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CTX-M-15 Extended-Spectrum β-Lactamase in a Shiga Toxin-Producing *Escherichia coli* Isolate of Serotype O111:H8

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We report the discovery of a CTX-M-15-producing *Escherichia coli* (STEC) of serogroup O111:H8, a major serotype responsible for human enterohemorrhagic *Escherichia coli* (EHEC) infections. In line with the recent CTX-M-15/O104:H4 *E. coli* outbreak, these data may reflect an accelerating spread of resistance to expanded-spectrum cephalosporins within the *E. coli* population, including STEC isolates.

Shiga toxin-producing *Escherichia coli* (STEC) comprises food-borne pathogens producing Stx1 and/or Stx2 (1). O157:H7 is the main serotype responsible for human infections, but O26:H11, O103:H2, O111:H8, and O145:H28 are also frequently incriminated (8). Ruminants are a major source of STEC, and transmission principally occurs through consumption of contaminated food but also through direct or indirect contacts with contaminated animals or persons (4).

Several studies reported on the antimicrobial resistance of STEC, but these pathogens have not been considered so far as a reservoir of extended-spectrum β-lactamases (ESBLs), one of the most widespread mechanisms of transmissible antimicrobial resistance in Gram-negative bacteria. ESBLs confer resistance to all β-lactams but cefoxitin and carbapenems and mostly belong to the TEM, SHV, and CTX-M families, with the last group demonstrating an epidemiological success in recent years (3). To our best knowledge, only five ESBL-producing STEC isolates have been reported so far, including three human isolates belonging to serogroup O26 and carrying either a $bla_{\text{CTX-M-3}}$ (10), $bla_{\text{CTX-M-18}}$ (14), or $bla_{\text{TEM-52}}$ gene (2), one chicken isolate belonging to serogroup O157 and carrying a $bla_{\text{CTX-M-2}}$ gene (23), and the highly virulent O104:H4 isolate harboring the $bla_{\text{CTX-M-15}}$ gene and responsible for the recent outbreaks in Germany and France (19).

In this study, ESBL production was detected in an *E. coli* isolate, 22207, recovered in 2008 through the National Network for the Surveillance of Resistance to Antimicrobials in Animals in France (Résapath; www.resapath.anses.fr) from the fecal contents of a calf which died after severe diarrhea at a farm. After identification using colony morphology and API 20E tests (bioMérieux, Marcy l'Etoile, France), susceptibility testing to 32 β-lactam and non β-lactam antimicrobials was performed by agar diffusion as recommended by the CA-SFM (www.sfm-microbiologie.fr), using *E. coli* ATCC 25922 as a control strain. *E. coli* isolate 22207 showed resistance to amoxicillin, ceftiofur (with standard double-disk synergy), ceftazidime, and aztreonam but was susceptible to cefoxitin and carbapenems. Additional resistances to streptomycin, kanamycin, tetracyclines, and sulfonamides were detected. PCR (see Table S1 in the supplemental material) and DNA sequencing (Beckman Coulter, London, United Kingdom) revealed the presence of a narrow-spectrum TEM-1 β-lactamase-encoding gene, in addition to a $bla_{\text{CTX-M-15}}$ gene preceded by the IS*Ecp*1 element (17).

Transferability of ESBL genes was tested by broth mating assays with *E. coli* K-12 J5 (*pro met azi*) used as a recipient strain on agar plates containing cefotaxime (10 mg/liter) and sodium azide (500 mg/liter). *E. coli* isolate 22207 was proved to transfer the ESBL phenotype by conjugation, together with resistance to streptomycin, kanamycin, and tetracyclines. The presence of the $bla_{\text{CTX-M}}$ and bla_{TEM} genes in the transconjugant was confirmed by PCR (15, 17). Using PCR-based replicon typing (PBRT) (5) and S1-pulsed-field gel electrophoresis (PFGE), the donor strain was shown to contain six plasmids of different sizes and replicon types, including F, FIB, B/O, P, and untypeable ones. However, only one of these plasmids (75 kb, untypeable) was found in the transconjugant and proved to carry the $bla_{\text{CTX-M-15}}$ gene, as shown by Southern blotting (data not shown).

E. coli isolate 22207 was shown to be of serotype O111:H8 and was assigned to phylogroup B1 (7, 18, 22). Virulence analysis using a DNA array (Identibac EC, Alere, France) and PCR (see Table S1 in the supplemental material) revealed that this strain codes for Stx1 (9) and not for Stx2 (6). This strain possessed the intimin-encoding gene *eae* (θ variant) (18). It was also positive for genes coding for the type III secretion system (T3SS) encoded by the locus of enterocyte effacement (LEE) pathogenicity island and for genes coding for different T3SS effector proteins encoded or not by the LEE (*tir*, *espA*, *espF*, *espJ*, *tccP*, and *cif*). In addition, genes encoding bacteriocins (Cba, CelB, Cma) were identified. The genetic element O island 122 (*pagC*, *nleB*, and *efa1* genes) associated with STEC capable of causing HUS and food-borne outbreaks (11) was also found by real-time PCR. Three genes previously found in plasmid pO157, i.e., *espP* (16), *e-hlyA* (21), and *msbB2* (12) encoding a serine protease, the EHEC hemolysin, and an acetyltransferase involved in lipid A biosynthesis, respectively, were also detected. The *espP*, *e-hlyA*, and *msbB2* genes were not

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located on the $bla_{CTX\text{-}M\text{-}15}$-carrying plasmid, as they were not detected in the transconjugant.

Here we showed the presence of a $bla_{CTX\text{-}M\text{-}15}$-carrying plasmid in an *E. coli* of serotype O111:H8 carrying the stx_1 and *eae* genes. This strain is likely to be the cause of the severe diarrhea that occurred before the death of the calf. More importantly, this strain shows the worrying combination of a major determinant of β-lactam resistance in humans with virulence genes typical of one of the major serotypes of STEC responsible for hemorrhagic colitis and hemolytic and uremic syndrome in human beings. In line with the recent CTX-M-15/O104:H4 *E. coli* outbreak, the emergence of such strains is of great concern. However, in this study, the $bla_{CTX\text{-}M\text{-}15}$ gene was located on a different plasmid with an incompatibility group other than that of the O104:H4 plasmid, suggesting that the two strains did not result from the dissemination of the same plasmid. On the other hand, CTX-M-15 enzymes are recurrently found in *E. coli* from cattle (13, 20), which are also a major reservoir of STEC. Consequently, CTX-M-15-producing STEC from cattle may expand in the future, and this may indicate a more widespread movement of $bla_{CTX\text{-}M}$ genes within the *E. coli* population, including STEC isolates.

Finally, the presence of a $bla_{CTX\text{-}M\text{-}15}$-carrying plasmid in a Shiga toxin-producing *E. coli* of serotype O111:H8 points out again the relationship between virulence genotypes, phylogenetic backgrounds, and resistance traits in pathogenic *E. coli*. It would be valuable to better understand which selective pressures may promote such combinations of virulent and highly resistant pathogens. Current rapid genetic technologies such as those used in this study should facilitate screening of these hazardous strains and help to study their occurrence or emergence in animals.

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