Identification and characterization of CTX-M-producing *Shigella* isolates in the United States

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Running title: CTX-M-producing isolates of *Shigella*

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Shigellosis is a major source of gastroenteritis throughout the world (14). Extended-spectrum β-lactamases (ESBLs), including cefotaximases (CTX-M), confer resistance to broad-spectrum cephalosporins (BSC) and significantly compromise the treatment options for shigellosis. Numerous ESBL’s 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, 3880 Shigella isolates were screened for antimicrobial susceptibility to 14-17 antimicrobials by broth microdilution (Sensititre®, Trek Diagnostics, Westlake, OH). Six isolates displayed decreased susceptibility (MIC ≥ 2 mg/L) to ceftriaxone (Table 1). The six case-patients included three males and two females 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 non-hospitalized patients, one was an asymptomatic adoptee from China and the second reported no travel. Two patients received antimicrobial therapy; ceftriaxone, cefotaxime and trimethoprimsulfamethoxazole 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\textsubscript{CTX-M-15} gene while two were positive for the bla\textsubscript{CTX-M-14} gene (Table 1). All four bla\textsubscript{CTX-M-15} isolates were PCR positive for non-ESBL bla\textsubscript{TEM-1} genes. Both bla\textsubscript{CTX-M-14} isolates were PCR positive for non-ESBL bla\textsubscript{OXA-1} genes and a single isolate was positive for both bla\textsubscript{TEM-1} and bla\textsubscript{OXA-1}. By pulsed-field gel
electrophoresis (PFGE) analysis, all three *S. sonnei* and all three *S. flexneri* demonstrated
distinct patterns (data not shown) (15).

All six *bla*$_{CTX-M}$ 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 *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 multi-locus
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

(https://pubmlst.org/plasmid) (7). Of the three *S. flexneri* plasmids, the *bla*$_{CTX-M-15}$-positive
was a 165 kb IncA/C plasmid, while the two *bla*$_{CTX-M-14}$-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 plasmids carrying
CTX-M-15 have been already described in *E. coli* and *Salmonella* from Australia, France
and the UK (3).

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

We thank the NARMS participating public health laboratories for submitting the isolates,
Evangeline Sowers for confirming the *Shigella* species, Anne Whitney for DNA
sequencing, Lisa Theobald and the rest of the PulseNet team, and Rebecca Howie for
their assistance. This work was supported by an interagency agreement between CDC
and the FDA Center for Veterinary Medicine.
References:


Table 1. Characterization of CTX-M-positive *Shigella* isolates, transformants and CTX-M-encoding plasmids.

<table>
<thead>
<tr>
<th>Isolate #</th>
<th><em>Shigella</em> species</th>
<th>State/Year</th>
<th>MIC (µg/ml)</th>
<th>Additional resistance profile(^a)</th>
<th>β-lactamase</th>
<th>Plasmid Size (kb)</th>
<th>Plasmid Inc type (Sequence Type)</th>
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<tr>
<td>DH10B</td>
<td>-</td>
<td>-</td>
<td>≤0.25</td>
<td>AMP, CHL, COT, FIS, GEN, TET, TIO</td>
<td>STR</td>
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<td>-</td>
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<tr>
<td>AM13291</td>
<td><em>flexneri</em></td>
<td>MA 2002</td>
<td>16</td>
<td>0.5 8 2</td>
<td>CTX-M-15</td>
<td>165</td>
<td>A/C</td>
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<tr>
<td>DH-13291</td>
<td>-</td>
<td>-</td>
<td>32</td>
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<td>165</td>
<td>A/C</td>
</tr>
<tr>
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<td><em>flexneri</em></td>
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<td>-</td>
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<tr>
<td>DH-19035</td>
<td>-</td>
<td>-</td>
<td>32</td>
<td>AMP, STR, TIO</td>
<td>CTX-M-14</td>
<td>75</td>
<td>FII</td>
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<tr>
<td>AM20369</td>
<td><em>sonnei</em></td>
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<td>64</td>
<td>8 64 8</td>
<td>CTX-M-15</td>
<td>-</td>
<td>-</td>
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<td>DH-20369</td>
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<td>-</td>
<td>&gt;64</td>
<td>AMP, STR, TIO</td>
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<td>II (ST31)</td>
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<tr>
<td>AM22451</td>
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<td>&gt;64</td>
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<td>CTX-M-15</td>
<td>-</td>
<td>-</td>
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<td>-</td>
<td>&gt;64</td>
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<td>AM22855</td>
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<td>CTX-M-15</td>
<td>-</td>
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<td>DH-22855</td>
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<td>&gt;64</td>
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<td>CTX-M-15</td>
<td>90</td>
<td>II (ST31)</td>
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<tr>
<td>AM26336</td>
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<td>AMP, STR, TIO</td>
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<td>75</td>
<td>FII</td>
</tr>
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</table>
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.

-: not applicable