Isolation and Genetic Characterization of a Coinfection of Non-O157 Shiga Toxin-Producing Escherichia coli

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A coinfection of O177:NM and O55:H7 Shiga toxin-producing Escherichia coli (STEC) was identified for a child with acute bloody diarrhea and hemolytic uremic syndrome by using culture and serotype-specific molecular reagents. The profile of O157-related genetic islands revealed that the O55:H7 isolate was highly similar to O157 STEC whereas the O177:NM isolate lacked several fimbrial O islands and non-locus-of-enterocyte-effacement effector determinants. However, both STEC serotypes are known to cause serious disease, and the significant repertoire of virulence determinants in both strains made it impossible to determine their individual contributions to the clinical symptoms.

In August 2006 in New Brunswick, Canada, a 4-year-old child was hospitalized with acute bloody diarrhea and hemolytic uremic syndrome (HUS). These are the characteristic clinical manifestations associated with Shiga toxin-producing Escherichia coli (STEC). In North America, strains of serogroup O157 that do not ferment sorbitol are the most frequently isolated STEC associated with HUS, partly because these strains can be distinguished from the benign E. coli flora present in clinical stool samples using differential media containing sorbitol. In contrast, most non-O157 STEC and some O157 strains do ferment sorbitol and therefore cannot be distinguished from the nonpathogenic fecal flora using this culture-based technique (6). Stool cultures collected from the hospitalized patient 3 to 5 days after onset of the first symptoms and 1 day after progression to bloody diarrhea did not yield O157 STEC, nor did they yield other diarrheal pathogens, such as Campylobacter. Using recently developed molecular tools, clinical samples were screened for STEC, and both O177:NM and O55:H7 strains were ultimately recovered. Both of these serotypes have previously been associated with bloody diarrhea and HUS (5), and further genetic characterization was performed to attempt to identify the virulence potential of the strains individually to determine which strain(s) resulted in the clinical symptoms.

The presence of Shiga toxin in stool samples was first detected with the Premier enzyme immunoassay (Meridian Diagnostics, Inc.). To isolate STEC strains, stool samples were screened for STEC, and both O177 through gnd-based molecular serogrouping and confirmed as O177:NM with traditional serotyping (Table 1). Accordingly, this isolate did not carry lpfA or the espZ-g1 allele associated with O157 but instead carried the espZ-g1 allele.

Template DNA prepared during stool enrichments was also characterized by real-time PCR (Table 1). Enrichments were cultured in BHI broth for 16 to 18 h at 37°C with rotation at 200 rpm. Bulk DNA was purified from 0.5 ml of broth culture using a Puregene genomic DNA purification kit (Gentra Systems, Minneapolis, MN), and the same broth was used for cytotoxicity assays to detect Shiga toxin. The stool enrichment contained the stx2 toxin determinant, and cytotoxicity could be neutralized in cell culture by Stx2 antiserum. Additionally, traits characteristic of O157 STEC, including the espZ-g1 allele, the H7 allele of fbc (14), and lpfA were detected in the stool enrichment, which conflicted with the observed genetic profile for the O177:NM strain isolated from the same patient (Table 1).

Because molecular characterization of the enriched stool preparation suggested the presence of a second pathogenic E. coli, a sorbitol-nonfermenting (SNF) strain was isolated and characterized using Light Upon eXtension (LUX) real-time PCR targeting the toxin determinants stx1 and stx2, molecular O serogrouping using the gnd locus, and subtyping of the locus of enterocyte effacement (LEE) using allele-specific LUX primers for espZ-β, espZ-γ, and ε (2, 3). SNF STEC strains commonly belong to serogroup O157, and to rapidly screen for this serogroup, a LUX assay was developed to target the lpfA locus (strain EDL 933 coding sequence Z5225 of O-island 154 [OI-154], previously observed exclusively in O157:H7 and O157:NM strains (12, 13). LUX oligonucleotides were 5'-GG [OI-154]-cggctAACTCTAACG [OI-154]-cggctAACTCTAACG with the penultimate nucleotide labeled with an Alexa-546 fluorophore and the lowercase nucleotides at the 5' end being those required for LUX primer hairpin formation but not present in the target sequence. The SNF strain was identified as O177 through gnd-based molecular serogrouping and confirmed as O177:NM with traditional serotyping (Table 1). Accordingly, this isolate did not carry lpfA or the espZ-g1 allele associated with O157 but instead carried the espZ-g1 allele.

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<table>
<thead>
<tr>
<th>Sample</th>
<th>Serogroup (gnd allele analysis)</th>
<th>Serotype</th>
<th>Presence of gene</th>
<th>Presence of ( \text{espZ} ) allele</th>
<th>Presence of lpfA OI-154</th>
</tr>
</thead>
<tbody>
<tr>
<td>SMAC isolate</td>
<td>06-5121</td>
<td>O177</td>
<td>stx(_1) -</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Stool enrichment</td>
<td>NA(^a)</td>
<td>O177:NM</td>
<td>stx(_2) +</td>
<td>-</td>
<td>+; ( \gamma_1 )</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>fliC(_{177}) -</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\(^a\) Molecular and serotyping tests are described in the text.

\(^b\) NA, not applicable.

\(^c\) \(-\), real-time PCR amplification signal did not cross cycle threshold; +, real-time cycle threshold exceeded.

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<table>
<thead>
<tr>
<th>Strain</th>
<th>Serotype</th>
<th>Presence of ( \text{stx}_1 )</th>
<th>Presence of ( \text{stx}_2 )</th>
<th>Presence of ( \text{espZ} ) allele</th>
<th>Presence of gene</th>
<th>Presence of fimbrial OI (^d)</th>
</tr>
</thead>
<tbody>
<tr>
<td>06-5231</td>
<td>O55:H7</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>OI-71, OI-122, OI-36</td>
<td>1, 47, 141, 154</td>
</tr>
<tr>
<td>06-5121</td>
<td>O177:NM</td>
<td>-</td>
<td>stx(<em>{2d}) stx(</em>{2c}) +</td>
<td>-</td>
<td>OI-71, OI-122, OI-36</td>
<td>1, 47, 141, 154</td>
</tr>
</tbody>
</table>

\(^d\) The methods utilized were previously described by Karmali et al. (7) and by K. K. Coombes and M. A. Karmali (unpublished).

encoded by the O177:NM strain have both been associated with HUS (10). If STEC pathogenicity for humans could be measured as the sum of virulence determinants, then the O55:H7 isolate encoded the larger arsenal. However, the O177:NM strain also encoded stx2, the LEE pathogenicity island, and significant components of OI-122.

The clinical significance associated with non-O157 STEC is likely understated due to the difficulties associated with microbial isolation. In this study, we implemented several recently developed molecular methods to comprehensively screen and isolate two non-O157 serotypes from clinical stool samples. To our knowledge, this is the first observation of more than one non-O157 STEC serotype isolated concurrently from a single patient. Simultaneous infections of O157 and non-O157 STEC have previously been observed (11), and serological assays have identified mixed STEC infections through immunological responses to O157 and other STEC serogroups (8). Additionally, during an outbreak of O111:NM STEC wherein a HUS patient had antibodies to O111, an O145 STEC strain was isolated from clinical samples, suggesting that both serogroups were involved in the infection (1). Although the O111 strain was not isolated from this patient, the outbreak-associated etiology of this strain and the positive serological diagnosis provided evidence that the O111 STEC strain was the significant pathogen. Otherwise, the routine identification of all STEC serogroups is not currently conducted in the majority of public health microbiology laboratories, but the methods utilized in this study allowed for enhanced detection capabilities beyond serogroup O157 and indicated the possibility of isolating multiple serotypes. Further comparative genetic analyses of the virulence gene dosing effect and comprehensive testing of clinical samples will be required to determine the importance and frequency of non-O157 STEC infections in regard to their potential as human pathogens.

Nucleotide sequence accession numbers. DNA sequences of the O55:H7 stx2, and O177:NM stx2, loci were deposited in GenBank under accession numbers EF584538 and EU086525, respectively.

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REFERENCES


