Extended-Spectrum-β-Lactamase-Producing *Escherichia coli* Isolate Possessing the Shiga Toxin Gene (*stx*₁*) Belonging to the O64 Serogroup Associated with Human Disease in India


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Shiga toxin-producing *Escherichia coli* (STEC) causes a spectrum of human sufferings, like bloody diarrhea and even life-threatening conditions, such as hemolytic uremic syndrome (HUS) (1). In addition to the serotype O157:H7, several serogroups of STEC have been isolated from severe outbreaks of human disease in different countries, including India (2, 3). Although STEC strains are not considered a reservoir of extended-spectrum β-lactamases (ESBLs), recently a few reports have been published on the association of STEC isolates with ESBL genes (4–10). The present study was undertaken to investigate the association of ESBL-producing STEC or enteropathogenic *E. coli* (EPEC) with human diarrhea in Mizoram, India.

*E. coli* strains were isolated and identified from 180 nonrepli-cating fecal samples from individual human patients with a history of diarrhea from different hospitals in Mizoram, India, during August 2011 to May 2012. Antimicrobial susceptibility testing and confirmation of ESBL production were done by double-disc synergy testing (DDST) (11). All the ESBL-producing isolates were serotyped for their O serogroups. The *bla*:*CTX-M-1* and/or *bla*:*SHV* gene was detected by PCR assay. The transmission of antibiotic resistance genes was determined by an *in vitro* conjugation assay (12, 13, 14). Multiplex PCR was done to detect the *stx₁*, *stx₂*, *eae*, and *ehoxA* genes (1). Isolates positive for ESBL and STEC marker genes were characterized by enterobacterial repetitive intergenic consensus sequence PCR (ERIC-PCR), repetitive extragenic palindromic PCR (REP-PCR), and randomly amplified polymorphic DNA PCR (RAPD-PCR) (15, 16).

A total of 333 *E. coli* isolates were recovered and identified from 180 fecal samples (Table 1). Altogether, 41/333 (12.31%) isolates were confirmed as ESBL producers by DDST; of these, 29 belonged to 3 serogroups (O64, O89, and O91) and the remaining 12 were untypeable. O64 (16/29) was the predominant serogroup. Altogether, 36/333 (10.81%) and 5/333 (1.5%) isolates were found to be positive for the *bla*:*CTX-M-1* and *bla*:*SHV* (*bla*:*SHV-12*), genes, respectively. The resistance trait from none of the isolates could be transferred to the recipient host by the conjugation method. Altogether, 8/333 (2.40%) and 3/333 (0.90%) isolates were recorded as STEC and EPEC, respectively. Two isolates (0.60%) belonging to serogroup O64 and positive for the *bla*:*CTX-M-1* gene were also positive for the *stx₁* gene. The two isolates revealed identical banding patterns by restriction endonuclease fragment analysis (REA), RAPD-PCR, REP-PCR, and ERIC-PCR.

Antimicrobial resistance among diarrheagenic bacteria, especially the production of ESBLs, has been rising worldwide since the early 1990s, albeit to a lesser extent in STEC isolates (7). A total of 12.31% of *E. coli* isolates were confirmed as ESBL producers, which is also in corroboration with reports by others worldwide (17–20). As in the present study, a higher prevalence of the *bla*:*CTX-M-1* gene than of the *bla*:*SHV* gene has been reported, which may be due to the wide use of extended-spectrum cephalosporins in India, especially ceftiraxone and cefotaxime, or it may be associated with the high mobilization of the encoding genes (21, 22, 23, 24). In a conjugation study, neither of the plasmids carrying any one of the target genes could be transferred horizontally to the recipient isolates. A similarly low transconjugation success was also reported by other workers in Switzerland (25) and Germany (12). Although the ESBL genes (*bla*:*CTX-M-1* and *bla*:*SHV*) could not be transferred horizontally to any recipient strains in this study, this kind of resistance could still worsen the health situation of patients.

Two STEC isolates belonging to serogroup O64 were found to be positive for the *bla*:*CTX-M-1* gene also. To our knowledge, only 7 reports of ESBL-producing STEC/EPEC isolates are available worldwide (5, 8, 9): 3 human isolates belonging to serogroup O26, 1 chicken isolate belonging to serogroup O157 (9), 1 human isolate belonging to serotype O157:H7 (10), 1 cattle isolate belonging to serogroup O111 (6), and 1 highly virulent O104 isolate (26). This is probably the first information on an association of serogroup O64 *E. coli* strains carrying both ESBL and STEC genes. Serogroup O64 is not considered a potential human pathogen (7), but detection of such organisms with a combination of ESBL and

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**TABLE 1** Distribution of serogroups, ESBL genes, and STEC- and/or EPEC-related genes among 41 ESBL-positive *E. coli* isolates

<table>
<thead>
<tr>
<th>Serogroup (no. of isolates)</th>
<th>With <em>bla</em>:<em>CTX-M-1</em></th>
<th>With <em>bla</em>:<em>SHV</em></th>
<th>STEC</th>
</tr>
</thead>
<tbody>
<tr>
<td>O64 (16)</td>
<td>15</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>O89 (6)</td>
<td>6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>O91 (7)</td>
<td>7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>UT (12)</td>
<td>8</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>Total (41)</td>
<td>36</td>
<td>5</td>
<td>2</td>
</tr>
</tbody>
</table>

* The 41 ESBL-positive (DDST) isolates were out of 333 isolates recovered from 180 fecal samples collected from different places in Mizoram, India. UT, untypeable. No EPEC isolates were found.
STEC marker genes is suggestive of a high propensity of this O serogroup to exist in the human and/or animal population.

It is thus concluded that this is the first report of an ESBL-producing E. coli isolate possessing a Shiga toxin gene (stx1) and belonging to serogroup O64 associated with human diarrhea. Future studies should be directed to find out the nature of the selective pressure that may promote such combinations of virulent and resistant pathogens.

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REFERENCES