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Ecology, Virulence Factors and Global Spread of *Vibrio parahaemolyticus*

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Abstract

Vibrio parahaemolyticus is part of the autochthonous microflora in estuarine and coastal marine environments and is associated with water, sediment and various aquatic animals ranging from tiny zooplankton to marine mammals. The ecology of this organism is affected by temperature, salinity, turbidity, and the presence of zooplankton, crustaceans and molluscs. Most environmental strains are non-pathogenic to man and human pathogenic strains are characterized by the ability to produce a thermostable direct haemolysin (TDH) and *tdh*-related haemolysin (TRH). The *tdh* and *trh* genes are present in genomic islands that have been possibly acquired by *V. parahaemolyticus* by lateral gene transfer. Strains of *V. parahaemolyticus* causing acute hepatopancreatic necrosis disease (AHPND) harbour a 70 kb conjugative plasmid carrying pirA and pirB genes encoding a binary *Photorhabdus* insect-related toxin A and B (PirAB). Genetically diverse strains of *V. parahaemolyticus* isolated in Asia seem to have acquired the 70 kb plasmid, while Central American AHPND strains can be distinguished from Asian strains based on PCR amplification of TN-3-like transposon. All AHPND-causing strains tested so far lack virulence factors associated with human pathogenic strains, suggesting that risk to human health due to these strains is negligible. Bacteriophage therapy has shown potential for management of AHPND.

Keywords: AHPND, *Vibrio parahaemolyticus*, plasmid, genetic exchange, virulence

Introduction

Vibrio spp. are heterogenous Gram-negative, comma-shaped bacteria that inhabit freshwater, estuarine and marine environments. Over 100 species are recognized, and they are associated with water, sediment and a whole range of aquatic organisms ranging from microplankton to aquatic birds and marine mammals all over the globe. Only a few species of *Vibrio* are pathogenic to humans.

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Vibrio cholerae is a freshwater species and consists of over 200 serotypes. Of these, only two serotypes, O1 and O139, are associated with the disease cholera. While most non-O1, non-O139 are non-pathogenic to man, there are some strains that may cause gastroenteritis or even extra-intestinal infections. *Vibrio parahaemolyticus* and *V. vulnificus* are two other species that are involved in human infections, the former causing gastroenteritis and the latter causing primary septicaemia, mainly in immunocompromised people, wound infections and cellulitis.

Some *Vibrio* spp. are pathogens of aquatic animals. *Vibrio anguillarum* and *V. salmonicida* (now renamed as *Aliivibrio salmonicida*) are pathogens of finfish causing vibriosis. Others like *V. harveyi, V. owensii, V. penaecida* and *V. parahaemolyticus* are pathogens of crustaceans. Eleven closely related bacteria are referred to as comprising the Harveyi clade, and these include *V. harveyi, V. alginolyticus, V. parahaemolyticus, V. campbellii, V. rotiferianus, V. mytili, V. natriegens, V. azureus, V. sagamiensis, V. owensii* and *V. jasicida* (Urbanczyk et al. 2013). Members of this clade include important pathogens of aquatic animals and are also used as models in studies related to bioluminescence, quorum sensing and biofilm formation. Members of the Harveyi clade share some virulence-associated genes, suggesting genetic exchange between these in the natural environment (Ruwandeepika et al. 2010).

Until recently, *V. parahaemolyticus* attracted attention mostly as a human pathogen transmitted through raw or inadequately cooked seafood. Oysters and other bivalves eaten raw have been the main vehicles, but even marine finfish have been associated with human infections in some parts of the world, like Japan. Continued outbreaks of seafood poisoning in different parts of the world led the Codex Alimentarius Commission to request the Food and Agriculture Organization of the United Nations/World Health Organization (FAO/WHO) to assess such risk to human health. The FAO/WHO risk assessment report (FAO/WHO 2011) presented models for growth of *V. parahaemolyticus* in seafood and for predicting the risk of illness. Noting the geographical variations in the levels of *V. parahaemolyticus* in seafood and considering the need to collect more data on this pathogen, an FAO/WHO expert meeting identified the possible end users of methodologies for detection/enumeration of *V. parahemolyticus*, performance characteristics of the methods and came up with guidance on selection and application of methods (FAO/WHO 2016).

Vibrio parahaemolyticus and its habitat

Vibrio parahaemolyticus is part of the autochthonous microflora of estuarine and coastal environments in tropical to temperate zones all over the world, and there is no correlation between the presence of this organism and faecal contamination of the environments (Joseph et al. 1982; Oliver and Kaper 2007). This organism has been isolated from water, sediment, plankton, various fish and shellfish species, and marine mammals (Joseph et al. 1982). Thus, *V. parahaemolyticus* is naturally present in fish and shellfish, including shrimp and molluscs, growing in harvesting areas. The level of this organism in the environment and in various fish and shellfish may vary depending on environmental and geographical factors.

Certain areas may have more favourable environmental conditions that support establishment, survival and growth, such as temperature, salinity, zooplankton abundance, tidal flushing, suspended sediments, nutrients, dissolved organic matter and dissolved oxygen (Kaneko and Colwell 1977; Garay et al. 1985; Venkateswaran et al. 1990). In temperate waters, the ecology is strongly influenced by temperature and salinity. In these environments, *V. parahaemolyticus* is often detected in warmer months, and the organism has been reported to survive in the sediment during winter (Kaneko and Colwell 1977; De Paola et al. 2003). However, in tropical waters, *V. parahaemolyticus* can be detected throughout the year with low counts being recorded during post-monsoon period, suggesting that salinity may influence the levels in tropical waters (Deepanjali et al. 2005). In the United States of America, a predictive model based on temperature and salinity has been developed, but further studies indicated that addition of chlorophyll a as an additional parameter increased the predictability (Urquhart et al. 2016).

Vibrio parahaemolyticus can adhere to chitin, and zooplankton may thus play an important role in the ecology of this organism. In a study conducted off the coast of Spain, over 80 % of *V. parahaemolyticus* biomass was associated with zooplankton. Although cnidarians accounted for only 2 % of zooplankton biomass, they accounted for 51.87 % variation in abundance of *V. parahaemolyticus* (Martinez-Urtaza et al. 2012). Unlike *V. cholerae*, copepods have a smaller effect on the abundance of *V. parahaemolyticus*, which was favoured by a decrease in primary production, possibly due to grazing pressure by enhanced abundance of zooplankton.

Vibrio parahaemolyticus can grow in sodium chloride concentrations ranging from 0.5 to 10 %, with optimal levels between 1 and 3 % (Colwell et al. 1984). Adsorption of *V. parahaemolyticus* on plankton or chitin-containing materials occurs with higher efficiency under conditions of estuarine salinity (Kaneko and Colwell 1977). Adherence to chitin seems to improve survival of the organism at low temperatures (Karunasagar et al. 1986). In tropical shrimp culture environments, *V. parahaemolyticus* is often present. This organism accounted for 0 to 27 % of the flora in water and sediment of shrimp ponds in India (Otta et al. 1999; Gopal et al. 2005). The level of *V. parahaemolyticus* in seafood may vary depending on the type of seafood and geographical location. In oysters from the Gulf Coast of the United States of America during warm months, a level such as $1.1x10^4$ cfu.100 g^{-1} has been reported, but in Pacific oysters, which grow at lower temperatures, the levels were $2.1x10^3$ cfu.100 g^{-1} (Drake et al. 2007). In Indian oysters, the levels range from 10^2–10^4 cfu.g^{-1} (Deepanjali et al. 2005). In shrimp, the levels range from undetectable to 10^4 cfu.g^{-1}, high counts being rare (Cann et al. 1981; Karunasagar et al. 1984). In finfish, levels of ~88 cfu.g^{-1} have been reported (Chan et al. 1989).

Vibrio parahaemolyticus as a human pathogen

Vibrio parahaemolyticus was first described in 1950 from an outbreak of gastroenteritis implicating “shirasu” (small semi-dried sardine) involving 272 people. Early studies in Japan indicated that human pathogenic strains induce haemolysis in a high salt blood agar medium called Wagatsuma agar, and this phenomenon was referred to as “Kanagawa phenomenon”.

The observed haemolysis has been attributed to a thermostable direct haemolysin (TDH). Further studies on a large collection of clinical and environmental strains showed that 96 % of clinical strains produce TDH, while only 1 % of the environmental strains produce this haemolysin (Joseph et al. 1982), suggesting that most of the environmental strains may not be pathogenic to man. TDH has ability to lyse erythrocytes of various species. It also exhibits cytotoxicity that is lethal to small experimental animals and causes increased vascular permeability in rabbit skin. Using isogenic mutants, it has been demonstrated that TDH has an important role in fluid accumulation in rabbit ileal loop. This has been confirmed by a more sensitive assay using rabbit ileal tissue mounted in Ussing chambers. Culture filtrate of TDH-positive strain induced an increase in short circuit current in these chambers, but this was not seen with culture filtrate of TDH-negative strains. The Ussing chamber activity was neutralized by antiserum to TDH, thus providing confirmation that the activity was due to this virulence factor.

Low prevalence of TDH-positive strains in the environment has been confirmed from different geographical regions. In the Gulf Coast of the United States of America, the percentage has been generally less than 1 %, but in the Pacific northwest, up to 3.2 % of strains could be TDH-positive (FAO/WHO 2011). Oysters from India were positive for *V. parahaemolyticus*, with 6–10 % carrying the *tdh* gene (Deepanjali et al. 2005; Raghunath et al. 2008). Some TDH-negative strains from clinical cases were found to produce a TDH-related haemolysin (TRH) (Honda et al. 1988). Presently, strains producing TDH and TRH are considered pathogenic to man. The levels of pathogenic strains in oysters have been found to be low. DePaola et al. (2003) indicated that the average number of TDH-positive *V. parahemolyticus* in oysters from Alabama was 2 cfu.g^{-1}, while in Chesapeake Bay, a level of 10 cfu.g^{-1} was noted (Parveen et al. 2008). Studies in the People's Republic of China indicated that although the average number of pathogenic *V. parahaemolyticus* in oysters was 0.5 cfu.g^{-1}, the number of pathogens increased to 10 cfu.g^{-1}in oysters that harboured more than 10^4 cfu.g^{-1} total *V. parahaemolyticus* (Han et al. 2015).

There are five sequence variants of the *tdh* gene (*tdh*1 to *tdh5*) and two sequence variants of the *trh* gene (*trh*1 and *trh*2) (Nishibuchi and Kaper 1995). Kanagawa phenomenon has been attributed to the expression of *tdh*2 gene, but most such strains contain both *tdh*1 and *tdh*5 genes. Sixteen percent of environmental Kanagawa-negative strains may contain *tdh*1 gene or other *tdh* genes. The *tdh*4 gene was found in a plasmid, and some *tdh* genes have been detected, albeit rarely, in *V. hollisae, V. mimicus,* and in non-O1/O139 *V. cholerae.*

There is about 69 % nucleotide similarity between *tdh* and *trh* genes. The latter may occasionally be found in *V. alginolyticus* and in *Aeromonas veronii* (Raghunath et al. 2010). Analysis of whole genome sequence of several environmental and clinical strains in this study indicate that all *V. parahaemolyticus* strains carry Type Three Secretion System (TTSS). While TTSS-1 is present in both clinical and environmental strains, TTSS-2 is associated with strains carrying *tdh* (TTSS-2α) and *trh* (TTSS-2β).

Diverse serotypes may be associated with human infections, but at the beginning of 1996, an ongoing surveillance in Kolkata indicated an increase in the incidence of gastroenteritis due to *V. parahaemolyticus*. Subsequent studies indicated that 50–80 % of the isolates belonged to the O3:K6 serotype and were genetically very similar. Within a few months, cases due to the same or closely related serotype were reported in Bangladesh, Viet Nam, Laos, Indonesia, Thailand, Republic of Korea and Japan. These isolates could be identified based on nucleotide sequence variation in *toxRS* region. Today, 21 serotypes are recognized as "serovariants" of O3:K6 serotype, and these have been found to be the causative agents of several outbreaks in Europe, Mozambique, and countries of North and South America (Nair et al. 2007). Although several publications refer to these strains as "pandemic", Nair et al. (2007) pointed out that this is misleading in the epidemiological sense because outbreaks have not affected an exceptionally high proportion of the population. Nevertheless, strains belonging to this group show clonality in molecular typing methods like arbitrarily primed (AP) polymerase chain reaction (PCR), ribotyping or pulse field gel electrophoresis (PFGE). Strains are characterized by presence of only the *tdh* gene (and not *trh* gene), some mismatches in nucleotides in the *tox*RS gene and an open reading frame ORF8 derived from a filamentous bacteriophage f237 (Nair et al. 2007).

Genome Plasticity in Vibrio parahaemolyticus

Analysis of the nucleotide sequence of the *tdh* and *trh* genes of several strains of *V. parahaemolyticus* indicates that their G+C content of about 30 % is much lower than the average G+C content of *Vibrio* chromosomes (46–49 %). This suggests that *tdh* and *trh* genes might have been acquired by *V. parahaemolyticus.* This is further supported by the observation that the *tdh* genes are flanked by insertion sequence-like elements (ISV) that are related to *IS*903 (Nishibuchi and Kaper 1995). Although *IS*903 is known to encode an active transposase, there has been no evidence to demonstrate that actual transposition of *tdh* gene occurs. However, this could be due to base changes and deletions that have occurred in the ISVs, which show only about 50 % identity with *IS*903. Park et al. (2000) demonstrated that the *trh* gene cluster also has lower (41 %) G+C content than *Vibrio* chromosome. This gene cluster contained *trh* gene, nickel transport operon and urease genes. The first gene in this cluster is a transposase gene flanked by an 18 bp inverted repeat on both sides. The next gene is the *trh* gene, followed by urease and nickel transport genes. Analysis of the whole genome sequence of *V. parahaemolyticus* O3:K6 strain RIMD 2210633 and other clinical strains has provided more insights into the genome plasticity of this organism. It is now well established that *V. parahaemolyticus* has two circular chromosomes. So far, nine genomic islands, VPaI-1 to VPaI-9, ranging in size from 10–81 kb have been identified in *V. parahaemolyticus* (Ceccarelli et al. 2013).

Generally, genomic islands are characterized by having G+C content that is different from the other portions of the chromosome and are flanked by transposase or integrase gene or direct repeat regions that suggest they have been acquired through lateral gene transfer. Features of genomic islands of *V. parahaemolyticus* are listed in Table 1.

Table 1. Features of genomic islands of *Vibrio parahaemolyticus.*

Genomic Island	Size	Features
VPaI-1	22.79 kb	• Has 24 open reading frames encoding proteins involved in DNA replication, transcription regulation, signal transduction, general metabolism, type 1 restriction modification complex and DNA methyltransferase (VP0394), which may be an additional colonization factor • 8 kb region containing genes VP0389-VP0392 is syntenic with chromosomal region in *V. vulnificus* CMCP6 and *Shewanella* sp MR7 • Has been reported to be unique to post-1995 pandemic strains, though this pathogenicity island may be missing in some pandemic strains • Inserted adjacent to tRNA-Met
VPaI-2	10 kb	• Encodes outer membrane proteins and revolvases • Present in both pandemic and non-pandemic strains • Inserted adjacent to tmRNA
VPaI-3	32 kb	• Encodes methyl-accepting chemotaxis proteins, considered unique to post-1995 pandemic strains • Inserted adjacent to tRNA-Ser
VPaI-4	17 kb	• Encodes putative pore-forming cytotoxin integrase and M proteins involved in bacterial surface virulence factors • Inserted adjacent to tRNA-Ser
VPaI-5	12 kb	• Encodes a phage-like protein
VPaI-6	27 kb	• Encodes putative colicin
VPaI-7	81 kb	• Located in chromosome-2 • Encodes Type Three Secretion System (TTSS) and *tdh* gene or *trh* gene, homologue of *E. coli* cytotoxic necrotizing factor, ADP ribosyl transferase, enterotoxin, proteins inhibiting MAPK signaling pathway, which prevent cytokine induction
VPaI-8	17 kb	• Encodes hypothetical proteins, integrases and homologues of KAP proteins
VPaI-9	22 kb	• Encodes excisionase, helicase, type 1 restriction modification system

In addition to genomic islands, mobile genetic elements have been reported to be associated with *V. parahaemolyticus*. Mobile genetic elements like plasmids and bacteriophages may contribute to expansion of the ecological niche of this organism by enhancing the environmental fitness. Plasmids ranging in size from 3.5 to 70 kb have been described in *V. parahaemolyticus*. While smaller plasmids are coding for hypothetical proteins, a 28.8 kb plasmid contained a gene with 98 % sequence identity with a gene found in the genomic island VPaI-6 associated with pandemic strains (Hazen et al. 2010). This protein has close similarity to a protein found in *V. harveyi* and the *rep* gene encoding the replication protein in this 28.8 kb plasmid (p22702B) has 85 % identity with *rep* of *V. campbelli* plasmid p09022, suggesting that plasmids may have roles in genetic exchange and lateral gene transfer in *Vibrio* spp. The 70 kb plasmid of *V. parahaemolyticus* carries virulence genes involved in causing acute hepatopancreatic necrosis disease (AHPND).

Filamentous bacteriophage f237 has been found in the genome of pandemic strain of *V. parahaemolyticus*. Some of the prophages detected in chromosome 1 or 2 of *V. parahaemolyticus* have similarity to prophages found in the genome of *V. cholerae* (Kalburge et al. 2014).

Vibrio parahaemolyticus as a shrimp pathogen

Strains of *V. parahaemolyticus* implicated in acute hepatopancreatic necrosis disease (AHPND) are unique in carrying a 70 kb plasmid (pVA1) harbouring the virulence genes, *pirA* and *pirB* encoding the binary protein *Photorhabdus* insect related (Pir) toxin (Lee et al. 2015). All AHPND-causing strains tested harboured this plasmid, although size may vary marginally. The plasmid pVA1 described by Lee et al. (2015) contains 45 open reading frames (ORF) with known function. These include five putative transposases, one putative ORF with homology to the toxin-antitoxin gene *pndA* associated with post-segregational killing (PSK) system, operon that encodes proteins (~30 % homology) to PirA and PirB toxins, a cluster of conjugative transfer genes and two plasmid mobilization genes. The *pirAB* operon has transposases both upstream and downstream suggesting that the operon can be acquired by lateral gene transfer. The PSK system ensures that only progeny containing the plasmid survive, since the stable PSK mRNA in a plasmid-negative strain will be translated into bactericidal *pndA* toxin.

The detection of conjugative elements in the plasmid pVA1 suggests the possibility of mobilization to other strains or even other *Vibrio* spp. Kondo et al. (2015) reported *pirA* and *pirB* genes and related plasmid sequences in a *Vibrio* causing AHPND that is close to *V. harveyi*. These genes have also been detected in AHPND-causing *V. owensii* strain SH14 (Liu et al. 2015). Furthermore, strains of *V. parahaemolyticus* carrying the 70 kb AHPND plasmid are not clonal, but genetically diverse, suggesting that the virulence plasmid has been acquired by several genotypes of *V. parahaemolyticus* by lateral gene transfer (Chonsin et al. 2016).

The copy number of the plasmids has been reported to range from 7–121 in different strains from Viet Nam and Mexico (Han et al. 2016). However, virulence of the strains did not correlate with plasmid copy number (Tinwongger et al. 2016). All the AHPND strains studied so far are negative for *tdh* and *trh* genes and the associated TTSS2 present in genomic island VPaI-7 of human pathogenic strains. Thus, AHPND strains are not considered to possess the ability to cause human infections. Genetic variations in the virulence plasmid from different geographical regions have been reported (Han et al. 2015).

The isolates from Mexico and Central American countries had a 4.2 kbp TN-3-like transposon, which was absent in isolates from Asia. A 9b p repeat region (small sequence repeat, SSR) was found within the ORF in the plasmids. Isolates from the People's Republic of China, Viet Nam and Thailand had 4SSR, and one isolate from Viet Nam had 5SSR, while Mexican strains had 6SSR. Analysis by PCR based on the sequence of TN-3-like transposon could distinguish Asian from American strains causing AHPND.

Global spread of pathogenic Vibrio parahaemolyticus

One of the most common explanation for the global spread of coastal pathogens is the role of ballast waters, but the spread of pandemic *V. parahaemolyticus* from Asia to South America cannot be explained by this theory. Association of *V. parahaemolyticus* with zooplankton has been shown to influence the distribution and population dynamics of this organism in offshore areas. Genetically related populations of *V. parahaemolyticus* were found in zooplankton from estuarine and offshore areas dispersed along 1500 km, suggesting that zooplankton may play a role in oceanic dispersal of this organism (Martinez-Urtaza et al. 2012). Further studies on pandemic strains support this view. The O3:K6 strain that caused a large number of cases in India in 1996 was previously described in Indonesia in 1995. In 1996 and 1997, several cases were reported from different countries in Asia, but the first cases by this serotype outside Asia was observed in Chile in 1997. Using El Niño data and genetic typing of *V. parahaemolyticus* strains involved in human cases in Peru and Chile, Martinez-Urtaza et al. (2008) presented evidence that the pandemic clone of O3:K6 arrived in Peru in 1997 from Asia with El Niño currents. The inflow of foreign zooplankton trapped in El Niño currents in different areas in Chile and Peru in 1997 was also reported by Sanchez et al. (2000). The studies of Martinez-Urtaza et al. (2008) showed that the emergence and pattern of dissemination of *V. parahaemolyticus* showed close correlation with the arrival and propagation of 1997 El Niño. The pandemic strain belonging to O3:K6 serovar arrived in Peru in 1997 and infections were reported in the northern part of the country, but spread southward along more than 1500 km of the coast until it reached the Chilean city of Antofagasta. The peaks of infection corresponded with the arrival of equatorial Kelvin waves. In 1997, El Niño affected the South American coast for about 6 months, and it has been suggested that recurrent invasion of tropical masses of water might have resulted in repetitive sources of populations of *V. parahaemolyticus* that would have established there. There was another episode of outbreaks in Chile in 2004. Genetic analysis of strains from Asia and South American strains from 1997 and 2004 outbreaks showed that the 1997 strains are very closely related to Asian strains (Ansende-Bermejo et al. 2010). Once established in the region, the strains may undergo genetic variations and be involved in genetic recombination with local strains.

Further evidence for the transcontinental spread of *V. parahemolyticus* has been presented by genetic analysis of strains involved in an outbreak in Galacia, Spain in 2012 (Martinez-Urtaza et al. 2016). The outbreak involved 100–114 people travelling in a food banquet cruise boat. Epidemiological studies using questionnaire identified the most probable vehicle as shrimp that was subjected to a short boiling time of 1–2 min then to rapid cooling using water and ice. The shrimp was imported from Argentina and was negative for *V. parahaemolyticus*, and this suggests that water used for cooling was the source of contamination. The strains isolated in this outbreak were positive for both *tdh* and *trh* genes that have never been reported from Europe before. Genetic typing of the strains using PFGE indicated a profile identical to that of strains involved in an outbreak in New York just a couple of months earlier. The mechanistic route of migration of strains from the United States of America to Europe has not yet been explained, but this study supports the hypothesis that *V. parahaemolyticus* can spread globally through oceanic routes.

Potential for control of Vibrio parahaemolyticus using bacteriophages

The emerging problem of antibiotic resistance in both human and animal pathogens has been driving the search for alternatives to antibiotics. Recently, there has been a surge of interest in using bacteriophages as therapeutic agents in human medicine, animal husbandry, aquaculture, agriculture and for biocontrol of food-borne pathogens to improve food safety. 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, 2×10^8 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. Some of the virulence factors found in bacteria, such as 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 World War II. However, treatment failures due to bacteria showing resistance to multiple antibiotics led to renewed interest in bacteriophage therapy. Bacteriophages are host-specific, hence they lyse only the target bacteria, unlike antibiotics, which suppress most members of the bacterial groups. Bacteriophage therapy would not suppress useful commensal bacterial flora that are required for the health of the animals, an action that would be a great advantage in aquaculture.

Use of bacteriophages for control of bacterial diseases in shrimp aquaculture has been documented. Vinod et al. (2006) tested bacteriophage therapy of larvae and postlarvae of giant tiger prawn (*Penaeus monodon* Fabricius 1798) in both laboratory microcosms as well as in hatcheries during a natural outbreak of luminous bacterial disease by adding bacteriophages to rearing water. In microcosms, larval survival was 25 % in the control compared with 85 % in bacteriophage treatment. In hatchery trials, the survival was 86 % with bacteriophages, 40 % with antibiotics and 17 % 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 % survival was obtained with bacteriophage treatment compared to 65–68 % with antibiotics (Karunasagar et al. 2007). These studies show the potential for bacteriophages to be effective alternatives to antibiotics in shrimp larval health management. 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 antibiotics and disinfection (Karunasagar et al. 1996). The ability of bacteriophages to bring about a 3-log reduction in *V. harveyi* growing in biofilms on high density polyethylene (HDPE) surfaces was demonstrated by Karunasagar et al. (2007). However, considering that the host range for selected phages was 65–70 % and the possibility that bacterial strains may develop resistance to bacteriophages, phage therapy with a consortium of phages would be necessary to ensure efficacy against a wide range of opportunistic bacterial strains.

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 (Pasharawipas 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 phages from the family Siphoviridae were able to lyse 65–70 % of the strains, indicating a broad host range. Bacteriophages used by Vinod et al. (2006) and Karunasagar et al. (2007) lacked the putative virulence gene carried by VHML; hence, concern regarding carriage of virulence gene would be minimal.

Application of bacteriophages to control human pathogenic *V. parahaemolyticus* has been attempted. Rong et al. (2014) reported reduction in *V. parahaemolyticus* population in oysters by use of bacteriophages during depuration. Jun et al. (2014) demonstrated reduction of levels of antibiotic-resistant pandemic strain of *V. parahaemolyticus* from 8.9 x 10^6 cfu.mL^{-1} to 1.4 x 10 cfu.mL^{-1} in spiked oysters after 72 h treatment with bacteriophage by bath immersion. Application of bacteriophages on oyster surface led to reduction of *V. parahaemolyticus* counts from 1.44 x 10^6 cfu.mL^{-1} to 1.94 cfu.mL^{-1} in 12 h. Lomeli-Ortega and Martinez-Diaz (2014) reported that two bacteriophages could reduce mortality of larval whiteleg shrimp (*Penaeus vannamei* Boone 1931) challenged with *V. parahaemolyticus*. However, these were not AHPND strains. Jun et al. (2016) demonstrated that bacteriophage pVp-1 lysed 20 of 22 AHPND strains from Asia and America, showing potential for the control of this disease in aquaculture systems.

Conclusion

Vibrio parahaemolyticus is a very versatile organism adapting to new ecological niches and new hosts by acquiring genes. In the case of human pathogenic strains that spread rapidly from Asia to South America, oceanic currents associated with El Niño might have played a role. These strains are genetically highly related and demonstrate even clonal features. All AHPND-causing strains so far tested lack virulence factors required to cause human infections, indicating negligible human health risk from these strains. These strains have unique 70 kb plasmid carrying virulence genes that have features of acquired genes. Genetically diverse strains of *V. parahaemolyticus* seem to have acquired AHPND virulence plasmid, and Asian strains can be distinguished from South American strains based on PCR amplification of TN-3-like transposon. Bacteriophages have potential applications in the management of AHPND.

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