Serotype diversity as a drawback in the surveillance of Shiga toxin-producing Escherichia coli infections in Brazil

Shiga toxin-producing Escherichia coli (STEC) strains have emerged worldwide as an important cause of gastrointestinal diseases and complications in humans (Nataro & Kaper, 1998). Diarrhoea and haemolytic uraemic syndrome (HUS) associated with STEC have also been reported in patients with acquired immunodeficiency syndrome (AIDS) (Garcia Lara et al., 2000; Suthienkul et al., 2001; Pouessel et al., 2004). Although O157 : H7 is the prominent STEC serotype, in the last decade many non-O157 STEC strains were also reported in diarrhoea-associated illnesses and complications. Some outbreaks caused by non-O157 STEC strains have already been reported, although in many countries these organisms are more frequently associated with sporadic cases of diarrhoea, haemorrhagic colitis and HUS (Brooks et al., 2004; Pradel et al., 2000).

In Brazil, human STEC infections have been mainly associated with cases of diarrhoea, especially in children (Guth et al., 2002, 2005; Irino et al., 2002; Vaz et al., 2004). Except for the study of Vaz et al. (2004), which described the identification of one O157 : H7 STEC strain isolated from an human immunodeficiency virus-infected (HIV) patient in the late 1990s, there are no other reports on STEC infection in patients with AIDS in Brazil. A predominance of O111 and O26 serotypes have been reported among STEC strains identified in São Paulo from 1976 through 1999 (Vaz et al., 2004), but continuous surveillance on the occurrence of STEC serotypes as agents of gastrointestinal infection has important implications for diagnostic procedures and the establishment of control measures. This report addresses the importance of not using serotyping alone as a predictor for the presence of STEC.

A total of 439 patients, comprising 337 children and 102 HIV adult patients with diarrhoea, were investigated for the presence of STEC in the western region of São Paulo State in 2000–2003. Stool samples from HIV patients were sent from six public centres for AIDS treatment, and 97.3 % of faecal samples from children were from one private paediatric hospital. A total of 2115 sorbitol-positive and -negative E. coli colonies were screened for STEC by colony hybridization assays with specific DNA probes for stx1 and stx2 (Vaz et al., 2004). STEC isolates were biochemically characterized as E. coli, and serotyped by standard methods (Ewing, 1986). STEC strains were identified in 5 of the 337 children (1.5 %) aged 0–5, and in 3 of the 102 HIV adult patients (2.9 %), but these differences were not statistically significant. The phenotypic and genotypic characteristics of the STEC strains are shown in the Table 1. The biochemical reactions presented by the STEC strains are in agreement with other studies which reported that only O157 : H7 was sorbitol-negative, while failure to decarboxylate lysine occurred only in O111 STEC strains (Farmer & Davis, 1985; Vaz et al., 2004). A diversity of serotypes was identified among the eight STEC strains isolated. Serotype O77 : H18, and untypable serotypes ONT : H2 (two strains) and ONT : H8 were described, for what is believed to be the first time, as being associated with human infections in Brazil. Nevertheless, serotypes O103 : H2, O111 : NM (non-motile), O118 : H16 and O157 : H7 were also

Table 1. Diversity of serotypes and virulence characteristics among STEC strains associated with human infections in Brazil in 2000–2003

<table>
<thead>
<tr>
<th>STEC serotype*</th>
<th>Patient (age)</th>
<th>Phenotypic and genotypic characteristic†</th>
<th>Sorbitol</th>
<th>LDC</th>
<th>Ehx</th>
<th>Cytotoxic activity</th>
<th>Intimin type</th>
<th>Virulence profile</th>
</tr>
</thead>
<tbody>
<tr>
<td>O111 : HNM</td>
<td>HIV+ (42 years)</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>0</td>
<td>stx1 eae</td>
</tr>
<tr>
<td>O157 : H7</td>
<td>HIV+ §(32 years)</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>γ</td>
<td>stx1 stx2c eae ehxA</td>
</tr>
<tr>
<td>ONT : H2</td>
<td>HIV+ (adult)</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>i</td>
<td>stx1 eae ehxA</td>
</tr>
<tr>
<td>O103 : H2</td>
<td>(1 year 6 months)$</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>i</td>
<td>stx1 eae ehxA</td>
</tr>
<tr>
<td>O118 : H16</td>
<td>(1 year 5 months)§</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>β</td>
<td>stx1 eae ehxA</td>
</tr>
<tr>
<td>O77 : H18</td>
<td>(4 years)$</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>stx1 stx2c ehxA</td>
</tr>
<tr>
<td>ONT : H8</td>
<td>(3 years)$</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>0</td>
<td>stx1 eae ehxA</td>
</tr>
<tr>
<td>ONT : H2</td>
<td>(9 months)$</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>i</td>
<td>stx1 eae ehxA</td>
</tr>
</tbody>
</table>

*NM, Non-motile; NT, non-typable.
†Sorbitol, fermentation of sorbitol; LDC, lysine decarboxylase; Ehx, production of enterohaemolysin.
§Hospitalized with diarrhoea.

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recovered from children and HIV patients, which suggests their possible persistence and circulation in our settings over time. Broad ranges of isolation of O157: H7 strains from HIV patients have been reported in other countries and geographical areas (Suthienkul et al., 2001; Gwavava et al., 2001). In contrast to previous data, which showed that 69% of the STEC strains isolated from children with diarrhea in São Paulo from 1976 through 1999 belonged to serotype O111: H8 and O111:NM (Vaz et al., 2004), in the present study these serotypes were only identified once from an HIV patient (Table 1). All STEC isolates presented cytotoxin activity, and except for the O111 strain all the others produced enterohaemolysin, which were identified as described elsewhere (Vaz et al., 2004).

The stx genotypes determined by RFLP-PCR (Cergole-Novella et al., 2006) showed that except for the O157: H7 strain that carried stx1, stx2c, the other strains harboured only stx1, and one strain carried both stx1, stx2c sequences. In addition, the intimin gene (eae) and the plasmid-encoding enterohaemolysin gene (ehxA) detected by PCR assays as described by Gannon et al. (1993) and Schmidt et al. (1995), respectively, were carried by most (7 of 8; 87.5%) of the strains (Table 1), with four intimin types being identified (Oswald et al., 2000). Even though the majority of the STEC strains isolated in this study exhibited characteristics of enterohaemorrhagic E. coli (EHEC), only the patient from whom the O157: H7 strain was isolated presented bloody diarrhea, whereas others had non-bloody diarrhea without complications.

In conclusion, these data demonstrate the occurrence of important STEC serotypes in children and HIV patients with diarrhea in Brazil, and confirm previous observations (Vaz et al., 2004; Guth et al., 2005) that non-O157 strains are more frequently associated with human infection than the O157: H7 serotype, which may have significant implications for the diagnostic procedures employed in clinical laboratories in Brazil. As the importance of these serotypes varies with geographical region, the search for STEC should rely on the detection of stx genes, rather than testing only for specific serotypes of the classical EHEC strains. Otherwise, four of the eight (50%) STEC strains currently identified would be missed.

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