Rapid spread of *Vibrio cholerae* O1 El Tor variant in Odisha, eastern India 2008-09


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Abstract

Emergence and spread of *V. cholerae* O1 El Tor variant causing severe diarrhea has been witnessed worldwide in recent years. In the state of Odisha, India, the spread of *V. cholerae* O1 El Tor variant was studied during outbreaks in 2008 and 2009. Analysis of 194 *V. cholerae* Ogawa strains revealed that gradually *V. cholerae* O1 El Tor variant strains are spreading throughout the state causing outbreaks replacing normal *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, amongst 206 serogroups of *V. cholerae* identified so far (19), only O1 and O139 serogroups are associated with clinical cholera (7). *V. cholerae* O1 is further classified into two biotypes, classical and El Tor (1), based on assays such as chicken cell agglutination (CCA), Voges-Proskauer (VP) reaction, sheep erythrocyte lysis and polymyxin B susceptibility test (7). Clinical manifestations of cholera are caused by cholera toxin (CT), the principal virulence factor encoded by the *ctxAB* of *V. cholerae* located on the CTX prophage integrated on the *V. cholerae* chromosome (7). The cholera toxin B subunit encoded by *ctxB* defines its biotype, i.e. a classical strain has classical type *ctxB* and an El Tor strain has El Tor type *ctxB*. *V. cholerae* O1 El Tor biotype has been the causative agent in seven recent pandemic while classical biotype caused the earlier pandemics.

According to the recently redefined bio-typing 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 as hybrid biotypes where as *V. cholerae* O1 with a typical El Tor phenotype (resistant to 50 units of polymyxin B
and positive for CCA and VP test) but carrying \textit{ctxB} classical (\textit{ctxB}^C) are designated El Tor variant (13). Using the mis-matching amplification of mutation assay (MAMA) polymerase chain reaction (PCR) for \textit{ctxB} alleles (8), it was shown that \textit{V. cholerae} O1 El Tor variants carrying \textit{ctxB}^C have emerged since 1992 (10) and subsequently have spread worldwide causing outbreaks of severe diarrhoea (15).

Cholera has been reported in Odisha, an eastern state of India with 30 districts over last two decades (2, 6, 12). In our previous study, we reported a large outbreak of cholera in three tribal districts of Odisha in 2007 caused by \textit{V. cholerae} O1 El Tor variant carrying \textit{ctxB}^C (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 \textit{V. cholerae} obtained during these outbreaks and describe the chronology of appearance of \textit{V. cholerae} O1 El Tor variants and its spread in Odisha.

During the study period, 202 rectal swabs were collected from hospitalized diarrhoea patients from 21 districts within 5 regions (northern, southern, coastal, western and central) of Odisha and 720 swabs were collected from patients in the Infectious Disease Hospital (IDH), Puri. Information relating to the origin of each rectal swab was collected by questionnaire asking: (1) when the diarrhea started, (2) clinical symptoms, (3) source of drinking water, (4) date of first case detected in the outbreak, (5) how many persons were affected and the duration of the outbreak and (6) the age group and sex of the affected patient. Samples were processed for the isolation of \textit{V. cholerae} following standard methods (9, 18). Slide agglutination was done with polyvalent O1 and mono-specific Ogawa/Inaba antiserum (DIFCO, USA) to confirm the serogroups. Isolates of \textit{V. cholerae} that agglutinated with O1 antiserum were biotyped using polymixin B
susceptibility (50U, Hi-media, Mumbai, India), CCA and VP tests following standard procedures (17).

The presence of virulence and surface antigen genes ctxA, tcpA, zot, ace and wbe were determined by using PCR assays (5, 14). MAMA PCR was performed to detect the ctxB classical and/or El Tor harbored by V. cholerae O1 serogroup using specific ctxB primer pairs (8).

The analysis of 922 rectal swabs revealed 194 (21%) were V. cholerae O1 Ogawa (Table 1). All the 194 V. cholerae O1 Ogawa strains were found to carry ctxA, tcpA (El Tor biotype), wbe, zot and ace that confirmed their toxin producing capacity and epidemic potential and the presence of wbe provided molecular evidence for 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 proportion in males and females (0.9:1).

Of the 194 V. cholerae strains, 178 (92%) were resistant to Polymixin B and positive for VP and CCA tests with the remaining 16 (8%) strains being susceptible to Polymixin 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, El Tor biotype. MAMA-PCR for ctxB of these 178 isolates revealed that 41 (23%) strains carried ctxB El Tor (ctxB<sup>E</sup>) indicating that these were typical El Tor biotype, 123 (69%) carried ctxB<sup>C</sup>; all having conventional El Tor phenotype implying El Tor variant and remaining 14 (8%) were found to carry ctxB<sup>C+E</sup> indicating hybrid strains according to the redefined biotyping scheme.
The remaining 16 (8%) *V. cholerae* O1 strains with Polymixin B sensitivity and being positive in VP and CCA tests were found to harbor the tcpA, El Tor biotype indicating that these strains belonged neither to El Tor nor to classical biotypes. MAMA-PCR for ctxB gene of these isolates revealed that 12 (75%) strains carried ctxB<sup>C</sup> and 4 (25%) strains carried ctxB<sup>E+C</sup> indicating hybrid strains. The Odisha strains of *V. cholerae* detected with hybrid biotype and ctxB<sup>E+C</sup> could be arbitrarily classified into 3 different hybrid groups (Table 1).

The spread of *V. cholerae* O1 El Tor variants confirmed from each site of sample collection are 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 variants/hybrid as indicated by arrows in the figure is speculation and derived from the pattern of appearance of variants/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 (4) had spread to Haiti, causing the 2010 Haitian cholera outbreak and since then spreading globally, replacing prototype El Tor (3). During 2008-09, the El Tor variant spread to most parts of Odisha, following the 2007 cholera outbreak. The first cholera outbreak began in Khurda, a coastal district, in May 2008 caused by El Tor variant that of the same clone of 2007 strains of El Tor variant (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 out of 30 districts in the state over the 8-month study period and *V. cholerae* O1 El Tor variant
dominated, replacing El Tor strains. However it is possible that the remaining districts
might have affected by cholera but were unreported. The data supported that *V. cholerae*
O1 El Tor variant was found circulating in Odisha subsequent to the 2007 cholera
epidemic.

The preponderance of adults affected by El Tor variant in our study, as well as in
other studies, clearly indicates that lack of immunity against *V. cholerae* O1, El Tor with
modified *ctxB* results in rapid spread, infecting new hosts as was seen during the spread
of *V. cholerae* O1, El Tor biotype and O139 among the non-immune population (16).

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, the remarkable genetic evolution of the *V. cholerae* producing
strains of mixed traits/phenotypes of the two biotypes have been documented over the
last decade. Beginning in late 1990s, newly emerged variants from Bangladesh have the
genetic make up of El Tor with *ctxB* only. In Odisha, between 1995 and 2006, isolates
of El Tor strains were detected with *ctxB* only (12) whereas some strains in the present
study are unique, in having *ctxB* gene of both classical and El Tor biotype. Similar
findings have been reported in other parts of India and in Thailand (17, 11). From the
observation of stepwise evolution of *V. cholerae* like classical 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 round the globe with respect
to cholera outbreaks, and their impact on treatment and public health measures.
Acknowledgements

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References


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Figure 1. Spread of *V. cholerae* O1 El Tor variant in Odisha. Arrows indicate probable spread is speculative and based on first isolation of *V. cholerae* O1 in a given area.
Table 1. Phenotypic and genotypic traits of *V. cholerae* O1 strains isolated during 2008-09 from different regions of Odisha.

<table>
<thead>
<tr>
<th>Geographical regions/IDH</th>
<th>Number of RS</th>
<th><em>V. cholerae</em> O1 n=194</th>
<th><em>V. cholerae</em> O1 n=178</th>
<th>PBR</th>
<th>PBS</th>
<th>PBS^c</th>
<th>PBS^e</th>
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<tr>
<td></td>
<td></td>
<td>PBR^c</td>
<td>PBS^c</td>
<td>ctxB^C</td>
<td>ctxB^e</td>
<td>ctxB^C+E</td>
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<tr>
<td></td>
<td></td>
<td>El Tor</td>
<td>El Tor variant</td>
<td>Hybrid1</td>
<td>Hybrid2</td>
<td>Hybrid3</td>
<td></td>
</tr>
<tr>
<td>Northern</td>
<td>47</td>
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<td>25</td>
<td>11</td>
<td>12</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Southern</td>
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<td>4</td>
<td>4</td>
<td>1</td>
<td>3</td>
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<td></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>
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<td>Western</td>
<td>26</td>
<td>7</td>
<td>7</td>
<td>1</td>
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<td></td>
</tr>
<tr>
<td>Central</td>
<td>17</td>
<td>10</td>
<td>7</td>
<td>3</td>
<td>1</td>
<td>6</td>
<td>3</td>
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<tr>
<td>IDH</td>
<td>720</td>
<td>120</td>
<td>113</td>
<td>7</td>
<td>24</td>
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<td>9</td>
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<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>
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<td></td>
<td>12 (75%)</td>
<td>4 (25%)</td>
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</tbody>
</table>

*IDH – Infectious Disease Hospital, Puri; RS – Rectal swabs; PBR – Polymyxin B resistant; PBS – Polymyxin B sensitive.*