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Quinolone-Resistant *Escherichia coli* O127a:K63 Serotype with an Extended-Spectrum-Beta-Lactamase Phenotype from a Food Poisoning Outbreak in China

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We report an atypical enteropathogenic *Escherichia coli* O127a:K63 strain with resistance to quinolones and extended-spectrum cephalosporins isolated from a 2010 food poisoning outbreak involving 112 adults in China. Two resistance genes [$bla_{\text{CTX-M-15}}$, *aac*(*6′*)*-Ib-c*] and five mutations (two in *gyrA*, two in *parC*, one in *parE*) coexisted in this enteropathogenic *E. coli* strain.

Food poisoning from multidrug-resistant strains of diarrheagenic *Escherichia coli* has received considerable public attention, especially since the large outbreak of hemolytic uremic syndrome caused by *E. coli* O104:H4 in Germany in 2011 (12). Extended-spectrum cephalosporins and quinolones are widely used for diarrheagenic *E. coli*, but *E. coli* isolates are increasingly resistant to these antimicrobials (11). Here, we report the isolation of an *E. coli* strain harboring virulence genes and genes conferring resistance to quinolones and extended-spectrum cephalosporins from a 2010 food poisoning outbreak involving 112 adult students in China.

On 16 September 2010, 112 students (18 to 23 years old) from a university in Zhengzhou, China, experienced diarrhea and other enterogastritis symptoms after eating in the same dining room the previous evening. Seventy-eight (70%) students were hospitalized, and 27 of these students experienced serious symptoms, including stomachache, fever, vomiting, and watery diarrhea (>5 times/day). Twenty-eight stool specimens were collected from these 27 inpatients within 24 h after admission for laboratory testing. The suspected pathogenic bacteria were isolated through standard procedures on conventional selective media, and the isolated strains were identified using a commercial biochemical test (API 20E system; bioMérieux Vitek, France). Serotyping was carried out by slide agglutination with a conventional *E. coli* antiserum kit (Denka Seiken, Tokyo, Japan). Sixteen strains of enteropathogenic *E. coli* serotype O127a:K63 were isolated from the stool specimens. Results of epidemiologic investigation and laboratory tests indicated that this food poisoning outbreak was due to the enteropathogenic *E. coli* O127a:K63.

One 20-year-old male patient had especially severe symptoms, including persistent abdominal cramps with severe paroxysmal pain and abnormal blood test results. Between 16 September and 27 September, he was hospitalized twice for diarrhea. His symptoms were controlled with intravenous administration of levofloxacin and cefpiramide sodium and supportive treatment, and he was released from the hospital. We followed up with this patient for 1 year and found that he often experienced stomachaches, hiccups, nausea, and other symptoms of chronic enterogastritis. Further clinical investigation excluded other chronic or immune-related diseases (e.g., cancer, hepatitis, and HIV infection); therefore, the chronic enterogastritis appeared to be related to the food poisoning caused by enteropathogenic *E. coli*.

The two suspected isolates obtained from stool specimens during this patient's hospitalizations were identified as enteropathogenic *E. coli* serotype O127a:K63, which was the same isolate obtained from 14 other inpatients who had serious symptoms. All 16 isolates were screened for virulence factors, including *eae*, *stx*, *bfpA*, and the *E. coli* adherence factor (EAF) plasmid, using PCR assays that have been described previously (1, 2). Reference strains of enteropathogenic *E. coli* (EPEC) and enterohemorrhagic *E. coli* harboring these virulence genes were used as positive controls. Antimicrobial susceptibility was tested by the disk diffusion method according to the Clinical and Laboratory Standards Institute guidelines, and MICs of ciprofloxacin, norfloxacin, cefotaxime, ceftriaxone, and nalidixic acid were determined by Etest strips (AB Biodisk, Solna, Sweden); *E. coli* ATCC 25922 was used as the quality control organism. Resistance genes *gyrA*, *gyrB*, *parC*, *parE*, *qnr*, *acc*, bla_{TEM}, and $bla_{\text{CTX-M}}$ were detected using previously described PCR methods (4, 5, 8). Bidirectional sequence data were obtained for these resistance and virulence genes.

The PCR and sequencing results indicated that the *E. coli* isolate harbored the virulence gene *eae*, which encodes the outer membrane protein intimin but not *stx*, *bfpA*, or the EAF plasmid, suggesting an atypical EPEC (6). Antimicrobial susceptibility tests showed that the isolates were completely resistant to cefotaxime, ceftriaxone, nalidixic acid, norfloxacin, ciprofloxacin, levofloxacin, amoxicillin, ampicillin, gentamicin, trimethoprim-sulfamethoxazole, and tetracycline and intermediately resistant to chloramphenicol but susceptible to imipenem. The results of MIC tests showed high-level resistance to ceftriaxone, cefotaxime, norfloxa-

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TABLE 1 Antimicrobial susceptibility profile (MIC) and resistance genes of 16 *E. coli* O127a:K63 isolates and the ATCC 25922 control[a]

Strain	MIC (mg/liter)					Additional resistance	Mutation			Other resistance genes	
	TX	CT	NX	CIP	NAL		*gyrA*	*parC*	*parE*	*aac*	bla_{CTX-M}
E. coli O127a:K63	>256	>256	>256	>32	>256	LEV, AMC, SAM, AMP, CN, SXT, TE	S83L, D87N	S80I, A108V	S458A	*aac*(6′)-*Ib-cr*	$bla_{CTX-M-15}$
ATCC 25922	0.032	0.023	0.047	0.012	1.5						

[a] TX, ceftriaxone; CT, cefotaxime; NX, norfloxacin; CIP, ciprofloxacin; NAL, nalidixic acid; LEV, levofloxacin; AMC, amoxicillin; SAM, ampicil; AMP, ampicillin; CN, cidomycin; SXT, trimethoprim-sulfamethoxazole; TE, tetracycline. Mutations are denoted by the amino acid substitution and the position number (e.g., S83L denotes substitution of a serine by a leucine at position 83).

cin, nalidixic acid, and ciprofloxacin (Table 1). Results of sequence analysis revealed that resistance to quinolone and extended-spectrum cephalosporin was mediated by the following five mutations and two resistance genes: two mutations in *gyrA* (Ser83→Leu, Asp87→Asn), two mutations in *parC* (Ser80→Ile, Ala108→Val), one mutation in *parE* (Ser458→Ala), and resistance genes *aac*(6′)-*Ib-cr* and $bla_{CTX-M-15}$.

EPEC causes diarrhea in infants and children (7) but rarely causes disease in adults (9). EPEC infection in adults in this food poisoning outbreak was probably due to the coexistence of *eae*-encoded intimin and multidrug resistance. Enteropathogenic *E. coli* O127a strains were primarily isolated from the environment (10), and our study is the first to report clinical isolates of atypical EPEC O127a:K63 serotype obtained from a food poisoning outbreak. In recent decades, an increasing number of *E. coli* isolates have acquired resistance to quinolones or extended-spectrum cephalosporins (5, 13). However, few studies have described *E. coli* isolates that simultaneously display resistance to these two antimicrobials, which was primarily due to several ESBL-producing resistant genes (3, 8). In contrast, the *E. coli* isolates in this study carried more resistant genes [i.e., two mutations in *gyrA* (Ser83→Leu, Asp87→Asn), two in *parC* (Ser80→Ile, Ala108→Val), one in *parE* (Ser458→Ala), and *aac*(6′)-*Ib-cr* and $bla_{CTX-M-15}$], which led to high-level resistance to both quinolones and extended-spectrum cephalosporins.

In conclusion, we report an atypical enteropathogenic *E. coli* O127a:K63 strain isolated from adults that displayed multidrug resistance, including high-level resistance to both quinolones and extended-spectrum cephalosporins. The emergence of atypical ESBL-producing EPEC with quinolone resistance from adults is problematic because of the difficulties in treatment and infection control. It is therefore important to strengthen surveillance of EPEC strains with multidrug resistance, especially quinolone-resistant ESBL-producing bacteria.

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REFERENCES

1. **Andrews N, et al.** 2000. The European Sero-Epidemiology Network: standardizing the enzyme immunoassay results for measles, mumps and rubella. Epidemiol. Infect. **125**:127–141.
2. **Aranda KR, Fagundes-Neto U, Scaletsky IC.** 2004. Evaluation of multiplex PCRs for diagnosis of infection with diarrheagenic *Escherichia coli* and *Shigella* spp. J. Clin. Microbiol. **42**:5849–5853.
3. **Cassier P, et al.** 2011. Cephalosporin and fluoroquinolone combinations are highly associated with *CTX-M* beta-lactamase-producing *Escherichia coli*: a case-control study in a French teaching hospital. Clin. Microbiol. Infect. **17**:1746–1751.
4. **Cattoir V, Poirel L, Rotimi V, Soussy CJ, Nordmann P.** 2007. Multiplex PCR for detection of plasmid-mediated quinolone resistance *qnr* genes in ESBL-producing enterobacterial isolates. J. Antimicrob. Chemother. **60**: 394–397.
5. **Donaldson SC, et al.** 2006. Molecular epidemiology of ceftiofur-resistant *Escherichia coli* isolates from dairy calves. Appl. Environ. Microbiol. **72**: 3940–3948.
6. **Hernandes RT, Elias WP, Vieira MA, Gomes TA.** 2009. An overview of atypical enteropathogenic *Escherichia coli.* FEMS Microbiol. Lett. **297**: 137–149.
7. **Jensen C, et al.** 2007. Attaching and effacing *Escherichia coli* isolates from Danish children: clinical significance and microbiological characteristics. Clin. Microbiol. Infect. **13**:863–872.
8. **Maeda Y, et al.** 2011. Molecular characterization and phylogenetic analysis of quinolone resistance-determining regions (QRDRs) of *gyrA*, *gyrB*, *parC* and *parE* gene loci in viridans group streptococci isolated from adult patients with cystic fibrosis. J. Antimicrob. Chemother. **66**:476–486.
9. **Nimri LF, Meqdam M.** 2004. Enteropathogens associated with cases of gastroenteritis in a rural population in Jordan. Clin. Microbiol. Infect. **10**:634–639.
10. **Pandey M, et al.** 2003. Association of cytolethal distending toxin locus *cdtB* with enteropathogenic *Escherichia coli* isolated from patients with acute diarrhea in Calcutta, India. J. Clin. Microbiol. **41**:5277–5281.
11. **Paterson DL.** 2006. Resistance in Gram-negative bacteria: Enterobacteriaceae. Am. J. Infect. Control **34**:S20–S28; discussion S64-S73.
12. **Rohde H, et al.** 2011. Open-source genomic analysis of Shiga-toxin-producing *E. coli* O104:H4. N. Engl. J. Med. **365**:718–724.
13. **Siddiqui AA.** 2008. Prevalence of quinolone-resistant urinary tract infections in Comanche County Memorial Hospital. J. Okla. State Med. Assoc. **101**:210–212.