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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′-CCATTTATCGCAATCAGATTAA-3′ AggR_R 5′-CAAGCATCTACTTTTGATATTCC-3′, AggR_P Cy5-CAGCGATACATTAAGACGCCTAAAGGA-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 GenIII 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 $bla_{\text{CTX-M}}$, 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

Table. Characteristics of human-derived ESBL-producing enteroaggregative *Escherichia coli* isolates from sources in Germany, the Netherlands, and the United Kingdom*

Isolate	Serotype†	ST	Cplx‡	Country	Source	Phylotype	*aggR*§	Plasmidic ESBL
ESBL-723	OR:H30	38	38	UK	Urine	D	+	CTX-M-15
ESBL-746	O125ac:H30	38	38	UK	Urine	D	+	CTX-M-15
ESBL-884	O19a:H30	38	38	UK	Urine	D	+	CTX-M-14
ESBL-831	O19a:H30	38	38	UK	Urine	D	+	CTX-M-14
ESBL-815	O19a:H30	38	38	UK	Blood	D	+	CTX-M-15
ESBL-26	O153:H30	38	38	Netherlands	Urine	D	+	CTX-M-51
ESBL-221	O92:H33	34	10	Germany	Feces	A	+	CTX-M-3
ESBL-45	O?:H26	58	155	Netherlands	Urine	B1	+/–	CTX-M-14
ESBL-46	O?:H-	694	None	Netherlands	Urine	A	+/–	CTX-M-15
ESBL-48	O15:H1	545	None	Netherlands	Urine	D	+/–	CTX-M-1
ESBL-64	O?:H23	224	None	Netherlands	Urine	B1	+/–	CTX-M-1

*All isolates were collected in 2009 (*4*). ESBL, extended-spectrum β-lactamase. ST, sequence type.
†H- not motile; O?, O unidentifiable; R, rough reaction.
‡Cplx–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.

(*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, the most common (55%) ST, was significantly associated with extraintestinal sites in the subset of 140 human isolates (Fisher exact test, $p<0.0001$).

In this study, we identified multidrug-resistant EAEC isolates belonging to ST38; the isolates had various somatic antigens and $bla_{\text{CTX-M}}$ genes (Table). The multiple somatic antigens, variety of antimicrobial drug–resistance scores, and variety of gene complements in this successful ST indicate multiple acquisitions of virulence markers, rather than clonal expansion from a single source (Table; online Technical Appendix Figure, http://wwwnc.cdc.gov/EID/article/20/11/13-1845-Techapp1.pdf).

In the MLST public database, which contained 5,143 *E. coli* entries in June 2013, ST38 is predominantly associated with urinary tract infections, but in-house MLST studies at the Gastrointestinal Bacteria Reference Unit, Public Health England, have shown that ST38 is a successful EAEC group. The presence of EAEC virulence factors, such as aggregative adherence fimbria AAFI and *aggR*, can mediate adherence of *E. coli* to bladder epithelial cells, but the virulence factors do not impart uropathogenic properties to all EAEC isolates (*10*). The ST38 strain described here probably originated from the gut and independently acquired the 2 phenotypes (uropathogenic *E. coli* [UPEC] and EAEC), which would suggest the emergence of a UPEC/EAEC hybrid strain. It seems likely that an ST38 *E. coli* strain adapted to EAEC plasmid carriage (a change that would help survival in the gut through increased adherence) has acquired UPEC virulence factors, facilitating the exploitation of an extraintestinal niche, the urinary tract.

Despite the characterization of numerous virulence factors, no single genetic feature currently defines EAEC or UPEC isolates. Because the EAEC ST38 strain had 4–7 ExPEC-associated virulence factors, we suggest that, on the basis of epidemiologic, microbiological, and molecular characteristics, the EAEC ST38 described in this study should be considered an ExPEC associated with uropathogenic infections. It is possible that the multidrug-resistant EAEC ExPEC group has expanded globally but is currently underreported. We therefore urge testing for the EAEC genotype in all clinical studies of *E. coli* pathotypes.

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.

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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*).