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ARTICLE

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Abstract

Current growth in aquaculture production is parallel with the increasing number of disease outbreaks, which negatively affect the production, profitability, and sustainability of the global aquaculture industry. Vibriosis is among the most common diseases leading to massive mortality of cultured shrimp, fish, and shellfish in Asia. High incidence of vibriosis can occur in hatchery and grow-out facilities, but juveniles are more susceptible to the disease. Various factors, particularly the source of fish, environmental factors (including water quality and farm management), and the virulence factors of *Vibrio*, influence the occurrence of the disease. Affected fish show weariness, with necrosis of skin and appendages, leading to body malformation, slow growth, internal organ liquefaction, blindness, muscle opacity, and mortality. A combination of control measures, particularly a disease-free source of fish, biosecurity of the farm, improved water quality, and other preventive measures (e.g., vaccination) might be able to control the infection. Although some control measures are expensive and less practical, vaccination is effective, relatively cheap, and easily implemented. In this review, the latest knowledge on the pathogenesis and control of vibriosis, including vaccination, is discussed.

Aquaculture remains the fastest-growing sector of high-protein resource. It contributes toward economic development and social stability worldwide, promoting nutritional standards and relieving poverty in some poor countries (Béné et al. 2016; FAO (Food and Agriculture Organization of the United Nations) 2016a,b). Marine and inland capture fisheries represent two-thirds of the total global supply of food fish, and the remaining one-third comes from aquaculture (Thompson and Subasinghe 2011). According to Sapkota et al. (2008), aquaculture is defined as the farming of aquatic organisms, including finfish and shellfish, by groups, individuals, or corporations using interventions such as medications, feed, controlled breeding, and containment to increase production. The industry provides more than 15% of animal proteins and other essential micronutrients, such as calcium, iron, vitamin A, and zinc, to one-third of the global population (Allison 2011).

Aquaculture is becoming an important activity that contributes to aquatic-sourced foods for national food security. Valuable species, such as farmed shrimp, marine fish, and freshwater fish, are set to be the commodities for the export market and monetary gain (Ng 2009; FAO (Food and Agriculture Organization of the United Nations) 2017). The Food and Agriculture Organization of the United Nations (FAO 2017) reported that the fishery trade has become an important source of income in many developing countries, for which current exports have risen, representing 54% of total fishery export value in 2014. It is further estimated that 56.6 million people are engaged in capture fisheries and aquaculture, with 94% located in Asia. Improvement in the wealth of the population increases the trend in the preference for fish as a source of protein. It is estimated that the demand for fish will reach 1.93 million metric tons by 2020 (Yusoff 2015). To meet the increasing demands, the rearing systems have changed from extensive to super-intensive farming, eventually resulting in sudden disease outbreaks (Shariff and Subasinghe 1993; Chinabut 2001; FAO 2017).

Bacterial diseases in aquaculture are known to affect both economic and social developments in many countries (Defoirdt et al. 2005; FAO 2012). Therefore, various health management strategies have been implemented to provide solutions to aquatic health problems, such as international codes, regional guidelines, new biosecurity measures, targeted research, and new strategies for diagnostics and therapeutics (Bondad-Reantaso et al. 2005; FAO 2017). Vibriosis is one of the most prevalent bacterial diseases affecting diverse marine fish and shellfish. According to Chong et al. (2011), approximately 66.7% of diseases reported in groupers *Epinephelus* spp. are vibriosis, affecting all stages of growth and leading to mortality for up to 50% of the fish (Liao and Leaño 2008; El-Galil and Mohamed 2012). Several species of Vibrionaceae have been associated with health problems of marine animals. A recent report revealed *Vibrio parahaemolyticus*, *V. alginolyticus*, *V. harveyi*, *V. owensii*, and *V. campbellii* as the most common species infecting farmed aquatic animals (Nor-Amalina et al. 2017). The Vibrionaceae also includes some *Photobacterium* species, such as *P. damselae* subsp. *damselae* (formerly known as *V. damsela*; Love et al. 1981), *P. damselae* subsp. *piscicida* (formerly known as *Pasteurella piscicida*; Janssen and Surgalla 1968), and *P. toruni*, the latter of which was recently isolated from diseased Redbanded Seabream *Pagrus auriga* in Spain (Labella et al. 2017).

Integration of good husbandry practices, such as quarantine, optimizing water quality, providing high-quality feed, breeding of disease-resistant broodstocks, and establishment of a vaccination program, have served as alternative strategies to combat fish diseases (FAO 2005; Pridgeon and Klesius 2012). Although species-specific vaccines have shown effective protection against those specific bacterial strains, the antigenic diversity of *Vibrio* strains and their serotypes has made the vaccines unable to elicit protection against multiple *Vibrio* infections, thus leading to slow progress in vaccine development (Li et al. 2010, 2014). It is crucial for the vaccine to provide long-lasting immunity and to be easily administered, safe for the fish, inexpensive to produce and license, and able to protect fish against all serotype variants of the agent (Leong and Fryer 1993; Leong et al. 1996). This article reviews the current information on the family Vibrionaceae, methods of vibriosis control, and preventive measures by vaccination in aquaculture farms.

THE FAMILY VIBRIONACEAE: CAUSATIVE AGENTS OF VIBRIOSIS

To date, a total of 143 species has been described in the Vibrionaceae, and they are classified into six genera: *Aliivibrio* (Urbanczyk et al. 2007), *Enterovibrio* (Thompson et al. 2002), *Grimontia* (Thompson et al. 2003), *Photobacterium* (Beijerinck 1889), *Salinivibrio* (Mellado et al. 1996), and *Vibrio* (Pacini 1854). Members of Vibrionaceae are autochthonous and ubiquitous aquatic microorganisms with versatile metabolic pathways.

Traditional identification of *Vibrio* was through phenotypic characterization. This method was reported to have several limitations: for example, dissimilar species could have vague phenotypes (e.g., the *V. alginolyticus* species group); strains of similar species could likewise have distinctive phenotypes (e.g., colony variation and enzyme activities); and there were sometimes inconsistencies in the results with the same strain and a lack of interlaboratory reproducibility (Amaral et al. 2014). Direct molecular detection utilizing PCR can likewise be applied but has some limitations in view of the cost and the facilities required for analysis; these constraints are particularly significant for field studies involving large numbers of samples (Choopun et al. 2002). Amaral et al. (2014) suggested that a reliable and conceivable alternative is to acquire phenotypic information directly from whole genome sequences.

The genus *Vibrio* alone now consists of 123 species with a complete description and validated nomenclature registered in the List of Prokaryotic Names with Standing in Nomenclature (http://www.bacterio.net/; Euzéby 1997; Parte 2013). *Vibrio* is widespread in the estuarine and coastal marine environments, and the species show seasonal dynamics in their populations (Thompson et al. 2004). In these environments, *Vibrio* spp. play a significant role in the degradation of organic matter (Damir et al. 2013) and, hence, regulate the dissolved organic carbon to higher trophic levels of the marine food web (Grossart et al. 2005; Al-saari et al. 2015). However, some members of the genus *Vibrio* are also opportunistic pathogens that have been associated with infections in humans and marine animals (Austin 2010).

Vibrio cholerae, *V. vulnificus*, and *V. parahaemolyticus* are among the most potent pathogenic species to humans, causing gastroenteritis, wound infections, and septicemia in susceptible hosts. Although other *Vibrio* species, including *V. mimicus*, *V. fluvialis*, *V. furnissii*, and *V. alginolyticus*, were also reported as human pathogens, the illnesses are generally less severe (Baker-Austin et al. 2018). Given the fact that vibrios are ubiquitous in the environment, reports on isolation of three species—namely *V. metschnikovii*, *V. cincinnatiensis*, and *V. carchariae*—were perhaps more indicative of asymptomatic colonization rather than infection (Bode et al. 1986; Jean-Jacques et al. 1981; Pavia et al. 1989; Dalsgaard et al. 1996; Morris and Acheson 2003). Thus, their significance as human pathogens requires more thorough research. Likewise, studies are also needed to determine the pathogenicity of *V. metoecus*, a close relative to *V. cholerae* that was initially recognized as a nonpathogenic environmental variant of *V. cholerae* (Choopun 2004; Kirchberger et al. 2014) but has recently been isolated from clinical specimens (ear and leg wound, blood and stool samples) in the United States (Orata et al. 2015). *Vibrio metoecus* is postulated to become a pathogen due to the constant interactions with *V. cholerae*, allowing a higher rate of interspecies gene exchange, including genes involved in pathogenicity, from *V. cholerae* to *V. metoecus* (Orata et al. 2015). Therefore, the role of *V. metoecus* as a human pathogen is worth further study.

Vibriosis is a bacterial disease caused by certain species of *Vibrio* and *Photobacterium*, infamously known as important marine pathogens in wild and captive-reared animals, including fish, crustaceans, mollusks, corals, and rotifers (Gomez-Gil et al. 2014a, 2014b). Due to the dynamic nature of *Vibrio* taxonomy in recent years, some disease-causing *Vibrio* spp. are now being recognized with new amended names, such as *Aliivibrio* spp. For instance, *A. salmonicida* described by Egidius et al. (1981) was formerly recognized as *V. salmonicida*, a known causative agent of coldwater vibriosis (Colquhoun and Sørum 2001) or the Hitra disease affecting Atlantic Salmon *Salmo salar* (Egidius et al. 1981). The term “vibriosis” is loosely defined as septicemia after infection by vibrios. It was formerly known as penaeid bacterial septicemia, penaeid vibriosis, luminescent vibriosis, or red leg disease (Aguirre-Guzma et al. 2004). This disease is regarded as an epizootic, described as a global outbreak of communicable disease temporarily occurring in limited geographical areas (Austin and Austin 2012). It was first depicted in eels but is now known globally in a variety of marine fish and shellfish. Noga (2010) claimed that all marine fish are probably susceptible to at least one *Vibrio* species; thus, vibriosis has become an economically important disease in marine fish culture, negatively affecting many fishes and other cultured animals. *Vibrio* spp. are recognized based on their cell morphology, physiology, and biochemistry, which are usually characterized via biochemical tests and molecular methods (Table 1).

TABLE 1. List of some general characteristics of members of the family Vibrionaceae (*Vibrio* and *Photobacterium*).

Characteristic	Description for *Vibrio* or *Photobacterium* species	References
Habitat	Exist in an aquatic environment, such as brackish water, rivers, ponds, wells, and seawater, usually associated with aquatic animals and plants.	Uchiyama (2000)
Gram staining	Gram-negative or a group of strains forming a curved rod shape.	Igbinosa and Okoh (2008), Nehlah et al. (2016)
General biochemical characteristics	Mostly are motile (some of the nonmotile species include *V. tritonius* and *V. vulnificus*), oxidase positive (excluding *V. metschnikovii*), and use ammonium salts as their source of nitrogen. Excrete various extracellular enzymes, including amylase, chitinase, gelatinase, and DNase.	Esteve et al. (2006), Igbinosa and Okoh (2008), Sawabe et al. (2013), Gomez-Gil et al. (2014a,b)
Isolation media	General: trypticase soy agar supplemented with 1–2% NaCl or marine agar. Selective: thiosulfate–citrate–bile salts–sucrose (TCBS) agar.	Thompson et al. (2004)
Growth on TCBS	Strains that are able to ferment sucrose will form yellow colonies, while the others are green. *Vibrio harveyi*, *V. alginolyticus*, *V. fluvialis*, and *V. cholerae* form yellow colonies. *Vibrio parahaemolyticus*, *V. vulnificus*, and *P. damselae* form green colonies. However, *V. tritonius* is unable to grow on TCBS.	Thompson et al. (2004), Shikongo-Nambabi et al. (2012), Sawabe et al. (2013)
NaCl tolerance	Require at least 0.5–3% NaCl and can tolerate up to 10% NaCl for growth. *Vibrio cholerae* and *V. mimicus* can grow without NaCl.	Jayasinghe et al. (2010), Arunagiri et al. (2013)
Optimum growth temperature	Vibrios grow best at temperatures between 15°C and 30°C, depending on the strain under analysis. Psychrophilic vibrios, such as *V. logei*, *V. wodanis*, and *V. salmonicida*, grow poorly at temperatures higher than 20°C.	Thompson et al. (2004)
Optimum growth pH	All species are acid-sensitive, growing best at neutral and alkaline pH values up to pH 9.0; therefore, both selective and enrichment media are at pH 8.0–8.8.	Igbinosa and Okoh (2008)
Identification through molecular methods	16S ribosomal RNA, housekeeping gene sequencing, amplified fragment length polymorphism, multilocus sequence analysis, fluorescence in situ hybridization, and restriction fragment length polymorphism.	Fukui and Sawabe (2008), Chatterjee and Haldar (2012)

VIBRIOSIS IN AQUACULTURE

Diseases have been recognized as one of the major constraints to the efficient production, development, and sustainable expansion of marine fish aquaculture (Harikrishnan et al. 2010). Disease in fish develops as a consequence of an interaction between host, pathogen, and external stressors, such as environmental degradation due to climate change, eutrophication in natural habitat, and poor culture systems and poor management of fish (Toranzo et al. 2005; Noga 2010; Huicab-Pech et al. 2016). The ability of bacteria to readily survive in an aquatic environment without being dependent on their host makes the bacterial disease a major issue in aquaculture, particularly in warmer water (<17°C) and at high salinity levels (30–35‰; Pridgeon and Klesius 2012). Warmer water is most conducive for the survival and multiplication of *Vibrio* spp., allowing the pathogens to disseminate rapidly between fish and making vibriosis a “summer” disease (Noga 2010; Sankar et al. 2012). Cheng et al. (2009) reported that an overcrowded environment with a high water temperature greater than 15°C could put fish under stress, leading to an immunocompromised state and increasing the susceptibility to bacterial infection. Thus, vibriosis is particularly a threat to the aquaculture industry in tropical countries.

Vibrio outbreaks in mollusks in many temperate and cold regions have consistently been linked to warming patterns that affect many cultivation sites, increasing the vulnerability to infection during summer and spring (González-Escalona et al. 2005; Martinez-Urtaza et al.

2008; Rowley et al. 2014; FAO 2017). Vibriosis was reported to cause extensive mortality in a wide range of bivalve mollusk larvae and some crustaceans in the Irish Sea and surrounding regions, although such climate-related outbreaks have not yet been observed (Rowley et al. 2014). In Malaysia and its neighboring countries with a year-round tropical climate of 28°C, vibriosis has been frequently recorded at many aquaculture farms. In many outbreaks, *V. harveyi* was most frequently isolated, followed by *V. parahaemolyticus*, *V. alginolyticus*, and *V. anguillarum*, affecting Barramundi Perch *Lates calcarifer*, Brown-marbled Grouper *Epinephelus fuscoguttatus*, Orange-spotted Grouper *Epinephelus coioides*, snappers *Lutjanus* sp., and hybrid grouper (Brown-marbled Grouper × Giant Grouper *E. lanceolatus*; Albert and Ransangan 2013). Previously, Noorlis et al. (2011) reported the prevalence of *V. parahaemolyticus* in tilapias *Oreochromis* spp. and Striped Catfish *Pangasianodon hypophthalmus*, two common taxa of freshwater fish sold in local hypermarkets. Another survey involving *V. parahaemolyticus* isolates from different shellfish (i.e., mud crab *Scylla serrata*, flower crab *Portunus pelagicus*, carpet clam *Paphia textile*, hard-shell clam *Meretrix meretrix*, and mud creeper *Cerithidea obtusa*) revealed a quite concerning scenario for the emerging resistance against common antibiotics used in aquaculture (Letchumanan et al. 2015). Table 2 lists the common *Vibrio* spp. that are detected in some aquaculture organisms. Similar vibrios were also found to affect diverse marine organisms elsewhere; the list of other pathogenic species was reviewed by Gomez-Gil et al. (2014b). The latest *Vibrio* infections in Asia were reported in Xiangshan Bay, China, and involved *V. alginolyticus*, *V. harveyi*, and *V. parahaemolyticus* in Large Yellow Croaker *Larimichthys crocea*, an important commercial finfish in China (Liu et al. 2016).

On the other hand, *Photobacterium damselae* is another primary pathogen causing vibriosis-like symptoms, a disease called photobacteriosis (Essam et al. 2016). Since its first isolation from skin ulcers of Blacksmiths *Chromis punctipinnis* (Love et al. 1981), the infection has spread onto various hosts, such as the Turbot *Scophthalmus maximus*, Rainbow Trout *Oncorhynchus mykiss*, Derbio *Trachinotus ovatus*, Speckled Longfin Eel *Anguilla reinhardti*, Gilthead Bream *Sparus auratus*, European Bass *Dicentrarchus labrax*, Buri *Seriola quinqueradiata*,

TABLE 2. Common *Vibrio* species detected in some aquaculture organisms.

Vibrio sp.	Hosts	Clinical signs/symptoms	References
V. harveyi	Barramundi Perch *Lates calcarifer*, Summer Flounder *Paralichthys dentatus*	Lethargy, tissue and appendage necrosis, slow growth, slow metamorphosis, body malformation, bioluminescence, muscle opacity, and mortality	Austin and Zhang (2006), Gauger et al. (2006), Ransangan et al. (2012), Talpur and Ikhwanuddin (2012), Talpur et al. (2013)
V. alginolyticus	Brown-marbled Grouper *Epinephelus fuscoguttatus*, Malabar Grouper *E. malabaricus*, Barramundi Perch, Pacific white shrimp *Litopenaeus vannamei*, snappers *Lutjanus* spp.	Exoskeleton ulcer disease and black gill diseases	Aguirre-Guzma et al. (2004), Cheng et al. (2005), Nurhidayu et al. (2012), Cai et al. (2013), Krupersha-Sharma et al. (2013), Yang et al. (2013), Nehlah et al. (2016), Abdullah et al. (2017)
V. parahaemolyticus	Indian prawn *Fenneropenaeus indicus*, red prawn *Solenocera sub-uda*, Pacific white shrimp, tilapias *Oreochromis* spp., Striped Catfish *Pangasianodon hypophthalmus*	Gastroenteritis, septicemia, and wound infections in humans	Aguirre-Guzma et al. (2004), Noorlis et al. (2011), Ransangan et al. (2012), Krishna Kumar et al. (2014), Letchumanan et al. (2015)
V. anguillarum	Barramundi Perch, Olive Flounder *Paralichthys olivaceus*	Colonizes the fish intestine, penetrates the intestinal wall, and causes a systemic infection resulting in disease and death	Kumar et al. (2008), Chu et al. (2015), Choi et al. (2016)

Redbanded Seabream, White Seabream *Diplodus sargus*, and Meagre *Argyrosomus regius*, as reviewed by Rivas et al. (2013). In the last few years, *P. damselae* was reported to affect newer cultured marine species in Spain; the geographical distribution of this bacterium is getting wider, and thus it is listed as an emerging pathogen (Labella et al. 2011). Currently, the species comprises two subspecies: *P. damselae* subsp. *damselae* and *P. damselae* subsp. *piscicida*. *Photobacterium damselae* subsp. *damselae* has been widely recognized as an opportunistic pathogen for fish and mammalian species (including humans), while *P. damselae* subsp. *piscicida* causes serious septicemia (called pseudotuberculosis) in a wide variety of marine fishes. A review by Andreoni and Magnani (2014) summarized that the disease caused by *P. damselae* subsp. *piscicida*, like many other vibrios, was so impactful in the world of mariculture since the bacterium has a ubiquitous distribution and widespread antibiotic resistance, which allows infection on a wide range of hosts, causing massive mortality and lacking an effective vaccine.

The emergence of novel bacteria that cause diseases leads to more damage to the aquaculture industry. Two outbreaks were reported during spring and autumn 2011 at an experimental research farm in southwest Spain, causing death in Redbanded Sea Bream. Labella et al. (2017) characterized the three strains presumed to be in the genus *Photobacterium* and reported a novel fish pathogen named *P. toruni* that was isolated from kidney and spleen of the diseased fish. Controversy on the taxonomic position and phylogenetic relations of the *Vibrio fischeri* group, located between the genus *Vibrio* and *Photobacterium* (Sawabe et al. 2007), which lasted for decades (Reichelt and Baumann 1973; Baumann and Baumann 1977; Baumann et al. 1980), was resolved by Urbanczyk et al. (2007), who demonstrated the phylogenetic and phenotypic distinctions of the *V. fischeri* group from other genera in the family Vibrionaceae. They proposed a novel genus, *Aliivibrio*. *Aliivibrio wodanis* (Lunder et al. 2000) has been associated with the winter ulcer disease observed in Atlantic Salmon for more than two decades (Lunder et al. 1995). Although *A. logei* was found to have a symbiotic relationship in light organs of several species of Mediterranean sepiolids and squids, the bacterium also has the ability to associate with salmonids reared at low temperatures and causes the Hitra disease (Benediktsdottir et al. 1998). Additionally, recent mass mortality of hybrid grouper (Giant Grouper × Brown-marbled Grouper) due to vibriosis-like disease was reported in East Java, Indonesia, and involved a mixed infection of *V. alginolyticus*, *V. harveyi*, and *Streptococcus iniae* (Arif et al. 2016). In Egypt, molecular monitoring of bacterial infection reported *V. alginolyticus*, *V. parahaemolyticus*, and *V. vulnificus* in Dusky Grouper (*Epinephelus marginatus*, Egyptian Sole *Solea aegyptiaca*, and Striped Mullet *Mugil cephalus* sampled from Lake Qarun and the Gulf of Suez (Abdelaziz et al. 2017).

Vibrio spp. are dominant in seawater aquaria, making up of 60% of the total heterotrophic bacteria (Nagasawa and Cruz-Lacierda 2004; Urakawa and Rivera 2006). The bacteria interact either symbiotically or pathogenically with their hosts. In the aquaculture industry, *Vibrio* spp. are serious opportunistic pathogens affecting cultured hosts, such as finfish, shellfish, and shrimp (Liu et al. 2016). *Vibrio* spp. that cause vibriosis are usually found on gills, skin, intestinal tracts, and other internal organs of the infected shellfish or fish. They can be found at normal colonization sites, such as on the skin and in the gastrointestinal tracts of fish, which would result in a mixed bacterial culture indicating that *Vibrio* may not necessarily be the primary agent of infection. Thus, a definitive diagnosis should include isolations from kidney and other organs with lesions (Noga 2010). In Japan, cultured species, such as the Japanese Eel *Anguilla japonica*, Buri, and Ayu *Plecoglossus altivelis*, are also highly susceptible to vibriosis due to the stress of low oxygen and high temperature, which are important factors influencing the spread of vibriosis among fish.

CLINICAL SIGNS OF VIBRIOSIS

Most often, the disease vibriosis starts with external changes. If untreated, the infection may become systemic, contributing to mortality. Species of *Vibrio* that cause vibriosis are among the most common bacterial pathogens of fish, usually resulting in clinical signs such as skin ulcers and systemic infections (Sonia and Lipton 2012). The clinical signs of vibriosis were first described in infected European Eels *Anguilla anguilla* in Norway and later in the UK (McCarthy 1976). They include lethargy, anorexia, abnormal swimming patterns or spinning, hemorrhagic lesions on the skin, ulcerative skin lesions, abdominal distension, exophthalmia (“pop-eye”), gill necrosis, darkened skin, and death. The respiratory signs include an elevated opercular rate, piping, and respiratory distress (Ransangan and Mustafa 2009).

Nagasawa and Cruz-Lacierda (2004) documented the effects of vibriosis on all life stages of grouper. Among the first clinical signs observed in infected grouper are anorexia or loss of appetite and skin discoloration. The affected fish become lethargic and tend to swim near the surface of the water while exhibiting abnormal swimming behavior. The skin ulcers may become hemorrhagic, and the erosion of the fins eventually becomes necrotic. Furthermore, exophthalmia and corneal opacity are among the common signs. Blood might accumulate in the abdominal cavity due to internal organ hemorrhage, while *V. carchariae* (junior synonym of *V. harveyi*) was found to cause gastroenteritis, with swollen intestine containing yellow fluid (Nagasawa and Cruz-Lacierda 2004). Coldwater vibriosis is evident from clinical signs of acute anemia and severe hemorrhages, particularly in the integument surrounding the caeca, kidney, and abdominal fat. Blood smears from affected fish contain vast numbers of bacteria (Toranzo et al. 2005). Rameshkumar et al. (2014) reported a case of vibriosis caused by *V. alginolyticus* in Cobia *Rachycentron canadum* juveniles. The affected animals exhibited signs of surfacing, sluggish swimming, and bilateral exophthalmia that ended in mortality. Histologically, the affected fish displayed fatty change in the liver, congested eyes, and acute glomerulonephritis in the kidney. Barramundi Perch infected with *V. alginolyticus* showed ulcerations and hemorrhages on the body surface (Sharma et al. 2012). Similarly, infection by *V. harveyi* produces signs of lethargy and lack of appetite, with shallow to deep ulcers, eventually leading to 80% mortality in Red Sea Surgeonfish *Acanthurus sohal* (Hashem and El-Barbary 2013). Skin depigmentation, hemorrhagic spots, and rotting fins were also reported. Postmortem examinations revealed congested liver, spleen, intestine, stomach, and kidney, with enlargement of the gallbladder (Hashem and El-Barbary 2013).

TRANSMISSION AND VIRULENCE FACTORS INFLUENCING VIBRIOSIS

It has been well documented that water is the primary mode of transmission of *Vibrio* spp. in fish. The bacteria use the skin, gills, and gastrointestinal tract as the portals of entry, but infection mostly develops by penetration through the fish skin (Frans et al. 2011). Physical and chemical barriers of mucus and epidermis act as the first barrier against invasion by the pathogens; thus, injury or damage of the mucosal layer would enhance the possibility of infection. Li et al. (2003) reported that mortality of Goldlined Seabream *Rhabdosargus sarba* when challenged with *V. alginolyticus* by immersion depended upon the integrity of the skin. Occasionally, oral ingestion also contributes to the development of vibriosis. Transmission through oral ingestion occurs when fish consume copepods and chironomids that act as natural reservoirs of *V. cholera*. Furthermore, fish that carry the bacteria also serve as a vector and disseminate the bacteria when consumed by other predatory animals, including migratory waterbirds (Senderovich et al. 2010). Another mode of infection involves the contamination of eggs by the parent fish (Marco-Noales et al. 2001). Although virulent properties in a pathogen play an important role in the development of fish disease, environmental and nutritional conditions are said to be more significant in influencing the severity of disease outbreaks (Jun and Woo 2003). Major factors that influence the development of vibriosis include chemical stress (composition of the diet, water quality, and pollution), physical stress (salinity and temperature), and biological stress (population density and existence of other macro- or micro-organisms; Huicab-Pech et al. 2016).

A sudden change in water temperature (particularly in early to mid-summer) that corresponds to the optimum growth temperature for *Vibrio* spp., poor water quality, fluctuations in salinity, and insufficient diet have been reported as possible triggers for outbreaks of vibriosis in fish. For example, Albert and Ransangan (2013) reported high mortality of various marine fishes at a net-cage farm during a period of high water temperatures and concluded that temperature could enhance the susceptibility of marine fish to vibriosis. Furthermore, schooling behavior prior to spawning can lead to stress from overcrowding, thus increasing the chance of spreading the disease. Fish overcrowding in floating cages might cause abrasions of the otherwise intact integument, triggering pathogen infestation (Li et al. 2003). Other factors that could increase the occurrence of vibriosis are the stress of transportation and transfer from freshwater to seawater, pollution, or handling, as observed in Atlantic Cod *Gadus morhua* (Gratacap 2008).

Identification of virulence factors is essential and can be used as an antigen for vaccine preparation and diagnosis of vibriosis (Cai et al. 2007). In general, the virulence factors of interest are the genes that give access to the pathogens, allowing them to infect and damage the host. They include adherence and entrance, the establishment and multiplication ability of the pathogen, the avoidance of host defenses, damage-causing ability, and exit from the infected host (Ruwandeepika et al. 2012). Wu et al. (2008) identified the five major virulence factors of pathogenic bacteria (Table 3). Many *Vibrio* spp. that are pathogenic to humans and aquatic vertebrates or invertebrates produce virulence factors such as capsular polysaccharides, adhesive factors, cytotoxins, lipopolysaccharides, and flagella (Ceccarelli et al. 2013). The first step in bacterial pathogenesis is through adherence to the host surface and is considered an important step. Furthermore, the presence of multiple flagella facilitates bacterial motility, allowing successful infection of the host. Chen et al. (2008) proved that the adhesion mechanism of *V. alginolyticus* is adhesion to mucus. Extracellular polysaccharides are secreted around the cell as a capsule or loose slime that allows vibrios to attach to the host cells and are significantly involved in immune evasion, as encapsulated pathogens exhibit increased resistance to phagocytosis and complement-mediated killing (Chen et al. 2010).

Extracellular products (ECPs) are also important virulence determinants in *V. harveyi* (Austin and Zhang 2006). The ECPs are usually associated with disease symptoms leading to the toxic effects that cause the death of infected fish (Méndez et al. 2012). They are of low molecular weight and are believed to constitute a lethal toxin

TABLE 3. Major virulence factors of *Vibrio* spp. and mechanisms of pathogenicity (Wu et al. 2008).

Virulence factor	Mechanism and function
Membrane proteins	Important roles in adhesion, colonization, and invasions, promoting adherence to host cell surfaces; responsible for resistance to antibiotics and intercellular communication.
Polysaccharide capsule	Surrounds the bacterial cell and has antiphagocytic properties.
Secretory proteins	Modify the host cell environment and are responsible for some host cell–bacteria interactions. Pathogenic bacteria normally use distinct secretion systems (most commonly types I–IV) to transport toxins from their cytoplasms into the host or extracellular environment.
Cell wall and outer membrane components	Mostly found in gram-negative bacteria. The major outer membrane glycolipid consists of lipopolysaccharides, and lipoteichoic acids might activate the host complement pathway and act as a potent inducer of inflammation.
Other virulence factors	These factors confer a selective advantage for persistence under environmental conditions. They also help the pathogen by increasing the resistance to antimicrobial agents and facilitating colonization in the host. Examples include biofilm-forming proteins and siderophores.

generated by *V. harveyi* (Montero and Austin 1999). Saeed (1995) demonstrated that the ECPs produced by *V. harveyi* were toxic to Brownspotted Grouper *Epinephelus chlorostigma* but not to Sobaity Seabream *Sparidentex hasta*, while Liu et al. (1996) successfully recovered ECPs from diseased giant tiger prawns *Penaeus monodon*. Based on these findings, the ECPs appear to exhibit strong proteolytic (caseinase), phospholipase, and hemolytic activities in virulent compared to nonvirulent bacterial strains (Liu et al. 1996). Soto-Rodriguez et al. (2003) challenged brine shrimp *Artemia franciscana* nauplii with exposure to different ECPs of *V. harveyi* and found significant correlations between naupliar mortality and the production of proteases, phospholipases, or siderophores but no correlations with lipase or gelatinase production, hydrophobicity, or hemolytic activity. Biofilms—a lifestyle of microbes involving ECPs—are important for the survival, virulence, and stress resistance of *Vibrio* spp. as well as a reservoir for pathogenic bacteria, which cause harmful effects on cultured organisms (Ruwandeepika et al. 2012). Another important virulence factor produced by many pathogenic bacteria is the lytic enzymes, which play a major role in destroying the host tissues, allowing the pathogen to acquire nutrients and to spread through the tissues (Malathi et al. 2014). The well-known enzymes produced by *Vibrio* spp. include hemolysins, proteases, lipases, and chitinases. Zhang and Austin (2000) studied a total of 21 *V. harveyi* isolates from different sources; in comparing the pathogenicity and putative virulence factors of the isolates, those authors discovered that the most pathogenic isolate (the VIB 645 strain) produced ECPs with the highest titer of hemolytic activity to fish erythrocytes. Cai et al. (2007) reported on the cloning and expression of the gene encoding an extracellular alkaline serine protease from a *V. alginolyticus* strain, and the toxic virulent product was lethal to Red Snapper *Lutjanus campechanus.*

Virulence-related genes in *Vibrio* strains are frequently located in the mobile genetic elements, such as prophages, transposons, plasmids, integrative and conjugative elements, and genetic islands. Thus, acquisition of these elements would result in the emergence of virulence even in the nonpathogenic strains (Gennari et al. 2012; Castillo et al. 2018), making *Vibrio* spp. opportunistic pathogens. Gennari et al. (2012) proposed that the nonpathogenic vibrios are a significant reservoir of virulence and fitness genes. Those authors screened a total of 156 nonpathogenic *Vibrio* strains isolated from water, plankton, sediment, and fish samples in the Venetian Lagoon for the presence of virulence and fitness genes commonly found in *V. cholerae* and *V. parahaemolyticus*; the results showed that 37% of the strains possessed at least one of the virulence/fitness genes. Similarly, Castillo et al. (2018) revealed that the prophage-like elements encoding possible virulence and antibiotic resistance are widely distributed among the 64 species of environmental vibrios, including those classified as nonpathogenic.

Recently, a group of researchers from Taiwan demonstrated such activity in *V. parahaemolyticus* excreting the *Photorhabdus* insect-related (Pir) toxins, which cause severe acute hepatopancreatic necrosis disease in penaeid shrimp (Lee et al. 2015). The bacterium possessed PirA and PirB proteins that were structurally similar to those of the *Bacillus* Cry insecticidal toxin-like proteins, despite the low sequence identity (<10%). Lee et al. (2015) had experimentally demonstrated the role of PirAB of *V. parahaemolyticus* (PirAB^{vp}) and postulated that the PirAB^{vp} complex might induce cell death by forming ionic pores in the cell membrane (resembling the *Bacillus* Cry insecticidal toxin-like mechanism), thus immediately destroying the host cells. More importantly, discovery of the virulence factors is crucial in understanding bacterial pathogenesis and interactions with the hosts (Wu et al. 2008). Therefore, identification of virulence determinants is crucial, especially in research projects that study novel targets for the drug or in vaccine development.

POTENTIAL CONTROL AND PREVENTION MEASURES AGAINST VIBRIOSIS

Since the development of vibriosis requires adherence, establishment, and multiplication of *Vibrio* spp. in the host, control and prevention should be implemented toward preventing these crucial steps of disease development. Therefore, to be effective, control and prevention of vibriosis require a combination of the various activities detailed below.

Preventing Establishment of Infection

Farm management.— Farm management should be tailored toward preventing the entrance and spread of *Vibrio* into the farm. This preventive measure is the most cost-effective method against diseases such as vibriosis (Defoirdt et al. 2005). To fight vibriosis, farmers should maintain good sanitation and management procedures (Bullock 1977). Leano and Paner (1998) supported this by concluding that good water and feeding management are very effective against *Vibrio*. Farmers need to improve water quality via water treatment and bioaugmentation and should reduce stress factors in the farm, such as high stocking densities, temperature, salinity, and handling (Defoirdt et al. 2005). However, development of an optimal environmental condition in fish farming is not easy and is not always economical; hence, there will always be a risk of disease infection. Implementation of effective biosecurity procedures is therefore always required (Fjalestad et al. 1993). One of the most important issues in biosecurity is the introduction of fish fry from disease-free hatcheries (M. Zamri-Saad, plenary talk at the International Conference on Marine Science and Aquaculture, 2017). Indeed, this is a critical issue in fish farming in Asia, where certified disease-free hatcheries are almost nonexistent, forcing farmers to introduce fish of unknown disease status and leading to outbreaks of disease, including vibriosis.

Breeding disease-resistant fish.— After the occurrence of disease in a farm, not all fish succumb to the disease, and they do not show the same disease severity. Some fish are not affected and could be used as a starting point for the selection of disease-resistant fish. Furthermore, fish belonging to different strains differ in their resistance to disease. Work on the development of disease-resistant fish may be started not only for those possessing high individual resistance (i.e., fish that remained healthy within a stock affected by the disease) but also with the development of breed groups of hybrid origin, distinguishable by higher resistance to a particular disease. To do this, prior knowledge of the genetic variation is required, and marker-assisted selection and genomic selection increase the accuracy when predicting the genetic merit of selection candidates (Yanez et al. 2014).

Systematic breeding of Atlantic Salmon for increased resistance against important diseases, such as furunculosis and infectious pancreatic necrosis, was attempted. Attempts were also made to breed Rainbow Trout for increased resistance against viral hemorrhagic septicemia and furunculosis. Results revealed the existence of indirect markers of disease resistance in both Rainbow Trout and Atlantic Salmon, which could be employed in selection for resistance against viral diseases in Rainbow Trout (Midtlyng et al. 2002). By focusing on resistance to one disease at a time, the rate of survival is at least 12.5% higher per generation (Gjedrem 2015). Unfortunately, selective breeding for disease resistance is currently used only to a small extent in aquatic species. In 2010, it was estimated that only 8.2% of aquaculture production was based on genetically improved stocks (Gjedrem 2015). Thus far, attempts have been made to breed fish with resistance against *Aeromonas hydrophila* but not fish with resistance to vibriosis (Das and Sahoo 2012).

Controlling the Spread of Infection

Once vibriosis is diagnosed on a farm, efforts must be made to control or reduce disease incidence with the hope of reducing the effects of the disease by using the methods described below.

Antibiotics.— For many years, treatments with antibiotics have been extensively used against bacterial infections in the aquaculture industry (Gram et al. 2001). For instance, florfenicol and oxolinic acid are mainly used to treat vibriosis in cod fry production in Norway (Frans et al. 2011). Other types of antibiotics, such as quinolones and flumequine, are widely used to treat many types of bacterial infection, including classical and coldwater vibriosis (Shao 2001). Defoirdt et al. (2011) stated that antibiotics such as chloramphenicol, cotrimoxazole, streptomycin, and erythromycin were used to combat *V. harveyi* infection in giant tiger prawns, but the bacterium became resistant and antibiotics were no longer effective. Furthermore, 75% of the antibiotics used in aquaculture farms leached into the surrounding environment through feces and uneaten medicated feed and accumulated in the sediment (Mary et al. 2004). Similarly, Xiong et al. (2015) detected high antibiotic concentrations in sediment and water samples collected in China. Thus, fish ponds are reservoirs for both antibiotic residues and resistance genes, leading to the development of antibiotic-resistant organisms, such as methicillin-resistant *Staphylococcus aureus* (Atyah et al. 2010). The antimicrobial drug build-ups in the edible tissues can cause allergies, toxic effects, changes in the intestinal microbial fauna, and acquisition of drug resistance. Chloramphenicol deposits in sustenance consumed by people can even result in aplastic sickliness, which causes intense bone marrow ailments. Nitrofuran antibiotics are known to cause tumors and numerous different illnesses (Citarasu 2012). This issue has grown into a public health matter, and further reinforcement of legal requirements and stricter national regulations on the use of antibiotic application in aquaculture sectors are being implemented (FAO 2016a,b). Recently, importation of food products that relate to the use of antibiotics is forbidden in certain countries, such as the European Union and Japan, due to safety concerns (Ma et al. 2010).

Green water technique.— The green water technique is a relatively new method wherein the aquatic organisms are cultured in rearing water with an abundance of microalgae (Defoirdt et al. 2005). Research has revealed that green water containing 8 bacterial isolates and 12 fungal isolates successfully inhibited the growth of *V. harveyi* in giant tiger prawns (Lio-Po et al. 2005). Another study by Tendencia and dela Peña (2003) found that seawater containing *Chlorella* sp. could control *V. harveyi* abundance in the seawater (Lio-Po et al. 2005). Nevertheless, this technique is yet to be widely used in fish farms.

Topical disinfectants.— Topical disinfectants include a wide range of chemicals, such as formalin, benzalkonium chloride, malachite green, copper sulphate, and many more, which are used mainly to remove external opportunistic bacteria, protozoans, and fungi (Mohamed et al. 2015). However, Punitha et al. (2008) reported that an overdose of chemicals such as formalin resulted in major gill damage, while repeated treatments with nitrofurazone caused ulcerative dermatitis in fish. In Malaysia, farmers use hypochlorites to disinfect the incoming water to control vibrios (Mohamed et al. 2015). Hypochlorites (e.g., sodium hypochlorite) can be harmful to humans if swallowed (MedlinePlus 2015). Some chemicals are banned from the aquaculture industry due to health and environmental concerns, and many governments have restricted the use of chemical products at aquaculture farms (Harikrishnan et al. 2011).

Medicinal plants.— For centuries, medicinal plants have been used as immunostimulants that enhance animals' resistance to infection, as the plants contain some chemical compounds that induce the activation of white blood cells (Van Hai 2015). The plants' active compounds, such as alkaloids, polyphenolics, terpenoids, phenolics, lectins, quinones, and polypeptides, are effective substitutes for antibiotics, chemicals, or synthetic compounds and vaccines (Talpur et al. 2013). In fact, they can facilitate growth and reduce stress, particularly for shrimp and finfish, and are safe for humans and the environment. The medicinal plants include spices, herbs, herbal extracts, traditional Chinese medicines, seaweeds, and commercial plant-derived products (Van Hai 2015). Medicinal plants, such as Indian ginseng *Withania somnifera*, were reported to reduce mortality of Greasy Grouper *Epinephelus tauvina* against infection by *V. harveyi* (Harikrishnan et al. 2012). Fish that were fed dietary ginger developed stronger non-specific immunity and subsequently a reduced susceptibility to *V. harveyi* infection. A study using garlic *Allium sativum* revealed that the plant has immunostimulant, therapeutic, and antimicrobial effects that increase the immunity against *V. harveyi*, leading to improved growth and survival (Talpur and Ikhwanuddin 2012). Similarly, Van Hai (2015) reported that Indian prawns *Fenneropenaeus indicus* were protected from *V. parahaemolyticus* infection after being fed with seaweed extracts.

Bacteriophage.— A bacteriophage is a virus that infects and replicates within a bacterium. It is composed of proteins that encapsulate a DNA or RNA genome, and it replicates within the bacterium after injection of its genome into the cytoplasm (McGrath and Van Sinderen 2007). Bacteriophages are ubiquitous viruses widely distributed in locations populated by bacterial hosts, such as soil or the intestines of animals. One of the dense natural sources for bacteriophages and other viruses is seawater (Wommack and Colwell 2000). Bacteriophages have been used for over 90 years as an alternative to antibiotics and are seen as a possible therapy against many bacteria (Keen 2012).

Various studies on bacteriophage use against *Vibrio* spp. revealed successful isolations of lytic bacteriophages (Kalatzis et al. 2016; Solís-Sánchez et al. 2016). An in vitro study revealed an over 90% decrease of the *Vibrio* population after 4 h of treatment with lytic bacteriophages. Thus, administration of bacteriophages to live feed (*Artemia* spp.) could selectively reduce the transfer of *Vibrio* from infected *Artemia* into fish hatcheries. Furthermore, vibriosis is mostly observed among fish larvae, leading to high mortality due to ineffective prophylactic strategies. Since vaccination is not feasible in larvae and antibiotics are ineffective against multi-drug-resistant bacteria, the potential for phage therapy to combat vibriosis in fish larvae production is great. It has been shown that directly supplying phages to the culture water could be an effective and inexpensive approach to reducing the negative impact of vibriosis in larviculture (Silva et al. 2014). Although phage therapy may represent a viable alternative to antibiotics for the inactivation of fish pathogenic bacteria, its use requires awareness of kinetics phenomena. The bacteriophages should have the potential to inactivate fish pathogenic bacteria without exerting major effects on the structure of natural bacterial communities in aquaculture waters (Pereiera et al. 2011). Nevertheless, Tan et al.

(2017) recently examined the diversity and interactions of 11 vibriophages on 24 strains of *V. anguillarum* and 13 strains of *Vibrio* sp. and found that the 11 phages covered all 37 tested *Vibrio* host strains, which represent considerable temporal (20 years) and geographical (9 countries) differences in the origins of isolation.

Enhancing the Host Defense System by Probiotics

Gatesoupe (1999) defined probiotics as dietary supplements made up of living microbes that remain alive as they enter the gastrointestinal tract and enhance the health of their host. Probiotics can be yeast, microalgae, gram-positive bacteria, or gram-negative bacteria (Harikrishnan et al. 2011). In the aquaculture system, application of probiotics or beneficial live microbes into feed supplements provides advantages by producing antagonistic compounds, competing for chemicals and attachment sites, modulating and stimulating the immune functions, and enhancing the microbial balance (Verschuere et al. 2000). Other common mechanisms or modes of action by the probiotic include assisting in feed conversion efficiency that improves live weight gain (Al-Dohail et al. 2009) and production of organic acids (formic acid, acetic acid, and lactic acid), hydrogen peroxide, and several other antagonist components (e.g., antibiotics, bacteriocins, siderophores, and lysozymes; El-Dakar et al. 2007). In recent years, probiotics have become an essential part of the practices for enhancing growth and disease resistance in cultured fish (Nayak 2010).

A spectrum of probiotic application is obviously more diversified in the aquaculture practice compared to terrestrial agriculture. Monospecies or multispecies probiotic supplements are commercially accessible for aquaculture practices (Decamp and Moriarty 2006; Ghosh et al. 2007). Overall, there has been significant success from the application of *Bacillus* spp. as a probiotic in aquaculture systems (Hong et al. 2005). Merrifield et al. (2010) found that administration of *Bacillus subtilis* and *B. licheniformis* in commercial feed for Rainbow Trout resulted in significant enhancement in the feed conversion ratio, specific growth rate, and protein efficiency ratio. In an experiment by Lauzon et al. (2010), *Enterococcus thailandicus* was administered with daily feed to Atlantic Cod for 55 d at a probiotic cell density of 10^7 colony-forming units/mL, which resulted in a significant increase in growth, survival, and feed efficiency and minor colonization over pathogenic *Vibrio* spp. Currently, *Pseudomonas fluorescens*, *Bacillus subtilis*, *Lactobacillus plantarum*, and *Streptomyces* are among the organisms that have been used as probiotics in aquaculture and have reduced the vibriosis-related mortality in fish (Frans et al. 2011; Touraki et al. 2012; Tan et al. 2016). Similarly, Sorroza et al. (2012) used a probiotic strain of *Vagococcus fluvialis* to fight infection by *Vibrio anguillarum*. Gram et al. (1999) found that *Pseudomonas fluorescens* strain AH2 succeeded in reducing the mortality of Rainbow Trout when infected with a pathogenic strain of *V. anguillarum*. Control tanks with only *V. anguillarum* immersed into the medium showed a cumulative mortality of 47% after 7 d, while juvenile Rainbow Trout supplemented with probiotic *P. fluorescens* AH2 had a reduced cumulative mortality level of 32%. Vaseeharan and Ramasamy (2003) demonstrated that application of *B. subtilis* led to 90% survival in giant tiger prawns challenged with *V. harveyi*.

Noh et al. (1994) found that lactic acid bacteria showed a positive effect as growth promoters for juvenile Israeli carp (a variant of Common Carp *Cyprinus carpio*) but not necessarily for seabass. Another study by Shariff et al. (2001) reported that the commercial *Bacillus* probiotic Primalac (Star-Labs/Forage Research, Clarksdale, Missouri) increased the growth performance and feeding efficiency of Persian Sturgeon *Acipenser persicus* juveniles but failed to increase the survival of giant tiger prawns.

Vaccine and Vaccination

The chemotherapy era has dominated the concept of disease prevention due to the availability of antimicrobial compounds as well as the low price of antibiotics and other drugs. It was only in the mid-1970s that greater interest began to be focused on the enhancement of the hosts' immunity to combat infection through the application of vaccines in fish farming. Nevertheless, prevention of fish bacterial disease via oral vaccination was first reported by Duff (1942). This was followed by successful vaccination against bacterial diseases, including vibriosis, in the 1970s and 1980s (Evelyn 1996). Since then, fish immunology has become an advanced science and has assisted researchers in better understanding fish diseases and the success of fish vaccine development in the aquaculture industry (Van Muiswinkel and Nakao 2014). Fish vaccination is defined as the administration of an antigen from the pathogen into targeted fish to elicit a specific immunoprotective response against the foreign agent after subsequent exposure to the same antigen. Vaccination is the best immunoprophylactic measure against disease and thus could enhance the survival of fish and increase the profitability of aquaculture (Wali and Balkhi 2016).

Studies on vaccine formulation, strategies for vaccination regimens, and protective efficacy of vaccines have gained much interest in fish vaccine development. Diagnosis and vaccination of fish diseases have become increasingly advanced and more relevant for application in the aquaculture industry. Before the use of vaccination spread worldwide throughout the aquaculture industry, fish vaccines were only available for salmonid species. Vaccination is important in large-scale commercial fish farming and is a key reason for the success of salmon cultivation (Sommerset et al. 2005). The improvement in salmonid farming after vaccination has boosted interest in culturing different

TABLE 4. List of fish vaccines commercially available for use in preventing vibriosis.

Pathogens[a]	Main hosts[a]	Type of vaccine[b]	Trade name[b]	Vaccination route[b]
Vibrio alginolyticus, *V. parahaemolyticus*, *V. vulnificus*, *V. anguillarum*	Most marine fish, salmonids *Oncorhynchus* spp., groupers *Epinephelus* spp., cods *Gadus* spp., Madai *Pagrus major*, Gilthead Sea Bream *Sparus auratus*, Olive Flounder *Paralichthys olivaceus*, Summer Flounder *P. dentatus*, amberjacks *Seriola* spp., halibut *Hippoglossus* spp., Yellowtails *Seriola lalandi*, seabasses *Lates* spp., Milkfish *Chanos chanos*, horse mackerels *Trachurus* spp., Cobia *Rachycentron canadum*, sole *Solea* spp., eels *Anguilla* spp., tilapias *Oreochromis* spp.	*V. anguillarum-ordalii* bacterin	Vibrogen 2	Immersion
		Inactivated *V. anguillarum* 01 and *V. ordalii* 02	AquaVac *Vibrio*, AquaVac *Vibrio* Oral Boost	Immersion/oral
		Inactivated strain *Listonella* (*V. anguillarum* biotype I and II) and *Photobacterium damselae* subsp. *piscicida*	AquaVac *Vibrio Pasteurella*	Injection
		Aeromonas salmonicida–*Vibrio anguillarum-ordalii-salmonicida* bacterin	Lipogen Forte	Injection
		Infectious salmon anemia virus vaccine–*Aeromonas salmonicida*–*Vibrio anguillarum-ordalii-salmonicida* bacterin	Forte VI	Injection

[a]Data adapted from Grisez and Tan (2005), Austin and Austin (2007), Pridgeon and Klesius (2012), and Singh et al. (2009).
[b]Data adapted from Bowker and Trushenski (2016) and Merck Animal Health (http://aqua.merck-animal-health.com/products/vaccines.asp).

kinds of marine fish species (Gudding and Van Muiswinkel 2013). Fish vaccination has also been used in Asia for Asian fish species, and vaccine research and development are being carried out extensively. Besides amberjacks *Seriola* spp. in Japan and Grass Carp *Ctenopharyngodon idella* in China, commercial vaccines have recently been launched for use in seabasses *Lates* spp., tilapias *Oreochromis* spp., and other species in some Southeast Asian countries (Clark et al. 2010). Many studies have used vaccination methods to fight diseases in groupers *Epinephelus* spp. Huang et al. (2014) vaccinated Orange-spotted Grouper against pathogenic *Streptococcus iniae* with a formalin-inactivated vaccine via intraperitoneal injection that resulted in 100% survival at 6 months postvaccination.

Since vertical transmission of diseases can occur, immunization of broodstock is important for producing specific-pathogen-free eggs. Fish immunization can be accomplished by injection, immersion, or oral administration (Chang et al. 2008); according to Gudding et al. (1999), most of the commercial vaccines are inactivated vaccines administered by injection or immersion. In Japan, the incidence of diseases in mariculture animals has dropped since the use of vaccines that have been proven effective against bacterial infections, such as *Lactococcus garvieae* in Yellowtail Jacks *Seriola lalandi*, and against viral infections, such as iridovirus in Madai *Pagrus major* (Bondad-Reantaso et al. 2005).

In general, few types of vaccine formulation are possible in fish vaccination. These include bacterin (Karsi et al. 2017; Nguyen et al. 2017), live attenuated (Hu and Sun 2011; Pridgeon et al. 2013; Gao et al. 2014; Huang et al. 2014; Li et al. 2015; Wang et al. 2015), DNA vaccine (Sepúlveda and Lorenzen 2016; Rauta et al. 2017), subunit (Zhang et al. 2008; Hamod et al. 2012; Nehlah et al. 2016), anti-idiotypic (Qin and Yan 2010), and toxoid vaccines (Sudhagar et al. 2016). Wali and Balkhi (2016) classified bacterial vaccines into eight types: inactivated soluble cell extracts, heat-inactivated whole cells, attenuated live cells, cell lysates, serum, purified subcellular components, subunit vaccines, and a mixture of bacterial components.

Bacterin and inactivated vaccines are commercially available and authorized for use against vibriosis in the aquaculture industry (Table 4). They are currently available for salmonids, cod/halibut, seabasses, seabream, Buri, and yellowtails (Sommerset et al. 2005). Besides powerful vaccine components, the methods of vaccine preparation and administration and the dose of vaccine introduced into the fish also affect the outcome of vaccination in inducing an immune response. Currently, vaccines prepared from formalin- or heat-killed, whole-cell bacteria are the most popular types of vaccine used in farmed fish, yielding good protection in a diversity of fish species worldwide.

Therefore, vibriosis can simply be controlled by vaccination, and vaccines that are based on inactivated bacterial pathogen have been proven to be efficacious in fish (Sommerset et al. 2005). Li et al. (2015) found that Silver Bream *Abramis bjoerkna* immunized with formalin-killed *V. alginolyticus* showed an enhanced lymphocyte count, agglutinating antibody titer, and macrophage phagocytic activity. The best protection is obtained by an injection method (Håstein et al. 2005). Development of vaccines against vibriosis in fish is encouraging due to the broad range of hosts and wide geographical distribution of *Vibrio* spp. A commercial vaccine known as AquaVac *Vibrio* (Intervet/Schering-Plough) was developed to prevent vibriosis caused by *V. anguillarum* types I and II and *V. ordalii* infections in Rainbow Trout and European Bass only. This vaccine is available in two choices: AquaVac *Vibrio* Immersion and Injection; and AquaVac *Vibrio* Oral. However, these vaccines do not offer protection from other species of *Vibrio*.

To date, modernization of bacterial vaccine strategies has led to the development of recombinant vector and DNA vaccines, which are trending among researchers. Recombinant vector vaccines combine parts of the microorganism that causes the disease with weakened pathogens, thus allowing for antigen production. The DNA vaccine involves immunization by inserting circular plasmid DNA containing the gene that encodes the protective antigen into host tissue to induce vulnerable and long-term host immune response to the protein (antigen) encoded by the gene vaccine (Pasnik and Smith 2005). Moreover, the subunit and live attenuated vaccines composed of the native antigenic structure have successfully provided significant protection in fish by mimicking the response induced after natural infection (Pore et al. 2009). For example, a recombinant bivalent vaccine, rOmpK+-rOmpW, was able to provide high protection against vibriosis (Nehlah et al. 2017). This supports previous researchers' suggestions (Jiang et al. 2003) of the possible superiority of bivalent antigen vaccines as opposed to single-antigen vaccines for achieving a better immunocompetence effect. Angelidis et al. (2006) demonstrated high protection efficacy of bivalent *V. anguillarum* serotype 01 and 02 vaccines in juvenile European Bass after immersion that yielded relative percent survival values ranging between 93% to 100%. Many previous researchers have proven both OmpK and OmpW to be effective vaccine candidates. Qian et al. (2008) and Nehlah et al. (2017) concluded that the conserved OmpK was an effective vaccine candidate against *V. alginolyticus* infection since it was able to protect vaccinated Large Yellow Croaker and hybrid groupers, respectively. Li et al. (2010) demonstrated the cross-protective property of recombinant OmpK against virulent *Vibrio* strains (*V. harveyi*, *V. alginolyticus*, and *V. parahaemolyticus*) in Orange-spotted Grouper. According to studies by Qian et al. (2007) and Mao et al. (2007), OmpW was proven to be an effective vaccine candidate against *V. alginolyticus* and *V. parahaemolyticus*, respectively, in Large Yellow Croaker.

Nevertheless, attempts to search for the most suitable vaccine candidates encounter problems due to the existence of diverse serotypes, so the search for the perfect strain is crucial for vaccine development (Swain et al. 2010; Lun et al. 2014). Although species-specific vaccines have effectively shown protection against a specific bacterial strain, the antigenic diversity of *Vibrio* strains and their serotypes has made these vaccines unable to elicit protection against multiple *Vibrio* spp., resulting in the slow progress of vaccine development against vibriosis (Li et al. 2010, 2014). Until recently, no vaccine was produced to prevent vibriosis caused by *V. vulnificus* except for Vulnivaccine, which is effective for eels (Anguilliformes) but still requires a booster (Esteve-Gassent and Amaro 2004). However, vaccinating eels with *V. vulnificus* serovar E can be infected by other serovars of the pathogen that become secondary pathogens, as no cross-protection exists between serotypes (Fouz and Amaro 2003).

Thus, development of a powerful and versatile vaccine that can fight against various *Vibrio* pathogens by eliciting protection against homologous and heterologous strains is needed both for hindering vibriosis infections and to avoid the exploitation of antibiotics in the aquaculture industry. As a start, the current advancement in molecular technologies would provide accuracy in identifying bacterial pathogenesis that would serve as valuable information to the farmer, diagnostician, fish pathologist, and taxonomist for developing a better vaccine. Comparative genome analysis of strains of a pathogenic bacterial species can be used to unravel the acquisition of mobile genetic elements related to virulence (Solheim et al. 2011; Castillo et al. 2018), while integration of genomic and phenotypic features enlightens the molecular evolution of the pathogenic *Vibrio* spp. (Castillo et al. 2018; Martinez-Urtaza et al. 2017). Multilocus and whole-genome sequence analyses are now becoming fundamental and should be routinely used and adequately accessible for survey and diagnostic purposes.

Furthermore, major constraints on the development of fish vaccines include the lack of understanding of fish immunology, the countless unlicensed vaccines, the overpricing of vaccines, and the stress of administration (Muktar et al. 2016). The stress induced during fish vaccination, especially by injection, contributes to severe postvaccination side effects. Thus, oral vaccination is considered the most practical for a large number of fish because it is nonstressful and user-friendly (Muktar et al. 2016).

CONCLUSIONS AND RECOMMENDATIONS

In conclusion, a combination of good aquaculture farm practices, selective utilization of controlling agents, and proper vaccination programs is key to improving fish health, reducing disease outbreaks, and decreasing the devastating economic impact on aquaculture farming. Aquaculture is growing more rapidly worldwide than all other food-animal-producing sectors, but the great challenge is the disease. With the harmful effect of vibriosis to aquaculture farms, farmers must wisely choose the best treatment to combat the disease. Although maintaining an optimum condition at the aquaculture farm is quite difficult, methods that are harmful to human health and the environment, such as excessive use of antibiotics and chemotherapeutants, should be avoided. We strongly suggest that fish farmers use a proper vaccine to combat vibriosis due to the greater effectiveness and safety of vaccines compared to antibiotics. However, development of new vaccines is crucial, especially those that are effective against different serotypes. Furthermore, additional study on the epidemiology of vibriosis, specific virulence determinants, pathogenicity, and immune response could generate valuable information to support an understanding of the impact of vaccination.

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