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Letters to the Editor

Identification and Characterization of CTX-M-Producing *Shigella* Isolates in the United States▽

Shigellosis is a major source of gastroenteritis throughout the world (14). Extended-spectrum β-lactamases (ESBLs), including cefotaximases (CTX-M), confer resistance to extended-spectrum cephalosporins and significantly compromise the treatment options for shigellosis. Numerous ESBLs have been described among *Enterobacteriaceae* (2, 8, 13); however, only a single CTX-M-producing *Shigella* isolate has been reported in the United States (10).

From 1999 to 2007, 3,880 *Shigella* isolates were screened for antimicrobial susceptibility to 14 to 17 antimicrobials by broth microdilution (Sensititre; Trek Diagnostics, Westlake, OH). Six isolates displayed decreased susceptibility (MIC ≥ 2 mg/liter) to ceftriaxone (Table 1). The six case-patients included three males and two females (gender information was unavailable for one patient), and the median age was 3 years (range, 1 to 8 years). Additional details were available for five patients. Three of the five (60%) were hospitalized, and one was admitted twice. One patient had an adopted sibling from Russia but had not traveled herself. The second patient traveled to a neighboring state prior to illness onset, and the third reported no travel. Of the nonhospitalized patients, one was an asymptomatic adoptee from China and the second reported no travel. Two patients received antimicrobial therapy: ceftriaxone, cefotaxime, and trimethoprim-sulfamethoxazole for one patient, azithromycin for the other patient.

PCR analysis was used to screen the six isolates for 13 different classes or groups of *bla* genes, and PCR results were confirmed by DNA sequencing (1, 5, 11, 12, 16, 18–21). Four isolates were positive for the $bla_{\text{CTX-M-15}}$ gene, while two were positive for the $bla_{\text{CTX-M-14}}$ gene (Table 1). All four $bla_{\text{CTX-M-15}}$ isolates were PCR positive for non-ESBL $bla_{\text{TEM-1}}$ genes. Both $bla_{\text{CTX-M-14}}$ isolates were PCR positive for non-ESBL $bla_{\text{OXA-1}}$ genes, and a single isolate was positive for both $bla_{\text{TEM-1}}$ and $bla_{\text{OXA-1}}$. By pulsed-field gel electrophoresis (PFGE) analysis, all three *S. sonnei* and all three *S. flexneri* isolates demonstrated distinct patterns (data not shown) (15).

All six $bla_{\text{CTX-M}}$ genes were determined to be plasmid encoded (6). The non-ESBL β-lactamases (OXA-1, TEM-1) did not transfer and were not encoded on the same CTX-M plasmids. All three *S. sonnei* plasmids and two of the *S. flexneri* plasmids harbored only the CTX-M-associated resistance. The remaining *S. flexneri* plasmid contained additional determinants conferring resistance to trimethoprim-sulfamethoxazole and gentamicin.

All three *S. sonnei* plasmids were incompatibility type IncI1 and approximately 90 kb in size (plasmid pulsed-field gel electrophoresis) (Table 1) (4). Plasmid multilocus sequence typing (pMLST) identified them as novel sequence types designated as ST31 complex. The plasmid from AM22451 contained several point mutations in one allele, necessitating the ST32 designation within the ST31 clonal complex (http://pubmlst.org/plasmid) (7). Of the three *S. flexneri* plasmids, the $bla_{\text{CTX-M-15}}$-positive plasmid was a 165-kb IncA/C plasmid, while the two $bla_{\text{CTX-M-14}}$-positive plasmids were identical 75-kb IncFII plasmids. CTX-M-14 and CTX-M-15 are the most common types of cefotaximases identified among *Shigella* isolates (9, 17, 22), and IncI1 plas-

TABLE 1. Characterization of CTX-M-positive *Shigella* isolates, transformants, and CTX-M-encoding plasmids[a]

Isolate no.	*Shigella* species	State, yr isolated	MIC (μg/ml)				Additional resistance profile	β-Lactamase	Plasmid size (kb)	Plasmid incompatibility type (sequence type)
			CRO	CAZ	CTX	FEP				
DH10B	—[b]	—	≤0.25	0.25	≤0.06	≤0.06	STR	—	—	—
AM13291	*flexneri*	MA, 2002	16	0.5	8	2	AMP, CHL, COT, FIS, GEN, TET, TIO	CTX-M-15, TEM-1, OXA-1	—	—
DH-13291	—	—	32	2	32	4	AMP, AUG, COT, GEN, TIO	CTX-M-15	165	A/C
AM19035	*flexneri*	WI, 2003	32	0.5	32	2	AMP, CHL, COT, FIS, NAL, STR, TET, TIO	CTX-M-14, OXA-1	—	—
DH-19035	—	—	32	2	64	4	AMP, STR, TIO	CTX-M-14	75	FII
AM20369	*sonnei*	MI, 2004	64	8	64	8	AMP, COT, FIS, NAL, STR, TET, TIO	CTX-M-15, TEM-1	—	—
DH-20369	—	—	>64	16	128	16	AMP, STR, TIO	CTX-M-15	90	I1 (ST31)
AM22451	*sonnei*	NH, 2005	>64	8	64	8	AMP, COT, FIS, STR, TET, TIO	CTX-M-15, TEM-1	—	—
DH-22451	—	—	>64	32	128	16	AMP, STR, TIO	CTX-M-15	90	I1 (ST32)
AM22855	*sonnei*	NC, 2005	64	8	64	8	AMP, COT, FIS, NAL, STR, TET, TIO	CTX-M-15, TEM-1	—	—
DH-22855	—	—	>64	16	64	16	AMP, STR, TIO	CTX-M-15	90	I1 (ST31)
AM26336	*flexneri*	NE, 2006	16	0.5	8	2	AMP, CHL, COT, FIS, GEN, NAL, STR, TET, TIO	CTX-M-14, OXA-1	—	—
DH-26336	—	—	32	4	64	4	AMP, STR, TIO	CTX-M-14	75	FII

[a] AMP, ampicillin; AUG, amoxicillin-clavulanic acid; CHL, chloramphenicol; CAZ, ceftazidime; COT, trimethoprim-sulfamethoxazole; CRO, ceftriaxone; CTX, cefotaxime; FEP, cefepime; FIS, sulfisoxazole; GEN, gentamicin; KAN, kanamycin; NAL, nalidixic acid; STR, streptomycin; TET, tetracycline, TIO, ceftiofur. Additional drugs tested: AMI, amikacin; CIP, ciprofloxacin; FOX, cefoxitin.
[b] —, not applicable.

mids carrying CTX-M-15 have been already described in *Escherichia coli* and *Salmonella* isolates from Australia, France, and the United Kingdom (3).

The emergence of CTX-M-producing *Shigella* isolates in the United States is concerning and necessitates continued resistance surveillance.

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