**Prevalence of enteropathogens of zoonotic significance in fish and fish products from Ludhiana**

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**ABSTRACT**

Bacteriological examination of 23 raw fresh water fish and 8 fish pakoda samples revealed incidence of *Escherichia coli*, *Salmonella*, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Proteus vulgaris*, *Enterobacter aerogenes*, *Enterobacter agglomerans* and unidentified gram negative bacilli in 9.6, 3.2, 19.3, 6.4, 6.4, 9.6, 3.2 and 9.6% samples, respectively. Three *E. coli* isolates belonging to serotypes O: 8 (2) and O: 106 (1) were detected and the only *Salmonella* isolate was serotyped as *Salmonella typhimurium*. All the 21 isolates of various enteropathogens were tested for enterotoxigenicity by rabbit ileal loop method and skin permeability factor assay, which demonstrated that 38.0% of the isolates were enterotoxigenic. Enterotoxigenic response observed in *S. typhimurium*, *E. coli*, *K. pneumoniae* and *P. mirabilis* was 100.0, 66.6, 66.6 and 50.0 per cent, respectively. Majority of these bacteria are important from human health point of view due to their pathogenicity and direct association with the onset of diarrhea in man.

**Introduction**

Members of *Enterobacteriaceae* especially *Escherichia coli* are frequent causes of food poisoning outbreaks due to their ability to produce one or more enterotoxin (Sharma et al., 2005). *E. coli* and *Klebsiella pneumoniae* are known to produce either a heat labile (LT) or a heat stable (ST) enterotoxin that can be produced individually or in combination (Sharma et al., 2003). *Salmonellae* are capable of producing a diarrheogenic toxin resembling to that of the LT produced by *E. coli* and *K. pneumoniae* (Klipstein and Engert, 1977), which in turn is antigenically analogous to the cholera toxin elaborated by *Vibrio cholerae*. Fish and fishery products are frequently contaminated with aerobic enteropathogens (Singh and Kulshrestha, 1993; 1994; Sharma et al., 1995) and on few occasions the consumption of these infected fish and their products have resulted in serious diarrheal illnesses (D’Aoust, 1989; Saxena et al., 1987). Thus, it becomes essential to determine the hygienic quality of fish and fishery products as sold in the market so as to safeguard the consumer’s health. In India adequate progress has been made in this area particularly for various sea...
foods and marine fishes, but the statistics are lacking regarding the hygienic and bacteriological quality of fresh water fish and their products particularly in Punjab where fish and its products are turning out to be a regular part in the human diet. Taking into consideration all the above facts, the present study was initiated with an attempt to isolate, identify and characterize various enteropathogens in fresh water fish and fish products marketed in Ludhiana. The study was also designed to detect the enterotoxigenic ability of the isolates by using rabbit ileal loop (RIL) method and rabbit skin vasopermeability factor (PF) assay.

**Materials and methods**

Thirtyone samples of fish (23) and fish pakoda (8) were procured at random from local fish market, retail meat shops and local vendors of Ludhiana. All the samples were immediately transferred to laboratory on ice after their collection under aseptic conditions in UV sterilized polythene sachets. All the samples were processed with in 3-4 hours after their collection. Isolation and identification of various aerobic enteropathogens was made on the basis of their morphological, cultural and biochemical characteristics as per the standards (Sharma et al., 2005). Presumptive Salmonella isolates were verified by slide agglutination test with Salmonella Poly 'O' Sera (IVRI, Izatnagar). Serotyping of E. coli and Salmonella isolates was made from National Salmonella and Escherichia Center, CRI, Kasauli (H.P). Cell free culture filtrates (CFCFs) (containing the putative enterotoxins) of all the isolates were prepared according to the method suggested by Singh et al. (1983). The filtrates were concentrated by transferring on to a dialyzing bag (Sigma) and placing on polyethylene glycol-6000 in a petri plate at 4°C. Ten fold concentrations of CFCFs were achieved in 4-6 hours and they were stored at 4°C till use. Enterotoxins were detected by RIL method and PF assay (Sharma et al., 2005). A dilation index (Volume of fluid accumulated in the loop in ml / length of the loop in cm) of 0.3 or above was considered as a positive enterotoxic response of the test isolate in RIL method, whereas a bluing zone with a diameter of 5 mm or more (to the nearest millimeter in two different directions) on the back of rabbit was treated to be a significant enterotoxic response in PF assay. In comparison, the standard enterotoxigenic E. coli strain (MTCC723) (IMTECH Chandigarh) was used as positive control in these biological assays.

**Results and discussion**

The prevalence rates of different aerobic enteropathogens isolated from fish and fish pakoda have been presented in Table 1. Out of 23 fresh fish samples, E. coli, Salmonella typhimurium, K. pneumoniae, Proteus mirabilis, P. vulgaris, Enterobacter aerogenes and some unidentified gram negative bacilli were detected in the percentages of 13.0, 4.3, 26.0, 8.7, 8.7, 8.7 and 8.7 respectively. Fish Pakoda samples also revealed E. aerogenes, E. agglomerans and unidentified gram-negative bacilli in percentages of 8.7 each. K. pneumoniae was found to be the predominant species whereas Salmonella was recorded lowest in fish and fish pakoda. The overall prevalence rate of 9.6% for E. coli in market fish of Ludhiana is slightly on the lower side when compared to the data quoted by other workers (Bachhil, 1987; Kumari et al., 2001). However, Singh and Kulshrestha (1994) reported low prevalence of E. coli in fresh water fish that support the findings of this study and also the absence of E. coli in fish pakoda encountered in this study is in agreement with the findings of the former. This could be the result of high heat treatment imparted to the product.
Prevalence of enteropathogens of zoonotic significance in fish and fish product

Table 1: Incidence and enterotoxigenicity of various enteropathogens isolated from fish and fish pakoda

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Organism isolated</th>
<th>Fish</th>
<th>Fish Pakoda</th>
<th>Total</th>
<th>Enterotoxigenic Positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td><em>Escherichia coli</em></td>
<td>3 (13.0)</td>
<td>0</td>
<td>3 (9.6)</td>
<td>2 (66.6)</td>
</tr>
<tr>
<td>2.</td>
<td><em>S. typhimurium</em></td>
<td>1 (4.3)</td>
<td>0</td>
<td>1 (3.2)</td>
<td>1 (100.0)</td>
</tr>
<tr>
<td>3.</td>
<td><em>Klebsiella pneumoniae</em></td>
<td>6 (26.0)</td>
<td>0</td>
<td>6 (19.3)</td>
<td>4 (66.6)</td>
</tr>
<tr>
<td>4.</td>
<td><em>Proteus mirabilis</em></td>
<td>2 (8.7)</td>
<td>0</td>
<td>2 (6.4)</td>
<td>1 (50.0)</td>
</tr>
<tr>
<td>5.</td>
<td><em>Proteus vulgaris</em></td>
<td>2 (8.7)</td>
<td>0</td>
<td>2 (6.4)</td>
<td>0</td>
</tr>
<tr>
<td>6.</td>
<td><em>Enterobacter aerogenes</em></td>
<td>2 (8.7)</td>
<td>1 (12.5)</td>
<td>3 (9.6)</td>
<td>0</td>
</tr>
<tr>
<td>7.</td>
<td><em>Enterobacter agglomerans</em></td>
<td>0</td>
<td>1 (12.5)</td>
<td>1 (3.2)</td>
<td>0</td>
</tr>
<tr>
<td>8.</td>
<td>Unidentified Gram Negative Bacilli</td>
<td>2 (8.7)</td>
<td>1 (12.5)</td>
<td>3 (9.6)</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>18 (78.2)</td>
<td>3 (37.5)</td>
<td>21 (67.7)</td>
<td>8 (38.0)</td>
</tr>
</tbody>
</table>

During its preparation, two different serotypes of *E. coli* viz., O:8 (2) and O:106 (1) were detected in this investigation. Saxena et al. (1987) also detected O:8 E. coli serotype from fresh water fish. This E. coli serovar is of great public health significance, being one of the most common human enterotoxigenic E. coli serovar (Levine, 1987). In addition, another E. coli serotype O:106 was found during this work, which is enteropathogenic to man as well (Sharma et al., 1995). The only *Salmonella* isolate detected from raw fish was serotyped as *Salmonella* typhimurium 4,5,12:i:1,2. A few earlier workers have documented the low prevalence of *Salmonella* in fresh water fishes (Singh and Kulshrestha, 1993). Nevertheless, *Salmonella* typhimurium is a well-known food poisoning organism of high zoonotic importance. From the view point of the daily increasing consumption of fish and its products by the local population and very low infectious dose of *Salmonella*, the incidence in fish (even if in low numbers) is of great concern for human health and thus it must be considered as a probable cause of food poisoning outbreak. *Klebsiella pneumoniae* is an opportunistic pathogen that has been associated with human diarrhea both in young and adults. Besides diarrhea, *K. pneumoniae* also causes various other systemic disorders in man. Wadstroem et al. (1976) isolated enterotoxigenic strains of *K. pneumoniae* from clinical cases of diarrhea and studied its role in food-borne illnesses. The isolation of *K. pneumoniae* from fresh water fish during this study is in agreement with the findings of Kumari et al. (2001). The low prevalence of *Proteus mirabilis* and *P. vulgaris* substantiates the findings of some earlier workers (Okafor and Nzeako, 1985; Kumari et al., 2001). Their presence may be of public health significance, as occasionally different Proteus species are known to provoke diarrhea, dysentery and other maladies in man. The isolation of Enterobacter aerogenes, *E. agglomerans* and unidentified gram-negative bacilli might be of little human health importance as they have rarely been accounted for food poisoning. However their presence might signal the fecal contamination of water. Results of enterotoxigenicity testing reveal that out of a total of 21 isolates of different species of enteropathogens, 8 (38.0%) were found to be enterotoxigenic. Highest enterotoxigenic response (100.0%) was observed in *Salmonella*, which was followed by *E. coli* (66.6%), *K. pneumoniae* (66.6%) and *P. mirabilis* (50.0%). None out of the rest of the isolates could demonstrate any significant enterotoxigenic response. Singh and Kulshrestha (1994) also illustrated that out of different *E. coli*
strains isolated from fresh water fish, 30.7% were enterotoxigenic. Singh and Kulshrestha (1992) have reported incidence of enterotoxigenic K. pneumoniae in fish and fish products. It can be concluded from the present findings that the presence of enterotoxigenic strains of E. coli, Salmonella typhimurium, K. pneumoniae is a potent human health hazard which may prove as the source for food poisoning outbreaks upon getting the conditions that are favourable for their growth and toxin production.

References


