Classical ctxB gene in *Vibrio cholerae* O1 and O56 serogroups from Kerala, South India

*Vibrio cholerae* is the causative agent of the life-threatening diarrhoeal disease cholera. Cholera toxin (CT), the major enterotoxin produced by *V. cholerae*, is responsible for profuse diarrhoea. The major biotypes of *V. cholerae* O1 (El Tor and classical) are differentiated on the basis of some phenotypic and genotypic traits (Safa et al., 2010). The genotypic traits are based on variations in tcpA, rstR (Safa et al., 2010) and hlyA gene sequence (Rivera et al., 2001). The tcpA, hlyA and rstR genes encode a major pilin subunit, a cytotoxic haemolysin and a repressor (which regulates the replication of phage DNA), respectively. Strains other than O1 and O139 (collectively called non-O1/non-O139) are ubiquitously distributed in aquatic environments and are not associated with epidemic cholera (Islam et al., 1994). However, sporadic cases of diarrhoea and extraintestinal infections caused by non-O1/non-O139 strains have been reported widely (Sharma et al., 1998; Cheng et al., 2004; Restrepo et al., 2006). Recently, many new genotypes of El Tor strains have emerged carrying variants of CT (Bhuiyan et al., 2009; Joshi & Albert, 2009; Taneja et al., 2009). These variants have spread to countries of Asia and Africa including India and Bangladesh (Safa et al., 2010). In the present study, the biotype-associated phenotypic and genotypic characteristics of recently isolated O1 and O56 strains from southern Kerala, India, were analysed. The ctxB genes from all the strains were amplified by using Phusion High-Fidelity DNA Polymerase (NEB) and ctxB-F and ctxB-R primers (Olsvik et al., 1993). The purified PCR products were 'A' tailed and subsequently ligated to the pGEM-T Easy vector and sequenced on both strands using T7 and SP6 primers with the BigDye Terminator kit (Applied Biosystems).

Four strains were analysed in the present investigation. Strains A199 and A217 were isolated from Vembanad Lake, Allapuzha district, whereas O1 strain A880 was isolated from a drinking water source in Allapuzha district, Kerala. MCV09, a clinical O1 strain, was isolated from a hospitalized patient admitted to Medical College Hospital, Trivandrum, Kerala. All the test strains were positive in the Voges–Proskauer test and for β-haemolysis of sheep erythrocytes and exhibited resistance to polymyxin B (Table 1). The O1 strains showed El Tor-specific tcpA and hlyA genes. However, O56 serogroup strains exhibited the El Tor-specific hlyA gene and a variant allele of the tcpA gene (GenBank accession no. EU362122). The strains of serogroup O56 showed both the classical and El Tor type of rstR gene whereas O1 strains showed only the El Tor type of rstR. This indicates the presence of two different copies of CTX prophages in O56 strains. Unexpectedly, ctxB gene sequencing in all test strains revealed 100% identity to ctxB of classical strain 569B (GenBank accession no. U25679). Hence according to the new genotyping scheme developed by Safa et al. (2010), the O1 and O56 serogroup strains investigated could be classified into ctxB genotype 1 since they possess the ctxB1 allele.

The El Tor strains isolated after 2001 in Bangladesh carried the ctxB1 allele, producing CT of the typical classical biotype, designated 'altered El Tor' (Nair et al., 2006). Recently, many altered El Tor strains have been reported from the northern and eastern parts of India (Raychoudhuri et al., 2009; Taneja et al., 2009). The classical strains are believed to be extinct and have not been reported in recent times. It has been suggested that toxigenic non-O1/non-O139 strains (for example *V. cholerae* O114) may represent an alternative reservoir for classical type phages (Udden et al., 2008). From our investigation, it is clear that O56 strains carried classical ctxB so we hypothesize that apart from O114, the strains of serogroup O56 might also act as a reservoir for classical type phages. However, further studies are required to validate this hypothesis. The continued presence of such strains in the aquatic environment and phage-mediated horizontal transfer may be the reason for the emergence of recent O1 El Tor strains with classical CTX phages. The pathogenicity of altered El Tor strains is not properly understood but it is well known that cholera caused by classical strains is more severe than that caused by El Tor strains (Kaper et al., 1995). In this context, such genetic changes in CT and clinical and environmental strains deserve attention. To our knowledge, this is the first report of altered El Tor strains from South India, and our results necessitate continuous surveillance of all clinical cholera cases as well as regular monitoring of aquatic systems.

**Acknowledgements**

The authors are grateful to Professor M. Radhakrishna Pillai, Director, RGC, for the facilities provided. We are grateful to Dr Ramani Bhai, Professor, Department of Microbiology, Government Medical College, Trivandrum, for providing *V. cholerae* strain MCV09 used in this study. The research fellowship provided by the Council for Scientific and Industrial Research (CSIR) to P.K. is greatly acknowledged.

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Table 1. Phenotypic and genotypic traits of the *V. cholerae* isolates

<table>
<thead>
<tr>
<th>Strain</th>
<th>Serogroup</th>
<th>Source</th>
<th>Phenotype</th>
<th>Genotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>MCV09</td>
<td>O1</td>
<td>Clinical</td>
<td>+</td>
<td>R</td>
</tr>
<tr>
<td>A880</td>
<td>O1</td>
<td>Environmental</td>
<td>+</td>
<td>R</td>
</tr>
<tr>
<td>A217</td>
<td>O56</td>
<td>Environmental</td>
<td>+</td>
<td>R</td>
</tr>
<tr>
<td>A199</td>
<td>O56</td>
<td>Environmental</td>
<td>+</td>
<td>R</td>
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