First Report of the Shiga Toxin 1 Gene in Sorbitol-Fermenting Escherichia coli O157:H-

Sorbitol-fermenting Escherichia coli O157:H- (SF O157) is an emerging pathogen, and several outbreaks have been detected in Europe over the last few years (12). SF O157 is described as an aggressive pathogen frequently giving rise to hemolytic uremic syndrome (HUS) (9). The reservoir and exposure routes of this highly pathogenic bacterium still remain unknown (12). The Shiga toxin genes (stx1 and stx2), which are located in temperate lambdoid bacteriophages, are major virulence factors in enterohemorrhagic E. coli (EHEC) pathogenesis (9). So far, the only stx gene detected in SF O157 is stx2_EDL933 (3, 7). However, SF O157 strains negative for the stx genes but otherwise with virulence profiles and phylogenetic backgrounds identical to those of stx2_EDL933 SF O157 have been isolated from patients with bloody diarrhea (BD) and HUS. These isolates have been named EHEC-LST (EHEC that lost Stx) (9). It has been suggested that a dynamic system in which SF O157 isolates gain and lose stx2_EDL933-carrying bacteriophages exists (2, 10).

During a routine virulence gene screening of EHEC at the National Reference Laboratory of Enteropathogenic Bacteria at the Norwegian Institute of Public Health, an SF O157 strain was isolated from a 68-year-old man with BD. The SF O157 strain was characterized by PCR analysis for the following genetic markers: stx1, stx2, eae (with a method modified from reference 4), ehxA, subA, saa, cdTB, nleB, StcE, and StcE_E103 (L. T. Brandal, C. Sekse, B.-A. Lindstedt, M. Sunde, I. Løbersli, A. M. Urdahl, and G. Kapperud, unpublished data), as well as rbfO157, fliC_H7, terE, and the Shigella resistance locus (SRL) (6). The expression of the stx genes was investigated by Immunocard STAT! EHEC as described by the manufacturer (Meridian Bioscience Europe). Furthermore, stx2 was subtyped (F. Scheutz, L. D. Teel, L. Beutin, D. Piérad, G. Buvens, H. Karch, A. Mellmann, A. Caprioli, R. Tozzoli, A. D. O’Brien, A. R. Melton-Celsa, S. Persson, and N. A. Stockbine, unpublished data). Two different multiple-locus variable-number tandem-repeat analyses (MLVA) were performed: one covering all E. coli strains (8) and another specific for SF O157 (I. løbersli, K. Haugum, L. T. Brandal, and B.-A. Lindstedt, unpublished data). Interestingly, the SF O157 strain isolated in the present study carried stx1 subtype stx1a, which was not previously described in SF O157. Additionally, we showed that stx2 was expressed. This might indicate that stx-negative SF O157 has the ability to transduce not only stx2_EDL933-carrying bacteriophages but also bacteriophages carrying stx1a. Similar findings have been reported for EHEC-LST strain O26:H11/H- (2). Furthermore, the SF O157 strain described here was compared with an SF O157 outbreak strain from a Norwegian outbreak in 2009 that affected 13 children, nine of whom developed HUS and one of whom died (11). Apart from the stx status, the virulence profiles of these two SF O157 isolates were identical (Table 1). Another novel finding in both isolates was the presence of nleB and StcE, neither of which has previously been described in SF O157 (1, 3, 5). It should be noted, however, that nleB is colocalized with efal1, sen, and pagC on the pathogenic O island 122 (O1122) and that the three latter genes have formerly been detected in SF O157 (1). Interestingly, both MLVA methods showed that the stx1a-positive SF O157 strain and the outbreak strain from 2009 were different (Table 1).

To our knowledge, this is the first report showing stx1 (subtype stx1a) in SF O157.

REFERENCES


Table 1: Characteristics of the stx1a-positive SF O157 strain compared to those of the SF O157 outbreak strain from 2009

<table>
<thead>
<tr>
<th>Isolate no.</th>
<th>Clinical data</th>
<th>Virulence profilea</th>
<th>MLVA profile</th>
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<tr>
<td></td>
<td></td>
<td>rbfO157</td>
<td>fliC_H7</td>
</tr>
<tr>
<td>1</td>
<td>Male, 68 years old, BD^</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>2009 outbreak strain causing HUS^</td>
<td>+</td>
<td>+</td>
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a In isolate 1, stx1 was found and the corresponding protein was expressed, whereas isolate 2 harbored stx2 with expression of the protein. No isolates carried subA, saa, or StcE_E103.
b Flagellar H7 is not expressed.
c SRL, Shigella resistance locus.
d Analyzed with an MLVA based on 10 genomic repeat-containing loci (8). The MLVA profile comprises arbitrarily assigned allele numbers based on PCR amplicon sizes for each of the 10 loci.
f BD, bloody diarrhea.
g HUS, hemolytic uremic syndrome.


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