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ORIGINAL ARTICLE

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

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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 Azide^R 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/sulbactam, ampicillin, piperacillin/tazobactam, cephalothin, cefoxitin, cefotetan, cefotaxime, cefepime, 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).

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

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: bla_{TEM}-gene (F-ATGAGTATTCAACATTTCCG and R-CTGACAGTTACCAATGCTTA), bla_{SHV}-gene (F-GGGTTATTCTTATTTGTCGC and R-TTAGCGTTGCCAGTGCTC) and bla_{CTX-M}-gene (F-TTTGCGATGTGCAGTACCAGTAA and R-CGATATCGTTGGTGGTGCCATA). 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 Azi^r) 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 a 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

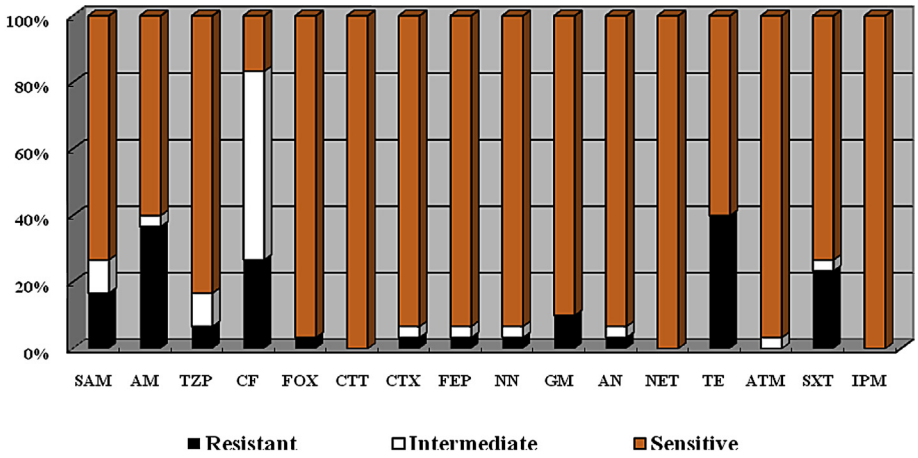

Figure 1. Antibiotic resistance patterns of *Escherichia coli* strains isolated from *Shigella sonnei* infected students in a gastroenteritis outbreak in 2004.

Azi^r [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 Azi^r and CTX-M-14 gene transfer from ESBL *E. coli* isolate to *E. coli* J53 Azi^r, 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 bla_{TEM} and $bla_{CTX\text{-}M}$-type genes, whereas no bla_{SHV} genes were detected in any of the isolates. Sequences of the bla_{TEM} 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.

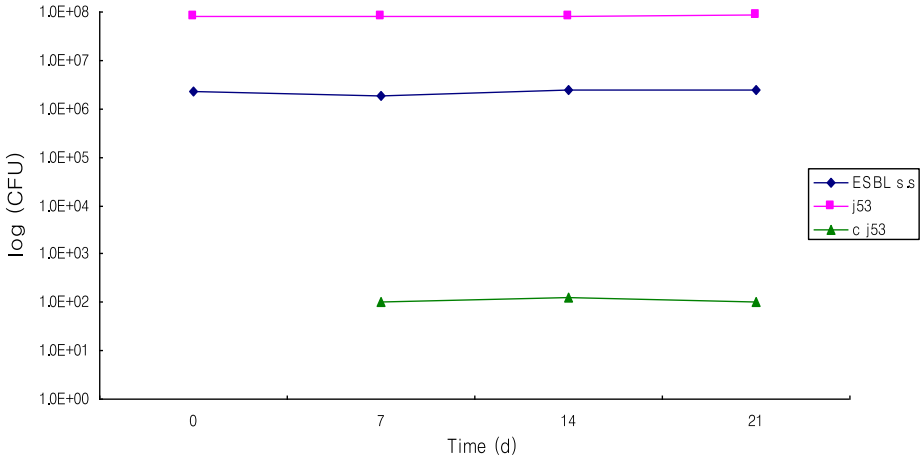

Figure 2. Bacterial counts in the feces of mice in *in vivo* conjugation experiments.

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 Azi^r at the similar frequency as conjugation between the ESBL *E. coli* and *E. coli* J53 Azi^r *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.

Acknowledgments

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References

1. van den Bogaard AE, Stobberingh EE. Antibiotic usage in animals: impact on bacterial resistance and public health. Drugs 1999 Oct;58(4):589–607.
2. Cho SH, Lim YS, Park MS, et al. Prevalence of antibiotic resistance in *Escherichia coli* fecal isolates from healthy persons and patients with diarrhea. Osong Public Health Res Perspect 2011 Jun;2(1):41–5.
3. Cho SH, Lim YS, Kang YH. Comparison of antimicrobial resistance in *Escherichia coli* strains isolated from healthy poultry and swine farm workers using antibiotics in Korea. Osong Public Health Res Perspect 2012 Sep;3(3):151–5.
4. Shin HH, Cho SH. Prevalence of antimicrobial resistance in *Escherichia coli* strains isolated from fishery workers. Osong Public Health Res Perspect 2013 Apr;4(2):72–5.
5. Peirano G, Sang JH, Pitondo-Silva A, et al. Molecular epidemiology of extended-spectrum-β-lactamase-producing *Klebsiella pneumoniae* over a 10 year period in Calgary, Canada. J Antimicrob Chemother 2012 May;67(5):1114–20.
6. Van der Bij AK, Peirano G, Goessens WH, et al. Clinical and molecular characteristics of extended-spectrum-beta-lactamase-producing *Escherichia coli* causing bacteremia in the Rotterdam area, Netherlands. Antimicrobial Agents Chemother 2011 Jul; 55(7):3576–8.
7. Burgess DS, Hall 2nd RG, Lewis 2nd JS, et al. Clinical and microbiologic analysis of a hospital's extended-spectrum β-lactamase-producing isolates over a 2-year period. Pharmacotherapy 2003 Oct;23(10):1232–7.
8. Bonnet R. Growing group of extended-spectrum β-lactamases: the CTX-M enzymes. Antimicrobial Agents Chemother 2004 Jan; 48(1):1–14.
9. Livermore DM, Canton R, Gniadkowski M, et al. CTX-M: changing the face of ESBLs in Europe. J Antimicrob Chemother 2007 Feb;59(2):165–74.
10. Paterson DL, Hujer KM, Hujer AM, et al. Extended-spectrum β-lactamases in *Klebsiella pneumoniae* bloodstream isolates from seven countries: dominance and widespread prevalence of SHV- and CTX-M-type β-lactamases. Antimicrobial Agents Chemother 2003 Nov;47(11):3554–60.
11. Pitout JDD, Church DL, Gregson DB, et al. Molecular epidemiology of CTX-M-producing *Escherichia coli* in the Calgary Health Region: emergence of CTX-M-15-producing isolates. Antimicrobial Agents Chemother 2007 Apr;51(4):1281–6.
12. Valverde A, Coque TM, Sánchez-Moreno MP, et al. Dramatic increase in prevalence of fecal carriage of extended-spectrum β-lactamase-producing *Enterobacteriaceae* during nonoutbreak situations in Spain. J Clin Microbiol 2004 Oct;42(10):4769–75.
13. Bauernfeind A, Grimm H, Schweighart S. A new plasmid cefotaximase in a clinical isolate of *Escherichia coli*. Infection 1990 Sep–Oct;18(5):294–8.
14. Edelstein M, Pimkin M, Palagin I, et al. Prevalence and molecular epidemiology of CTX-M extended-spectrum β-lactamase producing *Escherichia coli* and *Klebsiella pneumoniae* in Russian hospitals. Antimicrobial Agents Chemother 2003 Dec;47(12):3724–32.
15. Edelstein M, Pimkin M, Dmitrachenko T, et al. Multiple outbreaks of nosocomial salmonellosis in Russia and Belarus caused by a single clone of *Salmonella enterica* serovar Typhimurium producing an extended-spectrum β-lactamase. Antimicrobial Agents Chemother 2004 Aug;48(8):2808–15.

16. Pai H, Choi EH, Lee HJ, et al. Identification of CTX-M-14 extended-spectrum beta-lactamase in clinical isolates of *Shigella sonnei*, *Escherichia coli*, and *Klebsiella pneumoniae* in Korea. J Clin Microbiol 2001 Oct;39(10):3747−9.
17. Bae IK, Lee YN, Hwang HY, et al. Emergence of CTX-M-12 extended-spectrum β-lactamase-producing *Escherichia coli* in Korea. J Antimicrob Chemother 2006 Dec;58(6):1257−9.
18. Clinical and Laboratory Standards Institute (CLSI). Performance standards for antimicrobial susceptibility testing. Twenty-Second Informational Supplement. CLSI Document M100-MS19. Wayne, PA: CLSI; 2012.
19. Park SH, Choi SM, Lee DG, et al. Emergence of extended-spectrum β-lactamase-producing *Escherichia coli* as a cause of community-onset bacteremia in South Korea: risk factors and clinical outcomes. Microb Drug Resist 2011 Dec;17(4):537−44.
20. Chung HC, Lai CH, Lin JN, et al. Bacteremia caused by extended-spectrum-β-lactamase-producing *Escherichia coli* sequence type ST131 and non-ST131 clones: comparison of demographic data, clinical features, and mortality. Antimicrobial Agents Chemother 2012 Feb;56(2):618−22.
21. Rodríguez-Baño J, Alcalá JC, Cisneros JM, et al. Community infections caused by extended-spectrum beta-lactamase-producing *Escherichia coli*. Arch Intern Med 2008 Sep 22;168(17): 1897−902.
22. Peirano G, van der Bij AK, Gregson DB, et al. Molecular epidemiology over an 11-year period (2000 to 2010) of extended-spectrum β-lactamase-producing *Escherichia coli* causing bacteremia in a centralized Canadian region. J Clin Microbiol 2012 Feb; 50(2):294−9.