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Letters to the Editor

Characterization of Isolates from an Outbreak of Multidrug-Resistant, Shiga Toxin-Producing *Escherichia coli* O145 in the United States▽

Shiga toxin-producing *Escherichia coli* (STEC) is an important cause of food-borne illness, and several outbreaks of non-O157 serotype infections have been reported recently (10, 17). In 2010, a multistate outbreak of STEC O145:NM (nonmotile) infections was investigated and linked to shredded romaine lettuce (4). Twenty-six confirmed and five probable cases were reported from Michigan, New York, Ohio, Pennsylvania, and Tennessee. Eleven (35%) patients required hospitalization, and three (10%) developed hemolytic uremic syndrome (HUS). No deaths were reported.

Although antimicrobial treatment for STEC infections is not recommended, some patients receive antimicrobial treatment prior to the diagnosis, and identification of antimicrobial resistance may provide clues about potential sources of these infections (13, 14). Therefore, understanding antimicrobial resistance patterns among STEC isolates remains important.

Three representative outbreak isolates from ill patients were sent to the National Antimicrobial Resistance Monitoring System (NARMS) at the CDC for antimicrobial susceptibility testing (AST). MICs to 15 antimicrobial drugs were determined by broth microdilution (Sensititre; Trek Diagnostics, Westlake, OH). All three isolates displayed resistance to chloramphenicol, nalidixic acid, streptomycin, sulfisoxazole, and tetracycline and decreased susceptibility to ciprofloxacin (MIC = 0.25 μg/ml) (Table 1). Cell lysates were screened for antimicrobial resistance genes by PCR (7). All three isolates contained the *floR*, *strA*, *strB*, *sul2*, and *tetA* resistance genes. Sequence analysis confirmed that the gene content and orientation matched those of the *floR* accessory gene element found in some IncA/C plasmids from *E. coli* and *Salmonella* (2). PCR and sequence analysis of the quinolone resistance determining regions (QRDR) of *gyrA* and *parC* identified a mutation in *gyrA*, resulting in a serine-to-leucine amino acid change at position 83. PCR screening for plasmid-mediated quinolone resistance determinants (*qnrA*, *qnrB*, *qnrC*, *qnrD*, *qnrS*, *aac(6′)-Ib-cr*, and *qepA*) was negative (18).

TABLE 1. Antimicrobial susceptibilities of the *E. coli* O145 outbreak isolate and transformant

Antimicrobial agent	MIC (μg/ml) for[a]:	
	Original isolate	Transformant
Amikacin	2	1
Amoxicillin-clavulanic acid	≤1	4
Ampicillin	2	4
Cefoxitin	8	4
Ceftiofur	0.5	0.5
Ceftriaxone	≤0.25	≤0.25
Chloramphenicol	**>32**	**>32**
Ciprofloxacin	0.25	≤0.015
Gentamicin	0.5	≤0.25
Kanamycin	≤8	≤8
Nalidixic acid	**>32**	1
Streptomycin	**>64**	**>64**[b]
Sulfisoxazole	**>256**	**>256**
Tetracycline	**>32**	**32**
Trimethoprim-sulfamethoxazole	0.25	≤0.12

[a] One representative isolate and transformant are shown. MICs in bold are considered resistant as defined by CLSI interpretive standards, when available (8). For ceftiofur and streptomycin, the resistance breakpoints used are 8 and 64 μg/ml, respectively (6).

[b] The DH10B cell line is streptomycin resistant (*rpsL*) prior to transformation, but the transfer of *strA* and *strB* was confirmed by PCR analysis.

Plasmid DNA was purified from the three isolates and electroporated into laboratory DH10B cells. All three electroporations produced chloramphenicol-resistant transformants. AST of the transformants demonstrated that with the exception of nalidixic resistance and decreased susceptibility to ciprofloxacin, the resistance phenotype (chloramphenicol, sulfisoxazole, streptomycin, and tetracycline) successfully transferred, suggesting that these resistance genes were located on a single multidrug resistance (MDR) plasmid (Table 1). PCR analysis confirmed the transfer of the *floR*, *strA*, *strB*, *sul2*, and *tetA* genes. PCR-based plasmid incompatibility replicon testing performed on the transformants identified an IncA/C plasmid (3). Conjugation experiments were unsuccessful at transferring the plasmid into a recipient *E. coli* strain (J53, sodium azide resistant) (11).

Several reports have identified multidrug resistance among isolates of non-O157 *E. coli*; however, reports of outbreaks with multidrug-resistant non-O157 STEC infections are rare (5, 15). Although the resistance genes identified in this study are relatively common among enteric bacteria, it is worrisome that five out of six resistance determinants were associated with an IncA/C plasmid, a plasmid type that has a broad host range and has been found in both food animals and clinical members of the *Enterobacteriaceae* (1, 9, 12, 16). Continued surveillance and molecular characterization of outbreaks of multidrug-resistant infections are necessary to better understand the sources of these infections.

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