Vibrio parahaemolyticus- An emerging foodborne pathogen-A Review

S Nelapati, K Nelapati, B K Chinnam*

Veterinary College,
Sri Venkateswara Veterinary University, Tirupathi, Andhra Pradesh (AP) India
* Corresponding author email: bindukiranmayi@yahoo.com
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Abstract

Vibrio parahaemolyticus is a halophilic gram negative, motile, oxidase positive, straight or curved rod-shaped, facultative anaerobic bacteria that occur naturally in the marine environment. They form part of the indigenous microflora of aquatic habitats of various salinity and are the major causative agents for some of the most serious diseases in fish, shellfish and penacid shrimp. This human pathogen causes acute gastroenteritis characterized by diarrhea, vomiting and abdominal cramps through consumption of contaminated raw fish or shellfish. *V. parahaemolyticus* is the leading cause of gastroenteritis due to the consumption of seafood worldwide. The incidence of *V. parahaemolyticus* infection has been increasing in many parts of the world, due to the emergence of O_3:K_6 serotype carrying the *tdh* gene which is responsible for most outbreaks worldwide. The pathogenicity of *this organism* is closely correlated with the Kanagawa phenomenon (KP +) due to production of Kanagawa hemolysin or the thermostable direct hemolysin (*TDH*). The TDH and TRH (TDH-related hemolysin) encoded by tdh and trh genes are considered to be important virulence factors.
Key words: *Vibrio parahaemolyticus,* Kanagawa test, *tdh, trh,* sea foodborne pathogen, facultative anaerobic organism.

Introduction

Vibrio parahaemolyticus is a gram-negative bacterium that occurs naturally in the estuarine environment. This human pathogen is frequently found in seawater, sediments, plankton, finfish and shellfishes (Pavia *et. al.,* 1989) and 30 different marine species, including eel, crab, clams, oysters, lobsters, scallops, sardines, shrimp, and squid (Fishbein *et. al.,* 1974) and can cause acute gastroenteritis characterized by diarrhea, vomiting and abdominal cramps through consumption of contaminated raw fish or shellfish (Rippey, 1994). It also causes traveller's diarrhea, wound infection, ear infection and secondary septicemia in humans (Pavia *et. al.,* 1989). This organism was first identified as a causative agent of food-borne gastroenteritis after a large outbreak (272 illnesses and 20 deaths) associated with consumption of sardines in Japan in 1951 (Fujino *et. al.*, 1953). It forms part of the indigenous microflora of aquatic habitats of various salinity (Colwell *et. al.,* 1984) and are the major causative agents for some of the most serious diseases in fish, shellfish and penacid shrimp (Lee *et. al.,* 1996; Sugumar *et. al.,* 1998).

Shrimp culture has grown into a major economic industry worldwide. The rapid expansion of commercial culture of shrimp is threatened by vibrio infection affecting its survival and growth (Vandenberghe *et. al.,* 1999). The occurrence of this organism in fresh water has been construed as a fortuitous incidence that is probably related to tidal drift of organism from upper reaches of the river or its introduction by ambulatory cases or carriers (Sarkar *et. al.,* 1983).

The haemolytic activity of pathogenic strains of this organism on Wagatsuma's agar is popularly known as kanagawa phenomenon, which is due to Thermostable Direct Haemolysin (Honda and Iida, 1993). Some of the kanagawa

negative *V. parahaemolyticus* produces a toxin named TDH-related haemolysin (TRH) also causing gastroenteritis *(*Honda *et. al.*, 1988). The TDH and TRH encoded by tdh and trh genes are considered to be important virulence factors.

Enumeration of this organism from seafood is important in the context of current FDA guidelines, which stipulate that seafood should contain less than 10,000 cells per gram (McCarthy *et. al.,* 1999), but inadequacy of this is indicated by the outbreaks that occurred in the United States, despite the *V. parahaemolyticus* number being lower than the permissible limit (Nair and Hormazabal, 2005).

Prevelance and Zoonosis

V. parahaemolyticus is a marine bacterium that can cause seafood borne gastroenteritis and traveler's diarrhea in humans, after consumption of contaminated raw or partially cooked fish or shell fish, particularly oysters or exposure to a marine environment (Khan *et. al*., 2002).

V. parahaemolyticus was first isolated following food poisoning outbreaks in Japan in the early 1950s. In India *V. parahaemolyticus* was first isolated from a case of gastroenteritis by Chatterjee *et. al*. (1970) and about 10% of the cases of gastroenteritis in patients admitted to the Infectious Disease Hospital in Kolkata are due to *V. parahaemolyticus* (Deb *et. al.,* 1975). *Et*iological studies on acute diarrheal diseases in gang*et*ic plain areas have shown that gastroenteritis caused by *V. parahaemolyticus* ranks second to cholera in terms of incidence (Sakazaki *et. al*., 1971).

The survival of *V. parahaemolyticus* in seafoods subjected to chilling, freezing, heating, drying and smoking (Vanderzant and Nickelson, 1972; Karunasagar *et. al., 1*986) has been reported. This organism is known to be completely destroyed in cooked foods, especially in South Asian countries cooked with plenty of spices. Illness due to *V. parahaemolyticus* was reported in many countries like China, Indonesia, Vietnam, Australia, Great Britain, France, Germany, Italy, Canada and United States (CDC, 1998; CDC, 1999), but it is most prevalent foodborne pathogen in many Asian countries, where seafood is often consumed (Pan *et. al.,* 1997).

Following a social get-together some staff members of Christian Medical College and Hospital, Vellore involved in gastroenteritis due to V. parahaemolyticus and this has emphasized the public health hazard of this organism in India (Lalitha *et. al.,* 1983). *V. parahaemolyticus* accounts for about 70% of the gastroenteritis cases associated with seafood in Japan (Kaneko and Colwell, 1975), whereas Nishibuchi (2004) reported that this food poisoning are most common in Japan and Southeast Asia, although they occurred occasionally in other parts of the world. *V. parahaemolyticus* accounts for 35% to more than 50% of the bacterial food borne illness outbreaks occurring annually in Taiwan (Chiou *et. al*., 1991; Wang *et. al.,* 1996).

Matsumoto *et. al.* (2000) observed that the incidence of *V. parahaemolyticus* infection in recent years has been increasing in many parts of the world, due to the emergence of O_3:K_6 serotype carrying only the *tdh* gene that is responsible for most out breaks worldwide since, 1996. In recent years, outbreaks of *V. parahaemolyticus* infection have increased in Japan, Taiwan (Infectious Disease Surveillance Center, NIID, 1999; Chiou *et. al*., 2000) and United States, due to consumption of raw sea food contaminated with O_3:K_6 serovar (CDC, 1998; CDC, 1999). *Vibrios* other than *V. vulnificus* are estimated to cause approximately 5,000 food borne infections annually in the United States (Mead *et. al.,* 1999), with *V. parahaemolyticus* generally considered the leading cause of these infections (Hlady *et. al.,* 1993; Hlady and Klontz, 1996).

Molluscan-shell fish associated illness due to *V. parahaemolyticus* in the United States is occurring as individual cases or as small outbreaks (Hlady, 1997). Daniels *et. al*. (2000) reported 12 outbreaks of *V. parahaemolyticus* involving 84 individuals in the United States b*e*tween 1973 and 1993, whereas Depaola, *et. al*. (2000) reported four outbreaks involving 600 individuals from 1997 to 1998 due to consumption of raw or lightly cooked oysters.

Pathogenesis

The incubation period of *V. parahaemolyticus* is 3-24 h, usually about 10-15 h. The experimental dosages required for initiation of gastroenteritis in volunteer studies ranged from $2x10^5$ to $3x10^7$ CFU. The mechanism of pathogenicity of *has* not been defined or fully understood, but it was closely correlated with the Kanagawa phenomenon (KP+) due to production of Kanagawa hemolysin or the thermostable direct hemolysin (*TDH*) (Takeda, 1983; Nishibuchi *et. al*., 1989). However, Honda *et. a*l. (1988) reported that the clinical isolates of KP negative *V. parahaemolyticus* from traveler in Maldives newly produced hemolysin, named *TDH*-related hemolysin (*TRH*), which is also important virulence factor and possible cause of diarrhea.

Clinical isolates of *V. parahaemolyticus* most often produce either the *TDH* or *TRH* encoded by *tdh* and *trh* genes respectively (Nishibuchi and Kapar, 1995). A molecular study with *tdh- and trh*–specific DNA probes demonstrated a strong association of two genes with clinical strains, suggesting that *TRH* as well as *TDH* is an important virulence factor (Shirai *et. al.,* 1990; Okuda *et. al*., 1997b).

Virulence factors of Vibrio Parahaemolyticus:

I. Thermostable direct hemolysin (TDH): *TDH* was the first recognized virulence factor for *V. parahaemolyticus* and has been used as an important marker for identifying virulent strains (Okuda *et. al.,* 1997a; Cook *et. al.,* 2002). *TDH* was produced from the *tdh*2 gene rather than the *tdh*1 gene. The nucleotide homologies of *tdh*2 with *tdh*1, *tdh*3 and *tdh*4 were 97%, 98.6% and 98.6%, respectively (Nishibuchi and Kaper, 1985; Nishibuchi and Kaper, 1995).

Yoh *et. al.* (1991) studied the characteristics of TDH encoded by four representative *tdh* genes and showed different Electrophoretic mobilities under non-denaturing condition. All the gene products had hemolytic activities for various animal erythrocytes, stimulated vascular permeability in the rabbit skin, and were l*eth*al to mice, although their potencies were slightly different.

Nishibuchi *et. al*. (1992) demonstrated the link b*et*ween *TDH* and secr*et*ory diarrhea and concluded that the *TDH* encoding gene is able to induce intestinal chloride secretion. Raimondi *et. al.* (1995) demonstrated (using rabbit as animal model) that the *TDH* is one of the few enterotoxins produced by human pathogens, whose action is mediated by intracellular calcium. *TDH* also raises the cytosolic free calcium concentration [ca^{2+}] in non transformed rat intestinal IEC-6 cells (Fabbri *et. al.,* 1999). *TDH* has also cardiotoxic (Honda *et. al.,* 1976a) and cytotoxic (Sakazaki *et. al*., 1974) effects.

Honda *et. al.* (1991) characterized the Vp-*TDH*-like hemolysin or named Vp-*TDH*/I produced by a KP-negative clinical isolate (THO12) of *V. parahaemolyticus*, which has both similar and different characteristics to Vp-*TDH* (Miwatani and Takeda, 1976; Joseph *et. a*l., 1983) and Vp-*TRH* (Honda *et. a*l., 1988; 1989). Nakayama *et. al.* (1995) described the fourth type of hemolysin-Vp-*TDH*/II produced by a KP clinical isolate of *V. parahaemolyticus* (O13: K, untypable), which was bio-physico-chemically and immunologically similar, but not identical to Vp-*TDH*, Vp-*TRH* and Vp-*TDH*/I.

ii. Thermostable direct hemolysin- related hemolysin (TRH): Honda *et. al.* (1988) isolated a KP-negative *V. parahaemolyticus* strain from an outbreak of gastroenteritis in the Republic of Maldives in 1985, which produced *TRH*. Honda and Iida (1993) reported that purified Vp-*TRH* showed various biological activities, such as fluid accumulation in rabbit ilea loops, increase of rabbit skin vascular permeability, and cardiotoxicity on cultured myocardial cell. Vp-*TRH* played roles similar to Vp-*TDH* in the pathogenesis. A survey of 285 strains of *V. parahaemolyticus* revealed that the *trh*-positive strains had a strong association with gastroenteritis (Shirai *et. al.*, 1990).

Nishibuchi *et. al.* (1989) studied the gene encoding *TRH (trh* gene) and proposed that the nucleotide sequence of *trh* gene, like *tdh* gene, encoded the hemolysin subunit and the *trh* gene had significant nucleotide sequence homology with the *tdh* gene (68.4% with the *tdh*1 gene copy and 68% with the *tdh*2 gene copy).

Subsequently, Kishishita *et. al.* (1992) identified a variant of the *trh* gene (named *trh*2), which was 84% homologous to the *trh* gene (newly named *trh*1) and 54.8 – 68.8% homologous to the *tdh* gene, indicated that both the *trh*1 and *trh*2-carrying strains should be considered potentially virulent. Similar to *TDH*, *TRH* can induce chloride secr*et*ion in human colonic epithelial cells and is, therefore, considered a virulence factor of *V. parahaemolyticus* (Takahashi *et. al.,* 2000a; b).

iii. Other virulence factors: Taniguchi *et. al*. (1986) identified a thermolabile hemolysin that is Lecithin dependent hemolysin (LDH), which is one of the species specific gene fragments of *V. parahaemolyticus*. Taniguchi *et. al. (1990)* cloned a new thermostable hemolysin (ä -VPH) gene from a KP negative *V. parahaemolyticus* strain into vector pBR 322 in *E. coli* K12.

A number of possible virulence factors including a haemolysin, a Chinese hamster ovary (CHO) cell elongation factor and factors responsible for cytotoxicity, invasiveness and adherence (Blake *et. al.,* 1980). *V. parahaemolyticus* produce a novel siderophore named vibrioferrin under conditions of little or no iron (Yamamoto *et. al.,* 1994; Yamamoto *et. al.,* 1995). Yamamoto *et. al.,* (1999) reported higher levels of vibrioferrin in the nutrient-depleted culture of clinical isolates of *V. parahaemolyticus* than by isolates from food or environmental sources, when they were grown in a medium containing limited iron. They also reported that vibrioferrin under iron-limited conditions might contribute to the pathogenesis.

Symptoms

Although most oftenly it induces a self-limiting watery diarrhea, it occasionally causes bloody diarrhea and rarely sudden cardiac arrhythmia (Honda *et. al.,* 1976b). Clinical manifestations of *V. parahaemolyticus* include diarrhea, abdominal cramps, nausea, vomiting, head ache, fever and chills (Takeda, 1983). Dehydration, collapse, and abnormality on electrocardiograms have occurred in individual cases (Joseph *et. al.,* 1983; Honda and Iida, 1993; Carpenter, 1995).

Primary septicemia is reported in the individuals with chronic illness (Cook *et. al.,* 2002) and becomes life-threatening to people having underlying medical conditions such as liver disease or immune disorders. In addition, *V. parahaemolyticus* also can cause wound infection in people exposed to contaminated seawater (Bonner *et. al.* 1983; Murray *et. al.* 1998) and two deaths were reported among three cases of wound infections in Louisiana and Mississippi after Hurricane Katrina in 2005 (CDC, 2005).

Influence of season

The incidence of food poisoning in Japan caused by this organism is restricted to the summer months, very likely because of sensitivity of the organism to low temperatures (Kaneko and Colwell, 1973) and it can survive the winter in sediment, in scavenger fish and shell fish (Noguchi and Asakava, 1967), but the number of organisms isolated are less.

Kaneko and Colwell (1975) reported that when the temperature of seawater is below 13-15^{0}C, *V. parahaemolyticus* is rarely isolated and probably exists in a viable but non-culturable state (VBNC) and is not culturable on common media. Many researchers reported the abundance of *V. parahaemolyticus* during summer in temperate zone, when temperature was above 25^{o}C (CDC 1998; 1999; Khan *et. al.,* 2002), where as the organism is expected to be prevalent throughout the year in the tropical zone like Malaysia (Elhadi *et. al.,* 2004).

Deepanjali *et. al.* (2005) observed high levels of *V. parahaemolyticus* during the dry season b*et*ween January and May and decreased during post monsoon months. In tropical countries the seasonal cycle of the organism is correlated with rainy and dry seasons; the lowest numbers are found in rainy months, and the highest numbers are found in the dry season (Neumann *et. al.,* 1972).

Foodborne infection

Vibrio spp. have been isolated from seawater, sea mud, or sea foods in Asia, North

America, Australia, New Zealand, Africa, and Europe. It has been reported that vibrios are the predominant bacteria in the digestive tracts of oysters, clams and mussels (Sugita *et. a*l., 1981; Kueh and Chan, 1985), prawns (Yasuda and Kitao, 1980), and Artemia (Puente, *et. al.* 1992).

Fish: ICMSF has recommended an acceptability limit of 100/g for *V. parahaemolyticus* organism in some fishery products (ICMSF, 1974). Quadri and Zuberi, (1977) reported high percentage of kanagawa positive isolates (52.5%) from fish and shell fish samples of Karachi.

Abraham *et. al.* (1997) and Sanjeev (2002) reported that the incidence of *V. parahaemolyticus* in fresh, marine and brackish water fish in India varied from 35 to 55%. The incidence of this organism was reported high in faeces, least in external surface and moderate in gills of the fish (Sarkar *et. al.,* 1985; Sanjeev and Stephen, 1995). Nithya Quintol *et. al.* (2008a) observed that the incidence of *V. parahaemolyticus* contamination ranges from 15 to 46.66% of fish samples collected from different fish mark*et*s of West Bengal, India.

Prawns and shrimps: The most common *Vibrio* species found in farming phases of black tiger shrimp in India were *V. alginolyticus, V. parahaemolyticus* and *V. vulnifica*s (Bhaskar and S*et*ty, 1994). *V. parahaemolyticus* load of prawn harvested from the brackish water culture pond situated near Cochin was $2.4X10^4$ MPN/g (Sanjeev, 1999).

The acceptable level for the total no. of *V. parahaemolyticus* cells in fish fill*et*s and shocked shell fish for raw consumption is below 100 CFU/g of sample in Japan (Hara-kudo *et. al.,* 2001). Multiple resistant *V. parahaemolyticus* strains were more in prawn than in other samples (Pradeep and Lakshmanaperumalsamy, 1985). Vanderzant and Nickelson (1972) reported that *V. parahaemolyticus* has survived in shrimp tissue cooked at 80°C. Sanjeev and Stephen (1995) reported that 22.9% of the isolates from shell fish were found to be kanagawa positive *V. parahaemolyticus*.

Crabs: *V. parahaemolyticus* was identified as the causative agent in mortalities of blue crabs in Chesapeake Bay (Krantz *et. al.,* 1969) and gulf shrimp (Vanderzant *et. al.,* 1969) and has been isolated from sea water and sediment (Bartly and Slan*et*z, 1971).

The first outbreak of *V. parahaemolyticus* in the United States was recorded in 1971 in Maryland, where in 425 cases of gastroenteritis associated with consumption of improperly cooked crabs. (Molenda *et.* al., 1972).

Oysters: Vanderzant *et. al.* (1973) recovered *V. parahaemolyticus* from 70% of oyster samples from Galveston Bay collected over a 12-months period with counts as high as 10^4 CFU/g. Vibrio bacteria concentrate in the gut of oysters and other filter feeding molluscs (including clams, mussels, scallops) where these adhere and multiply, making them resistant to depuration and protect from external disinfectants (Tamplin and Capers, 1991). From 1997 to 1998, more than 700 cases of illness associated with eating raw oysters contaminated with *V. parahaemolyticus* were reported in California, Oregon, Washington, Connecticut, New Jersey, New York, and British Columbia of Canada (CDC, 1998; 1999).

Hlady *(*1997) reported that the average annual incidence of vibrio infection was 10.1 per 1,000,000 raw oyster consuming adults. Galveston Bay was closed to oyster harvesting from June to November 1998 because of the *V. parahaemolyticus* outbreak (Depaola *et. al.,* 2000). Cook *et. al. (*2002) reported that the TDH gene associated with *V. parahaemolyticus* virulence was d*et*ected in 4% lots of oysters in coastal and inland mark*et*s throughout the United States. The United State Food and Drug Administration has an established level of concern for total *V. parahaemolyticus* numbers and will consider enforcement action against the shipment of molluscan shell fish with high levels equals to or greater than a MPN count of 10,000/g (FDA, 1997).

Environment samples: Kaper *et. al.* (1981) reported that the occurrence of *V.parahaemolyticus* moderately halophilic organism in fresh water has been construed as a fortuitous incidence that

is probably related to tidal drift of the organism from the upper reaches of rivers or to its introduction by ambulatory cases or carriers. Sarkar *et. al.* (1983) reported that the incidence and counts of *V. parahaemolyticus* were consistently higher in association with plankton than with water and sediment samples (Sarkar *et. al.* 1985).

Sanjeev (1999) reported that the counts of *V. parahaemolyticus* were 460 MPN/ml and $2.4X10^4$ MPN/g in water and sediment respectively, collected from brackish water culture pond near Cochin, India and the distribution of *V. parahaemolyticus* in water and sediment of culture pond is dirrectly influenced by salinity. Bej *et. al.* (1999) reported that 100% of isolates were positive for *tlh* and 46.66% for *tdh* and 46.66% for *trh* genes, out of 15 environmental samples by multiplex PCR method.

Clinical samples: Epidemiological studies revealed the high incidence of human carriers of *V. parahaemolyticus* in Kolkata (Deb, *et. al.* 1975). Lee *et. al.,* (2003) described the isolation and characterization of two *V. parahaemolyticus* strains isolated either from a patient's stool sample or a diseased abalone with withering syndrome (Huang *et. al*., 2001) and their implication as the first evidence of a laboratory acquired zoonotic infection. Nithya Quintoil *et. al.* (2008b) reported that 13.33% of the stool samples collected from ailing fish handlers gave positive results for *V. parahaemolyticus* in Calcutta.

Processed foods: Anonymous (1998) studied the food borne outbreaks due to various vibrio species and reported that the percentage of outbreaks were 38.5% through ready to eat lunch box, 24.6% sea food and their products, 12.8% meat and meat products, 7.8% cakes and confectionaries, 17.3% vegetables, 7.3% cereals and 1.7% egg products. They also reported that the causative organisms account to *V. parahaemolyticus* (52.3%), V. cholerae (5.4%), Aeromonas hydrophila (4.1%), V. alginolyticus (3.8%), V.vulnificus (1.4%) and V. mimicus (0.8%).

Other products: Sanjeev and Stephen (1995) reported that all the strains of *V. parahaemolyticus* isolated from cooked, shucked clams were kanagawa negative and 50% of the isolated from mussels were kanagawa positive.

Lhafi and Kuhne (2007) analyzed 90 blue mussels samples from German Wadden Sea and reported that 74.4% of the samples contained vibrio species, among these 39.5% contain *V. parahaemolyticus*.

Isolation techniques:

i. Conventional media: USFDA recommended APW/APS broth with 3% Nacl with 16h enrichment for quantification of *V. parahaemolyticus* in 3-tube MPN procedure (FDA, 1984) and then plating on to selective medium, TCBS i.e. Thiosulfate Citrate-Bile salts-Sucrose agar for 24-48 h at 37^0C. *V. parahaemolyticus* cannot ferment sucrose and produce green colonies. So, this medium is widely used for isolation of *V. parahaemolyticus.* Detection of pathogenic *V. parahaemolyticus* is traditionally done by the Wagatsuma agar test for the Kanagawa reaction, which require fresh human or rabbit blood and tends to give false positive reaction also (Raghunath *et. al.,* 2008). Production of *TDH* is responsible for phenomenon (Sakurai *et. al.,* 1974), which is manifested as β-type haemolysis on a special blood agar called wagutsuma agar (Miyamoto *et. al.,* 1969).

ii. Special media: In order to provide results more rapidly with sensitivity similar to or greater than the conventional methods, several workers used different special media as detailed in Table.1.

iii. Membrane filtration (MF): Watkins *et. al.* (1976) developed MF method for the direct enumeration of *V. parahaemolyticus* (mVP) in sea water, which features overnight incubation at 41^oC on the selective agar, followed by in-situ testing for the fermentation of galactose and sucrose and the production of oxidase. Entis and Boleszczuk (1983) modified the mVP method by using hydrophobic grid membrane filters (HGMFs).

Table .1 Special media used for identification of *Vibrio parahaemolyticus*

Sr.No.	Media	References
1.	Glucose-mineral medium	Citarella and Colwell, 1970
2.	Marine sea water yeast extract agar	Colwell and Wiebe, 1970
3.	Tryptic soy agar or broth	Colwell and Wiebe, 1970
4.	SWYE	Kaneko and Colwell, 1973
5.	Horie arabinose ethyl violet broth (HAE, pH 9.0)	Beuchat, 1977
6.	Water blue-alizarin yellow broth (WBAY)	Beuchat, 1977
7.	Glucose Salt Teepol Broth (GSTB)	Twedt, 1978
8.	Biken agar no. 1	Honda *et. al.,* 1980
9.	Nutrient broth with eosin yellow (NBYE)	Hofer and Silva, 1984
10.	Modified arabinose ethyl violet Broth	Sarkar *et. al*., 1985
11.	Arabinose-Glucuronate (AG) medium	Miyamoto *et. al*., 1990
12.	Zoobells 2216E agar	Dhevendaren and Georgekutty, 1998
13.	Vibrio agar	Dhevendaren and Georgekutty, 1998
14.	Marine agar (Zoobells 2216E, Himedia)	Harish *et. al*., 2003
15.	Muller Hinton agar with added 1.5% Nacl	Shanthini *et. al.,* 2004
16.	Zobell's marine broth	Mishra *et. al*., 2009

iv. Latex agglutination test: The presence of TDH can be detected using a commercial latex agglutination kit. Rapid anti- *V. parahaemolyticus* outer membrane protein antibodies should be produced and latex particles sensitized with the affinity purified antibodies should be used as a reagent for the rapid identification of the bacterium (Chang *et al.,* 1994).

v. Enzyme Linked Immunomagnetic Sorbent Assay (ELISA): Honda *et. al*. (1985) developed ELISA for detection of *tdh* of *V. parahaemolyticus.* The method was sensitive but preparation of anti-*tdh*-alkaline phosphatase conjugate may be needed. Chen and Chang (1995) developed an ELISA for detection of two outer membrane proteins of *V. parahaemolyticus.* The detection limit of this assay was 10 ng/ml and need 18 hr for the enrichment step.

vi. Immunological methods: Honda and Finkelstein (1979) and Honda *et. al.* (1980) developed the modified Elek test by adding anti haemolysin serum to a well cut in agar medium near a colony grown on the medium and the immunohalo test was carried out by inoculating bacteria onto agar medium containing anti haemolysin antiserum or anti haemolysin immunoglobulin G.

Selective media for *V. parahaemolyticus*, BTB-teepol agar and modified arabinose-ammonium sulphate-cholate agar, were modified for use in immunological d*et*ection of the TDH produced by *V. parahaemolyticus* by using modified Elek test and immunohalo test (Honda *et. al*. (1982).

Tomoyasu (1992) developed a method that involved Immunomagnetic separation to isolate the specified K serovar of *V. parahaemolyticus* from a mixture of a large no. of bacteria with other K serovars by using antisera (anti-rabbit immunoglobulin G) against K antigens coated on super para magnetic polysterene beads and harvested using a magnetic concentrator.

Chen and Chang (1996) developed immuno-fluorescence microscopy method to targ*et* two specific outer membrane proteins 34 and 34KDa. All *V. parahaemolyticus* tested produced strong fluorescence under fluorescence microscopy, while only six of the other 63 bacteria generated weak to moderate fluorescence.

vii. Pulsed Field Gel Electrophoresis (PFGE): A PFGE method was developed for a molecular typing of *V. parahaemolyticus* (Wong *et. al.*, 1996), later the method has been used to type highly gen*et*ically diverse *V. parahaemolyticus* strains in sea food imported from Asian countries (Wong, *et. al.* 1999).

viii. Colony hybridization: Toxin gene elements in *V. parahaemolyticus* can be detected by colony hybridization (Nishibuchi *et. al.,* 1985; Banerjee *et. al.,* 2002) or bot hybridization (Yamamoto *et. al.,* 1992) using appropriate nucleic acid probes. Non-radioactive labeled oligonucleotide probes were used for binding *tlh* and *tdh* genes for

detection of total and pathogenic *V. parahaemolyticus* respectively (Deepanjali *et. al.,* 2005).

ix. Polymerase Chain Reaction: The PCR represents a rapid procedure with both high sensitivity and specificity for the immediate detection and identification of specific pathogenic bacteria from different food materials (Lantz *et. al.,* 1994; Hill, 1996). The advantage of PCR over conventional isolation is its ability to distinguish virulent and avirulent strains, saves time and hence molecular methods are valuable in such cases (Deepanjali *et. al.,* 2005).

Okura *et. al.* (2003) developed PCR based assay to identify pandemic group of *V. parahaemolyticus* by using oligonucleotide primer pair derived from the group specific sequence of an arbitrary primed-PCR fragment by yielding a 235-bp specific amplicon product.

Croci *et. al.* (2007) evaluated five PCR methods for the identification of *V. parahaemolyticus* to the species level by using 25 Vibrio reference strains and 163 isolates from fishery products, environmental sources and clinical samples using target genes of *toxR, gyrB, tlh* (tested with two protocols) and the fragment PR72H and they reported that the PCR assay targeting the *tox*R and *tlh* gene achieved the highest performance, where as the genes *gyr*B and PR72H fragments were less reliable.

Tada *et. al.* (1992) developed a polymerase chain reaction (PCR) protocol using DNA probes for specific detection of the *tdh* and *trh* genes of pathogenic *V.parahaemolyticus*. The procedure could detect both *tdh* and *trh* genes in 400 fg of cellular DNA derived from 100 cells. However, this PCR protocol requires enrichment as a pretreatment to detect pathogenic strains in fecal samples and cannot be used to detect non-pathogenic *V. parahaemolyticus* strains.

x. Improved PCR techniques:

Multiplex PCR (mPCR): Bej *et. al.* (1999) developed multiplex PCR to detect total and pathogenic *V. parahaemolyticus* targeting *tlh, tdh* and *trh* genes. Okura *et. al.* (2003) developed a multiplex PCR assay that distinguishes the pandemic group from other *V. parahaemolyticus* strains by yielding two distinguished PCR products for *tdh* gene and *tox*RS/new sequence.

Random Amplified Polymorphic DNA-PCR (RAPD-PCR): RAPD analysis is a commonly used method in PCR for typing and differentiation of bacteria and increasingly for the study of genetic relationships between strains and species of microorganisms, plants and animals (Oakey *et. al.,* 1998).

Bilung *et. al.* (2005) demonstrated that genotyping *V. parahaemolyticus* isolates by using RAPD-PCR is feasible for detection of various strains and genetic relatedness among *V. parahaemolyticus* strains.

Real-time PCR: Real time PCR shows the ability to process many samples with speed and consistency and single-tube amplification and confirmation of target sequences (McKillip and Drake, 2000). Real-time PCR can be used to detect the *tdh* gene (Black stone *et. al.,* 2003) and *tlh* gene (Kaufman *et. al.,* 2004) *in V. parahaemolyticus* using specific primer sets and fluorogenic probes.

Reverse transcriptase PCR: The development of reverse transcriptase PCR allows the detection of mRNA with advantage over other methods, such as high sensitivity, rapid turn-around time and use of total RNA instead of just poly (A)+ mRNA (Rappolee *et. al.*, 1988).

The mRNA is not expected to be detected in a VBNC bacterial cell because there is no growth activity of the cell under the VBNC stage. This makes the detection of mRNA a suitable means of identifying VBNC *V.parahaemolyticus*. A sample containing VBNC *V.parahaemolyticus* will produce PCR assay targeting *tlh* gene and a negative results by mRNA analysis (Siebert and Larrick, 1995).

Other PCR based methods: Wong and Lin (2001) developed three rapid PCR methods for typing of *V.parahaemolyticus* that is RS-PCR, REP-PCR and ERIC-PCR to avoid the use of random primers. Further they reported that REP-PCR is better over ERIC-PCR due to greater reproducibility.

Wong (2003) developed a ribotyping method in which genomic DNA was digested with 28 restriction enzymes separated and

hybridized with digoxigenin labeled cDNA probes derived from EcoI 16S and 23S RNA genes.

Prevention and Control

Since the infection primarily occurs via consumption of contaminated aquatic foods, the preventive measures include proper handling and cooking of marine and other aquatic foods prior to consumption and hygienic handling of products to avoid cross-contamination between raw and cooked products. Proper environmental hygienic measures should also be taken to avoid this infection i.e. the untreated sewage should not be discharged in the sea/rivers near coastal areas since most of the catch is made from these areas and aquatic foods should not be harvested form heavily contaminated water bodies.

Therefore considering the high prevalence of this infection, *V. prahaemolyticus* should be given considerable preference in diagnosing the infections and eliminating the transmission through aquatic products.

Conclusion

Since, *Vibrio parahaemolyticus* is a normal inhabitant of the marine environment, foods of marine origin like fish, shellfish, crustaceans and plankton may harbor this organism and the presence of the organism in seawater is heavily influenced by the season, occurring at the highest levels in the warmer months. Majority of the isolates from seawater are not of the human pathogenic kind (they are KP-). The pathogenic strains can be detected by Kanagawa Phenomenon (KP). Usually it induces a self-limiting watery diarrhea, however, the relatively rapid onset of disease (average 12 hours) suggests that some enterotoxins (TDH & TRH) are involved. Consumption of raw mollusks and cooked crustaceans and finfish are the most common food sources of *V. parahaemolyticus*. The emergence of O_3:K_6 serotype carrying the *tdh* gene is responsible for most out breaks worldwide O3:K6 and is majorly affecting the export trade. Keeping above factors in view, proper hygienic handling and cooking of marine and other aquatic food products should be strictly followed.

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