NOTE

On prevalence of vibrios in some marine fin and shell fishes

B. R. SINGH¹, M. P. SAXENA², S. B. KULSHRESHTHA² AND K. N. KAPOOR

Division of Veterinary Public Health, Indian Veterinary Research Institute,
Izatnagar — 243 122, India

ABSTRACT

The prevalence of *Vibrio parahaemolyticus* and *V. cholerae* in fishes, crustaceans and molluscs from various markets of India was studied. For this purpose 426 samples comprising 192 freshwater fish, 182 marine fish, 13 marine prawns, 13 freshwater prawns and 26 molluscs were analysed. In the study, 12 *V. parahaemolyticus*, 10 *V. cholerae* non O:1, one *V. fischeri* and 9 *V. alginolyticus* were isolated. Of these *V. parahaemolyticus* was isolated only from marine species. All the NAG vibrios isolated were negative for production of heat labile cholera toxin when tested with latex agglutination and coagglutination tests. The importance of these vibrios in diarrhoeal disorders in fish consumers has been suggested.

Attempts were made to study the prevalence of various vibrios in fin and shell fishes of both freshwater and marine origin, collected from various markets of India. For isolating vibrios, slime was swabbed from the skin/gills of fish and other seafoods purchased from fish markets of various regions of India (Table 1). Swabs were brought to laboratory in alkaline vibrio enrichment broth and 3% glucose salt teepol broth (Chatterjee *et al.*, 1970), for isolating *V. cholerae* and *V. parahaemolyticus* respectively. After 18-24 hr incubation at 37°C, cultures from both the tubes were streaked on TCBS agar (Hi-Media) plates. Suspected bluish green colonies for *V. parahaemolyticus* and yellowish colonies for *V. cholerae* were picked up on nutrient agar slants. Bacterial isolates were further characterised on the basis of morphological, biochemical and cultural characteristics (Baumann and Schubert, 1984). All vibrio isolates were sent to National Institute of Cholera and Enteric Diseases, Calcutta, for confirmation and serotyping.

For enterotoxigenicity testing, vibrios isolated from the specimens were grown in 100 ml conical flasks containing 10 ml of brain heart infusion broth (Hi-Media) at 37°C for 24 hr on a shaker water-bath (100 rpm). Broth cultures were centrifuged at 5,000 g for 30 minutes, then supernatant collected and filtered through 0.45 μm pore size membrane filters to make cell free culture supernatants (CFCS). The CFCS preparations were stored at 0-4°C till further testing within a week. Just a

Present address: 1. Department of Veterinary Microbiology, G. B. Pant University of Agriculture & Technology, Pantnagar — 263 145, India. 2. Kumar Pathologicals, Kanoongoyan, Bareilly, India.
day before testing CFCS were concentrated by dialysing against polyethylene glycol 6,000 (Hi-Media). Enterotoxicity of concentrated CFCS was determined by latex agglutination and coagglutination tests (Ito et al., 1983; Kapoor, 1989) by using anticholera toxin antibody coated latex beads and similarly coated protein A positive staphylococcal cells respectively. For performing the tests two drops each of CFCS preparations and antibody coated latex bead and staphylococcal cell suspensions were mixed separately on perlex glass agglutination plates, for 30 seconds and observed for clumping of beads/staphylococcal cells. For comparison, known cholera toxin preparation containing one PFU (permeability factor unit)/ml and sterilised normal saline solution (0.85%, pH 7.2) were used as positive and negative controls respectively.

From fresh water fish, marine fish, marine prawns and molluscs, 10 V. cholerae non 0:1 (NAG vibrios) were isolated but not from fresh water prawns (Table 2). Isolation of NAG vibrios from brackish water and fresh water fish might be due to disposal of sewage in natural waters (Barua and Burrow, 1974). Hence fish could carry enteropathogens including vibrios on their body surface and gills, from the surrounding contaminated waters. Apart from contamination of water, evidences are sufficient to support the view that vibrios are commonly present on fish and other aquatic animals, as aquatic animals are their natural reservoirs (Blake et al., 1980).

All the NAG vibrio isolates in the study could not be proved enterotoxigenic with serological techniques namely DAT and coagglutination test. However, both the serological tests could only detect heat labile cholera toxin and not the heat stable cytotoxic and cytotoxic enterotoxins (Takeda et al., 1991). By the results it cannot be concluded that NAG vibrio isolates in the study were non-pathogenic for human beings, unless tested in detail for other virulence factors as enterotoxins, haemolysins and colonization factors of the strains (Baumann and Schubert, 1984). Non 0:1 vibrios have been reported to cause cholera like disease in isolated cases as

<table>
<thead>
<tr>
<th>Collection centres of fish for isolation of vibrios</th>
<th>Number of isolation attempted</th>
<th>Types of vibrios isolated (Number of isolates)</th>
<th>% of positive samples to vibrios</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bombay</td>
<td>55</td>
<td>V. parahaemolyticus (3)</td>
<td>5.45</td>
</tr>
<tr>
<td>Bareilly</td>
<td>160</td>
<td>V. alginiticus (3)</td>
<td>5.45</td>
</tr>
<tr>
<td>Delhi</td>
<td>31</td>
<td>V. cholerae non 0:1</td>
<td>3.12</td>
</tr>
<tr>
<td>Delhi</td>
<td>31</td>
<td>V. fischeri (1)</td>
<td>0.62</td>
</tr>
<tr>
<td>Calcutta</td>
<td>19</td>
<td>None</td>
<td>0.00</td>
</tr>
<tr>
<td>Bijnere</td>
<td>34</td>
<td>V. cholerae non 0:1 (3)</td>
<td>15.85</td>
</tr>
<tr>
<td>Cochin</td>
<td>117</td>
<td>V. cholerae non 0:1 (2)</td>
<td>5.88</td>
</tr>
<tr>
<td>Cochin</td>
<td>117</td>
<td>V. parahaemolyticus (9)</td>
<td>7.69</td>
</tr>
<tr>
<td>Cochin</td>
<td>117</td>
<td>V. alginiticus (6)</td>
<td>5.13</td>
</tr>
</tbody>
</table>
well as in outbreak form (Hughes et al., 1978; De Paola et al., 1987). So the isolation of NAG vibrios in the present study is important from the health point of view of seafood consumers.

In the present study V. parahaemolyticus was isolated only from marine fish (Table 2). Further it was interesting to find that all the V. parahaemolyticus strains isolated were from the fish samples of Bombay and Cochin markets where most of the marine fish were in fresh condition compared to the cold stored marine foods available at Delhi and Calcutta fish markets. It may be also due to loss of V. parahaemolyticus during storage or lack of prevalence of this micro-organism in seafoods, but the first probability appears to be more relevant (Molenda et al., 1972). Vibriob parahaemolyticus food poisoning has been reported to be associated largely with the consumption of fresh marine foods because cold storage and processing of seafoods have detrimental effects on the survival of this organism in foods (Molenda et al., 1972).

Other vibrios isolated viz. V. fischeri and V. alginolyticus (Tables 1, 2) might be of little public health significance because these have been rarely reported to be associated with foodborne diseases. However, presence of V. alginolyticus is important because it frequently causes wound infections in fishermen and seashers (Blake et al., 1980). Vibriob alginolyticus has also been reported to cause diarrhoeal disease in human beings consuming contaminated fish (Blake et al., 1980). Hence it may be concluded that the presence of various vibrios in the aquatic foods of India should be viewed seriously in the light of a growing industry with vast export potentials.

References


