Possibility of CTX-M-14 Gene Transfer from *Shigella sonnei* to a Commensal *Escherichia coli* Strain of the Gastroenteritis Microbiome

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**Abstract**

**Objectives:** To investigated whether the CTX-M-14 gene could be transferred from a clinical *Shigella sonnei* strain to commensal *Escherichia coli* strain in the gastroenteritis microbiome.

**Methods:** *E. coli* strains were isolated from 30 stool samples of *S. sonnei* infected students in a gastroenteritis outbreak in 2004 and were characterized by antibiotic resistance analysis, *in vitro* conjugation and *in vivo* transfer of CTX-M-14 gene and molecular assays.

**Results:** One strain of *Escherichia coli* that had high levels of resistance to cefotaxime was isolated from a patient infected with *S. sonnei*. Isoelectric focusing showed that the *E. coli* and *S. sonnei* strains produced a β-lactamase with an isoelectric point of 8.1. Moreover, polymerase chain reaction analysis indicated that both strains possessed the same DNA sequences for CTX-M-14. The results of *in vitro* and *in vivo* conjugation showed that the efficiency of CTX-M-14 transfer from *S. sonnei* to *E. coli* was similar to CTX-M-14 transfer between *E. coli* strains.

**Conclusion:** The data suggest that the acquisition of the extended-spectrum β-lactamases gene by pathogenic bacteria in the human intestinal tract to commensal microbiome bacteria can cause serious infectious diseases.

1. Introduction

Antimicrobial resistance has become a global public health problem, which is caused by the over use of antibiotics [1–4]. Numerous studies have been published that describe the epidemiology and molecular characterization of the extended-spectrum β-lactamases (ESBLs) [5,6].

In the *Enterobacteriaceae*, resistance to ampicillin is mainly due to ESBLs such as TEM-1 and SHV-1 enzymes that hydrolytically cleave the β-lactam ring [7]. However, CTX-M-type ESBLs have recently acquired a major role as...
emerging resistance determinants to expanded-spectrum cephalosporins in Enterobacteriaceae [8]. In some epidemiological settings the prevalence of CTX-M-type enzymes can be even higher than that of the TEM- or SHV-type ESBL variants [9–12]. At present, the CTX-M family comprises >50 enzymes that have greater hydrolytic activity against cefotaxime than ceftazidime and can be subclassified to five major groups [8]. Escherichia coli is the first species in which CTX-M-type enzymes were identified as acquired ESBLs [13]. Dissemination of CTX-M-type enzymes in Klebsiella pneumoniae and Salmonella enterica has increasingly been reported [10,14,15].

In Korea, CTX-M-type enzymes have also been observed; for example, CTX-M-14 in a Shigella sonnei strain isolated during an outbreak of gastroenteritis in 2000 and CTX-M-12 from three clinical E. coli isolates [16,17].

The dissemination of ESBL may be due to the horizontal transfer of resistance plasmids. However, little is known about the transfer interspecies. In this study, to investigate the possible transfer of resistance plasmids, we have isolated fecal Escherichia coli strains from the patient infected with S. sonnei during an outbreak of gastroenteritis in 2004 and performed in vivo-transfer of the CTX-M-14 gene in a mouse model between the isolated fecal Escherichia coli strains and the clinical S. sonnei strain from a patient.

2. Materials and methods

2.1. Bacterial strains

For the test of antibiotic resistance in this study, 150 E. coli strains were isolated from 30 students of Chungju Elementary School in Korea (Table 1), who had visited hospital during a gastroenteritis outbreak in 2004. They were patients who had been infected by S. sonnei. E. coli BL21(DE3) was the host for cloning experiments. E. coli J53 AzideR and E. coli ATCC 25933 were used as a recipient strain for conjugative transfer and a minimal inhibitory concentration (MIC) reference strain, respectively.

2.2. Antimicrobial susceptibility testing

Antibiotic susceptibility of the isolates was tested by the disk diffusion method on Mueller–Hinton agar (bioMérieux, Marcy l’Etoile, France) and agar dilution methods according to the recommendations of the Clinical and Laboratory Standards Institute [18]. The following antibiotics were tested: ampicillin/sublactam, ampicillin, piperacillin/tazobactam, cephalothin, cefoxitin, cefotetan, cefotaxime, ceftipime, tobramycin, gentamicin, amikacin, netilmicin, tetracycline, aztreonam, trimethoprim/sulfamethoxazole, and imipenem. E. coli ATCC 25922 and E. coli ATCC 35218 were used as quality controls. MICs of β-lactams were determined alone or in combination with a fixed concentration of clavulanic acid (4 mg/L).

2.3. Isoelectric focusing

To determine the isoelectric point (pI), 5 mL of the condensed supernatant containing β-lactamase was loaded onto a Novex IEF Gel (pH 3–10; Invitrogen, Carlsbad, CA, USA) with an Xcell Surelock Mini-Cell system (Invitrogen). Running conditions were 100 V constant for 1 hour, 200 V constant for 1 hour, and 500 V for 30 minutes. The pI of the β-lactamase was measured by staining the gel with a 0.05% solution of nitrocefin (Oxoid, Basingstoke, UK).

2.4. Polymerase chain reaction

Searches for genes coding for ESBLs were performed by polymerase chain reaction (PCR) amplification with the specific primers as followed: blaTEM-gene (F-ATGAGTATCTTACATTTCCG and R-CTGACAGTTACCAATGCTTA), blaSHV-gene (F-GGTTATCTTTATTTGTCGC and R-TTACGCAGCCTGCATGCA and CTC) and blaCTX-M-gene (F-TTGGCATGTGCACTTACAGTAA and R-CTGATACGTGGGTTGCCATA). The templates for PCR amplification in clinical isolates were a whole-cell lysate. The PCR products were subjected to direct sequencing. Both strands of each PCR product were sequenced twice with an automatic sequencer (model 373A; Applied Biosystems, Weiterstadt, Germany).

2.5. In vitro filter mating

Conjugation experiments were performed by the filter mating procedure as follows. Donor (ESBL Shigella sonnei isolate) and recipient (E. coli J53 Azl) strains were grown with shaking in brain–heart infusion (BHI; bioMérieux) broth for 6 hours at 37°C, then 100 mL of the donor and the recipient strains were spread onto an 0.45 μm (pore size) nitrocellulose membrane filter (Millipore, Saint-Quentin, France) placed on top of BHI agar (bioMérieux). After 18 hours of incubation at 37°C, the cells were suspended in broth, diluted in sterile water supplemented with peptone in 10-fold series, and 100 mL of each dilution were plated on selective medium.

2.6. In vivo conjugation

For the in vivo conjugation, germ-free consanguineous C3H mice (mean weight, 25 g) were used. The germ-free mouse received the recipient strain E. coli J53

| Table 1. Information of collected stool samples from students in an outbreak in 2004 | Patients with dysentery |
|---|---|---|
| Age groups | Male | Female | Total |
| 12y | 13 | 13 | 26 |
| 13y | 2 | 2 | 4 |
| Total | 15 | 15 | 30 |
Azir [100 colony-forming-units (CFU)] and donor strain S. sonnei (20 CFU). Two types of transfer, i.e., CTX-M-14 gene transfer from ESBL S. sonnei isolate to E. coli J53 Azir and CTX-M-14 gene transfer from ESBL E. coli isolate to E. coli J53 Azir, were studied in this model. Fecal samples were collected from the mice in 1 week following the inoculation of the donor strain, then once/week for 3 weeks. Ten-fold dilutions were made in 0.85% saline and cultivated on CTX-M media. Bacterial counts were expressed as log (CFU) of feces.

3. Results

3.1. Characterization of antibiotic resistance and ESBL of E. coli strain isolated from a patient of ESBL S. sonnei outbreak

In total, 150 E. coli strains were isolated from 30 stool samples of S. sonnei infected students in a gastroenteritis outbreak in 2004. For the test of antibiotic resistance of the E. coli strains, agar dilution methods were used. The isolates showed high antibiotic resistance to ampicillin (36.7%), cephalothin (26.7%), tetracycline (40%), and trimethoprim/sulfamethoxazole (23.3%). However, among the isolates, no resistance to netilmicin or aztreonam was found (Figure 1).

One strain of E. coli was isolated from a student also had a high level of cefotaxime resistance similar to S. sonnei strains isolated from an outbreak in 2004. MIC analysis of both E. coli and S. sonnei strains showed resistant phenotype to ampicillin, ticarcillin, cefotaxime, cephalothin, nalidixic acid, and trimethoprim/sulfamethoxazole, but sensible phenotype to cefoxitin and ciprofloxacin. PCR amplifications using primers specific for ESBL-encoding genes revealed that both E. coli and S. sonnei isolates possessed both blaTEM and blaCTX-M-type genes, whereas no blaSHV genes were detected in any of the isolates. Sequences of the blaTEM PCR amplicons were 100% identical to the blaTEM-1 sequence. Sequence data from the amplicons of the CTX-M-1 cluster indicated the presence of CTX-M-14. The genetic organization of the CTX-M-14 gene was investigated by sequencing of the regions surrounding this gene.
Isoelectric focusing of the partially purified β-lactamase of *E. coli* BL21 (DE3) carrying plasmid pET30a-CTX-M-14 revealed a band with a pI value of 8.1.

3.2. Possibility of CTX-M-14 gene transfer from ESBL *S. sonnei* to *E. coli* in gastroenteritis microbiome

To confirm the possibility of transfer of CTX-M-14 from ESBL *S. sonnei* isolate to *E. coli* in gastroenteritis microbiome, we performed *in vitro* conjugation and *in vivo* transfer of CTX-M-14 gene in a mouse model between the ESBL *S. sonnei* strain and *E. coli* strains. Cefotaxime resistance was transferred from ESBL *S. sonnei* to *E. coli* J53 Azir at the similar frequency as conjugation between the ESBL *E. coli* and *E. coli* J53 Azir in *in vitro* conjugation and *in vivo* transfer (Figure 2). Transconjugants were detected in 1 week of inoculation with the donor strain, and persisted throughout the experiment. On Day 21, bacterial counts in the whole intestinal tract were similar to those found in fecal samples. PCR analysis of the transconjugants obtained *in vivo* showed that donor strain and transconjugants harbored CTX-M-14 gene. The transconjugants were found to be resistant to cefotaxime.

4. Discussion

In Korea, the rate of ESBL-producing *Enterobacteriaceae* has increased recently [19]. Several reports have shown that CTX-M-producing *E. coli* isolates are important causes of bloodstream infections, with the urinary tract as the most frequent infection site [20–22]. Our study described for the first time the possibility of the *in vivo* transfer of cefotaxime resistance from ESBL *S. sonnei* to *E. coli* in gastroenteritis microbiome. *In vivo* experiments revealed that the efficiency of CTX-M-14 transfer from *S. sonnei* to *E. coli* was similar to CTX-M-14 transfer between *E. coli* strains. Our model showed the risk of acquisition of the CTX-M-14 gene by pathogenic bacteria in the human intestinal tract to commensal microbiome bacteria. This transfer may occur, leading to the formation of a dangerous pool of ESBL *E. coli* capable of transmitting their resistance gene to other species. These data suggest that bacteria colonizing healthy individuals constitute a reservoir of new or known ESBL genes that could further evolve in the nosocomial setting and be responsible for future epidemic situations. Therefore, ongoing surveillance and investigations for the dynamic and fast evolving ESBL genes in intestine are needed.

Conflicts of interest

All contributing authors declare no conflicts of interest.

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