CTX-M–producing Non-Typhi Salmonella spp. Isolated from Humans, United States

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CTX-M–type β-lactamases are increasing among US Enterobacteriaceae isolates. Of 2,165 non-Typhi Salmonella isolates submitted in 2007 to the National Antimicrobial Resistance Monitoring System, 100 (4.6%) displayed elevated MICs (>2 mg/L) of ceftriaxone or ceftiofur. Three isolates (serotypes Typhimurium, Concord, and I 4,5,12:i–) contained bla<sub>CTX-M–5</sub>, bla<sub>CTX-M–15</sub>, and bla<sub>CTX-M–55/57</sub> respectively.

Severe non-Typhi Salmonella (NTS) infections are commonly treated with fluoroquinolones such as ciprofloxacin or, in children, with extended-spectrum cephalosporins such as ceftriaxone. The emergence of Salmonella spp. isolates that display resistance to extended-spectrum cephalosporins is of increasing public health concern. In the United States, almost all resistance to extended-spectrum cephalosporins among Salmonella spp. isolates is caused by AmpC-type β-lactamases; extended-spectrum β-lactamases (ESBLs), including cefotaximases (CTX-M), rarely have been reported. Likewise, among other Enterobacteriaceae isolates in the United States, CTX-M enzymes have been considered rare until recently.

In 2007, Lewis et al. reported that CTX-M was the predominant ESBL among Enterobacteriaceae isolates in a US health care system (San Antonio, TX) (1). Among the ESBL-producing isolates collected, CTX-M enzymes increased in prevalence from 25% in 2002 to 70% in 2006. The emergence was observed mainly in urinary tract isolates of Escherichia coli, and the predominating enzyme was CTX-M-15 (1). Similarly, a US study investigating clinical samples of Enterobacteriaceae submitted to The Hospital of the University of Pennsylvania (Philadelphia, PA) in 2007 reported that 48% of cephalosporin-resistant E. coli isolates were CTX-M positive (2). Furthermore, CTX-M enzymes, and CTX-M-15 in particular, were common among ESBL-producing Enterobacteriaceae isolates collected at 15 US medical centers participating in the Meropenem Yearly Susceptibility Test Information Collection Program during 2007 (3).

In the United States, the National Antimicrobial Resistance Monitoring System (NARMS) has systematically monitored antimicrobial susceptibility of NTS since 1996. This program is a collaborative effort between the Centers for Disease Control and Prevention (CDC), the Food and Drug Administration, and the US Department of Agriculture. Reports on increased prevalence of CTX-M enzymes among Enterobacteriaceae isolates in the United States prompted us to investigate CTX-M enzymes among NARMS NTS isolates collected from humans in 2007.

The Study

In 2007, public health laboratories in all US state health departments submitted every twentieth NTS isolated from humans to CDC for susceptibility testing by NARMS. MICs were determined by using broth microdilution (Sensititer, Trek Diagnostics, Westlake, OH, USA) and interpreted according to Clinical Laboratory Standards Institute criteria, where available.

Of the 2,165 human NTS isolates submitted to NARMS in 2007, a total of 100 (4.6%) displayed elevated MICs (>2 mg/L) of ceftriaxone or ceftiofur, extended-spectrum cephalosporins used in human and veterinary medicine, respectively. Genomic DNA prepared from the 100 isolates and a PCR screen obtained by using degenerate primers capable of detecting all CTX-M enzymes identified 3 positive isolates, including serotypes Typhimurium; I 4,5,12:i–; and Concord (4). Most (66%) of the remaining 97 isolates harbored a bla<sub>CMY</sub> gene.

The 3 CTX-M–producing Salmonella spp. infections occurred in 2 female patients (8 months of age and 72 years of age) and 1 male patient (1 year of age). Interviews were available for 2 patients, the 8-month-old (her parents) and the 72-year-old; in both instances, gastrointestinal symptoms with diarrhea were reported, and medical care was sought. Both patients received antimicrobial drug treatment (azithromycin and levofloxacin, respectively). The 72-year-old patient had traveled internationally before illness onset; the 8-month-old patient, who was infected with S. enterica serovar Concord, was an adoptee from Ethiopia.

All 3 isolates displayed resistance to β-lactams and extended-spectrum cephalosporins (Table). The S. enterica serovar Typhimurium and Concord isolates displayed additional multiresistance phenotypes. In addition, the serovar Typhimurium isolate displayed resistance to the quinolone nalidixic acid, a resistance phenotype associated with...
decreased susceptibility to fluoroquinolones. The serovar Typhimurium and Concord isolates showed decreased susceptibility to ciprofloxacin (MIC 0.25 mg/L and 0.125 mg/L, respectively). PCR for the plasmid-mediated mechanisms qnrA,B,S and aac(6′)Ib-cr showed a qnrA gene in the serovar Concord isolate. Sequencing confirmed qnrA1.

Group-specific PCR primers were used to characterize the presumed $bla_{CTX-M}$ genes (5). S. enterica serovar Concord and I 4,5,12:i– harbored group II enzymes, whereas the S. enterica serovar Typhimurium isolate harbored a group II enzyme. Sequencing showed $bla_{CTX-M-15}$ in the serovar Concord isolate, $bla_{CTX-M-5}$ in the serovar Typhimurium isolate, and $bla_{CTX-M-55/57}$ in the serovar I 4,5,12:i– isolate. Presence of other β-lactamase–encoding genes ($bla_{TEM}$, $bla_{SHV}$, $bla_{OXA}$, and $bla_{SOX}$) was investigated by using PCR (6–9). Amplification and sequencing confirmed a $bla_{OXA}$ and a $bla_{SHV}$ gene in the serovar Typhimurium and Concord isolates, respectively (7,8).

The genetic environment of each $bla_{CTX-M}$ gene was investigated by using PCR aimed at identifying insertion elements ISEcp1, IS26, and CR1 (formerly known as orf513) (10). Amplification and sequencing of the PCR products confirmed the ISEcp1 element upstream of each $bla_{CTX-M}$ gene (10). In addition to ISEcp1, an IS26 element was detected upstream of the $bla_{CTX-M-55/57}$ and $bla_{CTX-M-15}$ genes.

To determine whether the CTX-M enzymes were plasmid borne, we extracted and transformed plasmids into electrocompetent E. coli DH10B. The $bla_{CTX-M-55/57}$ gene transferred to E. coli; repeated attempts to transfer the $bla_{CTX-M-5}$ and $bla_{CTX-M-15}$ genes were unsuccessful. PCR amplification and plasmid pulsed-field gel electrophoresis confirmed the presence of the $bla_{CTX-M-55/57}$ gene on a 70-kb plasmid in the transformant. The plasmid was not typeable by PCR-based incompatibility/replicon typing.

Conclusions
We describe 3 CTX-M–producing isolates of NTS collected from humans in the United States during 2007. CTX-M–producing Salmonella spp. previously have been reported among the NARMS collection of human isolates. The first isolate was S. enterica serovar Typhimurium from a 3-month-old child in Georgia in 2003 (11). This infection was considered domestically acquired because the child’s family did not report a history of international travel. In addition, a CTX-M-15–producing isolate of S. enterica serovar Concord was identified among NARMS NTS collected in 2006 (12). However, in contrast to the previous case, this infection most likely was acquired abroad because the patient reported travel to Ethiopia in conjunction with illness onset.

At least 1 of the infections described in the present study probably was acquired abroad; the CTX-M-15–producing S. enterica serovar Concord isolate was isolated from an adopted child from Ethiopia. Thus, both instances of CTX-M–producing serovar Concord isolates identified in NARMS thus far have been associated with travel to Ethiopia. The emergence of CTX-M-15–producing serovar Concord infections among Ethiopian adoptees has been described previously (13). In addition, the emergence of serovar Concord isolates that produced CTX-M-15, SHV-12, and QnrA1 was recently described (14).

The fact that $bla_{CTX-M}$ genes commonly are located on plasmids and in conjunction with mobile genetic elements such as ISEcp1 most likely has contributed to their dissemination. The $bla_{CTX-M-55/57}$ gene that was transferable to E. coli in the present study was located on a 70-kb plasmid. The fact that the $bla_{CTX-M-5}$ and $bla_{CTX-M-15}$ gene did not transfer might suggest chromosomal locations. In fact, Fabre et al. found that most CTX-M–15–producing S. enterica serovar Concord isolates studied harbored the $bla_{CTX-M}$ gene on the chromosome (13).

The recently reported increase in CTX-M–producing Enterobacteriaceae in the United States raises concern. First, a reservoir of ESBLs and CTX-M genes among E. coli and Klebsiella spp. constitutes a risk factor for increased spread of resistance to other pathogenic bacteria, including Salmonella spp. Second, use of cephalosporins to treat serious ESBL–producing bacterial infections has been associated with high rates of treatment failure (15). Thus, an increase in CTX-M–producing Salmonella spp. strains is likely to directly affect treatment, especially among children for whom use of fluoroquinolones is contraindicated.
Moreover, the decreased susceptibility to ciprofloxacin of the serovar Typhimurium and Concord isolates in the present study raises concern about the emergence of isolates showing concurrent resistance to both extended-spectrum cephalosporins and fluoroquinolones. Continued surveillance of resistant bacteria, in combination with prudent use of antimicrobial agents in animals and humans, is crucial for limiting further spread of CTX-M–producing isolates of Enterobacteriaceae in the United States.

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References


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