Nosocomial Outbreak of Pediatric Gastroenteritis Caused by CTX-M-14-Type Extended-Spectrum β-Lactamase-Producing Strains of Salmonella enterica Serovar London

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CTX-M-14-type extended-spectrum β-lactamase was first detected in Salmonella enterica serovar London strains which were isolated from three hospitalized pediatric patients with gastroenteritis. The isolates had pulsed-field gel electrophoresis patterns identical to those of the previously isolated antimicrobial-susceptible strains from community-acquired gastroenteritis, suggesting the susceptible clone acquired the resistance.

Nontyphoidal salmonella (NTS) is one of the most important enteric pathogens even in developed countries, and it may also cause bloodstream and other extraintestinal infections for which antimicrobial therapy is required. Salmonella spp. are usually susceptible to many antimicrobial agents, but a recent increase of resistance has become a concern (18). Salmonella enterica serovar London is not a common serovar; the infection was rarely reported, but an epidemic occurred in Hungary in 1980 (9). Only 4 of 1,306 (0.3%) Salmonella isolates were of this serovar in 1998 in Korea (7). However, the number increased to 74 in the period from 2000 to 2001 (8), indicating a wide dissemination. In 2000, an infant formula-associated community-acquired gastroenteritis outbreak affecting 31 infants was caused by Salmonella serovar London (16), and even a rare endophthalmitis infection in a previously healthy 3-month-old infant was similarly caused (20). There were no reports of outbreaks of Salmonella serovar London infection in 2002 or 2003, but the National Institute of Health, Korea, reported that 23 of 632 (3.6%) Salmonella isolates were of this serovar in 2003 (http://dis.cdc.go.kr/cdmr/eng_cdmr.asp).

A small outbreak of gastroenteritis occurred, affecting three hospitalized pediatric patients during an 11-day period in June 2004. Serogroup E Salmonella strains with cefotaxime resistance were isolated from watery stool specimens, and this prompted us to determine a possible relationship between these strains and the recently reported Salmonella serovar London strains (8, 16) and to determine the mechanism of β-lactam resistance.

The serovar of the isolates was determined at the National Institute of Health, Korea. Antimicrobial susceptibility was tested by the disk diffusion method (14), and extended-spectrum β-lactamase (ESBL) was screened by the double-disk synergy test with amoxicillin-clavulanic acid versus cefotaxime and ceftazidime disks with distances of 15 mm from edge to edge. Etest strips and Etest ESBL strips (AB BIODISK, Solna, Sweden) were used to determine MICs of cefotaxime and ceftazidime and to confirm ESBL production, respectively. Isoelectric points (pIs) of the β-lactamases were determined as described previously (11). The bla TEM and bla SHV alleles were detected by PCR as described previously (2, 10). The primers shown in Table 1 were used to detect or sequence the bla CTX-M gene with the following conditions: 30 cycles of 94°C for 30 s, 54°C for 30 s, and 72°C for 45 s. Proteus mirabilis strain YMC 03/01/U226, which harbors the bla CTX-M-14 gene, was used as a positive control. The sequencing was performed as reported previously (11). Resistance transfer was tested by both broth- and plate-mating methods using an azide-resistant recipient, Escherichia coli strain J53. Pulsed-field gel electrophoresis (PFGE) of XbaI-restricted genomic DNA was performed according to the manufacturer’s instructions (Bio-Rad, Hercules, Calif.), and the patterns were visually compared with those of recently reported Salmonella serovar London strains (8, 16).

The three patients developed diarrhea 4, 17, and 22 days after hospitalization, respectively (Table 2). All three Salmonella isolates from the patients were identified as Salmonella serovar London. However, contrary to the previously reported strains (8), the isolates were resistant to ampicillin, cefotaxime, aminoglycosides, and trimethoprim-sulfamethoxazole. ESBL production was suspected by the double-disk synergy test, and the MICs for Etest, i.e., ≥256 μg/ml for cefotaxime, 0.25 μg/ml for cefotaxime-clavulanic acid, 3 to 6 μg/ml for ceftazidime, and 0.38 μg/ml for ceftazidime-clavulanic acid, suggested the enzyme being of the CTX-M type. All three isolates had β-

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lactamase bands of pI 5.4 and >8.0. The \( \text{bla}_{\text{TEM}} \) and \( \text{bla}_{\text{CTX-M}} \) alleles, but not the \( \text{bla}_{\text{SHV}} \) allele, were detected by PCR, and the sequences were identical to those of \( \text{bla}_{\text{TEM}-1} \) and \( \text{bla}_{\text{CTX-M-14}} \).

A majority of the ESBL-producing Salmonella isolates have been \textit{Salmonella enterica} serovar Typhimurium (4) and \textit{Salmonella enterica} serovar Enteritidis (17). Nosocomial infections caused by ESBL-producing NTS are not often reported (4). In Korea, a few isolates of TEM-52-type ESBL-producing NTS were reported, but they were not \textit{Salmonella} serovar London (10).

Among the six groups of CTX-M enzymes (1), the CTX-M-1, -2, and -9 groups have been reported in serovars Typhimurium, Virchow, Enteritidis, Kentucky, Infantis, and Oranienburg (1, 3, 5, 12, 19). However, to the best of our knowledge this is the first report of a CTX-M-14 enzyme-producing \textit{Salmonella} serovar London, which suggests a gradual spread of this resistance to various serovars of \textit{Salmonella}. ESBL genes have the potential to spread to other organisms because they reside on plasmids or in class 1 integrons (1, 3). Repeated attempts failed to transfer the cefotaxime resistance by conjugation, as was reported with other CTX-M-type ESBL-producing isolates (4).

The PFGE patterns of XbaI-digested genomic DNA of all three \( \text{bla}_{\text{CTX-M-14}} \)-positive isolates in this study were identical, suggesting an outbreak by a single clone (Fig. 1). We could not determine the origin of the outbreak. The pattern was also identical to that from community-acquired pediatric patients (8, 16), suggesting an identical clone had been spreading. However, it is interesting that our isolates were multidrug resistant, while the other strains reported were susceptible to multiple antimicrobial agents, including ampicillin. Acquisition of \( \text{bla}_{\text{TEM-52}} \) by NTS during hospitalization was documented in a previous study (9). The \textit{Salmonella} serovar London strains in this study also probably acquired the \( \text{bla}_{\text{CTX-M}} \) gene from other gram-negative bacilli. CTX-M-14 enzyme-producing \textit{Klebsiella pneumoniae} and \textit{E. coli} isolates were detected from blood at the same hospital (15).

Antimicrobial treatment for NTS gastroenteritis is generally not required, but treatment with ceftriaxone or trimethoprim-sulfamethoxazole is recommended if a patient is under six months or over 50 years of age or has underlying diseases (6). Two of our patients did not receive antimicrobial therapy for the gastroenteritis until the persistence of the organisms for 9 and 24 days was known, which provided ample opportunity to disperse the strains. Fluoroquinolone is not a recommended drug for pediatric patients, but a short treatment with oral ciprofloxacin is considered safe and allows a rapid recovery (13). The isolates were susceptible to nalidixic acid, and ciprofloxacin therapy eliminated the \textit{Salmonella} from two patients. The one remaining patient received cefazolin for the treatment of other conditions but did not receive any antimicrobial agent to treat gastroenteritis because she was discharged before the \textit{Salmonella} isolation report was available.

In conclusion, the CTX-M-14-type extended-spectrum \( \beta \)-lactamase was first detected in \textit{Salmonella} serovar London strains which were isolated from three hospitalized pediatric patients with gastroenteritis. The isolates had PFGE patterns identical to those of the previously isolated antimicrobial-susceptible strains from community-acquired gastroenteritis, suggesting the susceptible clone acquired the resistance.

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**Table 1. Primers used to detect and sequence CTX-M-type ESBL genes**

<table>
<thead>
<tr>
<th>PCR target</th>
<th>Utility</th>
<th>Primer</th>
<th>Nucleotide sequence (5‘ to 3’)</th>
<th>Position of primer</th>
<th>Expected size (bp)</th>
<th>GenBank accession no.</th>
<th>Source or reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>CTX-M</td>
<td>Detection</td>
<td>CTX-Uni-F</td>
<td>CVA TGT GCA GYA CCA GTA A</td>
<td>209–227</td>
<td>585</td>
<td>AJ416341</td>
<td>A. Bauernfeind*</td>
</tr>
<tr>
<td>CTX-M-1</td>
<td>Detection</td>
<td>CTX-Uni-R</td>
<td>ARG TSA CCA GAA YMA GCG G</td>
<td>775–793</td>
<td>500</td>
<td>X92506</td>
<td>This study</td>
</tr>
<tr>
<td>CTX-M-2</td>
<td>Detection</td>
<td>CTX-M-2 Gr-F</td>
<td>TCA ATG GGA CGA TGT CAC TG</td>
<td>350–366</td>
<td>360</td>
<td>X92507</td>
<td>This study</td>
</tr>
<tr>
<td>CTX-M-8</td>
<td>Detection</td>
<td>CTX-M-2 Gr-R</td>
<td>CGG TTC GGT AAA GTA GGT CAC</td>
<td>831–849</td>
<td>2236</td>
<td>AF189721</td>
<td>This study</td>
</tr>
<tr>
<td>CTX-M-9</td>
<td>Detection</td>
<td>CTX-M-8 Gr-F</td>
<td>AGA CGG TTC GCA ATC TGA C</td>
<td>789–807</td>
<td>207</td>
<td>This study</td>
<td></td>
</tr>
<tr>
<td>CTX-M-9</td>
<td>Detection</td>
<td>CTX-M-8 Gr-R</td>
<td>TGG CTG GGT GAA GTA AGT G</td>
<td>789–807</td>
<td>207</td>
<td>This study</td>
<td></td>
</tr>
<tr>
<td>CTX-M-14</td>
<td>Sequencing</td>
<td>CTX-M-14-F1</td>
<td>GAG TGT TGC TCT GTC CAT A</td>
<td>196–214</td>
<td>463</td>
<td>AJ416345</td>
<td>This study</td>
</tr>
<tr>
<td>CTX-M-14</td>
<td>Sequencing</td>
<td>CTX-M-14-R2</td>
<td>GAT GAA CGC TTT CCA ATG T</td>
<td>640–658</td>
<td>1347</td>
<td>AF252622</td>
<td>11</td>
</tr>
<tr>
<td>CTX-14-F</td>
<td>Sequencing</td>
<td>CTX 14S-R</td>
<td>TTA CAG CCC TTC CGT TGA G</td>
<td>224–244</td>
<td>131</td>
<td>This study</td>
<td></td>
</tr>
<tr>
<td>CTX-14-R</td>
<td>Sequencing</td>
<td>CTX 14S-R</td>
<td>AAA AAT GAT TGA AAG GTO GTT GT</td>
<td>162–182</td>
<td>124</td>
<td>This study</td>
<td></td>
</tr>
<tr>
<td>CTX-14-Seq-F</td>
<td>Sequencing</td>
<td>TGC TCC AAA GCC AAT ACG A</td>
<td>637–655</td>
<td>124</td>
<td>This study</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\* Personal communication.

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**Table 2. Clinical features of the gastroenteritis patients infected with ESBL-producing \textit{Salmonella} serovar London isolates**

<table>
<thead>
<tr>
<th>Case no.</th>
<th>Age/sex</th>
<th>Underlying disease</th>
<th>Department</th>
<th>Admission date</th>
<th>Date of \textit{Salmonella} culture/persistence</th>
<th>Treatment/outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>18 mo/male</td>
<td>Acute lymphocytic leukemia</td>
<td>Hematooncology</td>
<td>1 June 2004</td>
<td>1 June 2004</td>
<td>Ciprofloxacin/cured</td>
</tr>
<tr>
<td>2</td>
<td>4 yr/male</td>
<td>Ganglioneuroblastoma</td>
<td>Hematooncology</td>
<td>6 June 2004</td>
<td>28 June 2004</td>
<td>Ciprofloxacin/cured</td>
</tr>
<tr>
<td>3</td>
<td>3 mo/female</td>
<td>Meningocele</td>
<td>Neurosurgery</td>
<td>19 June 2004</td>
<td>23 June 2004</td>
<td>Cefazolin/unknown</td>
</tr>
</tbody>
</table>
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REFERENCES