaculture.

The application of bacteriophages to combat fish pathogens was investigated by Nakai and coworkers (Nakai *et al.*, 1999; Park *et al.*, 2000; Nakai and Park, 2002). They used bacteriophages belonging to Siphoviridae family isolated from the aquaculture environment. Oral administration of bacteriophages against *Lactococcus garvieae* to young yellowtails (*Seliora quinqueradiata*) resulted in 100 percent survival following intraperitoneal challenge with the pathogen compared with 10 percent survival in control groups (Nakai *et al.*, 1999). Oral administration of phage impregnated feed (mixture of two bacteriophages, one belonging to Myoviridae and another belonging to Podoviridae family) to ayu (*Plecoglossus altivelis*) brought down cumulative mortality to 22.5 percent compared with 65 percent in controls, following an oral challenge with *Pseudomonas plecoglossicida* (Park, 2000). In both studies, the authors used oral administration and this would be very convenient in aquaculture. Fish digestive tracts have a relatively high pH and therefore the acid sensitivity of phages would not be an issue in aquaculture (Nakai and Park, 2002). Examples of reported efficacy of bacteriophages in improving survival of fish/shrimp when challenged with pathogens are indicated in Table 6.

Imbeault *et al.* (2006) studied the application of bacteriophages in preventing furunculosis caused by *A. salmonicida* in farmed brook trout (*Salvelinus fontinalis*). In aquarium tanks, application of bacteriophages resulted in a 6 log reduction in the number of *A. salmonicida* and reduced the mortality from 100 percent to 10 percent. Phage resistant mutants were isolated, but they were susceptible to other phages and the investigators suggested the use of bacteriophage combinations to overcome the problem. Park and Nakai (2003) also noted that a combination of two bacteriophages gave a significantly higher protection to ayu (*Plecoglossus altivelis*) infected with *Pseudomonas plecoglossicida* compared with treatment with a single bacteriophage.

TABLE 6

Reported examples of bacteriophage therapy in fish and shrimp

Pathogen	Fish/Shrimp species	Route of administration	Observed effect	Reference
Lactococcus garvieae	Yellowtail (*Seliora quinqueradiata*)	Oral administration	Improved survival on challenge	Nakai *et al.*, 1999
Pseudomonas plecoglossicida	Ayu (*Plecoglossus altivelis*)	Oral administration	Improved survival on challenge	Park *et al.*, 2000
Aeromonas salmonicida	Brook trout (*Salvelinus fontinalis)*	Addition to tank water	Reduction in *A. salmonicida* in water, improved survival of fish	Imbeault *et al.*, 2006
Vibrio harveyi	Black Tiger shrimp (*Penaeus monodon*)	Addition to larval rearing tank water	Improved survival of post-larvae during a natural outbreak	Vinod *et al.*, 2006
Vibrio harveyi biofilm	Black Tiger shrimp (*Penaeus monodon*)	Addition to water	Reduction in bacterial density in biofilm, improved survival of post-larvae	Karunasagar *et al.*, 2007

One of the concerns regarding the use of bacteriophage therapy has been the possibility that certain phages may go into a lysogenic state and may be involved in gene transfer. Virulence genes have been associated with lysogenic bacteriophages. Bacteriophages against the shrimp pathogen *V. harveyi* may belong to the family Siphoviridae or Myoviridae (Oakey and Owens, 2000, Shivu *et al.*, 2007; Crothers-Stomps *et al.*, 2010). Generally members of Siphoviridae have been reported to be lytic phages (Vinod *et al.*, 2006; Shivu *et al.*, 2007; Karunasagar *et al.*, 2007; Crothers-Stomps *et al.*, 2010). A *V. harveyi* myovirus like phage (VHML) has been reported to be temperate and confer virulence to the host strains (Pasarawipas *et al.*, 2005). Shivu *et al.* (2007) tested the host range of a collection of *V. harveyi* phages against 180 isolates from different geographical regions. Three strains of siphoviridae family were able to lyse 65–70 percent of the strains, indicating a broad host range. Vinod *et al.* (2006) tested bacteriophage therapy of shrimp (*P. monodon*) larvae and post-larvae in both laboratory microcosms as well as in hatcheries during a natural outbreak of luminous bacteria disease. The bacteriophages were added to larval tanks. In microcosms, larval survival was 25 percent in the control and 85 percent with treatment. In hatchery trials, the survival was 86 percent with bacteriophages, 40 percent with antibiotics and 17 percent in controls (Vinod *et al.*, 2006). Bacteriophage treatment brought down counts of luminous bacteria in the tanks. In another hatchery trial during a natural outbreak of luminous bacteria disease, 86–88 percent survival was obtained with bacteriophage treatment compared with 65–68 percent with antibiotics (Karunasagar *et al.*, 2007). These studies show the potential for bacteriophages to be effective alternatives to antibiotics in shrimp larval health management. Bacteriophages used by Vinod *et al.* (2006) and Karunasagar *et al.* (2007) lacked the putative virulence gene carried by VHML and hence the concern regarding carriage of virulence gene would be minimal.

One of the problems in shrimp larval health management is the persistence of *V. harveyi* in the hatchery environment, by forming a biofilm that is resistant to antibiotic and sanitizer treatment (Karunasagar *et al.,* 1996). The ability of bacteriophages to bring about a 3 log reduction in biofilm cells of *V. harveyi* on high density polyethylene (HDPE) surfaces was demonstrated by Karunasagar *et al.*, (2007). This provides an additional advantage for bacteriophages in shrimp larval health management. However, considering that the host range for selected phages was 65–70 percent, and also considering the possibility that bacterial strains may develop resistance to bacteriophages, phage therapy with a consortium of phages would be necessary to ensure effectiveness with unknown strains causing disease outbreaks.

Biocontrol of pathogens using bacteriophages has already been commercialized in agriculture, aquaculture and in the food industry. Agriphage is a commercial product from OmniLytics Inc. to combat *Xanthomonas campestris* pv. *vesicatoria*, which causes bacterial spot disease in peppers and tomatoes, and *Pseudomonas syringae* pv. *tomato*, which causes bacterial speck disease in tomatoes. It has been registered by the United States Environmental Protection Agency in 2005 (US Environmental Protection Agency, 2010). OmnilLytics Inc. has also received US FDA approval for use of bacteriophages against *Escherichia coli* and *Salmonella* in live animals before slaughtering (Garcia *et al.*, 2010). In 2007, the US FDA approved Listex P100 from EBI Biosafety as GRAS (Generally Recognised As Safe) for use in all foods in which *Listeria* could be a risk (EBI Food Safety, 2010). ListShield from Intralytix has received US FDA and US EPA approval for use in ready-to-eat foods for control of *L. monocytogenes* (Intralytix, 2010). In India, Mangalore Biotech Laboratory (2010) has a commercial product for control of luminous bacteria in shrimp hatcheries.

CONCLUSIONS

Microbial diseases have been causing serious economic losses for the aquaculture industry, but there is a need to minimize the use of antimicrobials in aquaculture to avoid problems of residues and antibiotic resistance in food-associated bacteria. A number of alternatives are available for managing the health of animals in aquaculture systems. Implementation of good aquaculture practices would to a large extent reduce the health risk for animals in aquaculture systems. Tools like vaccines, immunostimulants and probiotics could be used for prevention of diseases depending on aquaculture systems and the risks involved. Biocontrol agents like bacteriophages could be used for both prevention as well as control in case of outbreaks. It could be recommended that the farmers use a risk based approach and decide on appropriate preventive or control strategies.

REFERENCES

Alam, S.M.N., Pokrant, B., Yakutipiyage, A. & Phillips, M.J. 2007. Economic returns of disease affected extensive shrimp farming in southwest Bangladesh. *Aquaculture International*, 15: 363–370.

Alday de Graindorge, V. & Griffith, D. 2001. Ecuador: national review on management strategies for major diseases in shrimp aquaculture. *In* R. Subasinghe, R. Arthur, M.J. Phillips & M. Reantaso, eds. *Thematic review on management strategies for major diseases in shrimp aquaculture*, pp. 17–19 [online]. library.enaca.org/Shrimp/Case/Global/CEBU/FinalCEBU.pdf

Balcazar, J.L., de Blas, I., Ruiz-Zarzuela, I., Cunningham, D., Vendrell, D. & Muzquiz, J.L. 2006. The role of probiotics in aquaculture. *Veterinary Microbiology*, 114: 173–186.

Bergh, O., Borsgeim, G., Bratback, S. & Heldal, M. 1989. High abundance of viruses found in aquatic environments. *Nature*, 340: 467–468.

Bricknell, I. & Dalmo, R.A. 2005. The use of immunostimulants in fish larval aquaculture. *Fish and Shellfish Immunology*, 19: 457–472.

Burridge, L., Weis, J., Cabello, F. & Pizzaro, J. 2008. *Chemical use in salmon aquaculture: a review of current practices and possible environmental effects* [online]. www.worldwildlife.org/what/globalmarkets/aquaculture/WWFBinaryitem8842.pdf

Burridge, L., Weis, J., Cabello, F., Pizzaro, J. & Bostick, K. 2010. Chemical use in salmon aquaculture: a review of current practices and possible environmental effects. *Aquaculture*, 306: 7–23.

CAC. 2006. *Codex Alimentarius Commission, Maximum residue limits for veterinary drugs in foods.* CAC/MRL 02-2006. Rome, FAO. 31 pp.

CAC. 2010. *Codex Alimentarius Commission Procedural Manual, 19th Editon.* Rome, WHO/FAO. 191 pp.

Chanratchakool, P., Fegan, D.F. & Phillips, M.J. 2001. Thailand: national review on management strategies for major diseases in shrimp aquaculture. *In* R. Subasinghe, R. Arthur, M.J. Phillips & M. Reantaso, eds. *Thematic review on management strategies for major diseases in shrimp aquaculture*, pp. 85–90 [online]. library.enaca.org/Shrimp/Case/Global/CEBU/FinalCEBU.pdf

Crothers-Stomps, C., Hoj, L., Bourne, D.G., Hall, M.R. & Owens, L. 2010. Isolation of lytic bacteriophage against *Vibrio harveyi*. *Journal of Applied Microbiology*, 108: 1744–1750.

Evelyn, T.P.T. 1997. A historical review of fish vaccinology. *In* R. Gudding, A. Lillehaug, P.J. Midtlyng & F. Brown, eds. *Fish vaccinology*, pp. 3–12. Developments in Biological Standardization 90. Basel, Switzerland, Karger.

EBI Food Safety. 2010. *Regulatory position of ListexTM P100* [online]. www.ebifoodsafety.com/en/listex-regulatory.aspx

FAO. 2007. *The State of World Fisheries and Aquaculture, 2006*. Rome. 180 pp.

FAO. 2009. *The State of World Fisheries and Aquaculture, 2008*. Rome. 176 pp.

FAO/OIE/WHO. 2003. *Report of the First Joint FAO/OIE/WHO Workshop on the non-human antimicrobial usage and antibiotic resistance: Scientific assessment*. 37 pp.

FAO/OIE/WHO. 2006. *Report of A Joint FAO/OIE/WHO Expert Consultation on Antimicrobial Use in Aquaculture and Antimicrobial Resistance*. Geneva, Switzerland, WHO. 97 pp.

FAO/WHO. 2002 *Joint FAO/WHO Working Group Report on Drafting Guidelines for the Evaluation of Probiotics in Food* [online]. ftp://ftp.fao.org/es/esn/food/wgreport2.pdf

FAO/WHO. 2004. *Report of Joint FAO/WHO Technical Workshop on residues of veterinary drugs without ADI/MRL* [online]. www.fao.org/docrep/008/y5723e/y5723e00.htm

Fueller, R. 1989. Probiotics in man and animals. *Journal of Applied Bacteriology*, 66: 365–378.

Hai, V.N., Buller, N. & Fotedar, R. 2009. The use of customised probiotics in the cultivation of western king prawns (*Penaeus latisulcatus* Kishinouye 1896). *Fish and Shellfish Immunology*, 27: 100–104.

Imbeault, S., Parent, S., Lagace, M., Uhland, C.F. & Blais, J.-F. 2006. Using bacteriophages to prevent furunculosis caused by *Aeromonas salmonicida* in brook trout . *Journal of Aquatic Animal Health*, 18: 203–214.

Intralytix. 2010. ListShieldTM. In: *Intralytix* [online]. www.intralytix.com/Intral_Products_ListShield.htm

Karunasagar, I. 2011. Alternatives to antibiotics in aquaculture. *In*: *Proceedings of FAO Expert Workshop on Improving Biosecurity through Prudent and Responsible Use of Veterinary Medicines in Aquatic Food Production*. Rome, FAO.

Karunasagar, I. & Karunasagar, I. 1999. Diagnosis, treatment and prevention of microbial diseases of fish and shellfish. *Current Science*, 76: 387–399.

Karunasagar, I., Shivu, M.M., Girisha, S.K., Krohne, G. & Karunasagar, I. 2007. Biocontrol of pathogens in shrimp hatcheries using bacteriophages. *Aquaculture*, 268: 288–292.

Karunasagar, I., Vinod, M.G., Bob Kennedy, M.D., Atnur, V., Deepanjali, A., Umesha, K.R. & Karunasagar, I. 2005. Biocontrol of bacterial pathogens in aquaculture with emphasis on phage therapy. *In* P. Walker, R. Lester & M.G. Bondad-Reantaso, eds. *Diseases in Asian aquaculture V*, pp. 535–542. Manila, Fish Health Section, Asian Fisheries Society.

Khoa, L.V., Hao, N.V. & Huong, L.T.L. 2001. Vietnam: national review on management strategies for major diseases in shrimp aquaculture. *In* R. Subasinghe, R. Arthur, M.J. Phillips & M. Reantaso, eds. *Thematic review on management strategies for major diseases in shrimp aquaculture*, pp. 91–94 [online]. library.enaca.org/Shrimp/Case/Global/CEBU/FinalCEBU.pdf

Liu, C.H., Chiu, C.S., Ho, P.L. & Wang, W.W. 2009. Improvement of growth performance of white shrimp Litopenaeus vannamei by a protease producing probiotic, *Bacillus subtilis* E20, from natto. *Journal of Applied Microbiology*, 107: 1031–1041.

Magnadottir, B. 2006. Innate immunity of fish (overview). *Fish and Shellfish Immunology*, 20: 137–151.

Magnadottir, B. 2010. Immunological control of fish diseases. *Marine Biotechnology*, 12: 361–379.

Mangalore Biotech Laboratories. 2010. *Lumi Nil MBL* [online]. mangalorebiotech.com/Luminil_MBL.pdf

Moriarty, D. 1998. Control of luminous *Vibrio* species in penaeid aquaculture ponds. *Aquaculture*, 164: 351–358.

Nakai, T. & Park, S.C. 2002. Bacteriophage therapy of infectious diseases in aquaculture. *Research in Microbiology*, 153: 13–18.

Nakai, T., Sugimoto, R., Park, K.H., Matsuoaka, S., Mori, K., Nishioka, T. & Maruyama, K. 1999. Protective effects of bacteriophage on experimental *Lactococcus garviae* infection in yellowtail. *Diseases of Aquatic Organisms*, 37: 33–41.

Nayak, S.K. 2010. Probiotics and immunity: a fish perspective. *Fish and Shellfish Immunology*, 29: 2–14.

Oakey, H.J. & Owens, L. 2000. A new bacteriophage VHML isolated from a toxin producing strain of *Vibrio harveyi* in tropical Australia. *Journal of Applied Microbiology*, 89: 702–709.

Park, S.C., Shimamura, I., Fukunaga, M., Mori, K. & Nakai, T. 2000. Isolation of bacteriophages specific to a fish pathogen Pseudomonas plecoglossicida as a candidate for disease control. *Applied and Environmental Microbiology*, 66: 1416–1422.

Pasaharawipas, T., Thaikua, S., Sriurairatana, S., Ruandpan, L., Direkbusaarakum, S., Manopvisetcharean, J. & Flegel, T.W. 2005. Partial characterization of a novel bacteriophage of *Vibrio harveyi* isolated from shrimp culture ponds in Thailand. *Virus Research*, 114: 63–69.

Qi, Z., Zhang, X., Boon, N. & Bossier, P. 2009. Probiotics in aquaculture of China – current state, problems and prospects. *Aquaculture*, 290: 15–21.

Rengpipat, S., Phianphak, W., Piyatiratitivorakul, S. & Menasaveta, P. 1998. Effects of a probiotic bacterium in black tiger shrimp *Penaeus monodon* survival and growth. *Aquaculture*, 167: 301–313.

Sakai, M. 1999. Current research status of fish immunostimulants. *Aquaculture*, 172: 63–92.

Salinas, I., Cuesta, A., Esteban, M.A. & Meseguer, J. 2005. Dietary administration of *Lactobacillus delbrueckii* and *Bacillus subtilis*, single or combined, on gilthead seabream cellular innate immune responses. *Fish and Shellfish Immunology*, 19: 67–77.

Shivu, M.M., Rajeeva, B.C., Girisha, S.K., Karunasagar, I., Krohne, G. & Karunasagar, I. 2007. Molecular characterization of *Vibrio harveyi* bacteriophage isolated from aquaculture environments along the coast of India. *Environmental Microbiology*, 9: 322–331.

Shoemaker, C.A., Klesius, P.H. & Bricker, J.M. 1999. Efficacy of a modified live Edwardsiella ictaluri vaccine in channel catfish as young as 7 days post hatch. *Aquaculture*, 176: 189–193.

Sommerset, I., Krossoy, B., Biering, E. & Frost, P. 2005. Vaccines for fish in aquaculture. *Expert Reviews in Vaccines*, 4: 89–101.

Umesha, K.R., Bob Kennedy, M.D., Manja Naik, B., Venugopal, M.N., Karunasagar, I. & Karunasagar, I. 2006. High prevalence of dual and triple viral infections in black tiger shrimp ponds in India. *Aquaculture*, 258: 91–99.

Umesh, N.R., Chandra Mohan, A.B., Ravibabu, G., Padiyar, P.A., Phillips, M.J., Mohan, C.V. & Vishnu Bhat, B. 2010. Shrimp farmers in India: Empowering small-scale farmers through a cluster-based approach. *In* S.S. De Silva & B.F. Davy, eds. *Success stories in Asian aquaculture*, pp 41–66. New York, USA, Springer.

US Environmental Protection Agency. 2010. Bacteriophages of *Xanthomonas campestris* pv. *vesicatoria* (006449) and *Pseudomonas syringae* pv. *tomato* (006521). Fact Sheet. In: *United States Environmental Protection Agency* [online]. www.epa.gov/oppbppd1/biopesticides/ingredients/factsheets/factsheet_006449-006521.htm

Valderhaug, L. 2008. *Disease threats in fish farming in general; Special focus on sea lice and ISA in Chile* [online]. www.pharmaq.no/Presentations/2008/Disease_threats_fish_farming_in_general_Glitnir_27_05_2008.pdf

Verschuere, L., Rombaut, G., Sorgeloos, P. & Verstraete, W. 2000. Probiotic bacteria as biological control agents in aquaculture. *Microbiology and Molecular Biology Reviews*, 64: 655–671.

Vinod, M.G., Shivu, M.M., Umesha, K.R., Rajeeva, B.C., Krohne, G., Karunasagar, I. & Karunasagar, I. 2006. Isolation of *Vibrio harveyi* bacteriophage with a potential for biocontrol of luminous vibriosis in hatchery environments. *Aquaculture*, 255: 117–124.

Wei, Q. 2002. Social and economic impacts of aquatic animal health problems in aquaculture in China. *In* J.R. Arthur, M.J. Phillips, R.P. Subasinghe, M.B. Reantaso & I.H. MacRae, eds. *Primary aquatic animal health care in rural, small-scale, aquaculture development*, pp. 55–61. FAO Fisheries Technical Paper No. 406. Rome, FAO. 390 pp.

Witteveldt, J., Cifuentes, C.C., Vlak, J.M. & van Hulten, M.C. 2004a. Protection of *Penaeus monodon* against whitespot syndrome virus by oral vaccination. *Journal of Virology*, 78: 2057–2061.

Witteveldt, J., Vlak, J.M. & van Hulten, M.C. 2004b. Protection of *Penaeus monodon* against whitespot syndrome virus using a WSSV subunit vaccine. *Fish and Shellfish Immunology*, 16: 571–579.

Zapata, A., Diez, B., Cejalvo, T., Gutierrez-De Frias, C. & Cortes, A. 2006. Ontogeny of the immune system of fish. *Fish and Shellfish Immunology*, 20: 126–136.