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.

**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 isolationa</th>
<th>Obtained from rectal swabs</th>
<th>V. cholerae O1 (n = 194)</th>
<th>PBR (n = 178)</th>
<th>CBS El Tor variant</th>
<th>CBS*E hybrid 1</th>
<th>CBS*E hybrid 2</th>
<th>CBS*E hybrid 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Northern</td>
<td>47</td>
<td>25</td>
<td>11</td>
<td>2</td>
<td>1</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>Southern</td>
<td>26</td>
<td>4</td>
<td>1</td>
<td>3</td>
<td>3</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>Coastal</td>
<td>86</td>
<td>22</td>
<td>3</td>
<td>16</td>
<td>3</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>Western</td>
<td>26</td>
<td>7</td>
<td>1</td>
<td>6</td>
<td>3</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Central</td>
<td>17</td>
<td>10</td>
<td>3</td>
<td>1</td>
<td>9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IDH</td>
<td>720</td>
<td>120</td>
<td>24</td>
<td>80</td>
<td>9</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>922</strong></td>
<td><strong>194 (21)</strong></td>
<td><strong>178 (92)</strong></td>
<td><strong>16 (8)</strong></td>
<td><strong>14 (8)</strong></td>
<td><strong>12 (75)</strong></td>
<td><strong>4 (25)</strong></td>
</tr>
</tbody>
</table>

* IDH, Infectious Disease Hospital, Puri, India.
* 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 duration of the outbreak, and (vi) the age group and sex of the affected 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 antisera (Difco, BD, United States) to confirm the serogroups. Isolates 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 procedures (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

FIG 1 Spread of *V. cholerae* O1 El Tor variant strains in Odisha State. Arrows indicate speculative probable spread, based on the first isolation of *V. cholerae* O1 in a given area. Dates of isolation are given as day.month.year.
strains were found to carry ctxA, tcpA (El Tor biotype), wbc, zot, and ace, which confirmed their toxin-producing capacity and epidemic 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 agewise 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<sup>E</sup>, indicating that these were the typical El Tor biotype, 123 (69%) carried ctxB<sup>C</sup>, all of which had the conventional El Tor phenotype, implying the El Tor variant, and the remaining 14 (8%) were found to carry ctxB<sup>C</sup> and ctxB<sup>E</sup> (ctxB<sup>C+E</sup>), 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<sup>E</sup> and 4 (25%) strains carried ctxB<sup>C+E</sup>, indicating hybrid strains. The Odisha strains of V. cholerae detected with hybrid biotype and ctxB<sup>C+E</sup> 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 caused the 2007 cholera outbreak in Odisha (16) had spread to Haiti, causing the 2010 Haitian cholera outbreak and, since then, have spread globally, replacing El Tor strains. The first cholera outbreak began in Khurda, a coastal district, in May 2008, caused by El Tor variant strains that were of the same clone as the 2007 El Tor variant strains (data not shown), and spread to most areas. In 2009, Kalahandi District (western part of Odisha) and Rajnagar Block in Kendrapada District (eastern part of Odisha) were affected by large cholera outbreaks. Cholera was reported from 21 of 30 districts in the state over the 8-month study period, and the V. cholerae O1 El Tor variant dominated, replacing El Tor strains. However, it is possible that the remaining districts might have been affected by cholera that went unreported. The data support the fact that V. cholerae O1 El Tor variant strains were found circulating in Odisha subsequent to the 2007 cholera epidemic.

The preponderance of adults affected by El Tor variant strains in our study, as well as in other studies, clearly indicates that lack of immunity against V. cholerae O1 El Tor with modified ctxB<sup>E</sup> results in rapid spread, with infection of new hosts as was seen during the spread of V. cholerae O1 El Tor biotype and V. cholerae O139 among the nonimmune population (18). However, immunity 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<sup>E</sup> only. In Odisha, during 1995 and 2006, isolates of El Tor strains were detected with ctxB<sup>C</sup> 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 ctxE, 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.


