Rapid Spread of *Vibrio cholerae* O1 El Tor Variant in Odisha, Eastern India, in 2008 and 2009


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The emergence and spread of *Vibrio cholerae* O1 El Tor variant strains causing severe diarrhea has been witnessed worldwide in recent years. In the state of Odisha, India, the spread of the *V. cholerae* O1 El Tor variant strains was studied during outbreaks in 2008 and 2009. Analysis of 194 *V. cholerae* O1 Ogawa strains revealed that *V. cholerae* O1 El Tor variant strains are spreading gradually throughout the state, causing outbreaks replacing typical *V. cholerae* O1 El Tor biotype strains.

*Cholera* is a major concern worldwide, occurring as epidemic and pandemic forms caused by *Vibrio cholerae*, a Gram-negative bacterium. Until recently, among 206 serogroups of *V. cholerae* identified so far (1), only O1 and O139 serogroups are associated with clinical cholera (2). *V. cholerae* O1 is further classified into two biotypes, classical and El Tor (3), based on assays such as chicken cell agglutination (CCA), Voges-Proskauer (VP) reaction, sheep erythrocyte lysis, and polymyxin B susceptibility test (2). Clinical manifestations of cholera are caused by cholera toxin (CT), the principal virulence factor encoded by *ctxAB* of *V. cholerae*, located on the CTX prophage integrated on the *V. cholerae* chromosome (2). The cholera toxin B subunit encoded by *ctxB* defines its biotype, i.e., a classical strain has classical-type *ctxB* (*ctxB<sup>C</sup>*) and an El Tor strain has El Tor-type *ctxB* (*ctxB<sup>E</sup>*) (4). *V. cholerae* O1 El Tor biotype has been the causative agent in seven recent pandemics, while the classical biotype caused the earlier pandemics.

According to the recently redefined biotyping scheme, *V. cholerae* O1 strains carrying mixed phenotypes of classical and El Tor biotypes (susceptibility to 50 units polymyxin B and positive for CCA and VP test) are designated hybrid biotypes, whereas *V. cholerae* O1 strains with a typical El Tor phenotype (resistant to 50 units of polymyxin B and positive for CCA and VP test) but carrying *ctxB<sup>EE</sup>* are designated El Tor variant (4). Using the mismatch amplification of mutation assay (MAMA) PCR for *ctxB* alleles (5), it was shown that *V. cholerae* O1 El Tor variant strains carrying *ctxB<sup>C</sup>* have emerged since 1992 (6) and have subsequently spread worldwide, causing outbreaks of severe diarrhea (7).

Cholera has been reported in Odisha, an eastern state of India with 30 districts, over the last 2 decades (8–10). In our previous study, we reported a large outbreak of cholera in three tribal districts of Odisha in 2007 caused by a *V. cholerae* O1 El Tor variant carrying *ctxB<sup>C</sup>* (10). Subsequently, outbreaks of cholera were observed during 2008 and 2009 in different parts of Odisha. The present study was designed to characterize the isolates of *V. cholerae* obtained during these outbreaks and describe the chronology of the appearance of *V. cholerae* O1 El Tor variant strains and its spread in Odisha.

During the study period, 202 rectal swabs were collected from hospitalized diarrhea patients from 21 districts in 5 regions (northern, southern, coastal, western, and central) of Odisha, and 720 swabs were collected from patients in the Infectious Disease Hospital (IDH), Puri, India. Information relating to the origin of

**TABLE 1** Phenotypic and genotypic traits of *V. cholerae* O1 strains isolated during 2008 and 2009 from different regions of Odisha State, India

<table>
<thead>
<tr>
<th>Region or location of isolation&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Obtained from rectal swabs</th>
<th>P. B. R.</th>
<th>P. B.</th>
<th><em>ctxB&lt;sup&gt;E&lt;/sup&gt;</em> EL Tor variant</th>
<th><em>ctxB&lt;sup&gt;EE&lt;/sup&gt;</em> hybrid 1</th>
<th><em>ctxB&lt;sup&gt;EE&lt;/sup&gt;</em> hybrid 2</th>
<th><em>ctxB&lt;sup&gt;EE&lt;/sup&gt;</em> hybrid 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Northern</td>
<td>47</td>
<td>26</td>
<td>25</td>
<td>11</td>
<td>3</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>Southern</td>
<td>26</td>
<td>4</td>
<td>4</td>
<td>3</td>
<td>1</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>Coastal</td>
<td>86</td>
<td>27</td>
<td>22</td>
<td>5</td>
<td>16</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>Western</td>
<td>26</td>
<td>7</td>
<td>7</td>
<td>1</td>
<td>6</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>Central</td>
<td>17</td>
<td>10</td>
<td>7</td>
<td>3</td>
<td>6</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>IDH</td>
<td>720</td>
<td>120</td>
<td>113</td>
<td>24</td>
<td>80</td>
<td>9</td>
<td>4</td>
</tr>
<tr>
<td>Total</td>
<td>922</td>
<td>194 (21)</td>
<td>178 (92)</td>
<td>16 (8)</td>
<td>41 (23)</td>
<td>123 (69)</td>
<td>14 (8)</td>
</tr>
</tbody>
</table>

<sup>a</sup> IDH, Infectious Disease Hospital, Puri, India.

<sup>b</sup> PBR, polymyxin B resistant; PBS, polymyxin B sensitive.
each rectal swab was collected by questionnaire, asking (i) when
the diarrhea started, (ii) what clinical symptoms were present, (iii)
the source of drinking water, (iv) the date of the first case detected
in the outbreak, (v) how many persons were affected and the du-
ration of the outbreak, and (vi) the age group and sex of the af-
fected patient. Samples were processed for the isolation of
*V. cholerae* following standard methods (11, 12). Slide agglutination was
done with polyvalent O1 and monospecific Ogawa/Inaba anti-
sersum (Difco, BD, United States) to confirm the serogroups. Iso-
lates of *V. cholerae* that agglutinated with O1 antiserum were bio-
typed using polymyxin B susceptibility (50-U discs; Hi-media,
Mumbai, India), CCA, and VP tests following standard proce-
dures (13).

The presence of virulence and surface antigen genes ctxA, tcpA,
*zot*, *ace*, and *wbe* was determined by using PCR assays (9, 14).
MAMA PCR was performed to detect the ctxB classical and/or El
Tor type harbored by *V. cholerae* O1 serogroup isolates, using
specific ctxB primer pairs (5).

The analysis of 922 rectal swabs revealed that 194 (21%) were
*V. cholerae* O1 Ogawa (Table 1). All 194 *V. cholerae* O1 Ogawa
strains were found to carry ctxA, tcpA (El Tor biotype), wbe, zot, and ace, which confirmed their toxin-producing capacity and epidemiologic potential, and the presence of wbe provided molecular evidence for the O1 serogroup. The results indicated that toxigenic V. cholerae O1 Ogawa was the causative agent of cholera during the study period. An age wise distribution showed that most of the cholera cases were adult (89%), with nearly the same proportions of males and females (0.9:1).

Of the 194 V. cholerae strains, 178 (92%) were resistant to polymyxin B and positive for VP and CCA tests, with the remaining 16 (8%) strains being susceptible to polymyxin B and positive for VP and CCA tests. Biotyping suggested that all 178 strains were prototype El Tor, also evident by the presence of tcpA of the El Tor biotype. MAMA PCR for ctxB of these 178 isolates revealed that 41 (23%) strains carried ctxB*, indicating that these were the typical El Tor biotype, 123 (69%) carried ctxB+, all of which had the conventional El Tor phenotype, implying the El Tor variant, and the remaining 14 (8%) were found to carry ctxB* and ctxB* (ctxB*+), indicating hybrid strains according to the redefined biotyping scheme.

The remaining 16 (8%) V. cholerae O1 strains with polymyxin B sensitivity and VP and CCA test positivities were found to harbor tcpA of the El Tor biotype, indicating that these strains belonged neither to El Tor nor to classical biotypes. MAMA PCR for the ctxB gene of these isolates revealed that 12 (75%) strains carried ctxB* and 4 (25%) strains carried ctxB*+ctxB*, indicating hybrid strains. The Odisha strains of V. cholerae detected with hybrid biotype and ctxB*+ctxB* could be arbitrarily classified into 3 different hybrid groups (Table 1).

The spread of V. cholerae O1 El Tor variant strains, confirmed from each site of sample collection, is shown in Figure 1. The date of appearance of V. cholerae O1 El Tor variant strains, confirmed at each site, was obtained from the data sheets from the hospitals concerned. The spread of El Tor variant/hybrid strains indicated by arrows in the figure is speculative and derived from the pattern of appearance of variant/hybrid strains over time in a specified area.

Recently, it was reported that the new mutant El Tor variant strains with increased virulence that causing the 2007 cholera outbreak in Odisha (16) had spread to Haiti, causing the 2010 Haitian cholera outbreak and, since then, have spread globally, replacing the classical cholera outbreak subsequent to the super cyclone in Orissa, India. Epidemics of V. cholerae O1 El Tor biotype and O139 among the nonimmune population (18). However, immuno factors coupled with poor hygiene, lack of safe drinking water, and improper disposal of waste and excreta may have assisted the swift spread of the new variant in Odisha.

Interestingly, remarkable genetic evolution of the V. cholerae-producing strains of mixed traits/phenotypes of the two biotypes has been documented over the last decade. Beginning in the late 1990s, newly emerged variant strains from Bangladesh have the genetic makeup of El Tor with ctxB* only. In Odisha, between 1995 and 2006, isolates of El Tor strains were detected with ctxB* only (10), whereas some strains in the present study are unique in having ctxB genes of both the classical and El Tor biotype. Similar findings have been reported in other parts of India and in Thailand (13, 19). From the observation of stepwise evolution of V. cholerae, such as classical biotype with classical ctxB, El Tor with El Tor ctxB, El Tor variant with classical ctxB, and hybrid with classical ctxB and El Tor ctxB, surveillance programs should be closely monitored to document the future evolution of V. cholerae strains and their subsequent spread around the globe, with respect to cholera outbreaks and their impact on treatment and public health measures.

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REFERENCES
15. Reference deleted.