An update on the microbiology and epidemiology of enteropathogenic *Escherichia coli* in England 2010–2012

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Historically, enteropathogenic *Escherichia coli* (EPEC) are a well-known cause of outbreaks of infantile diarrhoea associated with morbidity and mortality in England. The aim of this study was to provide an update on the microbiology and epidemiology of strains of EPEC in England between 2010 and 2012. A wide range of *E. coli* serogroups were identified, with the most common being *E. coli* O145, O49 and O157. Few isolates (9%) had additional virulence factors (specifically *bfp*, *vtx2f* and *espT* genes) and the majority were classified as atypical EPEC. The majority of cases (86%) were among children. This included a significantly higher percentage (17.4%) of cases aged 0–12 months when compared with cases of other common gastrointestinal pathogens (*P*<0.001). No outbreaks were reported during this period; however, the data indicated that EPEC are still an important cause of sporadic cases of infantile diarrhoea in England.

**INTRODUCTION**

Enteropathogenic *Escherichia coli* (EPEC) were first recognized as a cause of infantile diarrhoea in the 1940s (Bray, 1945) and were associated with outbreaks in hospitals and nurseries in England throughout the 20th century (Taylor, 1970; Smith *et al.*, 1996). Initially, EPEC were defined as belonging to a limited number of *E. coli* serogroups known to be associated with diarrhoeal disease, including O114, O119, O127, O128 and O142, and were identified in the laboratory using antisera raised to these specific serogroups (Smith *et al.*, 1996). Later, Kaper (1998) defined EPEC as diarrhoeagenic *E. coli* (DEC) producing characteristic histopathology, known as attaching and effacing (AE) lesions, on intestinal cells and not producing verocytotoxin. The formation of the AE lesions on the gut mucosa destroys the microvilli, resulting in a mild to severe, persistent diarrhoea often accompanied by abdominal pain. The genes necessary for the formation of AE lesions are located on a 35 kb chromosomal region called the locus of enterocyte effacement (LEE) (McDaniel *et al.*, 1995; Chen & Frankel, 2005). One of these genes, *eae* (*E. coli* attaching and effacing), encodes an outer membrane adhesion protein called intimin and is the target for PCR assays used for the detection of EPEC.

Isolates harbouring the *eae* gene identified at the Gastrointestinal Bacteria Reference Unit (GBRU; Health Protection Agency, London) are tested further using a real-time PCR assay targeting putative pathogenicity genes *bfp*, *vtx2f* and *espT* (see Methods). Typical EPEC (tEPEC) strains possess a plasmid, designated EAF for *E. coli* adherence factor (Cravito *et al.*, 1979), whereas atypical EPEC (aEPEC) do not (Trabulsi *et al.*, 2002). Located on the EAF plasmid is the *bfp* gene cluster containing 14 genes, encoding type IV bundle-forming pili (Bfp) (Clarke *et al.*, 2003). *Vtx2f* is a variant *Vtx2* subtype originally associated with pigeons. Recently, *Vtx2f* has been detected among clinical isolates from children with diarrhoea, sometimes showing severe clinical symptoms (Prager *et al.*, 2009). The *vtx2f* gene is not detected by conventional *vtx2* PCR assays and a separate assay is required (Scheutz *et al.*, 2012). Bulgin *et al.* (2009) found EspT in a subset of invasive EPEC strains. EspT is a member of the WxxxxE family which comprises important virulence factors, essential for intracellular *Salmonella* survival and for *Shigella* cell invasion.

EPEC are not routinely sought in frontline diagnostic laboratories in England. However, the GBRU receives, on average, 930 verocytotoxigenic *E. coli* (VTEC) isolates each year for confirmation and typing, of which approximately 4% are identified as EPEC (positive for the *eae* gene but negative for the *vtx* genes). A small number (<10 per year) are isolated at the GBRU from faecal specimens associated with cases of bloody diarrhoea or haemolytic uraemic
syndrome found to be negative for *Salmonella*, *Campylobacter*, *Shigella* and *E. coli* O157 at the local hospital laboratory. The aim of this study was to describe the serogroups, pathogenicity profiles and epidemiology of strains of EPEC identified at the GBRU between 2010 and 2012.

**METHODS**

**Bacterial strains.** The EPEC strains described in this study were identified at the GBRU from isolates or from faecal samples submitted by frontline diagnostic microbiology laboratories in England between 2010 and 2012. Control strains for the real-time PCRs were E2348 (O127:H6) for the *eae* and *bfp* genes, 4900294 (O128:H17), which has the *vtx2f* gene, and E110019 (O111:H19), which harbours the *espT* gene.

**Bacterial culture of faeces.** At the reference laboratory, each faecal specimen was streaked onto MacConkey agar and sorbitol MacConkey agar with and without cefixime and tellurite and incubated at 37 °C overnight. A small loop of faeces was inoculated into modified tryptone soya enrichment broth (mTSB) and incubated at 37 °C overnight for DNA extraction.

**DNA extraction from isolates and faecal specimens.** Isolates were grown in nutrient broth with shaking at 37 °C for 4 h, and a 10 μl sample was diluted in 450 μl distilled water and boiled for 15 min. For the faecal specimens, after overnight incubation, DNA was extracted from mTSB using Instagene (Bio-Rad). Briefly, 50 μl enrichment broth was added to 950 μl distilled water and boiled for 30 min and boiled for 20 min. Finally, the sample was recentrifuged as above and the supernatant removed to a clean tube.

**Target detection by PCR.** DNA extracts were tested using the routine VTEC real-time PCR detecting the *vtx1*, *vtx2*, *eae* ( intimin) and O157 *rpfB* genes, as described previously (Jenkins et al., 2012). Specifically, for this study, for all specimens positive for *eae* ( intimin), 10–20 colonies were picked from the MacConkey plate and retested for the presence of the *eae* gene. Only those colonies harbouring the *eae* genes were identified biochemically, serotyped and characterized further using a real-time PCR for detection of the *bfp*, *vtx2f* and *eae* genes on a Rotorgene Q (Qiagen). The primers and probes, designed for this study using Primer Express software are shown in Table 1. The amplification parameters were 95 °C for 5 min, followed by 95 °C for 15 s and 60 °C for 60 s, for 40 cycles, with the cycle threshold set at 0.05.

**Results**

**Microbiology.**

Between January 2010 and December 2012, 109 isolates of EPEC were detected at the GBRU (2010, 30; 2011, 41; 2012, 38). Subsequently, the *bfp* gene, associated with tEPEC, was detected in five of these isolates; two carried the *vtx2f* genes and three had *espT* (Table 2). The most common *E. coli* serogroups identified were O145 (17), O49 (five), O157 (five), O125 (four), O26 (four) and O126 (three). Thirty-three of the strains were designated ‘O’ unidentifiable as they did not serotype with any of the known somatic ‘O’ antigens in the typing scheme. Five isolates of *E. coli* O157 included in this study were negative for all *vtx* gene subtypes, failed to phage type using the VTEC O157 typing phages and were sorbitol-fermenting strains.

**Epidemiology.**

Descriptive analyses were performed on 92 of the 109 cases whose isolates are described above. Cases were excluded if they did not reside in England (five cases), if they also had VTEC isolated in the same time frame (seven cases) or if they were isolated during a research study (five cases).

Clinical data were available for 69 (75%) cases. Two cases were reported as asymptomatic and no clinical information

<table>
<thead>
<tr>
<th>Designation</th>
<th>Sequence (5′→3′)</th>
<th>Size of product (bp)</th>
<th>Position (nt)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bfp_F</td>
<td>GCATCATTCCGTGTGG</td>
<td>117</td>
<td>858–875</td>
</tr>
<tr>
<td>Bfp_R</td>
<td>GGACCATGATTATCATA</td>
<td>974–951</td>
<td></td>
</tr>
<tr>
<td>Bfp_Probe</td>
<td>FAM-CCGCTTCCTGAACGCTGTGGTGG-BHQ1</td>
<td>877–900</td>
<td></td>
</tr>
<tr>
<td>Vtx2f_F</td>
<td>AGTCTTTCTCGATCTTCGG</td>
<td>121</td>
<td>745–764</td>
</tr>
<tr>
<td>Vtx2f_R</td>
<td>CCTGTTCCCAAATCTGGC</td>
<td>865–846</td>
<td></td>
</tr>
<tr>
<td>Vtx2f_Probe</td>
<td>JOE-AGT GTTGCCGCTCATCCTTAATTGCCAC-BHQ1</td>
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</tr>
<tr>
<td>espT_F</td>
<td>GGTGTTCAGCTCGGAAGT</td>
<td>80</td>
<td>294–312</td>
</tr>
<tr>
<td>espT_R</td>
<td>AAATCATGTGATGGATGGATGT</td>
<td>373–352</td>
<td></td>
</tr>
<tr>
<td>espT_probec</td>
<td>CY5-TTATGGCGTCTGTTGATG-BHQ3</td>
<td>313–337</td>
<td></td>
</tr>
</tbody>
</table>

*Table 1. Primers and probes designed for the real-time multiplex EPEC pathogenicity PCR assay*
For just over half (n=36) of cases, clinical details were extracted from the VTEC ESQ, while for the remaining 33, symptoms reported on the laboratory referral form were extracted from the LIMS. The majority reported diarrhoea (64/69; 93%), including bloody diarrhoea in 22 (32%) cases. Abdominal pain was reported for 22 (32%) cases, although this was mostly among adults. Fever and nausea/vomiting were reported in 15 (22%) and 13 (19%) cases, respectively. Where reported, the median duration of symptoms was 11 days (range 1–52 days), which compares with 7 days for VTEC. Four cases were hospitalized, two cases were reported as haemolytic uraemic syndrome, and one infant fatality (associated with EPEC serogroup O145) was recorded. The majority of cases (86%) were in children (aged <15 years), including a high percentage (17.4%) in infants (0–12 months of age). Relative to other common bacterial causes of gastrointestinal disease, including Campylobacter sp., Salmonella sp. and VTEC O157 (Fig. 1), the proportion of cases in infants was significantly higher (P<0.001).

**DISCUSSION**

Much of the literature on EPEC epidemiology in England describes the limited number of tEPEC serogroups associated with the large outbreaks of infantile diarrhoea that occurred throughout in the 20th century (Smith *et al.*, 1996; Jenkins *et al.*, 2003). In this study, aEPEC were more common and were associated with a much wider variety of serogroups, confirming that the use of specific antisera raised to the tEPEC serogroups would no longer be a suitable approach for detecting EPEC in the diagnostic laboratory. Few isolates (9%) had additional virulence factors, supporting the evidence from other countries that in recent years aEPEC has become more commonly associated with diarrhoeal disease than typical strains (Ochoa & Contreras, 2011) and that vtx2f and, in particular, espT-positive strains are relatively rare (Arbeloa *et al.*, 2009; Hernandes *et al.*, 2009).

A high proportion of cases reported bloody diarrhoea, a symptom not generally associated with tEPEC infection, although it has been described in patients with aEPEC. Bielaszewska *et al.* (2008) suggested that aEPEC strains associated with bloody diarrhoea were originally VTEC but had lost Vtx-encoding phages during infection. In support of this theory, during this study, a strain of VTEC O103 (harbouring the vtx1 gene) and a strain of EPEC O103 (negative for vtx genes) were isolated from the same patient. Although the median duration of symptoms was

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**Table 2. Details of cases with EPEC harbouring additional pathogenicity genes**

<table>
<thead>
<tr>
<th>Case</th>
<th>Year</th>
<th>Serogroup</th>
<th>Age (years)</th>
<th>Sex*</th>
<th>Clinical details†</th>
<th>Additional information</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2010</td>
<td>O126</td>
<td>5</td>
<td>M</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>2</td>
<td>2011</td>
<td>O111</td>
<td>1</td>
<td>F</td>
<td>A</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>2011</td>
<td>O7</td>
<td>1</td>
<td>F</td>
<td>ND</td>
<td>Travel to Hungary</td>
</tr>
<tr>
<td>4</td>
<td>2011</td>
<td>O55</td>
<td>58</td>
<td>M</td>
<td>ND</td>
<td>Travel to the Middle East</td>
</tr>
<tr>
<td>5</td>
<td>2012</td>
<td>O32</td>
<td>1</td>
<td>M</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>2010</td>
<td>O145</td>
<td>ND</td>
<td>M</td>
<td>ND</td>
<td>Sibling of case 6</td>
</tr>
<tr>
<td>7</td>
<td>2010</td>
<td>O145</td>
<td>ND</td>
<td>M</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>2012</td>
<td>O49</td>
<td>46</td>
<td>ND</td>
<td></td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>2012</td>
<td>O85</td>
<td>1</td>
<td>D, F</td>
<td></td>
<td>Travel to Somalia</td>
</tr>
<tr>
<td>10</td>
<td>2012</td>
<td>O39</td>
<td>ND</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*M, male; F, female.
†ND, No data; D, diarrhoea; A, asymptomatic; F, fever.

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![Fig. 1. Age group distribution of cases of common gastrointestinal pathogens reported by GBRU in 2010–2012.](http://jmm.sgmjournals.org/1533)
11 days, many cases reported being symptomatic for longer and an association of aEPEC with prolonged diarrhoea (lasting longer than 14 days) has been described (Afset et al., 2004).

Certain studies have shown that EPEC can be isolated from both symptomatic and asymptomatic subjects (Tompkins et al., 1999; Jenkins et al., 2006), casting doubt on its association with gastrointestinal disease and making interpretation of laboratory results difficult. However, in common with historical data and data from studies from other countries (Ochoa & Contreras, 2011), the results of this study show a specific age-association in EPEC cases.

In England, the national standard methods for the investigation of faecal specimens for bacterial pathogens (http://www.hpa.org.uk/sm) describe the detection of VTEC O157 but do not include the detection of non-O157 VTEC and other DEC, such as EPEC. However, a number of laboratories in England are adopting a molecular approach for the detection of enteric pathogens and many in-house and commercial assays include targets for DEC. Improved protocols for the detection of EPEC directly from faecal specimens will facilitate a better understanding of the pathogenicity and epidemiology of EPEC in England. Meanwhile, the results of this study suggest that EPEC isolated from symptomatic infants less than 12 months old should be regarded as a significant pathogen.

REFERENCES


