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CTX-M-55-Type Extended-Spectrum β-lactamase-Producing Shigella sonnei Isolated from a Korean Patient Who Had Travelled to China

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We report a case of CTX-M-55-type extended-spectrum β-lactamase (ESBL)-producing Shigella sonnei infection in a 27-year-old Korean woman who had traveled to China. The patient was admitted to the hospital due to abdominal pain, watery diarrhea, and fever (39.3°C). S. sonnei was isolated from her stool specimens, and the pathogen was found to be resistant to cefotaxime due to CTX-M-55-type ESBL. Insertion sequence (IS) Ecpl was found upstream of the blaCTX-M-55 gene. The blaCTX-M-55 gene was transferred from the S. sonnei isolate to an Escherichia coli J53 recipient by conjugation. Pulsed-field gel electrophoresis and Southern blotting revealed that the blaCTX-M-55 gene was located on a plasmid of approximately 130 kb.

Key Words: CTX-M-55, Shigella sonnei, ESBL

INTRODUCTION

Shigellosis is very rare in Korea, but people who travel to certain countries may acquire the infection. The bacteria are excreted through feces and are transmitted by ingestion of contaminated food and water [1]. To reduce the severity of the clinical course of illness and the duration of fecal excretion of bacteria, the WHO recommends ciprofloxacin as the first-line treatment for shigellosis [2, 3]. Ampicillin and trimethoprim-sulfamethoxazole are no longer sufficiently effective due to increases in the bacterial resistance to these drugs [4]. Because of concerns regarding the adverse effects of ciprofloxacin in children and an increase in the resistance to ciprofloxacin, third generation cephalosporins are frequently used as alternative treatments [2, 4]. However, Shigella species producing CTX-M-type β-lactamase are frequently being reported in some parts of the world [1, 5-7]. CTX-M-type β-lactamase is one of the most common extended-spectrum β-lactamases (ESBL), and it confers resistance to all β-lactams except cephamycins and carbapenems [8]. The spread of CTX-M type ESBL-producing Shigella species has become of concern worldwide because the increasing circulation of this resistant strain further narrows the choice of effective antibiotics. In Korea, only CTX-M-14-producing Shigella sonnei have been reported [9-11].

We report the isolation of CTX-M-55-producing S. sonnei from a Korean woman who was admitted to the hospital due to abdominal pain, watery diarrhea, and fever (39.3°C) immediately after having traveled to China.
CASE REPORT

A 27-yr-old woman was admitted to the hospital with watery diarrhea, abdominal pain, and fever (39.3°C) on the day of her return from a 4-day trip to China. Laboratory investigation showed a peripheral blood leukocyte count of 8.2×10⁹/L with 80% polymorphonuclear cells and a C-reactive protein level of 107 mg/L. Stool samples were inoculated on MacConkey and SS agar (BD Diagnostics Systems, Sparks, MD, USA) and incubated at 37°C for 20 hr, and colorless medium-sized colonies developed. The bacterium was identified as *S. sonnei* using the Vitek2 GN ID card (bioMérieux, Marcy l’Etoile, France); a positive slide agglutination test with a *Shigella* serogroup D antiserum (Joong Kyeom Co., Anshan, Korea) confirmed the identification.

The pathogen’s antimicrobial susceptibility was initially determined by the VITEK 2 AST N131 card (bioMérieux, Marcy l’Etoile, France). ESBL production was confirmed by the double disk synergy test using cefotaxime (30 μg), ceftazidime (30 μg) and amoxicillin-clavulanic acid (20/10 μg) disks (BD Diagnostics Systems) [12]. Minimum inhibitory concentrations (MICs) were determined by the CLSI agar dilution method or by the Etest (Table 1). The isolate was resistant to cephalothin, aztreonam, and cefotaxime, but susceptible to cefoxitin and intermediate to ceftazidime. The patient was treated with oral ciprofloxacin, after which her symptoms began to improve. She was discharged 4 days after admission.

To determine the genetic characteristics of the ESBL, PCR and sequencing were carried out as described previously [13]. These investigations identified the ESBL as being encoded by *bla*<sub>CTX-M-55</sub>, which is identical to *bla*<sub>CTX-M-57</sub> [14]. The *bla*<sub>CTX-M-55</sub> gene and surrounding regions were investigated by PCR and sequencing [12], and insertion sequence (IS)<sub>Ecp1</sub> was found to be upstream of *bla*<sub>CTX-M-55</sub>. The *bla*<sub>CTX-M-55</sub> gene was successfully transferred from the *S. sonnei* isolate to an *Escherichia coli* J53 (azide<sup>R</sup>) recipient by plate mating. MacConkey agar containing cefotaxime (2 μg/mL) and sodium azide (100 μg/mL) was used as the selective media. The β-lactam resistance patterns of the *S. sonnei* isolate and the transconjugant were similar, but the MICs of ceftazidime and aztreonam for the transconjugant were increased 2-fold, from 8 μg/mL and 24 μg/mL to 16 μg/mL and 48 μg/mL, respectively (Table 1).

To estimate the size of the plasmid harboring *bla*<sub>CTX-M-55</sub>, genomic DNA from *S. sonnei* isolate and its transconjugant were digested with S1 nuclease and separated by pulsed-field gel electrophoresis [15]. Southern blotting was performed on the agarose gels by in-gel hybridization with a *bla*<sub>CTX-M-55</sub> probe labeled with digoxigenin (Roche Diagnostics, Indianapolis, IN, USA) [15]. This experiment showed that *bla*<sub>CTX-M-55</sub> was located on a plasmid of approximately 130 kb in each of the strains (Fig. 1). Replicon typing of the plasmid by PCR was unsuccessful [16].

Table 1. Antimicrobial susceptibility of the clinical isolate, transconjugant, and *Escherichia coli* J53 recipient determined by the Etest or the agar dilution method

| Antibiotics                  | Shigella sonnei | Transconjugant | Escherichia coli J53 |
|------------------------------|-----------------|----------------|----------------------|
| Ampicillin                   | >256            | >256           | 4                    |
| Piperacillin<sup>*</sup>      | >128            | >128           | 1                    |
| Ampicillin-sulbactam<sup>*</sup> | 16              | 16             | 8                    |
| Aminocillin-clavulanic acid  | 6               | 6              | 6                    |
| Cephalothin                  | >256            | >256           | 4                    |
| Cefoxitin                    | 4               | 8              | 2                    |
| Cefotaxime                   | 128             | 128            | 0.064                |
| Ceftazidime                  | 8               | 16             | 0.125                |
| Cefepime                     | 8               | 12             | 0.064                |
| Aztreonam                    | 24              | 48             | 0.125                |
| Ciprofloxacin<sup>*</sup>    | 0.12            | 0.03           | 0.032                |
| Trimethoprim-sulfamethoxazole| >32             | 0.032          | 0.032                |
| Imipenem                     | 0.25            | 0.25           | 0.25                 |
| Meropenem<sup>*</sup>        | 0.03            | 0.03           | 0.064                |
| Colistin<sup>*</sup>         | 0.5             | 0.5            | 0.25                 |

*MIC was determined by the agar dilution method. Abbreviation: MIC, minimum inhibitory concentration.

![Fig. 1. Pulsed-field gel electrophoresis of the S1 nuclease-digested genomic DNA (A) and Southern blot hybridization with the *bla*<sub>CTX-M-55</sub> probe (B). The arrow indicates plasmids in the *Shigella sonnei* isolate (lane 1) and the transconjugant (lane 2).](image-url)
DISCUSSION

CTX-M β-lactamases are plasmid-mediated ESBLs that are typically found to have high hydrolytic activity against cefotaxime [17]. During the last 2 decades, CTX-M β-lactamases have rapidly spread though the world and 133 different CTX-M β-lactamases are now recognized [14]. CTX-M-55 was first reported in ESBL-producing Escherichia coli and Klebsiella pneumoniae in Thailand during 2004 and 2005. That study reported 7 CTX-M-55-producing isolates (6 E. coli and 1 K. pneumoniae) [18]. Thereafter, CTX-M-55-producing Enterobacteriaceae isolates have been reported in both humans and animals in several countries [1, 19-23]. Interestingly, most of these studies have been conducted in China, which suggests that CTX-M-55-producing Enterobacteriaceae, including S. sonnei, are widely disseminated throughout China [1]. CTX-M-55 differs from CTX-M-15 only by a single amino acid substitution (valine for alanine) at position 80 (Ala80Val). Therefore, CTX-M-55 is expected to have similar hydrolytic activity as CTX-M-15 and to exhibit increased catalytic efficiency against cefazidime as well as cefotaxime [24].

Previous studies have reported that CTX-M-55-producing isolates generally show high resistance to cefazidime (MIC range from 32 to > 256 μg/mL) [13, 18, 25, 26]. However, 2 of 3 CTX-M-55-producing S. sonnei isolates from China were susceptible to cefazidime [1]. Our isolate also had a relatively low MIC for cefazidime (8 μg/mL), which was classified as intermediate sensitivity according to the CLSI breakpoints [27].

The blaCTX-M genes can be transferred to other bacteria more readily than other plasmid-mediated bla genes because IS6103, which is frequently found upstream of the blaCTX-M genes, has an important role in the mobilization and expression of blaCTX-M genes [23, 28]. In our isolate, the blaCTX-M-55 gene was flanked by IS6103 and both were located on the plasmid.

Tängdén et al. [29] reported that 24 of the 100 travelers, who had been studied, had recently been infected with ESBL-producing E. coli after a trip to a foreign country. Five of the 21 patients who completed a 6-month follow-up were found to be infected with ESBL-producing strains. A study analyzing the duration of colonization with ESBL-producing E. coli in patients with travelers’ diarrhea found that 10 of the 41 patients carried ESBL-producing E. coli at the first follow-up (3–8 months), and 4 patients still carried ESBL-producing E. coli after 3 yr [30]. The results from these studies indicate that travel to a foreign country plays an important role in the acquisition of and infection with ESBL-producing Enterobacteriaceae. Furthermore, these ESBL-producing Enterobacteriaceae can disseminate through the population. Specifically, the dissemination of new antimicrobial resistance genes may lead to major healthcare issues. In order to prevent the inflow of ESBL-producing Enterobacteriaceae, it is important to monitor suspected carriers of ESBL-producing organisms.

In summary, we report a case of CTX-M-55-producing S. sonnei from a Korean patient who had just returned from China, suggesting that the infection was acquired during the travel.

Authors’ Disclosures of Potential Conflicts of Interest

No potential conflicts of interest related to this article were reported.

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