Original Article

Clonal Spread of Enterotoxigenic *Escherichia coli* O128:H45 Strain in the Neonate Unit

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**SUMMARY:** Enterotoxigenic *Escherichia coli* (ETEC) has the potential to cause nosocomial infantile diarrhea in a hospital setting. We detected 12 ETEC serotype O128:H45 isolates from diarrheal neonates in our neonatal unit from July through October 2012. These infants developed hospital-acquired and epidemiologically related diarrhea. Pulsed-field gel electrophoresis analysis and multilocus sequence typing of these 12 isolates suggested that a specific clone of ETEC serotype O128:H45-CS21-ST2332 caused nosocomial diarrhea among neonates. Of concern, this ETEC clone strain was resistant to multiple drugs, particularly third-generation cephalosporins.

**INTRODUCTION**

Enterotoxigenic *Escherichia coli* (ETEC) is an important cause of diarrhea in infants and young children in developing countries (1). Approximately 280–400 million ETEC-associated diarrhea cases occur annually in children younger than 5 years old (2). A median of 13% (range 3–39%) of diarrheal specimens were positive for ETEC in 51 studies conducted in children (3). Birth cohort studies of infants in developing countries suggest that children aged > 6 months may be at increased risk owing to not being exclusively breastfed and the introduction of supplemental foods resulting in increased consumption of contaminated water or foods (4–6). ETEC is also an important causative agent responsible for nosocomial infantile diarrhea in hospital settings (7–9). Occasional nosocomial outbreaks caused by ETEC among neonates have been documented (10–12). The role of ETEC as a cause of infectious diarrhea is under-recognized in our country because ETEC diagnostic methods are unavailable in the clinical laboratories. Since July 2012, the diagnostic testing of diarrheagenic *E. coli* (DEC) was included in the routine surveillance program of infectious diarrhea by the public health laboratory of Shanghai Municipal Center for Disease Control and Prevention (CDC). Children’s Hospital of Fudan University was selected as one of the 4 surveillance sentinel sites for infectious diarrhea. We noted a clonal spread of ETEC O128:H45 among neonates with nosocomial diarrhea. This study retrospectively described the phenotypic and genotypic profiles of ETEC O128:H45 isolates causing nosocomial neonatal diarrhea.

**MATERIALS AND METHODS**

The stool samples collected from the diarrheal infants in the neonate units were routinely screened for enteric pathogens. *Shigella* spp., *Salmonella* spp., *Yersinia enterocolitica*, *Campylobacter jejuni* and *C. coli*, *Aeromonas* spp., *Vibrio* spp., and rotaviruses were screened as previously described (13,14). For identifying DEC, stool swab specimens were incubated overnight on an SSI enteric medium plate (State Serum Institute; Copenhagen, Denmark). Five medium-sized colonies (lactose-fermenting or non-fermenting) were selected and pooled for DNA extraction and multiplex PCR assay using the SSI-DEC-PCR kit (State Serum Institute) at the CDC laboratory. Agarose gel electrophoresis was performed to confirm the target bands of virulence genes. Subsequently, individual colonies from positive ETEC samples were analyzed using separate PCR assays to screen the exact colony containing *eltA* encoding heat labile toxin, *stIA* and *stIB* encoding porcine heat-stable toxin (STp) and human heat-stable toxin (STh), respectively, as previously described (15). ETEC colonies enriched for toxic genes were used for the biochemical tests and preserved at −80°C in Brain Heart Infusion medium (Difco; Detroit, MI, USA) containing 30% glycerol to further characterize the serotypes, colonization factors, and genotypes. The isolates were O:H-serotyped using commercial antisera kits (State Serum Institute). The isolates were subtyped by pulsed-field gel electrophoresis (PFGE) using the restriction enzyme *XbaI* and genotyped by multilocus sequence typing (MLST) using standard protocols (16,17). Detection of 19 different colonization factors (CFs) was performed by multiplex PCR as previously described (15). In addition, 6 putative virulence factors for ETEC (*clyA*, *eatA*, *leoA*, *tibC*, *tia*, and *east-l*) were analyzed as previously described (18).
Antimicrobial susceptibility testing of ETEC was performed using the minimum inhibitory concentrations (MICs). Antimicrobial classes and MIC resistance breakpoints were defined using the criteria established by the Clinical and Laboratory Standards Institute (19). The clinical data were obtained from the hospital medical records and reviewed by a principal investigator.

RESULTS

Thirteen neonates had ETEC exclusively isolated in the stool samples during the period of July 14 to October 14, 2012. One ETEC isolate was detected in July, 4 isolates in August, 7 isolates in September, and 1 isolate in October. In 1 case, rotavirus was co-detected. Afterward, no ETEC was isolated in the submitted stool samples from the neonate unit. Of 13 neonates with ETEC infection, 11 cases were considered to have developed hospital-acquired diarrhea because diarrhea occurred 3 days after hospital admission or within 3 days of discharge; 1 case developed diarrhea 1 day after exposure to her sister who developed diarrhea on the day she was discharged from the neonate unit; 1 case developed diarrhea at home and then was admitted to the hospital for fever and loose stool. The 13 ETEC-infected infants (6 girls and 7 boys) were aged between 6 and 63 days on admission. Eleven infants with hospital-acquired diarrhea and 1 secondary case were formula-fed; 1 infant was breast-fed with formula supplementation before developing diarrhea at home. Thirteen diarrheal infants passed frequent watery stools or loose stools (5–12 times/24 h), 4 had mucus in the stools and 1 had visible blood in the stool. Four infants experienced vomiting, 7 had mild fever (38–39°C) for 1–4 days, and 3 had moderate dehydration. Thirteen neonates received parenteral antibiotics such as third-generation cephalosporins or ampicillin-sulbactam. All ETEC-infected infants fully recovered 3–5 days after the onset of diarrhea.

Detailed serotyping of ETEC isolates showed that 11 infants with hospital-acquired diarrhea and 1 infant exposed to her sibling with hospital-acquired diarrhea excreted ETEC O128:H45. However, the ETEC isolate recovered from the infant with home-acquired diarrhea was non-O128:H45 serotype. Twelve ETEC O128:H45 isolates that were positive for STh-gene, also had identical or almost identical PFGE patterns (Fig. 1) and the same sequence type (ST2332), which were distinctly different from 1 ETEC non-O128:H45 strain that was positive for STp-gene and had sequence type ST182. All 12 ETEC O128:H45 isolates expressed CS21, clyA, and eatA, and 10 isolates also expressed CFA/I. However, 1 ETEC non-O128:H45-ST182 isolate expressed CS6, clyA, eatA, leoA, tia, and east-1. All 13 ETEC isolates were lactose-fermenting. All 13 ETEC isolates were susceptible to gentamicin, kanamycin, amikacin, chloramphenicol, ofloxacin, ciprofloxacin, and levofloxacin; resistant to nalidixic acid, tetracycline, ampicillin, streptomycin, sulfisoxazol, trimethoprim-sulfamethoxazol; and had intermediate-resistance to amoxicillin-clavulanic acid. However, 12 ETEC O128:H45 isolates were also resistant to cefotaxime, cepfepime, and ceftazidime.

Detailed information of these 13 infants and 13 ETEC isolates is summarized in Table 1.

DISCUSSION

Neonatal nosocomial diarrhea is a big concern in our setting because of the shortage of healthcare providers and the admission overload in neonate units. The findings of this study indicate that ETEC is a neglected pathogen causing nosocomial diarrhea among neonates. Currently, as the ETEC diagnostic methods are not widely available in clinical laboratories, ETEC-associated nosocomial infection cannot be recognized in a timely manner, and consequently, a cluster outbreak could occur. ETEC is primarily spread through contaminated food or water, and is often linked to outbreaks of gastrointestinal illness (20,21). Although 12 cases of ETEC O128:H45 infection seemed to be temporally unrelated, 12 ETEC O128:H45 isolates were verified to be genetically related based on their indistinguishable PFGE pattern and MLST genotype, which is distinctly different from the 1 ETEC non-O128:H45 isolate recovered from an infant with home-acquired diarrhea. Thus, we reason that an epidemiologically related ETEC clone, that is, ETEC O128:H45-CS21-ST2332 strain, was responsible for nosocomial diarrhea in the neonate unit. Several hospital-acquired outbreaks of diarrhea in infants and children caused by ETEC have been described (7–12). We could not trace the source of

![Fig. 1. PFGE-Xba profiles of 12 ETEC serotype O128:H45 isolates and 1 non-O128:H45 ETEC isolate. Dendrograms and degrees of similarity (% values) constructed using the unweighted pair group method with arithmetic mean (UPGMA) are shown.](image-url)
### Table 1: Informations of ETEC-infected diarrheal cases and serotypes, genotypes, virulence, and resistance phenotypes of each isolates

<table>
<thead>
<tr>
<th>Patient number</th>
<th>Isolation date</th>
<th>Age (days)</th>
<th>Sex</th>
<th>Frequency of diarrhea</th>
<th>Strain type</th>
<th>Serotype</th>
<th>MLST number</th>
<th>ST number</th>
<th>Resistance-phenotype</th>
</tr>
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<tr>
<td>1</td>
<td>SH12E032</td>
<td>Aug. 21</td>
<td>Male</td>
<td>26</td>
<td>Formula M</td>
<td>ST2322</td>
<td>STh</td>
<td>CS21</td>
<td>TET-AMC-AMP-SXT-NAL-FEP-CTX-TMP-CAZ-S3</td>
</tr>
<tr>
<td>2</td>
<td>SH12E033</td>
<td>Aug. 27</td>
<td>Male</td>
<td>17</td>
<td>Formula M</td>
<td>ST2322</td>
<td>STh</td>
<td>CS21</td>
<td>TET-AMC-AMP-SXT-NAL-FEP-CTX-TMP-CAZ-S3</td>
</tr>
<tr>
<td>3</td>
<td>SH12E071</td>
<td>Aug. 9</td>
<td>Male</td>
<td>13</td>
<td>Formula F</td>
<td>ST 2322</td>
<td>STh</td>
<td>CS21</td>
<td>TET-AMC-AMP-SXT-NAL-FEP-CTX-TMP-CAZ-S3</td>
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<tr>
<td>4</td>
<td>SH12E166</td>
<td>Sep. 7</td>
<td>Male</td>
<td>12</td>
<td>Formula F</td>
<td>ST2322</td>
<td>STh</td>
<td>CS21</td>
<td>TET-AMC-AMP-SXT-NAL-FEP-CTX-TMP-CAZ-S3</td>
</tr>
<tr>
<td>5</td>
<td>SH12E168</td>
<td>Sep. 12</td>
<td>Male</td>
<td>10</td>
<td>Formula F</td>
<td>ST2322</td>
<td>STh</td>
<td>CS21</td>
<td>TET-AMC-AMP-SXT-NAL-FEP-CTX-TMP-CAZ-S3</td>
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<tr>
<td>6</td>
<td>SH12E173</td>
<td>Sep. 7</td>
<td>Male</td>
<td>12</td>
<td>Formula F</td>
<td>ST2322</td>
<td>STh</td>
<td>CS21</td>
<td>TET-AMC-AMP-SXT-NAL-FEP-CTX-TMP-CAZ-S3</td>
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<tr>
<td>7</td>
<td>SH12E179</td>
<td>Sep. 28</td>
<td>Male</td>
<td>6</td>
<td>Formula F</td>
<td>ST2322</td>
<td>STh</td>
<td>CS21</td>
<td>TET-AMC-AMP-SXT-NAL-FEP-CTX-TMP-CAZ-S3</td>
</tr>
</tbody>
</table>

**Other virulence factor**

- CyaA/LeaE-LeoA-Tia-East-1
- CFA/I-CfaI-ClyA-EatA
- STh, human heat-stable toxin

**Resistance-phenotype**

- TET, tetracycline
- AMC, amoxicillin-clavulanate
- AMP, ampicillin
- SXT, trimethoprim-sulfamethoxazole
- NAL, nalidixic acid
- FEP, cefepime
- CTX, cefotaxime
- TMP, trimethoprim
- CAZ, ceftazidime
- S3, sulfisoxazol
- STR, streptomycin

* represents medium resistance.

ETEC-Associated Diarrhea among Neonates

In the middle of July 2012 in Shanghai, an ETEC O128:H45-ST2332 clone was detected in a neonate unit. This ETEC-related nosocomial outbreak in the neonate unit emphasizes the necessity for clinical laboratories to include ETEC in routine diagnostic capabilities. Utilization of standardized molecular methods such as multilocus sequence typing (MLST) and virulence factor sequencing will be crucial for the identification of new ETEC clones and the development of effective therapeutic strategies.
REFERENCES


