Isolation and Characterization of Enteropathogenic *Vibrio parahaemolyticus* from Fish along the Coastal Parts of Gujarat

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**ABSTRACT**

The production of a family of haemolysins-thermostable direct haemolysin (Vp-TDH) and Vp-TDH-related haemolysin (Vp-TRH) has been reported in clinical isolates of *Vibrio parahaemolyticus*. The work was aimed to study the occurrence of *V. parahaemolyticus*, in fishes sold in the domestic markets of Gujarat and incidence of *Vibrio parahaemolyticus*. A total of 155 fish samples comprising fin fishes and prawns were collected from various fish markets in and around Anand (Gujarat), India. Eighteen samples were found to be positive for *V. parahaemolyticus*. Pathogenicity of the isolates was confirmed by Kanagawa phenomenon. Antibiogram revealed 88% isolates to be sensitive to chloramphenicol, followed by 72.22% to ciprofloxacin and 66.66% to cephalothin. Multiple antibiotic resistance index was found to be 0.4, indicating higher resistance acquired by the isolates. The present study demonstrated the need to adopt marine fish safety measures for the products meant for human consumption.

**Keywords:** Antibiotic resistance, fin fishes, prawns, *Vibrio parahaemolyticus*

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**Introduction**

The West coast of India is by far the most important area so far as fisheries production is concerned, accounting for over 70 per cent of national production. Gujarat’s contribution to fish production is the third largest in India followed by West Bengal and Kerala and second largest in marine fish production after Kerala (GIDB, 2006). Vibrios of seafood origin have attracted increasing attention from time to time as it is found to be one of the most important cause of human food poisoning. Earlier reports revealed food poisoning due to the consumption of seafood contaminated with *Vibrio* spp., particularly *Vibrio parahaemolyticus* (Wong et al., 2000). Fish and fishery products constitute an important food component for a large section of world population, more so in developing countries, where fish forms a cheap source of protein (Karunsagar et al., 2004). The majority of outbreaks have also been epidemiologically traced to the consumption of fishes and shellfishes originating from warm coastal waters.

The isolation of Kanagawa positive strains of *V. parahaemolyticus* from food samples is significant because it is generally believed that Kanagawa positive strains are encountered in cases of gastroenteritis (Okuda et al., 1997). Although *V. parahaemolyticus* forms the common cause of gastroenteritis transmitted by contaminated seafood consumption, the true incidence was not known probably due to underreporting of cases and lack of proper study. Keeping in view the above points in the present investigation an attempt was made to study incidence of *Vibrio parahaemolyticus* along with enteropathogenic potential.
Materials and Methods

A total of 155 samples comprising finfish (95) and prawn (60) were purchased from retail fish markets in and around Anand of Gujarat, India. Immediately after collection, the samples were placed in the sterile polythene bags and brought to the laboratory in icebox within 2-3 h for isolation of the organism.

Isolation and identification of *V. parahaemolyticus*

Different organs of fish samples were analysed for the presence of *V. parahaemolyticus* such as muscle (n = 68), intestine (n = 55), and gill (n = 32) for selective isolation of *V. parahaemolyticus*.

A loopful of culture from APW after 18-24 h enrichment was streaked onto thiosulfate citrate bile salt sucrose agar (TCBS) and incubated at 37°C for 24 h. The characteristic large colonies (3-4 mm) with light blue or green centers on TCBS were regarded as presumptive *V. parahaemolyticus* and further subjected to morphological, cultural and biochemical characterization. A series of biochemical tests as per BAM, USFDA method (Kaysner and DePaola, 2004) were used for the identification of *Vibrio* isolates.

Kanagawa test

The Kanagawa reaction was carried on Wagatsuma agar using 2% human RBCs. Loopfuls of overnight grown culture of *V. parahaemolyticus* isolates were spot inoculated onto Wagatsuma agar plates and incubated at 37°C for 24 h. The α-haemolysis of human RBCs after 24 h incubation was interpreted as positive for Kanagawa reaction (Beauchat, 1982).

Antibiogram

In this study, all 18 isolates were screened for their sensitivity to 12 antibiotics viz., ampicillin, penicillin, chloramphenicol, ciprofloxacin, cephalothin, tetracycline, oxytetracycline, erythromycin, gentamicin, streptomycin, vancomycin and rifampicin.

Antibiotic sensitivity of the *V. parahaemolyticus* cultures was determined by the standard disk diffusion method (Bauer et al., 1966). The multiple antibiotic resistance (MAR) index was determined as the ratio between the number of antibiotics for which the organism is resistant and the total number of antibiotics used (Kasper et al., 1990).

Results and Discussion

Examination of 155 raw finfish and prawn samples resulted in the isolation of 18 (11.61%) *V. parahaemolyticus* isolates. The presence of *V. parahaemolyticus* was maximum in intestine (9, 16.36%) followed by muscle (7, 10.29%) and gills (2, 6.25%).

Being halophilic organism the occurrence of *V. parahaemolyticus* in the coastal area is not uncommon. The present overall isolation rate in marine samples was 14.28%, which appeared to be similar to the findings Wong (1995). As compared to the present findings, lower level of incidence (10.0%) was reported by Rajapandiyan et al., (2009), whereas higher level of incidence (48.5%) of *V. parahaemolyticus* in marine fish has been reported by Subhashini and Krishnaiah (2010). The present findings suggested that the gastrointestinal tract of the marine water fishes and freshwater fishes provide a unique microcosm for the proliferation of *V. parahaemolyticus*.

Out of 18 *V. parahaemolyticus* isolates, 2 were found to be positive for Kanagawa reactions. Kanagawa positive strains contain a thermostable direct haemolysin (TDH), which might be responsible for gastroenteritis syndrome by *V. parahaemolyticus* (Miyamoto et al. 1969).

The incidence of Kanagawa positive strains of *V. parahaemolyticus* marine ecosystems stresses the need for hygienic handling of sea foods at every stage. Honda et al. (1988) identified a TDH-related haemolysin (TRH) from Kanagawa negative strains of *V. parahaemolyticus* and this TRH was immunologically similar but not identical to TDH. Therefore, it appeared to be evident that the Kanagawa negative strains of *V. parahaemolyticus* also produce some toxic materials, which may play some role in the pathogenicity.
Table 1. Antibiogram of *V. parahaemolyticus* (n= 18) isolated from fish

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<tr>
<td>Sensitive</td>
<td>8 (44.44)</td>
<td>3 (16.66)</td>
<td>16 (66.66)</td>
<td>12 (72.22)</td>
<td>13 (11.11)</td>
<td>2 (5.55)</td>
<td>0 (11.11)</td>
<td>1 (22.22)</td>
<td>4 (61.11)</td>
<td>11 (61.11)</td>
<td>3 (16.66)</td>
<td>0</td>
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<tr>
<td>Intermediate</td>
<td>2 (11.11)</td>
<td>1 (5.55)</td>
<td>2 (11.11)</td>
<td>4 (22.22)</td>
<td>5 (16.66)</td>
<td>3 (5.55)</td>
<td>0 (22.22)</td>
<td>1 (16.66)</td>
<td>2 (44.44)</td>
<td>3 (16.66)</td>
<td>8 (44.44)</td>
<td>0</td>
</tr>
<tr>
<td>Resistant</td>
<td>8 (44.44)</td>
<td>14 (77.77)</td>
<td>0 (11.11)</td>
<td>2 (72.22)</td>
<td>0 (100)</td>
<td>13 (100)</td>
<td>18 (88.88)</td>
<td>16 (66.66)</td>
<td>12 (22.22)</td>
<td>4 (38.88)</td>
<td>7 (38.88)</td>
<td>18 (100)</td>
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Ampicillin (A); Penicillin (P); Chloramphenicol (C); Cephalothin (CEP); Ciprofloxacin (CF); Tetracycline (T); Oxytetracycline (O); Rifampicin (R); Erythromycin (E); Gentamicin (G); Streptomycin (S); Vancomycin (VA)

In the present study, *V. parahaemolyticus* isolates were found variably resistant to the antibiotics tested (Table 1). All the isolates showed highest sensitivity towards chloramphenicol (88.88%), followed by ciprofloxacin (72.22%), cephalothin (66.66%), gentamicin (61.11%) and ampicillin (44.44%). The pattern clearly indicated that the high per cent of *Vibrio* isolates were resistant to oxytetracycline and vancomycin (100%), followed by rifampicin (88.88%), penicillin (77.77%), tetracycline (72.22%) and erythromycin (66.66%).

The occurrence of antibiotic resistant *V. parahaemolyticus* might be associated with indiscriminate or uncontrolled use of antibiotics to contain fish or shrimp diseases, faecal and industrial pollution of water bodies (Watkins and Cabelli, 1985).

In the present study MAR was found to be 0.4, which was similar to the findings of Chakraborty *et al.* (2008) indicating higher resistance acquired by the isolates. Isolation of Kanagawa positive strains of *V. parahaemolyticus* in food samples is a cause for concern.

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References


