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Evidence of Evolving Extraintestinal Enteroaggregative Escherichia coli ST38 Clone

To the Editor: Several clones of extended-spectrum β-lactamase (ESBL)–producing extraintestinal pathogenic Escherichia coli (ExPEC) have globally expanded their distribution, including multilocus sequence types (MLSTs) ST38, ST131, ST405, and ST648 (1). ExPEC infections often originate from the patient’s own intestinal flora, although the degree of overlap between diarrheagenic E. coli and ExPEC pathotypes is unclear. Relatively little is known about antimicrobial drug resistance in the most common diarrheagenic E. coli groups, including enteroaggregative E. coli (EAEC), and bacterial gastroenteritis is generally managed without use of antimicrobial drugs.

The ability of diarrheagenic E. coli to cause extraintestinal infections has been shown in previous studies: a study among children in Nigeria linked EAEC to uropathogenic clonal group A (2), and a study in Brazil showed that EAEC markers were present in 7.1% of the E. coli isolates from urinary tract infections (3). Neither of these studies identified clonal lineages of EAEC specifically associated with extraintestinal infections.

We conducted this study to establish the presence and characteristics of ESBL-producing EAEC in a well-defined collection of ESBL-producing isolates (4). The isolates were from human and animal sources in Germany, the Netherlands, and the United Kingdom. The study was conducted at Public Health England during January–April 2013.

DNA from 359 ESBL isolates (4) was screened for the presence of the EAEC transport regulator gene (aggR), located on the EAEC plasmid, by using a real-time PCR assay and the following primers and probe: AggR_F 5′-CCATTATCGCAATCAGATTAA-3′ AggR_R 5′-CAAGCATCATTATGATTTCC-3′, AggR_P Cy5-CAGCGATACATTAAGAC-GCCTAAAGGA-BHQ. The amplification parameters were 50°C for 2 min, 95°C for 2 min, and 40 cycles at 95°C for 10 s and at 60°C for 20 s. Isolates positive for aggR were confirmed to be E. coli by using the Omnilog GelIII MicroPlate (Biolog, Hayward, CA, USA). Serotyping was done by using standard methods (5).

The phylogroup was determined for each isolate, and isolates were then assigned to 1 of the 4 major E. coli groups: A, B1, B2, and D (6). A microarray was used to detect ESBL genes, such as bilm, at the group level, as previously described (4). The antimicrobial drug susceptibilities of EAEC isolates were determined by using the agar incorporation method, as described in the British Society for Antimicrobial Chemotherapy guidelines (7).

Virulence factors associated with intestinal and extraintestinal infection
Our findings show the potential for EAEC, previously considered a gut pathogen, to cause extraintestinal infection. We suggest that the UPEC/EAEC pathotype may be an evolving clonal group. In particular, a single sequence type, ST38, was associated with multidrug resistance and with urinary tract infection in humans.

Acknowledgments

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(8) and with EAEC were investigated as previously described (9). We assigned a virulence score (total number of virulence factor genes detected; maximum possible score 22) and a resistance score (total number of drug classes; maximum score 11) to each isolate.

We isolated 11 EAEC from humans. Eight of the EAEC were isolated from urine specimens, and 1 was isolated from a blood culture; 63% belonged to phylogroup D (Table). EAEC ST38, which contained 5,143 ESBL-Producing enteroaggregative Escherichia coli isolates from sources in Germany, the Netherlands, and the United Kingdom*  

<table>
<thead>
<tr>
<th>Isolate</th>
<th>Serotype†</th>
<th>Cpx‡</th>
<th>Country</th>
<th>Source</th>
<th>Phytotype</th>
<th>aggR§</th>
<th>Plasmidic ESBL</th>
</tr>
</thead>
<tbody>
<tr>
<td>ESBL-723</td>
<td>O125a:H30</td>
<td>58</td>
<td>Netherlands</td>
<td>Urine</td>
<td>D</td>
<td>+</td>
<td>CTX-M-15</td>
</tr>
<tr>
<td>ESBL-746</td>
<td>0125a:H30</td>
<td>38</td>
<td>UK</td>
<td>Urine</td>
<td>D</td>
<td>+</td>
<td>CTX-M-15</td>
</tr>
<tr>
<td>ESBL-884</td>
<td>O125a:H30</td>
<td>38</td>
<td>UK</td>
<td>Urine</td>
<td>D</td>
<td>+</td>
<td>CTX-M-15</td>
</tr>
<tr>
<td>ESBL-831</td>
<td>O125a:H30</td>
<td>38</td>
<td>UK</td>
<td>Urine</td>
<td>D</td>
<td>+</td>
<td>CTX-M-15</td>
</tr>
<tr>
<td>ESBL-815</td>
<td>O125a:H30</td>
<td>38</td>
<td>UK</td>
<td>Blood</td>
<td>D</td>
<td>+</td>
<td>CTX-M-15</td>
</tr>
<tr>
<td>ESBL-26</td>
<td>0125a:H30</td>
<td>38</td>
<td>Netherlands</td>
<td>Urine</td>
<td>D</td>
<td>+</td>
<td>CTX-M-15</td>
</tr>
<tr>
<td>ESBL-221</td>
<td>O92:H33</td>
<td>34</td>
<td>10 Germany</td>
<td>Feces</td>
<td>A</td>
<td>+</td>
<td>CTX-M-3</td>
</tr>
<tr>
<td>ESBL-45</td>
<td>O7:H26</td>
<td>58</td>
<td>155 Netherlands</td>
<td>Urine</td>
<td>B1</td>
<td>+/-</td>
<td>CTX-M-14</td>
</tr>
<tr>
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<td>0?:H-</td>
<td>694</td>
<td>None Netherlands</td>
<td>Urine</td>
<td>A</td>
<td>+/-</td>
<td>CTX-M-15</td>
</tr>
<tr>
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<td>O15:H1</td>
<td>545</td>
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<td>Urine</td>
<td>D</td>
<td>+/-</td>
<td>CTX-M-14</td>
</tr>
<tr>
<td>ESBL-64</td>
<td>O7:H23</td>
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<td>None Netherlands</td>
<td>Urine</td>
<td>B1</td>
<td>+/-</td>
<td>CTX-M-14</td>
</tr>
</tbody>
</table>

*All isolates were collected in 2009 (4). ESBL, extended-spectrum β-lactamase. ST, sequence type. †H- not motile; O?, O unidentifiable; R, rough reaction. ‡Cpx-ST complex comprising single-locus variants. §aggR, enteroaggregative E. coli regulatory gene; +, positive in screen and isolates; -, negative in screen and isolates; +/-, positive in screen but negative in isolates, indicating unstable plasmid.

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Resolution Threshold of Current Molecular Epidemiology of Diphtheria

“The fox who longed for grapes, beholds with pain
The tempting clusters were too high to gain;
Grieved in his heart he forced a careless smile,
And cried, “They’re sharp and hardly worth my while.””

(Aphra Behn, 1687, after Aesop’s The Fox and the Grapes)

To the Editor: Diphtheria is an extremely rare disease in Europe but remains a major health issue in developing countries (1–3). In recent years, steady progress has been made toward understanding the factors of pathogenicity of its causative agent (Corynebacterium diphtheriae). In contrast, remarkable advances in its basic genomics have not been sufficiently translated into the molecular epidemiology of diphtheria. A recent report by Zasada (4) offers an apt opportunity to take a new look at this issue.

The current genotyping repertoire of C. diphtheriae includes several methods but those most frequently used are classical ribotyping and pulsed-field gel electrophoresis (PFGE). More recently, a multilocus sequence typing (MLST) scheme for C. diphtheriae was developed (5). Compared with ribotyping, PFGE, and other methods based on analysis of banding profiles, MLST results are digital, unambiguous, and portable. MLST discrimination of 150 isolates from 18 countries and spanning 50 years was “in accordance with previous ribotyping data, and clonal complexes associated with disease outbreaks were clearly identified by MLST” (5).

In the report by Zasada (4), all 3 recommended methods (PFGE, MLST, and ribotyping) were used to genotype 25 nontoxigenic C. diphtheriae isolates from Poland. The author concluded that these isolates “represent a single clone despite isolation … in different part of the country over a 9-year period” and raised the question of whether a single clone of C. diphtheriae is circulating in Poland (4). These isolates are related genetically, but do they represent a truly single clone or might they be further discriminated? Their circulation in Poland may be caused by their high pathogenicity, but also (or instead) it might reflect their endemic, historical prevalence in this country. I believe that these questions are unlikely to be answered by the internationally agreed-upon methods for C. diphtheriae typing because of their insufficient resolution: the discriminatory power of MLST does not exceed that of ribotyping (5).
Evidence of Evolving Extraintestinal Enteroaggregative *Escherichia coli* ST38 Clone

Technical Appendix

Technical Appendix Figure. Virulence factors and antimicrobial drug resistance gene content of Enteroaggregative *Escherichia coli* (EAEC) isolates, grouped by phylogroup.