2. Review of literature

2.1. Incidence of HPVs in fish and fish products

*Vibrio* species are natural inhabitants of marine aquatic environments of both temperate and tropical regions, with most human infections acquired by exposure to such environments or to foods derived from them (Kelly *et al.*, 1991, Colwell and Huq, 1994). Currently, 12 *Vibrio* species viz. *V. alginolyticus*, *V. carchariae*, *V. cholerae*, *V. cincinnatiensis*, *V. damselae*, *V. fluvialis*, *V. furnissii*, *V. hollisae*, *V. metschnikovii*, *V. mimicus*, *V. parahaemolyticus* and *V. vulnificus* are known to cause or to be associated with human infections. (Kelly *et al.*, 1991; Dalsgaard *et al.*, 1996,a). *Vibrio* spp. mainly associated with intestinal disease may represent health hazards when present in seafood meant for consumption, whereas extra intestinal disease, especially wound infections, can occur after exposure to the aquatic environments and handling of fish. Many *Vibrio* spp. are pathogens to humans and have been implicated in food borne disease (Table 1).

Table 1. Association of *Vibrio* spp. with different clinical syndromes

<table>
<thead>
<tr>
<th>Species</th>
<th>Gastro-enteritis</th>
<th>wound infection</th>
<th>Ear infection</th>
<th>Primary septicemia</th>
<th>Secondary septicemia</th>
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<tr>
<td><em>V. cholerae</em> O1</td>
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<td><em>V. cholerae</em> non- O1</td>
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<td><em>V. mimicus</em></td>
<td>++</td>
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<td><em>V. fluvialis</em></td>
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<tr>
<td><em>V. parahaemolyticus</em></td>
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<td><em>V. alginolyticus</em></td>
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<td><em>V. cincinnatiensis</em></td>
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<td><em>V. hollisae</em></td>
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<td><em>V. vulnificus</em></td>
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<td><em>V. furnissii</em></td>
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<td><em>V. damselae</em></td>
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<td><em>V. metschnikovii</em></td>
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<td><em>V. carchariae</em></td>
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</table>

+++ = frequently reported, ++ = less common (6-100 reports); + = rare (1-5 reports), and (+) = association is unclear. Pavia *et al.* (1989).
Several authors have reported the incidence of HPVs in seafoods from different parts of the world. In India (Bandekar et al., 1982; Karunasagar et al., 1987; Prasad and Rao, 1994; Thampuran et al., 1997; Sanjeev et al., 2000; Deepanjali et al., 2005), Bangladesh (Huq et al., 1980), U.K (Lee et al., 1981), Malaysia (Cann and Taylor, 1981; Elhadi et al., 2004), Indonesia (Lesmana et al., 2002), United states (Blake, 1984; Colwell, 1984; Depaola et al., 1994; Wright et al., 1996; Hlady, 1997; Hackney et al., 1988; Gooch et al., 2002), Japan (Sakazaki, 1983; Alam et al., 2002), Senegal (Schandevyl et al., 1984), Hong Kong (Chan et al., 1989), Sri Lanka (Foneska and Widarapathirama, 1990), Philippines (Aklani-Rose et al., 1990), Taiwan (Wong et al., 1992, 1995 and 2000), China (Yano et al., 2004), France (Hervio-Heath et al., 2002) Netherlands (Veenstra, 1994), Spain (Sunen et al., 1995; Arias et al., 1999; Castro et al., 2002), Iran (Hosseini et al., 2004), Israel (Bisharat and Raz, 1996), Denmark (Dalsgaard, 1998), Italy (Ripabelli et al., 1999; Baffone et al., 2000; Maugeri et al., 2000; Cavallo and Stabili, 2002; Parisi et al., 2004).

Incidence of HPVs from a variety of seafoods collected from fish markets (Sanjeev and Stephen, 1993; Sunen et al., 1995; Elhadi et al., 2004; Parisi et al., 2004) as well as from factory processed raw, cooked, peeled, packed and frozen products (Cann and Taylor, 1981; Sanjeev et al., 2000) have been reported. These include those foods that may be consumed raw (cuttlefish, oysters), partially cooked (steamer clams, mussels) or fully cooked (finfish and shellfish) (Buck, 1991). Occurrence of these species from raw samples collected at sea from commercial vessels (Cann and Taylor, 1981; Jaksic et al., 2002) and also from farmed mussels, shrimps and clams (Maugeri et al., 2000; Bhaskar et al., 1998; Sanjeev, 1999; Castro et al., 2002) has also been reported.

V. parahaemolyticus, V. vulnificus, V. damsela and V. alginolyticus may also be fish pathogens (Ruangpan and Kitao, 1991; Liu et al., 2000; Lee et al., 2003). Anderson et al. (1988) reported that vibriosis caused 70-95% reduction in the expected harvests in some farms in Malaysia. Vibrio spp. have been isolated from a variety of environmental samples including water, sediment and plankton (Colwell, 1984; Ayres and Barrow, 1978; Aiyamperumal et al., 1994; Montanari et al., 1999; Hervio-Heath et al., 2002). It has been reported that vibrios are the
predominant bacteria in the digestive tracts of oysters, clams, mussels (Sugita et al., 1981; Kueh and Chan, 1985), Prawn (Yasuda and Kitao, 1980; Oxley et al., 2002) and fish (Okuzumi and Horie, 1968, Sera and Ishida, 1972, Depaola et al., 1994).

*Vibrio* spp. have also been isolated from water showing a broad range of salinities and varying pH values (Dalsgaard, 1998). He reported that a positive correlation exists between water temperature and the number of human pathogenic vibrios isolated as well as the number of reported infection. Such seasonality is particularly noted for *V. parahaemolyticus* and *V. vulnificus* (Oliver and Kaper, 1997; Alam et al., 2002). Due to the halophilic nature and marine source of *Vibrio* spp raw seafood is naturally contaminated and is the main food responsible for infection (Desmarchelier, 2003).

*V. alginolyticus, V. cincinnatiensis, V. damsel, V. fluvialis, V. furnissii, V. metschnikovii, V. parahaemolyticus* and *V. vulnificus* were the major species isolated during the study. *V. cincinnatiensis* *V. fluvialis, V. furnissii* and *V. parahaemolyticus* were selected for detailed study for which a detailed review is provided.

### 2.2. *V. cincinnatiensis*

*V. cincinnatiensis*, Latin adjective derived from the society of Cincinnati from which the city of Cincinnati, Ohio, was named (Brayton et al., 1986). The organism described was isolated from a 70-year-old male patient with bacteremia and meningitis at the University of Cincinnati hospital (Bode et al., 1986). He had a 24 h. history of lethargy, disorientation, and altered mental status. There was no history of diarrhea, rashes, exposure to seafood or contact with salt water. Although the patient drank alcohol heavily on occasion, he had no liver disease. Physical examination revealed a temperature of 103°F (39.4°C). Laboratory data reported normal hepatic enzymes, leukocytes of 13,200 cells per mm$^3$, hemoglobin of 14.5 g/dL, and a platelet count of 194,000 mm$^3$. Blood and cerebrospinal cultures were inoculated into blood agar plates, and pure cultures of *V. cincinnatiensis* grew from both samples. This was the first reported case of *Vibrio* sp meningitis. Therapy was begun with ampicillin (day 1) and continued with moxalactam for the next 9 days. Recovery was uneventful, representing the
first successful treatment of *Vibrio sp.* meningitis in an adult (Bode *et al.*, 1986). Wuthe *et al.* (1993) have reported the isolation of one strain of *V. cincinnatiensis* from the stool specimen of an immunocompromised elder patient suffering from enteritis and two strains from the rennin stomachs of aborted bovine fetuses.

*V. cincinnatiensis* is a gram-negative non-sporforming rod, measuring approximately 0.7 by 2.0 μm. Overnight incubation at 25°C and 35°C produces round, smooth, glossy colonies (1 to 2 mm in diameter) that are cream colour on nutrient agar and yellow on thiosulphate citrate bile salts sucrose agar. Single polar flagella are observed attached to cells grown on solid and liquid media (Brayton *et al.*, 1986).

Facultatively anaerobic, sodium chloride is required for growth, ferments glucose, trehalose, sucrose, D-celllobiose, D-mannose, m-inositol, salicin, and L-arabinose. Catalase, oxidase, amylase, chitinase and DNase are produced. Gelatinase, urease, alginate, caseinase, lecithinase and elastase are not produced. Positive for lysine decarboxylase, Ortho-nitrophenyl-β-d-galactopyranoside and Voges-Proskauer. Negative for ornithine decarboxylase, arginine dihydrolase and indole production. Sensitive to 150 μg of vibriostatic agent 0/129 (2, 4- diamino 6, 7- di isopropyl pteridine). The DNA base composition is 45 mol % guanine + cytosine (Brayton *et al.*, 1986).

In a study on the phenogram Brayton *et al.* (1986) showed that *V. cincinnatiensis* possessed closest relationships, i.e. > 70 % similarity with *V. diazotrophicus* and *V. nereis*. All three organisms required NaCl for growth, were positive for cytochrome oxidase, reduced nitrate, fermented sucrose, trehalose, and celllobiose, and were sensitive to 150 μg of 0/129. All were gelatinase negative.

According to MacDonnell and Colwell (1985), the nucleotide base sequence of the 5sr RNA of *V. cincinnatiensis* shares a recent common ancestor with *V. gazogenes* (98.3 % sequence homology), which in turn shares a common ancestry with *V. mimicus, V. fluvialis* and *V. metschnikovii*.

Information on the incidence of *V. cincinnatiensis* in seafood is scanty, although there are reports on the isolation of this species from seafoods, coastal waters and zooplanktons. Ripabelli *et al.* (1999) studied the bacterial pathogens in
mussels (*Mytilus galloprovincialis*), and showed that 48.4 % of samples contained vibrio pathogens and *V. cincinnatiensis* was isolated from 3.2 % of the samples analyzed. Cavallo and Stabili (2002) observed a selective retention of HPVs viz., *V. cincinnatiensis, V. hollisae* and *V. vulnificus* in mussels (*Mytilus galloprovincialis*) from the Mar Piccolo of Taranto (Ionian sea, Italy). *V. cincinnatiensis* was the dominant species isolated from frozen fish products meant for export from Kerala and Tamil Nadu (Sanjeev *et al.*, 2000). Occurrence of *V. cincinnatiensis* in coastal waters of Cochin has been reported by Thampuran *et al.* (1997). Heidelberg *et al.* (2002 & 2002. a) indicated the occurrence of *V. cincinnatiensis* in association with zooplanktons, in the water samples collected from the chop tank river in Chesapeake Bay. Heidelberg *et al.* (2002, a) in another study observed the abundance of *V. cincinnatiensis* during cooler months although the species accounted for less than 0.1 to 3 % in the water samples collected from the chop tank river in Chesapeake Bay. Mao *et al.* (2001) have reported the isolation *V. cincinnatiensis* from diseased mud crabs with different symptoms, cultured in marine ponds of various districts in Ningbo area.

2.3. *V. fluvialis*

*Vibrio fluvialis* was first identified in 1975 in Bahrain in a patient with diarrhea, and was initially designated as group F vibnos (Furniss *et al.*, 1977). In 1980, the Center for Disease Control renamed the organism as group EF6 (Huq *et al.*, 1980). This organism was responsible for an epidemic involving more than 500 patients in Bangladesh (Huq *et al.*, 1980) and has also caused diarrheal disease especially in Bahrain, Bangladesh and Indonesia (Furniss *et al.*, 1977; Joseph *et al.*, 1983). This organism can be misidentified as *Aeromonas* because of similar biochemical reactions in identification scheme and with *V. alginolyticus*, especially because of its tolerance to 8 to 10 % NaCl concentration (Joseph *et al.*, 1978; Furniss *et al.*, 1977; Seidler *et al.*, 1980; Lee *et al.*, 1981)

Lee *et al.* (1981) have done a detailed study on the taxonomy of *V. fluvialis*. Earlier these organisms were frequently isolated from the estuarine environments and were referred to as 'marine-aeromanads' but later designated them group F (Furniss *et al.*, 1977). A numerical taxonomical study of *Vibrio metschnikovi* and related organisms demonstrated that group F strains formed a distinct phenon
and probably constituted a new species or genus (Lee et al., 1978). Phenotypically these organisms appeared to be intermediate between Aeromonas and certain species of vibrios, such as V. anguillarum. In another study Lee et al. (1978) have shown that the minimum inhibitory concentration (MIC) of 2, 4-diamino - 6-7-diisopropyl pteridine (0/129) phosphate for group F organisms was 10-50 μg ml⁻¹. This is similar to that of some species of the genus Vibrio but different from that of strains of the genus Aeromonas which have a MIC ≥ 320 μg 0/129 phosphate ml⁻¹ and the species V. anguillarum which has an MIC of 1-5 μg 0/129 phosphate ml⁻¹ (Lee et al., 1978).

Phenon 1, described by Lee et al. (1981) corresponds to strains designated group F by Lee et al. (1978) and includes strains described as group F strains isolated in 1975 from a patient whose diarrhea was contracted in Bahrain. Mol % (G+C) studies of phenon 1 strains have shown that it forms two sub clusters (1 a and 1 b) or sub phenons (Lee et al., 1981). The values were found to be in the range 49.3-50.6 mol % (G+C) with a mean of 50.0 and concluded that sub-phenons 1 a and 1 b are biovars of a single species. Jensen et al. (1980) have obtained similar values for a number of group F strains in the range of 50.5 - 51.0 mol % (G + C). Group EF6 strains have a mol % G+C of 50 and DNA relatedness tests indicate that all the EF6 strains tested belong to a single species (Brenner et al., 1979). Jensen et al. (1980) in another study examined strains of both groups and confirmed that they are synonyms. Lee et al. (1981) proposed the inclusion of Phenon 1 in the genus Vibrio and given the name V. fluvialis (belonging to a river) and this would require modification of the genus definition to include aerogenic strains.

Vibrio fluvialis [synonyms group F (Furniss et al., 1977), group EF6 (Huq et al., 1980)] is gram negative short rods, axis straight or curved, sides usually parallel, rods rounded, occurring singly, in pairs and occasionally in short chains of 3 to 4 organisms, may be pleomorphic. Motile by means of single polar sheathed flagellum in liquid media. On solid media lateral, unsheathed flagella of shorter wavelength may be produced. Sodium chloride may be required for growth and the optimum concentration for growth is 1-3 % (w/v). Colonies on TCBS agar are yellow, shiny smooth round, domed and entire may be mucoid and are 2-3 mm in
diameter after 24 h at 37 ± °C. Pigments not produced, facultative anaerobe, metabolism of glucose is positive, gas may be produced. Kovacs oxidase positive, reduce nitrate to nitrite. Grow on simple mineral media on a variety of organic carbon sources.

The species may be divided into biovar I and II. Biovar I strains are widely distributed throughout the aquatic environments particularly in brackish and estuarine waters but other sources have included shellfish and sewage. Biovar II strains occur in the same aquatic habitat as biovar I but unlike them they are rarely, if ever, isolated from human faeces. They have, however, been isolated from faeces of cattle, pigs and rabbits (Lee et al., 1981).

*V. fluvialis* has been reported as the etiologic agent in diarrheal illness in Asia, the Middle East, Africa, Eastern Europe, Great Britain, and United States (Huq et al., 1980; Tacket et al., 1982; Bellet et al., 1989; Hodge et al., 1995). Since 1981, 14 cases of enterocolitis associated with *Vibrio fluvialis* have been reported in the United States (Kolb et al., 1997) two of these cases occurred in infants (Bellet et al., 1989; Hickman et al., 1984) and 11 of 14 occurred in Florida (Klontz and Desenclos, 1990). 10 of these 14 patients reported eaten shellfish 1 to 7 days before onset of symptoms (Hodge et al., 1995; Klontz et al., 1994; Klontz and Desenclos, 1990).

The largest experience with *V. fluvialis* infection was reported by Huq et al. (1980) in Bangladesh and involved more than 500 patients, half of whom were young children under 5 years of age, between October 1976 and November 1977. The clinical syndrome described in that study included diarrhea (100 %), vomiting (97 %), abdominal pain (75 %), moderate to severe dehydration (67 %), and fever (35 %), in 75 % patients, blood and leukocytes were found on microscopic examination of stools. According to Kolb et al., 1997, *V. fluvialis* should be included among potential bacterial pathogens causing severe gastroenteritis in infants and known exposures to seafood or coastal waters is not a pre-requisite to *V. fluvialis* infections especially in infants. Hickman et al. (1984) reported a similar unremarkable exposure history in a case of *V. fluvialis* in a one-month-old female infant with bloody stools. Bellet et al. (1989) isolated *V. fluvialis* from a four-week-old female infant with diarrhea, exposure history was significant.
for the mother’s consumption of crabs on the day of labor. *V. fluvialis* rarely causes wound infections or primary septicemia (Hlady and Klontz, 1996; Varghese et al., 1996) although the species has been isolated from wound infection in Hawaii (Seidler et al., 1980).

*V. fluvialis* has been frequently isolated from brackish and marine waters and sediments in the United States (Joseph et al., 1983). From shellfish and water in the Pacific Northwest, Hickman et al. (1984), Klontz and Desenclos (1990), Klontz et al. (1994), Hodge et al. (1995) reported the isolation of *V. fluvialis* in New York Harbor, nearby sewage dumpsites and from shellfish in Louisiana. Thampuran et al. (1997) have reported the occurrence of *V. fluvialis* in coastal waters of Cochin (India). Maugeri et al. (2000) have observed that *V. fluvialis* was the most frequently recovered species in water and mussel samples collected from two brackish lakes, used as mussel farms in Sicily (Italy).

Sunen et al. (1995) have reported the incidence of *V. fluvialis* in 2.04% of mussels and 13.8% of clams purchased from retail outlets in the North of Spain. Sanjeev et al. (2000) have observed the incidence of *V. fluvialis* in 2.09% of frozen fish products meant for export. *V. fluvialis I* and *V. fluvialis II* were isolated from seafood and aquacultured food available in Taiwan (Wong et al., 1992). *V. fluvialis I* was the major species found in oysters and clams and showed an incidence of 68.8% and 78.6% respectively (Wong et al., 1992). Matte et al. (1994) reported the incidence of *V. fluvialis* in oysters (*Crassostrea gigas*) (27%) originating from the southern coast of the state of Sao Paulo-Brazil. Most probable number (MPN 10^3) obtained for the species was 3-150 (Matte et al., 1994).

Venkateswaran et al. (1989) have isolated *V. fluvialis* from surface waters and sediment samples of the freshwater Ohta river. Gianelli et al. (1984) have reported the occurrence of *V. fluvialis* in shellfish in shores of the Adriatic Sea or purchased from retail shops. This was the first reported incidence of *V. fluvialis* in fishery products of the Mediterranean area.

Incidence of *V. fluvialis* in seawater environment has been reported by Rodriguez and Hofer (1986) in Brazil, Shinoda et al. (1985) in Japan and Schandevyl et al. (1984) from Senegal. *V. fluvialis* was found positive for chitinase and chitobiase
activities (Osawa and Koga, 1995). *V. fluvialis* was the most frequently isolated *Vibrio* species from shellfish bred in nurseries located in the Ebro river delta (Montilla et al., 1994).

Chang et al. (2001) isolated a strain of *V. fluvialis* from the diseased *Paraliethys olivaceies* supplied by fish farm of Xunshan fisheries Group Company of Rengcheng city in July 1988. Six strains of *V. fluvialis* were isolated from diseased black tiger shrimp, *Penaeus monodon* Fabricius (Ruangpan and Kitao, 1991). Engelbrecht et al. (1996) found *V.fluvialis* as potential active spoilers on fresh cape Hake and other south Atlantic fish species (Kingklip, Monk, Angel fish and Gurnard). *V. fluvialis* was the predominant microorganisms isolated from the haemolymph of diseased American Lobsters (*Hemarus americanus*) harvested from the Atlantic coastal waters (Tall et al., 1999). It was reported that *V. fluvialis* was responsible for a mysterious disease that has killed 1000 of Maine and New Brunswick lobsters during the past years (Anon, 1999). Guner et al. (1997) have reported the isolation of *V. fluvialis* from fresh potatoes.

2.4. *V. furnissii*

The biogroup 2 strains of *V. fluvialis* that produced gas from the fermentation of carbohydrates were named *V. furnissii* by Brenner et al. (1983). The separation of *V. furnissii* from *V. fluvialis* was supported by studies of DNA relatedness (Brenner et al., 1983).

Strains of the organism now classified as *V. fluvialis* were first described by Furniss et al. in 1977. These organisms, designated group F, were isolated in 1975 from a patient with diarrhea in Bahrain, from patients with diarrhea in Bangladesh and from shellfish and estuarine waters in England. Group F required salt and have a number of properties compatible with or mid way between those of vibrios and aeromonads. In a numerical taxonomy study Lee et al. (1978) showed that group F strains were a distinct phenon that probably represented a new species, and this group contained two subgroup on the basis of gas production during fermentation of glucose.

Huq et al. (1980) studied large number of strains associated with an outbreak of diarrhea in Bangladesh as well as strains isolated from patients with diarrhea in
Indonesia, strains from sewage in Brazil and US strains that had been called group EF-6 in the special bacterial reference activity at the centers for disease control. By both phenotypic tests and DNA relatedness, they found that the organism was closer to the genus *Vibrio* than to the genus *Aeromonas*. All of their strains produced no gas from the fermentation of glucose (were anerogenic) and formed a single DNA relatedness group. Thus, the EF6 group appeared to be identical to group F.

Group F strains isolated from several parts of the world were compared phenotypically and genetically by Seidler et al. (1980). They confirmed and extended the observation that group F was more closely related to *Vibrio* than to *Aeromonas*. They further showed that the aerogenic group F strains were in a different DNA relatedness group from the anaerogenic strains, and they recommenced that the two biogroups be considered as two separate species with in the genus *Vibrio*.

The genetic and phenotypic tests indicate that the aerogenic strains formerly included in *V. fluvialis* represent a new species in the genus *Vibrio* and Brenner et al. (1983) proposed the name *V. furnissii* for the new species in the genus *Vibrio*, in honour of A.L. Furniss, Maidstone Public Health Laboratory, Maidstone, England, for his role in the classification of *V. fluvialis* and for his many contributions to the knowledge of the genus *Vibrio* (Brenner et al., 1983).

*V. furnissii* is a gram negative, straight to slightly curved rod that is motile by means of polar flagella. It is NaCl requiring, oxidase positive, nitrate positive organism that ferments D – glucose and other carbohydrates with the production of acid and gas, has 50 mol % guanine + cytosine in its DNA (Brenner et al., 1983).

*V. furnissii* has been isolated from river, estuarine water, marine molluscs and crustacean throughout the world (Oliver and Kaper, 1995). Matte et al. (1994) have reported 19 % incidence of *V. furnissii* in oysters (*Crassostrea gigas*) 19 % originating from the southern coast of the state of Sao Paulo-Brazil. Wong et al. (1992) found a relatively small percentage (7 to 12 %) of the oysters, clams, shrimps and crabs they examined. Thampuran et al. (1997) have reported the incidence of *V. furnissii* in the intestinal contents of fish collected from Cochin and
the percentage varied from 25.9 to 32.9 %. Sanjeev et al. (2000) indicated the occurrence of \textit{V.fumissii} in frozen fish and fish products (1.05 \%) collected from processing factories situated in Kerala and Tamil Nadu meant for export.

The largest documented outbreaks of \textit{V.fumissii} were reported in 1969, when this species was isolated during an investigation of two outbreaks of acute gastroenteritis in American tourists returning from the Orient (Anon, 1969). In the first outbreak 23 of 42 elderly passengers returning from Tokyo developed gastroenteritis, one woman died and two other persons required hospitalization. Food histories implicated shrimp and crab salad and or the cocktail sauce served with the salads. \textit{V.fumissii} was recovered from seven stool specimens. The second outbreak affected 24 of 59 persons returning from Hong Kong (Anon, 1969). Nine persons were hospitalized. Food vehicle was not identified, but \textit{V. fumissii} was isolated from at least five fecal specimens. In 1994, during a cholera surveillance program in Peru, \textit{V.fumissii} was isolated from 14 patients, 6 with diarrhea and 8 without symptoms (Dalsgaard et al., 1997). Magalhaes et al. (1993) isolated sixteen strains of \textit{Vibrio fumissii} from 16 Brazilian patients with diarrhea. Lesmana et al. (2002) have reported the isolation of small numbers of \textit{V.fumissii} strains along with \textit{V. parahaemolyticus} and \textit{V. fluvialis} from patients with acute diarrhea in North Jakarta, Indonesia. Although \textit{V.fumissii} had been isolated from diarrheal patients, its role as an enteric pathogen still remains unclear.

Symptoms described by Brenner et al. (1983) for the gastroenteritis outbreaks described above included diarrhea (91 to 100 \%), abdominal cramps (79 to 100\%), nausea (65 to 89 \%) and vomiting (39 to 78 \%). There were no reports of fever onset of symptoms occurred between 5 and 20 h with the patients recovering within 24 h. Neither the infectious dose of \textit{V.fumissii} nor the susceptible population is known.

\textit{Vibrio fumissii} is also pathogenic to fishes. Esteve et al. (1995) reported for the first time the isolation of \textit{V.fumissii} strains from a European eel culture system, which are pathogenic to eels (\textit{Anguilla anguilla}). Ahsan et al. (1992) isolated 14 strains of \textit{V. fumissii} from different ulcerated areas of eel. Their observation clearly establishes the enterotoxicity of these organisms. Sung et al. (2001)
isolated *V. furnissii* from cultured tiger shrimp (*Penaeus monodon*) and pond water. Studies of Cantoni *et al.* (2001) have shown the incidence of *V. furnissii* in brined vegetable (*Ocimum basilicum*).

2.5. *V. parahaemolyticus*

*Vibrio parahaemolyticus* as it is now known was first isolated by Fujino *et al.*, in 1951 and designated *Pasteurella parahaemolytica*. The organism caused gastroenteritis in 272 persons resulting in 20 deaths in Osaka, Japan. The fry of sardine boiled in salt water and sold and eaten in the half dried/fried state (Shirasu) was the contaminated food product eaten by all who had acute gastroenteritis. Halophilic nature of this organism was first indicated by Takikawa (1958) and classified the species as *Pseudomonas enteritis*. Miyamoto *et al.* (1961) noted the serological differences between this organism and *Pseudomonas* and proposed the generic name *Oceanomonas*.

Sakazaki *et al.* (1963) were the first to present a detailed description of *V. parahaemolyticus*, based on the differences of growth in peptone water containing 7 and 10 % NaCl, Voges- Proskauer reaction and fermentation of sucrose, arabinose and cellobiose. They recognized three subgroups, subgroup 1 and 2 were designated as *V. parahaemolyticus* and subgroup 3 resembled *V. anguillarum*, which did not grow in 7 or 10 % NaCl. Subgroup 2 grew in 7 and 10 % NaCl, whereas subgroup 1 grew in 7 % NaCl only.

Zen-Yoji *et al.* (1965) confirmed the differences between subgroup 1 and 2 and reported the differences in pathogenicity between the two groups. Sub group 1 was isolated frequently from patients with unidentifiable enteritis and subgroup 2 was not pathogenic to man. Sakazaki (1968, a) reexamined 100 cultures of each subgroup and confirmed the results reported by Zen-Yoji *et al.* (1965). Cultures of subgroup 2 grew in 10 % NaCl, fermented sucrose and produced acetoin, whereas those of subgroup 1 did not. Because of these differences, he proposed the specific name *alginolyticus* for subgroup 2 (biotype 2). The organism of subgroup 1 (biotype 1) continued to be classified as *V. parahaemolyticus*.

Morphologically *V. parahaemolyticus* is gram-negative rods exhibiting pleomorphism. Slight curved, straight, coccid and swollen forms can be
observed. All strains of \textit{V. parahaemolyticus} are motile by means of a single polar flagellum. In broth cultures, the vibrio has a single polar flagellum but on the surface of nutrient agar young cultures may possess peritrichous flagella. On agar plates most cultures appear as smooth, moist, circular, opaque colonies with entire edges. Rough variants have been reported in pure cultures by Tewedt \textit{et al.} (1969). A swarming phenomenon occurs in some instances, when low concentrations of agar are used. This diminishes with increased concentration of agar.

More than five decades have passed since the first report by Fujino \textit{et al.} (1951) that implicated \textit{Vibrio parahaemolyticus} as the cause of an outbreak of seafood poisoning. Since then voluminous literature has accumulated from the investigators worldwide.

\textit{V. parahaemolyticus} inhabits the marine and brackishwater environments and it is therefore associated with fishes harvested from these environments. Although this halophilic organism was first isolated more than 50 years ago, it has remained practically unknown elsewhere for sometime. At first it was thought to be limited to Japan and Far East. But during the last 30-40 years, it has been isolated from different species of fish, shellfish and marine environments such as bottom sediments and plankton. This has been reported from many countries viz. Brazil (Matte \textit{et al.}, 1994), Italy (Maugeri \textit{et al.} (2000), USA (Florida) (Hlady, 1997; Ellison \textit{et al.}, 2001), USA (Liston, 1973), U.K. (Barrow, 1974), Hong Kong (Chan \textit{et al.} (1989), Philippines, Taiwan, Hong Kong, Singapore and Japan (Sakazaki, 1969; Wong \textit{et al.}, 2000 and Alam \textit{et al.}, 2002), Panama, West Africa and Indonesia (Beuchat, 1977; Lesmana \textit{et al.}, 2002), Malaysia (Cann and Taylor, 1981; Elhadi \textit{et al.}, 2004) and in India (Chatterjee \textit{et al.}, 1970; Chatterjee and Neogy, 1972; Nair \textit{et al.}, 1975, Victor and Fred. 1976; Natarajan \textit{et al.}, 1979; Lall \textit{et al.}, 1979; Nair \textit{et al.}, 1980, Karunasagar and Mohankumar, 1980; Pradeep and Lakshmanaperumalasamy, 1984; Sanjeev and Iyer, 1986; Sanjeev and Stephen, 1993; Karunasagar \textit{et al.}, 1990, Prasad and Rao, 1994,a; Thampuran \textit{et al.}, 1997; Sanjeev at \textit{et al.}, 2000, Deepanjali \textit{et al.}, 2005). The earlier work on \textit{V. parahaemolyticus} has been extensively reviewed by Sakazaki (1969), Lee (1973), Liston (1973), Sakazaki (1973), Barrow (1974) and Joseph \textit{et al.} (1983).
In 1973 an international conference on *V. parahaemolyticus* was held in Tokyo (Anon, 1974).

De et al. (1977) showed the incidence of *V. parahaemolyticus* in marine fishes of Calcutta to be 35.2 %. Natarajan et al. (1979) reported 36.8 % occurrence in fishes from brackishwater environments. Karunasagar and Mohankumar (1980) found that the incidence varied from 8.33 to 33.3 %. The studies of Nair et al. (1980) revealed that 35.6 % of the freshly harvested fishes from the estuarine waters, 40.6 % fishes of mangroves, 37.5 % of freshly caught brackishwater fishes and 44 % fishes from market showed the incidence of *V. parahaemolyticus*. Sanjeev and Iyer (1986) reported the occurrence of *V. parahaemolyticus* in 55.9 % of the market fish samples and 2 out of 15 cooked clam meat samples. Sanjeev and Stephen (1993) showed the incidence of *V. parahaemolyticus* in marine fresh finfish and shellfish varied from 67 to 92 %, whereas in fish products it was less (3.69 to 30.23 %). Sanjeev and Stephen (1993), Karunasagar et al. (1990) reported that *V. parahaemolyticus* was the most commonly encountered halophilic pathogenic vibrio in market samples of fish and shellfish and showed 69 % incidence of the organism. Prasad and Rao (1994, a) have reported the incidence of *Vibrio parahaemolyticus* from fresh and frozen prawns and fishes of Kakinada coast. Thampuran et al. (1997) were able to isolate *Vibrio parahaemolyticus* in coastal waters and fishes of Cochin. Deepanjali et al. (2005) in a study of the oysters along the south west coast of India, detected vibrios in 93.87 % of the samples, and the densities ranged from <10 to 10⁴ organisms per gram. They could also detect pathogenic *V. parahaemolyticus* from 10.2 % of the samples. Sanjeev et al. (2000) reported 9.42 % occurrence in fish products collected from processing factories situated in Kerala and Tamil Nadu meant for export.

In a study on halophilic vibrios in seafood from Hong Kong markets Chan et al. (1989) reported that bivalve shellfish were more frequently and more heavily contaminated with vibrios and in particular with *V. parahaemolyticus* and *V. alginolyticus*. [Mussels (4.6x10⁴ g⁻¹), oysters (3.4x10⁴ g⁻¹) and clams (6.5x10³ g⁻¹)]. This differs from the observation of Molitoris et al. (1985) who found mackerel, shrimps and squids to be the most frequently contaminated with *V. parahaemolyticus* and *V. alginolyticus* in Indonesia. Elhadi et al. (2004) in a
survey of seafood markets and super markets reported the incidence of *V. parahaemolyticus* in 4.7% of the samples consisting of shrimp, squid, crab, cockles and mussels. Matte *et al.* (1994) analyzed vibrio species in oysters (*Crassortrea gigas*) originating from the southern coast of the state of Sao Paulo Brazil. Most Probable Number (MPN 10^-2 g) obtained for *V.parahaemolyticus* was <3-1, 200 and showed an incidence of 77%.

Wong *et al.* (1995) isolated *V.parahaemolyticus* from 36.0% frozen raw or semi prepared seafoods such as peeled shrimp, fish and shrimp dumplings. Sunen *et al.* (1995) reported 30.23% incidence of *V.parahaemolyticus* in samples consisting mussels and clams purchased from retail outlets in Spain. Hase *et al.* (1997) have shown the incidence of *V. parahaemolyticus* in 21.1% of raw seafood samples and 23.3% of environmental samples collected from Osaka (Japan) seafood market. Baffone *et al.* (2000) have reported 14.8% incidence of *V. parahaemolyticus* in fresh seafood products in Italy. Jaksic *et al.* (2002) in a study of seafood samples collected along the sea side in Croatia reported 9.40% incidence of *V. parahaemolyticus*. Wong *et al.* (1992) isolated *V.parahaemolyticus* from 22.8% freshwater clams in Taiwan. Joseph *et al.* (1983), Sarkar *et al.* (1985), Venkateswaran *et al.* (1989) have also isolated *Vibrio parahaemolyticus* from freshwater samples of India and Japan. Rashid *et al.* (1992) have reported the incidence of *V. parahaemolyticus* in frozen shrimps imported from South East Asia and Mexico.

Aiyamperumal *et al.* (1994) reported the occurrence of *V. parahaemolyticus* in 14.2% of finfish, 14.5% of prawns, 23.8% of crabs and 34.7% of bivalves of coastal waters of Tuticorin. Sanjeev and Stephen (1993) have shown that densities of *V. parahaemolyticus* in estuarine shellfish were found to be much higher compared with shellfish from the Arabian Sea. This is in agreement with the findings of Nair (1985) with respect to fish from the Bay of Bengal. Similar results were reported by Kaneko and Colwell (1974), Vargu and Heritle (1975).

In man *V. parahaemolyticus* usually causes diarrhea, occasionally dysentery like or gastroenteritis of sudden onset varying from mild to severe. The mortality rate is less than 10%. There is very little information regarding the dose response in the 5-9 log_{10} region (FDA CFSAN, 2000). *V.parahaemolyticus* and closely related
organisms have also been isolated occasionally from infected skin or tissue lesions in bathers and fish handlers (Roland, 1970).

In an outbreak in US *V. parahaemolyticus* infections comprised 59% with gastroenteritis, 8% with septicaemia and 34% with wound infections (Daniels et al., 2000, a). Infections in outbreaks resulted in diarrhea being the most common symptoms, often associated with abdominal cramps, nausea and vomiting (Daniels et al., 2000, a). It is a self-limiting infection generally lasting only for a few days with little evidence of spread of the infection from one person to another (Barrow and Miller, 1976). Tamura et al. (1993) have reported an isolated case of reactive arthritis in Japan following *V. parahaemolyticus* infection. A death due to *V. parahaemolyticus* infection following consumption of oysters was reported in New South Wales in 1992 (Kraa, 1995).

There is a link between contamination of seafoods and sea temperature with 89% of oysters associated with disease coming from waters >22°C (Daniels et al., 2000). Numbers of *V. parahaemolyticus* are higher in seafoods harvested when the water is warmer (DePaola et al., 1990). Cook et al. (2002) in a study observed that geometric means for *V. parahaemolyticus* in oysters harvested from the Gulf of Mexico in one study were 7.2 MPN g⁻¹ in winter, 1,330 in Spring, 5,150 in Summer and 500 in the Autumn (Cook et al., 2002).

The organism has been isolated frequently from cases of food poisoning accounting for about 40-60% of all cases of bacterial food poisoning in Japan (Honda and Lida, 1993). In a survey of vibrio infections associated with raw oyster consumption in Florida during the period 1981-1994, *V. parahaemolyticus* was found to be most often identified vibrio species in patients (29%) with gastroenteritis (Hlady, 1997). It is one of the most important causative agents of food poisoning in Japan and was considered a local problem until recently, but it has now been recognized in many countries especially in South East Asia (Chatterjee et al., 1970, Sakazaki et al., 1974). These workers reported the isolation of *V. parahaemolyticus* from up to 15% patients with diarrhea in Calcutta.

The vibrios have also been isolated from infection of the hands and feet, eyes and ears of the person who have been in contact with marine shore areas. The
disease caused by *V. parahaemolyticus* infection is more prevalent in countries that consume large quantities of seafood and that frequently consume raw seafood (e.g. Japan). Reports on outbreak of infection due to *V.parahaemolyticus* are available from different parts of the world. From USA (Summer et al., 1971; Hlady and Klontz, 1996; Olsen et al., 2000; Daniels et al., 2000, a; CDC, 2001), Japan and Taiwan (Lee et al., 2001), Australia (Batley et al., 1970; Davey, 1985; Kraa, 1995), Sweden (Lindqvist et al., 2000); Thailand (Tangkanakul et al., 2000) and UK (Scoging, 1991) Nigeria (Chigbu and Iroegbu, 2000) and Indonesia (Lesmana et al. 2002).

*V. parahaemolyticus*, Kanagawa phenomenon positive, are those mostly associated with disease. This phenomenon represents the production of a thermostable direct haemolysin (TDH). In a study of clinical, marine and shellfish isolates Yam *et al.* (2000) found that all of the 38 clinical isolates were TDH/KP+ve, while only 0.85 of coastal water and 2.5 % of shellfish isolates were TDH/KP+ve. Their suggestion was that it is more important to determine the TDH/KP status of *V.parahaemolyticus* present in foods than it is to enumerate them. Of 115 Japanese clinical isolates 7 % possessed (TDH and/or the TDH-related haemolysin and urease) all three genes (Lida *et al.*, 1998).

Matsumoto *et al.* (2000) have reported the emergence of a new serotype O3: K6 described as a pandemic clone was first appeared in Bangladesh. This clone posses a high infection frequency and capacity to spread globally (Matsumoto *et al.*, 2000; Daniels et al., 2000, a and Wong *et al.*, 2005). From India Deepanjali *et al.* (2005) have reported the incidence of the pandemic strain from oysters along the south west coast of India. It was also shown that this serotype exhibits increased adherence and cytotoxicity in tissue culture, and this may contribute to the enhanced pathogenic potential of strains of this serotype (Yeung *et al.*, 2002).

### 2.6. *V. alginolyticus*

*V. alginolyticus* is a halophilic vibrio first recognized as being pathogenic in humans in 1973 (Zen-Yoji *et al.*, 1973). *V. alginolyticus* was originally classified as biotype 2 of *V. parahaemolyticus* was reclassified as a separate species by Sakazaki (1968, a). The ecological niche occupied by *V. alginolyticus* is similar to that of *V. parahaemolyticus*. Seawater is the normal habitat for *V. alginolyticus*,
and it has been isolated from seawater and seafood in many parts of the world (Baross and Liston, 1970; Kampelmacher et al., 1972; Vasconcelos et al., 1975; Hosseini et al., 2004). *V. alginolyticus* was the dominant species isolated from coastal waters of Cochin (Thampuran et al., 1997). *V. alginolyticus* was isolated from mussels (Matte et al., 1994, a: Mugeri et al., 2000), Clams (Sunen et al., 1995), Oysters (Matte et al., 1994). Reports are available on the incidence of this species in seafood obtained from markets in Hong Kong (Chan et al., 1989) and Malaysia (Elhadi et al. 2004). This species was isolated from frozen seafoods mainly from shrimps and crabs (Wong et al., 1995; Sanjeev et al., 2000; Jaksic et al., 2002). Wong et al. (1992) have reported the incidence of this species in aquacultured foods comprising oysters and grass shrimps in Taiwan.

Studies by Baross and Liston (1970) in oysters of Washington State showed that *V. alginolyticus* was rarely found in winter, but counts rose rapidly with increasing water temperature, and the organism was abundant in summer, and the minimum growth temperature for *V. alginolyticus* is 8°C.

*V. alginolyticus* is reported to have been isolated from extra intestinal sites from exposure to seawater, mostly infection of the ear, eye, hand, leg, lung, blood and burns (Rubin and Tilton, 1975; English and Lindberg, 1977; Olsen, 1978; Hansen et al., 1979; Hollis et al., 1976; Pien et al., 1977). Tubiash et al. (1970) have reported the association of *V. alginolyticus* with bacillary necrosis of larval and juvenile bivalve molluscs. Resistance to tetracycline and chloramphenicol has been reported in a few isolates of *V. alginolyticus*, but all strains appeared to be sensitive to ciprofloxacin (French, 1990).

2.7. *V. damsela*

*Vibrio damsela* is a halophilic gram-negative bacillus similar to *V. vulnificus* that strictly causes soft tissue infections following exposure of wounds to brackishwater or injury by saltwater animals (Barber and Swygert, 2000) *V. damsela* infections can be fulminant and are frequently fatal even in immunocompetent hosts. Of the 16 cases of *V. damsela* infection reported between 1982 and 1996, 4 were fatal (Fraser et al., 1997). Thampuran et al. (1997) have reported the isolation of *V. damsela* from coastal waters and fishes of Cochin. Sanjeev et al. (2000) reported the incidence of this species in iced
prawns. Elhadi et al. (2004) have reported the incidence of the species in seafood samples collected from markets and supermarkets in Malaysia. Hosseini et al. (2004) have reported the isolation of the species from shrimp caught off the coast of Iran.

2.8. *V. metschnikovii*

*V. metschnikovii*, an oxidase-negative and nitrate negative species, of the genus *vibrio* was first described in 1888 by Gamaleia (1888), redefined in 1978 (Lee et al., 1978), and extensively characterized in 1988 by Farmer et al. (1985), is rarely isolated in human infections. Most nonhuman strains of this species were isolated from river water, sewage, cockles, clams, oysters, prawns and lobsters (Lee et al., 1978), crabs and shrimps (Farmer et al., 1985), fish (Hansen et al., 1989) and scallops (Buck, 1991). Thampuran et al. (1997) have reported the isolation of *V. metschnikovii* from coastal waters of Cochin. Elhadi et al. (2004) have reported the incidence of the species in seafood samples collected from markets and supermarkets in Malaysia.

Although human isolates have been recovered from blood, urine, a foot wound (Farmer et al., 1985; Farmer et al., 1988), gall bladder, and bile duct (Jean-Jacques et al., 1981), as well from feces (Lee et al., 1978). Only one well documented clinical observation concerning a case of bacteremia in a patient with an inflamed gallbladder has been described (Janda et al., 1988). *V. metschnikovii* was isolated from 5 infants with diarrhea during a cholera surveillance programme in Peru (Dalsgard et al., 1996). All isolates were identified within a 10-day period. No common source of infection was found and no additional isolates of the organism were identified in the following year. Lesmana et al. (2002) have reported the isolation of *V. metschnikovii* from patients with acute diarrhea in Jakarta, Indonesia.

2.9. *V. vulnificus*

This species has been identified as a halophilic “Lactose – positive” marine vibrio (Hollis et al., 1976). *V. vulnificus* is an indigenous bacterium in marine and coastal water environments and is responsible for wound infection and primary septicemia after ingestion of raw seafood, especially oysters or contact with
seawater (Høi et al., 1998; McLaughlin, 1995). The species *V. vulnificus* can be divided into biotypes 1 and 2 on the basis of differences in biochemical and serological properties (Tison et al., 1982). Biotype 1 strains are associated with human disease, whereas biotype 2 strains are pathogenic for fish, especially eels (Amaro and Biosca, 1996).

*V. vulnificus* is widespread in the environment and has been isolated from estuarine waters of most U.S. coastal states (Oliver et al., 1982; 1983; Tilton and Ryan, 1987; Pfeffer et al., 2003). Thampuran et al. (1997) have reported the incidence of *V. vulnificus* in coastal waters of Cochin. In a survey conducted in Karnataka on the west coast of India, Karunasagar et al. (1990) observed the incidence of *V. vulnificus* in fish and shellfish samples collected from the market. *V. vulnificus* was the dominant species isolated from fishes along Kakinada in the eastern coast of India (Prasad and Rao, 1994, a). Sanjeev et al. (2000) have reported the occurrence of this species in frozen fish and fish products collected from Kerala and Tamil Nadu meant for export. Yamo et al. (2004) have reported the isolation of *V. vulnificus* from live seafood samples consisting of razor clams, giant tiger prawns and mantis shrimp samples collected from the markets in coastal cities of China.

Schandevyl et al. (1984) have reported the isolation of *V. vulnificus* from marine fish in Senegal, Africa. Vaseeharan and Ramasamy (2003) in a study of the *Penaeus monodon* rearing hatcheries in India, reported the isolation of *V. vulnificus* from shrimp eggs, post larvae, rearing tank water, source seawater and feed.

The presence of *V. vulnificus* in water and shellfish is seasonal, being most prevalent when the water temperature is high i.e. >20°C (Kelly, 1982). Low salinity (0.5-1.6%) also favours the presence of *V. vulnificus* in seawater (Kelly, 1982). Some strains of *V. vulnificus* show bioluminescence, and these strains may also be pathogenic (Oliver et al., 1986).

Food borne infection may result after consuming contaminated raw or undercooked seafood, particularly oysters and clams, with illness usually starting 16-48 h after ingestion. The organism penetrates the intestinal tract and produces primary septicemia. The illness usually begins with malaise, followed by chills,
fever, and prostration. Vomiting and diarrhea are uncommon, but sometimes occur shortly after chills and fever. Hypotension is present in approximately 33% of the cases (Blake et al., 1979; Hollis et al., 1976). Persons with known liver disease, particularly those patients with cirrhosis, are at high risk for \textit{V. vulnificus} primary septicemia. (Vollberg and Herrara, 1997). \textit{V. vulnificus} can also cause skin infection when open wounds are exposed to warm seawater. These skin infections may lead to cellulitis, ulceration, necrotizing fasciitis, and sepsis (Klontz et al., 1988; Howard and Leib, 1988; Oliver, 2005).

\subsection{2.10. Effect of washing on HPVs}

Information regarding the effect of washing on HPVs is scanty. According to Shewan (1977) most of the bacteria adhering to the slime and skin surface could be washed away with water. Several methods have been evaluated for their effectiveness in reducing initial microbial population in seafoods. High-pressure washes have been demonstrated to be effective in reducing bacterial populations on the surface of whole fish by removing the slime layer in seafoods (Mayer and ward, 1991). Karthikeyan \textit{et al.} (1999) reported that washing and chlorine disinfections reduced the total plate counts and faecal coliform counts in cultured shrimp from 6.31 log \text{g}^{-1} to the tune of 0.68-1.25 log units. These results corroborate the observations of Vanderzant and Nickelson (1972) and Peranginangin and Suparna (1992). Thampuran and Gopakumar (1990) in a study of the impact of handling practices on the microbial quality of shrimp (\textit{M. dobsoni}) observed that washing resulted in a decrease in the total bacterial load, while the percentage of reduction due to washing was 50.54 to 80.71, on the basis of the count in Sea water agar (SWA). Shucking and washing appear to result in an overall decline in vibrios viz \textit{V.cholerae}, \textit{V.parahaemolytius} and \textit{Lac+} vibrios (Mary and Gregory, 1984). Akalani-Rose \textit{et al.} (1990) in a study of the prawns harvested from prawn farms observed that \textit{V.parahaemolyticus} was always detected in newly harvested prawns where levels varied from $10^2-10^3$ cfu \text{g}^{-1} and washing at the pond site reduced the count slightly. Codex Committee on Food Hygiene (CCFH, 2002) in a review, principally concerning \textit{V. parahaemolyticus} has stressed the need for effective washing of seafood after harvest and during seafood preparation with disinfected seawater or potable water.
2.11. Effect of chilling on HPVs

Reports generally have indicated that vibrios are sensitive to cold. Studies of Muntada-Garriga et al. (1995) indicates that high numbers of *V. parahaemolyticus* can be inactivated at chill temperatures; the time of total inactivation depends on the initial number of microorganisms and incubation temperature. Enteropathogenic vibrios grow over the range 5 to 43°C with an optimum at 37°C. There is variation both between species and among strains within species. Both food type and incubation temperature affected survival of vibrios at low temperatures (Corrales et al., 1994).

Cook and Ruple (1992) have shown that *V. vulnificus* held at temperatures of 4°C and 0°C underwent a time dependent decrease in number of recoverable cells. The time required for the bacterium to reach undetectable levels (MPN<3g⁻¹) may exceed the usual storage of 14 days for shucked oyster meats and 21 days for shell stock oysters. Gooch et al. (2002) have reported a 0.8 log₁₀ decrease in number of *V. parahaemolyticus*, when the oysters were chilled at 3°C after 14 days. Andrews et al. (2000) on the contrary have observed that low temperature pasteurization of raw oysters in ice was very effective in reducing *V. vulnificus* and *V. parahaemolyticus* from >100000 to non detectable levels in less than 10 min of processing. Kaysner et al. (1989) in a controlled study of *V. vulnificus* in oyster shellstock found that the organism survived up to 2 weeks at 2°C, whereas *V. parahaemolyticus* has been observed to survive storage in shell stock oysters for at least 3 weeks at 4°C (Oliver and Kaper, 1997). To reduce the consumer exposure to *V. vulnificus* the oyster shellstock must be cooled immediately after harvest to eliminate the post harvest growth of the organism (Cook, 1997). Quevedo et al. (2005) observed that although rapid chilling by immersion of unwashed whole oysters in ice for 3 h generally declined the *V. vulnificus* numbers, the method cannot be relied upon because of the relatively small decline in *V. vulnificus* number and the possibility of concomitant increases in fecal coliform and total bacterial contamination.

Some workers insisted that inactivation of *V. parahaemolyticus* occurred more rapidly when the organism was chilled (1- 7°C) than when it was frozen at -2 to -30°C (Beuchat, 1977; Johnson and Liston, 1973). Quite the reverse was
observed by Matches et al. (1971) who observed that temperatures below 8°C will usually stop growth but it has been observed that the organism can still survive. *V. parahaemolyticus* has been reported to undergo an initial rapid drop in survival (Ca. 99%) when incubated on whole shrimp at 3, 7, 10 or -18°C, although survivors remained at the end of the 8th day of study (Oliver and Kaper, 1997). Similar results were observed by Vasudevan et al. (2002) when the fish fillets were chilled. Bradshaw et al. (1974) found that vibrios grow well at 18°C, in cooked shrimp and crab, but their numbers declined from 0.5 to 1 log at 10°C and below during 48 h holding period.

Wide discrepancies in views may be due to the fact that the organism becoming non-culturable rather than non-viable. Oliver and Wanucha (1989) have observed that while cells rapidly lose culturability at 5°C or 10°C, a significant proportion remained viable and metabolically active.

2.12. Effect of storage at low temperature (4°C and 10°C) on HPVs

Although the vibrios are sensitive to cold; seafoods have also been reported to be protective for vibrios at refrigeration temperatures (Oliver and Kaper, 1997). Wong et al. (1994) reported that *V. mimicus*, *V. fluvialis* and *V. parahaemolyticus* survived well at low temperature (10°C and 4°C) although it showed 1 to 2 log reduction. In shell stock oysters, *V. parahaemolyticus* was observed to survive for at least 3 weeks with little or no decrease in numbers and it has been shown to grow slowly at 10°C in oyster homogenate (Johnson et al., 1973; Thompson and Thacker, 1973). Minimal temperatures reported for *V. parahaemolyticus* multiplication was 5°C (Beuchat, 1973) and 8°C (Baross and Liston, 1970) in artificial media and 10°C in oyster homogenate (Thompson and Thacker, 1973).

Vanderzant and Nickelson (1972) subjected their gulf coast isolates of *V. parahaemolyticus* strain to 0, 3, 7, 10 and -18°C in whole and homogenized shrimp. The loss of viability in shrimp homogenate was not as great as in the whole shrimp and more than 2 log reduction was observed in 8 days. It is also interesting to note that the strains Vanderzant and Nickleson studied was more readily inactivated at 3°C than at -18°C. Twedt (1989) reported that the initial concentration of *V. parahaemolyticus* suspended for 48 h in peptone broth with
3 % NaCl or raw fish held under refrigeration (4°C) declined from 1 to 4 log10.

Similar observations were made in subsequent investigations when *V. parahaemolyticus* was held at low temperature in shrimp (Bradshaw et al., 1974), oysters (Goatcher et al., 1974; Johnson and Liston, 1973; Beuchat, 1977), homogenized and filleted fish (Covert and Woodburn, 1972; Johnson and Liston, 1973; Matches et al., 1971) and crab meat (Beuchat, 1977; Johnson and Liston, 1973).

Oliver (1981) reported rapid and dramatic decrease in cell viability when *V. vulnificus* cells were incubated at 4°C in oyster homogenates. Similar observation was made when the organism was incubated in shrimp homogenate at 4°C (Boutin et al., 1985 and Hopkins and Modlin, 1985). It has been shown that *V. vulnificus* can multiply in post harvest shellfish if they are held at temperatures above 10°C (Oliver, 1989).

At 6 ± 2°C *V. parahaemolyticus* and *V. vulnificus* continued to survive till the end of storage period (90 days) in fish homogenate, although the organism showed a 5 log reduction (Sudha et al., 2003). They also reported that in 3 % salt solution and tryptic soya broth (TSB), *V. vulnificus* could survive only upto 14 days at 6.0± 2°C while *V. parahaemolyticus* remained viable for a longer period of upto 60 days. Vasudevan et al. (2002) observed an initial reduction (100- 1000 fold) of *V. parahaemolyticus* in fish fillets when stored at 4 and 8°C although the decline in numbers was less pronounced than when the fillets were frozen. Kaysner et al. (1989) indicated the presence of large numbers of cells of endogenous *V. vulnificus* in oysters after 7 days at both 0.5 and 10°C. Covert and Woodburn (1972), Temmyo (1966) have reported that the addition of NaCl to the suspending medium (upto 12 % NaCl) conferred a stabilizing effect on the organism.

### 2.13. Effect of freezing and frozen storage (-40°C and -20°C) on HPVs

Muntada-Garriga et al. (1995) studied the survival of *V. parahaemolyticus* in oyster meat homogenate at various temperatures i.e. -18°C and -24°C with different loads i.e. $10^2$, $10^4$, $10^5$ and $10^7$ ml$^{-1}$. In all cases, the numbers of *V. parahaemolyticus* were a logarithmic function of log time, and the study indicates
that high numbers of *V. parahaemolyticus* can be inactivated at low temperatures.

Covert and Woodburn (1972) studied the interaction of temperature and NaCl concentration in affecting the survival of *V. parahaemolyticus* in trypticase soya broth and shrimp homogenate. Temperature of \(-5 \pm 1\)°C and \(-18 \pm 1\)°C reduced the number of viable organism regardless of the NaCl concentration. Fish homogenate was protective as compared with tryptic soya broth.

Vanderzant and Nickelson (1972) reported 2 log reduction in counts of *V. parahaemolyticus* after 8 days storage at \(-18\)°C. Thompson and Thacker (1972) observed that oysters held at \(-20\)°C for more than 2 weeks seldom contained viable *V. parahaemolyticus* cells. *V. vulnificus* is also sensitive to freezing and rapid die off (6 logs in 40 days) was observed at \(-20\)°C (Oliver, 1981; Boutin et al., 1985). Matches et al. (1971) inoculated *V. parahaemolyticus* in fish homogenate and observed that the log reduction values of 2.2 to 6.2 at \(-18\)°C were attained in 12 to 19 days, and the same reduction values at \(-34\)°C were reached before 12th day.

Sudha et al. (2003) have reported the complete elimination of *V. vulnificus* from fish muscle homogenate within 3 months of storage at \(-18\)°C ± 1 whereas *V. parahaemolyticus* survived the period indicating better survival capacity for this pathogen. Parker et al. (1994) showed that oyster samples individually injected with *V. vulnificus* to a level of approximately \(1 \times 10^5\) cfu g\(^{-1}\) and vacuum packaged and frozen stored at \(-20\)°C had significant effect on decreasing the *V. vulnificus* count to approximately \(1 \times 10^1\) cfu g\(^{-1}\).

Balasundari et al. (1997) observed that vibrios remained viable in edible oysters (*Crassostrea madrasensis*) after 5 months of storage at \(-18\)°C in both antioxidants treated and untreated samples. Cook and Ruple (1992) were able to isolate *V. vulnificus* from oysters frozen at \(-20\)°C for 12 weeks although freezing and storage of pure cultures of *V. vulnificus* at \(-20\)°C reduced the number of culturable cells more quickly than holding the cultures at 0° C. *V. parahaemolyticus* survived freezing at \(-20\)°C for 7 weeks in fish fillets (Vasudevan et al., 2002). Wong et al. (1994) studied the survival of psychotropic
*V. mimicus*, *V. fluvialis* and *V. parahaemolyticus* in culture broth at low temperature and found that the strains survived well at 10°C, 4°C and −30°C and could probably enhance the risk of vibrios in seafood. Johnston and Brown (2002) in a study of *V. parahaemolyticus*, *V. vulnificus* and *V. cholerae* found cells become non culturable over a period of time at 4°C. According to them, these cells in their changed morphological form would not be detected in fish or seafood products by the current vibrio detection methods and freezing at −20°C had no effect in reducing cell numbers.

### 2.14 Effect of drying on HPVs

The most common method of utilization of fish in India is as fresh fish followed by cured and dried fish (Prasad and Rao, 1994). It is estimated that over 32 % of Indian marine fish catch is consumed as cured/dried form (Thomas and Balachandran, 1989). Studies on quality of commercial dry fish, both of west and east coasts have been reported (Kalaimani et al., 1988; Basu et al., 1989, Thomas and Balachandran, 1989). But, not much information is available on the effect of drying on HPVs. Sakazaki (1983) have reported that *Vibrio parahaemolyticus* is very sensitive to drying. Temmyo (1966) noticed that *Vibrio parahaemolyticus* exposed to desiccation on inoculated membrane filters or on flat board surfaces died rapidly. Venugopal *et al.* (1984) in a study of dried fishes reported the incidence of *Vibrio parahaemolyticus* in dried white bait to a level of \(2 \times 10^4\). In another study, they reported that sun drying for 4 days completely inactivated *V. parahaemolyticus* inoculated into salted and unsalted white bait. Chitu *et al.* (1977) reported isolation of *V. parahaemolyticus* from salted herring and roe in Rumania. Rank *et al.* (1988) have indicated the survival of *V. hollisae* in dried salt fish.

### 2.15 Effect of blanching on HPVs

Enteropathogenic vibrios are not heat resistant and are readily destroyed by cooking. Resistance depends on several factors, including heating menstrum and physiological condition (Varnam and Evans, 1996). There is considerable variation between species and information is neither complete nor in some cases, is fully reliable. Raw seafoods are mostly implicated in out breaks of food
poisoning, but if they are heated at 100°C shortly before consumption, infection
with *V.parahaemolyticus* never occur (Sakazaki, 1983).

The commercial practice of heat shocking oysters in boiling water to facilitate
opening reduced counts of *V.parahaemolyticus* and often non-*V. cholera* vibrios
to undetectable levels (Hackney et al., 1980). Chang et al. (2004) in a study
revealed that when *V.parahaemolyticus* were heat shocked at 42°C for 15, 30 or
45 min, it caused an increased demand for NaCl during recovery from heat injury.
They also reported that heat shock generally increased the survival of the test
organism during subsequent exposure to 47°C. Isolation of *V. hollisae* from fried
fish in the absence of evidence of post-process contamination suggests the
possibility of a higher level of heat resistance than other species (Lowry et al.,
1986).

Delmore and Crisley (1979) observed D values for *V.parahaemolyticus* in clam
homogenate of 0.70 min at 49°C, 0.54 min at 51°C, 0.31 min at 53°C, and 0.24 min
at 55°C. This study shows that a relatively mild heating process kills the
organism. Supporting these findings is the work of Goldmintz et al. (1974), who
demonstrated that steaming clams, for 5 and 15 min (internal temperature 88 °C
and 95 °C respectively) reduced *V.parahaemolyticus* population by 6 log cycles.
However, steaming does not provide enough heat to kill vibrios or other
pathogens (Hackney and Dicharry, 1988).

In peptone with 3 % NaCl medium 3 - 4 log₁₀ decline of *V.parahaemolyticus* was
observed in 5 min at 55°C, at 60°C the decline was 7 log₁₀ (Temmyo 1966). At
65°C 3-4 log₁₀ reduction in count in crabmeat- soybroth-3 % NaCl was reported
by Goldmintz (1974). Vanderzant and Nickelson (1972) observed 6 log₁₀ decline
at 100°C in 1 min in shrimp homogenate supplemented with 3 % NaCl. The rate
of inactivation appeared to be curvilinear in these instances (Goldmintz, 1974).

Heating oysters for 10 min in water at 50°C proved adequate to reduce
*V.vulnificus* to a nondetectable level (Cook and Ruple, 1992). This treatment
does not impart a noticeable cooked appearance or taste to the oysters and may
be employed as a strategy to improve the safety of raw oysters. Hesselman et al.
(1999) described a technique involving dipping oysters in tanks with water at
67°C for around 5 min followed by spraying with cold water for around one min
to assist in shucking. When combined with market chain procedures such as chilling, packing and cold storage, \textit{V. vulnificus} was reduced by 2 - 4 logs, depending on the original contamination level. The technique has also proved effective in other bivalves.

\textit{Ama et al.} (1994) found \textit{V. vulnificus} cells were more sensitive to heating at 50°C than at 40°C. However the cells were more resistant to heating in oyster or fish homogenate than in buffers at comparable temperature and destruction was rapid at lower pH. \textit{Covert and Woodburn} (1972) observed that resistance to death is enhanced when cells are heated in substratum containing sodium chloride and at pH near neutrality.

\textit{Wong et al.} (2002) found that logarithmically grown \textit{V. parahaemolyticus} cells heat shocked at 42°C for 30 min were more resistant to thermal inactivation at 47°C than were unshocked cells. They also observed the production of thermostable direct haemolysin, the major virulence factor in \textit{V. parahaemolyticus}, was enhanced in the cells heat shocked at 42°C but not in those heat shocked at 37°C. More recently, \textit{Andrews et al.} (2003) studied the effect of heating on \textit{V. parahaemolyticus} O3:K6, a pathogenic strain with enhanced heat resistance. The researchers found that 6 min heating at 50-52°C reduced a 4 log contamination level to undetectable levels (<0.3 MPN g⁻¹). When the pathogen was present at levels of 5-6 logs, a heating time of 22 min was required to reach non-detectable levels.

2.16. Effect of sodium chloride on HPVs

The vibrios grow very poorly or not at all on or in media lacking salt (Sakazaki, 1983). Most species grow well over the range between the lower and upper growth limits, growth reducing markedly towards the upper limit. The optimum for growth in pure culture is Ca 3 %, but may be higher in mixed cultures due to the inhibition of competing microorganisms at higher concentrations (Varnam and Evans, 1996). In practice, the upper limit is of little relevance since all species are able to grow in marine foods with the exception of acid-preserved or heavily salted and dried fish. \textit{V. hollisae} is able to survive in dried salt fish (\textit{Rank et al.}, 1988).
The minimum salt concentration of *V. parahaemolyticus* limiting multiplication in substrates is 0.5 % (Sakazaki and Shinoda, 1986). The organism is readily inactivated in distilled water, with 90 % of the cells inactivated at between 0.9 and 4.4 min (Lee, 1972) good to fair growth occurs at all salt concentrations between 0.5 and 8 %, with luxuriant growth appearing at the optimal concentration of 3 % NaCl (Sakazaki and Shinoda, 1986). Covert and Woodburn (1972) found NaCl appeared to be protective to the cells of *V. parahaemolyticus* in tryptic soya broth and fish homogenate at 48 ± 1°C. The optimum concentration for growth of *V. parahaemolyticus* may be influenced by the nature of medium employed (Sakazaki, 1983).

The minimum growth temperature reported for *Vibrio parahaemolyticus* is 5°C (Beuchat, 1973; Katoh, 1964), the lower limit is affected by pH and salt concentration (Beuchat, 1973). The minimum pH reported for *V. parahaemolyticus* to allow growth at 5°C in tryptcase soy broth with 3 % NaCl was 7.3, when salt concentration was increased to 7 %, the minimum pH rose to 7.6 (Beuchat, 1973).

Kelly (1982) reported that no growth of *V. vulnificus* took place at less than 0.1 % or greater than 5 % NaCl, and optimal growth in 1-2 % NaCl. Oliver and Wanucha (1989) have observed an optimal NaCl concentration between 1 % and 3 % for *V. vulnificus* although 0.5 % NaCl present in many routine laboratory media provides for very good growth. Marco-Noales (1999) studied the effect of salinity and temperature on long-term survival of the Eel pathogen *V. vulnificus* Biotype 2 (Serovar E). According to them the optimal temperature for survival was dependant on the salinity.

### 2.17. Effect of pH on HPVs

*Vibrios* grow over a pH range from 5.6 to 9.6, but it grows best at 7.6 to 8.6 (Anon, 1972). The optimal pH range of *V. parahaemolyticus* varies from 7.5 to 8.5 (Sakazaki *et al.*, 1963). Although *V. parahaemolyticus* has been reported as growing at pH 4.8 (ICMSF, 1980), they are generally sensitive to pH values below 7.0.
All the 79 cultures of *V. parahaemolyticus* tested in a study grew well in media with initial pH values from 5 to 11 (Twedt et al., 1969). Kondo et al. (1960) have reported that *V. parahaemolyticus* is more sensitive to acid than *E. coli* and growth is completely inhibited at pH 4.5 to 5.0. The vibrios may be killed in vinegar within 1 h and in 0.5 % acetic acid within several minutes (Kondo et al., 1960). However, raw fish or shellfish with vinegar, which is widely used in Japan, has frequently caused food poisoning due to *V. parahaemolyticus*. It is probable that proteins in rich foods interfere with the action of vinegar acid on the vibrio (Kodama, 1967).

Vanderzant and Nickelson (1972) studied the survival of *V. parahaemolyticus* in shrimp homogenates at various pH values. In homogenates adjusted to pH 1, 2, 3 and 4 no survivors could be detected. At pH 5.0, a sharp drop in viable count took place immediately, with no survivors detectable after 15 min. All species of enteropathogenic vibrios grow well at alkaline pH value, the upper limiting values being pH 10-11 (Varnam and Evans, 1996).

Ama et al. (1994) observed exponential inactivation rates of *V. vulnificus* at pH 6.5, 6.0, 5.5 during heating and the organisms were inactivated most at pH 5.5 than at any other rates. Yeung and Boor (2004) have reported enhanced survival of acid adapted (pH 5.5) log-phase cells of *V. parahaemolyticus* at pH 3.6, compared to cells not previously exposed to pH 5.5.

### 2.18. Effect of Chlorine on HPVs

The use of chlorine as a disinfectant has been one of the most important public health practices for the prevention of waterborne diseases over the past 100 years (Anon, 2000). Chlorine is used in fish processing sector as a water disinfectant and is probably the most wide spread disinfectant in use. Its uses include washing fishery products, addition to water for making ice for chilling fish, and in water for thawing frozen products. It is also used in water to cool canned fish after retorting to prevent “leaker” spoilage. The codex fish and fishery products committee recommended upto 10 mg l\(^{-1}\) chlorine in water that comes in contact with fishery products and upto 100 mg l\(^{-1}\) in water for cleaning equipment and facilities (Anon, 2000).
Venugopal et al. (2000) studied the concentration and contact time required by the commonly used sanitizer, hypochlorite for killing and reducing the cells of *V. parahaemolyticus* in phosphate buffered saline (PBS) and in association with fish. A minimum level of 0.5 ppm of available chlorine was able to reduce the count of both Kanagawa Positive (K+) and Kanagawa negative (K) *V. parahaemolyticus* in PBS by 90% within 5 min and complete killing of both was achieved in 20 and 30 min, respectively. In fish artificially contaminated with K+ *V. parahaemolyticus* and exposed to 10 and 20 ppm available chlorine complete destruction of the cells was observed within 10 min, but at 30 ppm, the time required was only 5 min. Venugopal et al. (1999) in another study reported the effect of sanitizers on *V. parahaemolyticus* in biofilms on stainless steel surface and hypochlorite at 100 and 200 μg ml⁻¹ for 5 min showed a reduction in numbers by only 2-3 log units and failed to completely inactivate biofilm cells.

Studies of Gray and Hsu (1979) have indicated the effectiveness of both chlorine and idophore in killing *V. parahaemolyticus* cells. The inhibitory or lethal activity depends on the amount of free available chlorine in the solution that comes in contact with microbial cells. Free chlorine disinfects by chemically disrupting bacterial cell walls and membrane through oxidation of a chemical group known as the thiol group (WHO, 1998). The exposure of microbial cells to chlorine was also known to cause disruption of cellular enzyme system (Wyss, 1961), protein synthesis (Benarde et al., 1967), oxygen uptake and oxidative phosphorylation (Venkobackar and Rao, 1977) resulting in death or inactivation of cells.

To minimize chlorine waste and optimize its efficient use, chlorine concentration in sanitizing solutions should be monitored (Suslow, 2000). The concentration of the fast acting, antimicrobial hypochlorous acid, the chemical species providing free available chlorine to disinfect solutions, is a function of pH, between pH 6.5 and 7.0, hypochlorite exists as 80-95 % of the free chlorine concentration (Suslow, 2000). The type and form of microorganism will also influence the antimicrobial effectiveness of chlorine disinfectants (Odlang, 1981). Mir et al. (1997) have reported that the gram-positive strains were more resistant to chlorine than gram-negative strains and the behaviour of some of them in the presence of chloramphenicol suggests either the synthesis of unique proteins or aggregation of the bacteria as mechanisms of resistance to inactivation.
2.19. Effect of Chlorine dioxide on HPVs

The bactericidal properties of chlorine dioxide (ClO$_2$) have been known since the beginning of this century, but it has been used in sanitation only since the 1950's (Masschelein, 1979). It has about 2.5 times the oxidation capacity of chlorine (Benarde et al., 1967). Chlorine dioxide has been shown to produce bactericidal effect equivalent to seven times its concentration of chlorine in poultry processing water (Lillard, 1979). Chlorine dioxide maintains its bactericidal activity longer than chlorine. Parts of its disinfection capacity are attributed to the chlorite resulting from the reduction of ClO$_2$ (Masschelein, 1979). The bactericidal activity of ClO$_2$ decreases with lower temperatures (Ridenour and Armbruster, 1949) and is not affected by high pH or the presence of ammonia or nitrogenous compounds (White, 1972). Chlorine dioxide is also less reactive than chlorine with organic compounds and its use is preferred, where high organic loads are encountered (White, 1972).

The Food and Drug Administration (FDA) on March 3, 1995, amended the food additive regulations to provide a 3 ppm residual chlorine dioxide for controlling microbial populations in poultry processing water (FDA, 1995). Information is limited regarding the usefulness of ClO$_2$ in seafood processing. The use of chlorine dioxide is less common in fish processing, probably because of its instability and the hazards involved in handling and transportation (Lin et al., 1996). However, it is used and has been shown to be effective in killing a large number of microorganisms, including some that are resistant to treatment with chlorine and to extend the storage time of many foods, including fishery products. Information regarding effect of ClO$_2$ on HPVs is scanty.

Shin et al. (2004) found initial load of food borne pathogens viz., E. coli, S. typhimurium and L. monocytogenes was reduced by antimicrobial ice containing chlorine dioxide and the lowered microbial level was maintained during treatment. They also reported that the application of antimicrobial ice is a simple and effective method for the safe preservation of fish. Puente et al. (1992) observed that sterile seawater treated with lower concentration of chlorine dioxide (less than 47 μg l$^{-1}$ Cl) had no effect on the shrimp, but inhibited the growth of V. parahaemolyticus and in sewage contaminated seawater chlorine dioxide levels.
at 285-2850 μg l\(^{-1}\) necessary for the inactivation of *V. parahaemolyticus* and any native bacteria, also destroyed the artemia culture.

### 2.20. Antibiotic sensitivity of HPVs

Antibiotics and other chemotherapeutic agents are commonly used in fish farm either as feed additives or immersion baths to achieve either prophylaxis or therapy (Li *et al.*, 1999). However, extensive use of these drugs has resulted in an increase drug-resistant bacteria as well as R-plasmids (Son *et al.*, 1997; Saitanu *et al.*, 1994). Furthermore, many species of halophilic vibrios have become recognized as potential human pathogens causing serious gastroenteritis or severe wound infection upon exposure to contaminated seafood and/or seawater (French *et al.*, 1989). In recent years, vibriosis has become one of the most important bacterial diseases in maricultured organisms, affecting large number of species of fish and shellfish (Woo *et al.*, 1995; Wu and Pan, 1997). Elucidation of the antimicrobial susceptibilities of potential pathogenic vibrios will be important for prophylaxis and treatment of vibrio infections in human beings and in cultured marine organisms.

In EU member states, only four or five antimicrobial agents are licensed for use in finfish culture. In the USA, Canada and Norway, regulatory control is equally vigorous. But in many countries there is either no or no effective control on the use of antibiotics in food fish or shellfish species (Alderman and Hastings, 1998). Li *et al.* (1999) viewed that different vibrio strain had similar antibiotic resistance profiles. He tested the antibiotic sensitivity of seven *Vibrio* species viz *V. alginolyticus, V. vulnificus, V. parahaemolyticus, V. logei, V. pelagius II, V. fluvialis* and *V. mediterranei* by the agar dilution method. All isolates were sensitive to streptomycin, nalidixic acid, rifampicin and ceftriaxone and almost all were sensitive to chloramphenicol (98 %), sulphamethoxazole (98 %) and ceftazidime (96 %). A large number of strains were found to be resistant to ampicillin, amikacin, kanamycin, trimethoprin and cefuroxime. French *et al.* (1989) reported similar antibiotic susceptibility profiles for *V. alginolyticus, V. parahaemolyticus and V. vulnificus* in a clinical and environmental setting. Ottaviani *et al.* (2001) studied the antimicrobial susceptibility of potentially pathogenic halophilic vibrios isolated from seafood, and confirmed that all isolates were uniformly sensitive to...
chloramphenicol, impenem, and meropenem but resistant to lincomycin. Joseph et al. (1978) observed that *V.parahaemolyticus* and *V.alginolyticus* produce β-lactamase and are resistant to ampicillin but are inhibited by tetracycline and chloramphenicol. Zanetti et al. (2001) have reported similar result and observed the frequency of resistance to β-lactams unexpectedly high among vibrio species.

Susceptibility to antibiotics differs among vibrios species. Bode et al. (1986) in a successful treatment of vibrio meningitis caused by *V.cincinnatiensis* have reported the sensitivity of the species towards gentamycin, tobrimycin, chloramphenicol, tetracycline, ticarcillin, ampicillin and moxalactam. Morris and Black (1985) suggested an empiric therapy with tetracycline or chloramphenicol, in combination with aminoglycoside in suspected vibrio sepsis until results of susceptibility testing are available.

Lee et al. (1981) while studying the taxonomy of *V. fluvalis* have reported sensitivity towards kanamycin, streptomycin sulphonamide, tetracycline and trimethoprin. Brenner et al. (1983) have reported similar antibiotic patterns for *V.fumissii* and *V fluvalis*. They observed sensitivity of the species towards chloramphenicol, nalidixic acid, tetracycline and kanamycin and very much resistant towards penicillin and ampicillin. Sanjeev (1999) has reported the antibiotic sensitivity of *V. parahaemolyticus* from a brackishwater culture pond. All the 250 strains were found sensitive towards chloramphenicol, 68.4% were sensitive to gentamycin, and 18% were sensitive to tetracycline and 16.8% to streptomycin. None of the strains were found sensitive towards penicillin and polymyxin-B. Similar results were reported by Pradeep and Lakshmanaperumalasamy (1985). They observed the antibiotic sensitivity of 120 strains of *V.parahaemolyticus* isolated from water, sediment, plankton, fish and prawns of Cochin backwaters. They also noted higher resistance to ampicillin exhibited by isolates from fish and prawns and none of them were sensitive to kanamycin. Prawns contained more multiple resistant *V.parahaemolyticus* than others samples.

Hollis et al. (1976) have reported sensitivity of *V. vulnificus* strains towards ampicillin, chloramphenicol, tetracycline and gentamycin. Similar results were
reported by Sanjeev and Mukundan (2003) while studying the antibiotic sensitivity of *V. vulnificus* strains isolated from iced and frozen fishery products.

### 2.21. Haemolytic activity of HPVs

Association between haemolysin production and virulence of *V. parahaemolyticus* has been noted by a number of workers. However, the role of haemolysin in the virulence is not clear. Lot of information is available on the haemolytic activity of *V. parahaemolyticus*. However, information regarding *V. fluvialis*, *V. furnissii* and *V. cincinnatiensis* is scanty.

Kato *et al.* (1965) found that vibrio strains isolated from diarrheal stool gave a haemolytic reaction on autoclaved brain heart infusion agar containing 5 % human blood, 3 % sodium chloride and 0.001 % crystal violet, whereas the strains isolated from marine sources were non-haemolytic. This medium was modified by Wagatsuma (1968) to give more clear-cut haemolysis by *V. parahaemolyticus* and the test was named "Kanagawa reaction". Among the virulence factors of *V. parahaemolyticus*, a close correlation between the production of thermostable direct haemolysin (TDH) and human pathogenicity was established by Miyamoto *et al.* (1969). For these authors, 96.5 % of the strains isolated from patients stools produced a thermostable haemolysin, while 99.0 % of those isolated from the marine environment did not. A simple means of revealing this haemolysin is to use the Wagatsuma medium, a blood agar in which strains with β-haemolysis are called KP⁺ve (Kanagawa phenomenon⁺ve), and those which are non-haemolytic are termed KP⁻ve (Miyamoto *et al.*, 1969; Blake *et al.*, 1980).

Although *V. parahaemolyticus* has been recognized as an important cause of gastrointestinal disease associated with the consumption of seafood, not all strains of this species are considered to be truly pathogenic (Nichibuchi and Kaper, 1995). TDH is a major virulence determinant of K⁺ve *V. parahaemolyticus* and that the K⁺ve phenotype makes a good marker for virulent strains (Nichibuchi and Kaper, 1995). Tdh genes have also been demonstrated in some strains of *V. mimicus*, *V. cholerae non-O1, non-O139* and in all strains of *V. hollisae* (Nichibuchi and Kaper, 1995).
However, recently a K⁺ve V. parahaemolyticus strains that produce a toxin TDH related haemolysin (TRH) was found associated with gastroenteritis (Suthienkul et al., 1995), and it appears that both TDH and TRH haemolysin are important virulence factors in the pathogenesis of V. parahaemolyicus (Suthienkul et al., 1995). According to Zhang and Austin (2005) there are four haemolysin families in Vibrio spp., namely the TDH (Thermostable Direct Haemolysin) family, the HIYA (El Tor Haemolysin) family, the TLH (Thermolabile Haemolysin) family and the δ-VPH (Thermostable Haemolysin) family.

Haemolysins act on erythrocytes membranes thus lysing the cells which lead to the freeing up of iron- binding proteins namely haemoglobin, transferring and lactoferrin (Zhang and Austin 2005). Lang et al. (2004) have reported that haemolysin induces cation permeability and activates endogenous gardos K⁺ve channels, consequences include break down of phosphatidyl serine asymmetry, which depends at least partially on cellular loss of K⁺ve. The pore-forming activity of haemolysin is not restricted to erythrocytes, but extends to a wide range of other cell types including mast cells, neutrophils, and polymorpho nuclear cells and enhances virulence by causing tissue damage (Zhang and Austin 2005).

Osawa et al. (1996) examined the ability of V. parahaemolyticus to hydrolyze urea, with specific reference to the presence of the thermostable direct haemolysin gene (tdh) and the gene for thermostable related haemolysin (trh) and suggested that urea hydrolysis is not a reliable marker for identifying tdh- carrying V. parahaemolyticus strains but may be a marker for trh- carrying strains. Kelly and Stroh (1989) reported that clinical isolates of V. parahaemolyticus obtained from patients with locally acquired gastroenteritis in Canada hydrolyzed urea, but none of the isolates were kanagawa haemolysin positive as determined by the in vitro plate test.

Quadri and Zuberi (1977) were perhaps the first to report a very high percentage of K⁺ve isolates (52.5 %) from fish and shellfish samples from Karachi, Pakistan. Karunasagar and Mohankumar (1980) observed 25 % incidence of K⁺ve strains in the environment around Mangalore. Sanjeev (1999) in a study of brackishwater culture pond isolated 12.4 % of K⁺ve V. parahaemolyticus strains. Malathi et al. (1988) have reported the isolation of V. parahaemolyticus and V. vulnificus strains
capable of producing haemolysins from seafoods. Bandekar et al. (1982) observed 12 % K\textsuperscript{+}ve strains among isolates from shrimp in Bombay. Hara-kudo et al. (2003) have reported the prevalence of pandemic tdh- positive *V. parahaemolyticus* O3:K6 from 10 % of shellfish samples in Japan.

High incidence of K\textsuperscript{+}ve strains among isolates form houseflies led Chaterjee (1980) to speculate that flies might be involved in carrying K\textsuperscript{+}ve strains from human excreta. The high incidence of K\textsuperscript{+}ve strain in the environment in India and Pakistan remain unexplained. Uchimura *et al.* (1993) observed high prevalence of thermostable direct haemolysin like toxin in *V. mimicus* strains isolated from diarrheal patients.

Douet *et al.* (1992) showed that TDH is haemolytic against erythrocytes of various animal species (including human erythrocytes, equine erythrocytes are the most resistant) and cytolytic against cultured mammalian cells.

97 % of *V. fluvialis* and 65.55 % of *V. parahaemolyticus* strain isolated from seafood and aquacultured foods in Taiwan showed haemolytic activity (Wong *et al*., 1992). In another study Wong *et al.* (1993) have reported the thermostable haemolytic activity of *V. fluvialis* strain after being heated at 100°C but not at 60°C. Chikahira and Hamada (1988) provided an extensive description of the toxic products produced by nine environmental strains of *V. fumissii*. Magalhaes *et al.* (1993) isolated sixteen strains of *V. fumissii* from 16 Brazilian patients with diarrhea and found that most were haemolytic on blood agar.

Zhang and Austin (2005) reported that pathogenic vibrio species were capable of producing various virulence factors consisting of enterotoxin, haemolysin, cytotoxin, protease, lipase, phospholipase, siderophore, adhesive factor and/or haemagglutinins. Results obtained by Baffone *et al.* (2001) corroborates the above view, they observed vibrio strains consisting of *V. alginolyticus*, *V. parahaemolyticus*, *V. cholerae non-O1*, *V. vulnificus*, *V. fluvialis*, *V. fumissii* and *V. metschnikovii* were in general positive for lipase and gelatinase activity (100 %), haemolytic activity (7.2 %), urease activity (19.2 %), adhesiveness (63 %), cytotoxicity (57.6 %). 23 % of the strains gave positive results in the ileal loop test in rats and 23 % showed the ability to infect the laboratory animals and suggested that pathogenicity of vibrios could be the result of a combination of factors.