Pathogenic and pandemic *Vibrio parahaemolyticus* detection in fish and shellfish isolates

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In this study, one hundred and seventy isolates of *V. parahaemolyticus* from marine water fish and shellfish were analyzed by PCR for the presence of *tdh* and *trh* virulence genes and *toxRS* region pandemic gene. The *tdh* and *trh* gene was detected in 55.3% and 0.59% of isolates respectively; whereas, *toxRS* region was detected in 34.1% isolates. Findings of the present study suggested that marine water fish and shellfish isolates of *V. parahaemolyticus* harbor pathogenic as well as pandemic genes which may serve as source for clinical infection through food chain.

**[Keywords: Vibrio parahaemolyticus, tdh-gene, trh-gene, toxRS region, GS-PCR].**

Introduction

*Vibrio parahaemolyticus* is a halophilic Gram-negative pathogenic bacterium, a natural inhabitant of temperate and/or tropical estuarine, coastal as well as marine environment and can be found in crabs, shrimps, fish, oysters, mussels and other seafoods1,2. It is one of the major causes of gastroenteritis, associated with consumption of raw or undercooked fish and shellfish3 and responsible for approximately 25% of total food-borne diseases in relation to other *Vibrio* species4. It accounts for about 10% of the gastroenteritis associated with seafood in Kolkata, India5 and its detection in acute human diarrheal cases ranged between 3.5–23.9%6.

*V. parahaemolyticus* isolates implicated in food poisoning outbreaks were found to harbor two major virulence factors viz., thermo stable direct haemolysin (TDH) encoded by the *tdh* or TDH-related hemolysin (TRH) encoded by *trh* gene or both7. More than 90% of clinical *V. parahaemolyticus* isolates possess *tdh* gene8, however, *tdh* and *trh* gene were rarely detected in the environmental isolates of *V. parahaemolyticus*9, European Commission guidelines also suggested the consideration of these virulent factors such as *tdh* and *trh* for judging seafoods10. The globally disseminated pandemic clone of *V. parahaemolyticus* exhibits unique sequence within the *toxRS* operon, which encodes trans-membrane proteins involved in the regulation of virulence-associated genes. Molecular typing technique named group-specific PCR (GS-PCR) can detect nucleotide variations within the 1364bp *toxRS* region unique to the pandemic clone11. Thus, the present study was undertaken to identify the occurrence of pathogenic and pandemic *V. parahaemolyticus* in fish and shellfish by characterizing their virulence genotypes and pandemic clone genotype.

Materials and Methods

A total of 170 VP-toxR PCR confirmed *V. parahaemolyticus* isolates of fish and shellfish samples collected from Kolkata market were analyzed for the presence of virulence genes (*tdh* and *trh*) and pandemic gene (*toxRS* region) by PCR (Table 2). The reference strains Vp-Kx-V138 (*tdh* gene & *toxRS* region) and VP-230 (*trh* gene) were obtained from National Institute of Cholera and Enteric Diseases, Kolkata.

The reference strains and *V. parahaemolyticus* isolates were revived in nutrient broth supplemented with 3% NaCl and used for DNA extraction by modified boiled cell method12. PCR was standardized using three sets of primer P1, P2 & GS-VP (Table 1)11, 13 to detect the presence of virulent genes (*tdh* and *trh*) and pandemic gene (*toxRS* region),
The PCR reaction mixture for amplification of all targeted genes consisted of 5.0 µl of DNA template, 2.5 µl of 10X PCR buffer (500 mM KCl, 100 mM Tris-HCl, pH-8.3; 15 mM MgCl₂), 2 µl dNTP (2.5 mM dNTP each mix.), 2.0 µl (10 pmol/µl) of each primers, 0.3 µl (1 unit) of Taq DNA polymerase and de-ionised water to make final volume up to 25 µl.

Amplification conditions for tdh gene consisted of 30 cycles with denaturation at 94ºC for 90s, annealing at 50ºC for 90s and extension at 72ºC for 90s whereas, trh gene was amplified with 30 cycles consisting of denaturation at 94ºC for 60s, annealing at 55ºC for 90s and extension at 72ºC for 90s. Cyclic conditions for toxRS region (GS-PCR) consisted of 25 cycles of denaturation at 96°C for 60s, annealing at 45°C for 120s and extension at 72°C for180s, with a final extension for 7 min at 72°C for each reaction. The PCR products were resolved by electrophoresis in 1.2% agarose gel along with 0.5% ethidium bromide and documented under gel documentation system.

Results and Discussion

One hundred and seventy V. parahaemolyticus isolates obtained from fish and shellfish were screened for virulence marker genes tdh, trh and pandemic marker gene toxRS region by PCR (Table 2). Specific amplicon size of 199bp, 250bp and 651bp were identified after gel electrophoresis with reference strains as well as isolates positive for tdh, trh and toxRS region, respectively (Fig. 1).

![Fig.1- Standardization of PCR for detection of tdh, trh and toxRS region of V. parahaemolyticus.](image)

The PCR analysis showed that tdh gene was present in 55.3% (94/170) isolates which was in agreement with the previous studies which reported that 50% to 74% V. parahaemolyticus isolates from dietary fishes, shellfishes and oysters were tdh positive14,15,16. On the contrary to the findings of present study, lower frequency (0 to 17%) of tdh positive V. parahaemolyticus were detected in environmental and seafood samples in several studies 17,18,19,20,21.

In contrast to tdh gene, trh gene was detected only in 0.59 % (1/170) isolates of V. parahaemolyticus. This finding was in accordance with other study, reporting only 0.87% isolates as trh positive22. On the other hand, noticeably higher (59.3%) incidences of trh positive V. parahaemolyticus were reported from oysters by Deepanjali et al (2005)21. The present study reflects that the occurrence of tdh and trh gene in the isolates, from the region under study, was contrary to each other and was in accordance to the finding of Deepanjali et al. (2005)21. Hence, it could be concluded that screening of tdh gene should be preferred to trh gene for identification of pathogenic V. parahaemolyticus in the region Kolkata, India.

The toxRS operon of pandemic strains contains a unique sequence (toxRS) encoding trans-membrane proteins involved in the regulation of virulence associated genes and an isolate possessing both tdh and toxR is considered as pandemic11. In the present study by GS-PCR it was found that 34.1% (58/170) V. parahaemolyticus isolates harbored toxRS region. This study also showed that 61.7% (58/94) tdh positive isolates was positive for toxRS region, whereas none of the tdh negative or trh positive isolates was found positive for pandemic gene. Thus it could be concluded that 34.1% V. parahaemolyticus isolates from marine water fish and shellfish were pathogenic as well as pandemic, however, 21.2% isolates were only pathogenic V. parahaemolyticus.
Table 2: Distribution of virulence and pandemic gene in *Vibrio parahaemolyticus* isolates

<table>
<thead>
<tr>
<th>Fish and shellfish species</th>
<th>No. of isolates used</th>
<th>Virulence genes detection</th>
<th>Pandemic gene detection</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td><em>tdh</em> gene</td>
<td><em>trh</em> gene</td>
</tr>
<tr>
<td><em>Lates calcarifer</em></td>
<td>31</td>
<td>19</td>
<td>01</td>
</tr>
<tr>
<td><em>Lophius piscatorius</em></td>
<td>28</td>
<td>15</td>
<td>Nil</td>
</tr>
<tr>
<td><em>Penaeus monodon</em></td>
<td>31</td>
<td>16</td>
<td>Nil</td>
</tr>
<tr>
<td><em>Liza parsia</em></td>
<td>28</td>
<td>13</td>
<td>Nil</td>
</tr>
<tr>
<td><em>Ompok pabda</em></td>
<td>30</td>
<td>20</td>
<td>Nil</td>
</tr>
<tr>
<td><em>Pampus chinensis</em></td>
<td>22</td>
<td>11</td>
<td>Nil</td>
</tr>
<tr>
<td>Total</td>
<td>170</td>
<td>94</td>
<td>01</td>
</tr>
</tbody>
</table>

**Conclusion**

The presence of pathogenic and pandemic *V. parahaemolyticus* isolates in marine water fish and shellfish indicated the potential risk of globally present gastroenteritis. However, in spite of high prevalence (~34%) of pandemic clone of *V. parahaemolyticus* in fish and shellfish of the marine environment, this organism had been isolated and reported in average frequency in routine gastroenteritis and considered as a source of limited incidences of sizeable epidemic gastroenteritis which needs further study.

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**References**


