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# Characterization of IncF plasmids carrying the $bla_{CTX\text{-}M\text{-}14}$ gene in clinical isolates of *Escherichia coli* from Korea

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**Objectives:** The purpose of this study was to investigate the molecular epidemiology of CTX-M-14-producing *Escherichia coli* clinical isolates from Korea.

**Methods:** A total of 138 non-duplicate *E. coli* clinical isolates showing reduced susceptibility or resistance to ceftazidime and/or cefotaxime were included in the study. Resistance genes, genetic environment, R plasmid size and replicon type, sequence type (ST) and XbaI-macrorestriction patterns were determined.

**Results:** Among 138 isolates, 35 were found to carry the $bla_{CTX\text{-}M\text{-}14}$ gene. The IS*Ecp1* element was identified in the upstream region of the $bla_{CTX\text{-}M\text{-}14}$ gene in 32 isolates. The $bla_{CTX\text{-}M\text{-}14}$ gene was located on an IncF plasmid in 21 isolates, on an IncA/C plasmid in 1 isolate, on the chromosome in 8 isolates and on both the chromosome and an IncF plasmid in 5 isolates. The most prevalent ST was ST405 ($n=8$), followed by ST354 ($n=4$), ST38 ($n=3$), ST69 ($n=3$) and the intercontinental ST, ST131 ($n=3$). PFGE and multilocus sequence typing experiments demonstrated no major clonal relationship among the CTX-M-14-producing isolates.

**Conclusions:** The $bla_{CTX\text{-}M\text{-}14}$ gene was probably mobilized by IncF plasmids, which can readily spread in *E. coli*, causing horizontal dissemination of the resistance gene in Korea.

**Keywords:** replicon sequence typing, IncA/C plasmid, sequence type 131

## Introduction

CTX-M-14 is one of the most successful plasmid-borne extended-spectrum β-lactamases (ESBLs) in the Enterobacteriaceae, especially in *Escherichia coli*, and shows worldwide distribution.[1] The rapid dissemination of $bla_{CTX\text{-}M}$ genes involves plasmid or strain epidemics, as well as mobile genetic elements such as IS*Ecp1*-like elements.[1,2] Recent reports on the intercontinental dissemination of *E. coli* clone of sequence type (ST) 131 harbouring CTX-M-15 emphasize the importance of epidemiologic studies on strains producing CTX-M enzymes.[3,4]

The $bla_{CTX\text{-}M\text{-}14}$ gene is mainly associated with IS*Ecp1*-like elements,[2] although two cases of the $bla_{CTX\text{-}M\text{-}14}$ gene in association with a complex class 1 integron containing IS*CR1* on a plasmid from clinical isolates of *E. coli* have been described in Korea.[5,6] The $bla_{CTX\text{-}M\text{-}14}$ gene has been found in diverse *E. coli* clones of ST10, ST23, ST131, ST155 and ST359 and on plasmids of diverse incompatibility (Inc) groups, such as IncA/C, IncHI2, IncF and IncK.[7–9] The recent development of a replicon sequence typing method enables us to compare IncF plasmids from different bacterial isolates more precisely.[10] Here we describe the characteristics of IncF plasmids carrying the $bla_{CTX\text{-}M\text{-}14}$ gene in *E. coli* isolates of diverse STs recovered at hospitals in Korea.

## Materials and methods

### Bacterial strains

The 138 non-duplicate *E. coli* isolates in this study were isolated in 11 different hospitals in Korea between May and June 2005 and identified using either a Vitek GNI card (bioMérieux, Marcy l'Étoile, France) or a MicroScan GN combo card (Dade Behring, West Sacramento, CA, USA).

### Antimicrobial susceptibility testing

Antimicrobial susceptibilities were tested by disc diffusion on Mueller–Hinton (MH) agar (Difco, Cockeysville, MD, USA) and by the agar dilution method according to the interpretative criteria proposed by the CLSI.[11] MICs of β-lactams were determined alone or in combination with a fixed concentration of clavulanic acid (4 mg/L). The double-disc synergy

test for confirmation of ESBL activity was carried out as described previously.[12] *E. coli* ATCC 25933 was used as an MIC reference strain.

### β-Lactamase gene identification

Detection of genes coding for plasmid-borne ESBLs and AmpC β-lactamases was performed by PCR amplification with primers as described previously.[12,13] The PCR products were subjected to direct sequencing with an automated DNA sequencer (model 3730xl; Applied Biosystems, Weiterstadt, Germany).

### Analysis of genetic elements surrounding the $bla_{CTX\text{-}M\text{-}14}$ gene

Genetic organization of the $bla_{CTX\text{-}M\text{-}14}$ gene was investigated by PCR and sequencing of regions surrounding the gene.[14] The internal IS*Ecp1* forward and $bla_{CTX\text{-}M\text{-}14}$ reverse primers were used to investigate regions upstream of the $bla_{CTX\text{-}M\text{-}14}$ gene, and forward primers of $bla_{CTX\text{-}M\text{-}14}$ and reverse primers of IS*903*, *orf3*, *orf477*, *orf1005*, d-*iron* and *mucA* genes were used downstream of the $bla_{CTX\text{-}M\text{-}14}$ gene.

### Transfer of resistance

Conjugation experiments were carried out between the donors and the azide-resistant recipient strain *E. coli* J53. Transconjugants were selected on MH agar plates supplemented with cefotaxime (2 mg/L) and sodium azide (100 mg/L).

### PCR-based replicon typing of plasmids

Plasmid replicon typing of the isolates carrying the $bla_{CTX\text{-}M\text{-}14}$ gene was performed by a PCR-based method with 18 pairs of primers as described by Carattoli *et al.*[15]

### PFGE

Plugs containing whole genomic DNA from the *E. coli* isolates were digested with XbaI, I-CeuI or S1 nuclease. DNA fragments were separated by PFGE using a CHEF-DRII device (Bio-Rad, Hercules, CA, USA). The PFGE conditions of XbaI-macrorestriction analysis were 6 V/cm for 20 h with pulse times ranging from 0.5–60 s at a temperature of 14°C. The pulse times for S1 nuclease and I-*Ceu*I restriction analysis were 9–90 s. Lambda ladder (Bio-Rad) was used as a DNA size marker. Tiff format gel images were exported to Molecular Analyst Fingerprinting Software (version 3.2; Bio-Rad) for analysis. Comparisons for *E. coli* isolates were made by using the band-based dice coefficient. Dendrograms were generated using the unweighted pair group with arithmetic averages method with 1.0% position tolerance, and DNA relatedness was calculated based on the criteria of Tenover *et al.*[16]

### Southern blotting

Plasmids linearized with S1 nuclease or I-*Ceu*I-digested chromosomal DNA were blotted onto nylon membranes (Bio-Rad) and hybridized with probes specific for the $bla_{CTX\text{-}M\text{-}14}$, $bla_{TEM\text{-}1}$ or 16S rRNA genes. Probe labelling, hybridization and detection were performed with the DIG DNA Labeling and Detection Kit (Roche Diagnostics, Indianapolis, IN, USA) following the manufacturer's protocols.

### Multilocus sequence typing (MLST)

MLST was performed on CTX-M-14-producing *E. coli* isolates using seven conserved housekeeping genes (*adk*, *fumC*, *gyrB*, *icd*, *mdh*, *purA* and *recA*) following protocols at http://mlst.ucc.ie/mlst/dbs/Ecoli.

### Replicon sequence typing of IncF plasmids

Replicon sequence typing was performed on IncF plasmids carrying the $bla_{CTX\text{-}M\text{-}14}$ gene in *E. coli* isolates for the genes encoding replicons FII, FIA and FIB following protocols at http://pubmlst.org/plasmid/.

### Nucleotide sequence accession numbers

Nucleotide sequence data reported in this paper are available in the GenBank nucleotide database under accession number EU136399 (*E. coli* SCE0510).

## Results and discussion

### Description of clinical isolates and ESBL genotype

Among 138 ESBL-producing *E. coli* isolates, 35 (25.4%) carried the $bla_{CTX\text{-}M\text{-}14}$ gene (Table 1). Other types of ESBL genes, including $bla_{CTX\text{-}M\text{-}3}$ ($n=7$), $bla_{CTX\text{-}M\text{-}9}$ ($n=8$), $bla_{CTX\text{-}M\text{-}12}$ ($n=1$), $bla_{CTX\text{-}M\text{-}15}$ ($n=58$), $bla_{CTX\text{-}M\text{-}57}$ ($n=1$), $bla_{SHV\text{-}2a}$ ($n=8$), $bla_{SHV\text{-}12}$ ($n=17$) and $bla_{TEM\text{-}52}$ ($n=4$), were also detected. ESBL genes were not detected in eight isolates with an ESBL phenotype. Genes encoding AmpC β-lactamases were detected in 24 isolates carrying the ESBL genes: the $bla_{CMY\text{-}2}$ gene in 14 isolates, the $bla_{CMY\text{-}10}$ gene in 1 isolate and the $bla_{DHA\text{-}1}$ gene in 9 isolates. The $bla_{TEM\text{-}1}$ gene was detected in 28/35 isolates carrying the $bla_{CTX\text{-}M\text{-}14}$ gene, some of which also harboured $bla_{SHV\text{-}12}$ ($n=1$) and $bla_{CMY\text{-}2}$ ($n=6$) (Figure 1). One isolate (HYE0515) carried β-lactamase genes $bla_{TEM\text{-}1}$, $bla_{SHV\text{-}2a}$ and $bla_{CTX\text{-}M\text{-}15}$, and seven isolates carried $bla_{CTX\text{-}M\text{-}14}$ only. MICs of β-lactams for CTX-M-14-producing isolates are shown in Table 1.

### Strain typing

PFGE of XbaI-digested DNA showed a genetic similarity <85% for most of the 35 CTX-M-14-producing isolates, which were considered to be unrelated (Figure 1). Although the isolates from different hospitals were heterogeneous, as seen on the dendrogram, some isolates recovered in the same hospital showed an identical PFGE pattern, suggesting possible small clonal outbreaks in these hospitals. MLST experiments identified 15 unique STs in 35 isolates and discovered a new ST, ST2026 (6-4-225-247-9-1-20). The most prevalent ST was ST405 ($n=8$), followed by ST354 ($n=4$), ST38 ($n=3$), ST69 ($n=3$) and the intercontinental ST, ST131 ($n=3$).

### Genetic contexts of the $bla_{CTX\text{-}M\text{-}14}$ gene

IS*Ecp1*-like elements were identified in the upstream region of the $bla_{CTX\text{-}M\text{-}14}$ gene in 32/35 isolates. The 32 isolates were classified into four different types according to their genetic environment: type I ($n=20$), type II ($n=10$), type III ($n=1$) and type IV ($n=1$) (Figure 2). Type I was characterized by IS*Ecp1*-like elements in the upstream region and IS*903* in the downstream region of the $bla_{CTX\text{-}M\text{-}14}$ gene. Type II showed an identical structure to type I in the upstream region of the $bla_{CTX\text{-}M\text{-}14}$ gene,

**Table 1.** Characteristics of *E. coli* isolates carrying the $bla_{CTX\text{-}M\text{-}14}$ gene

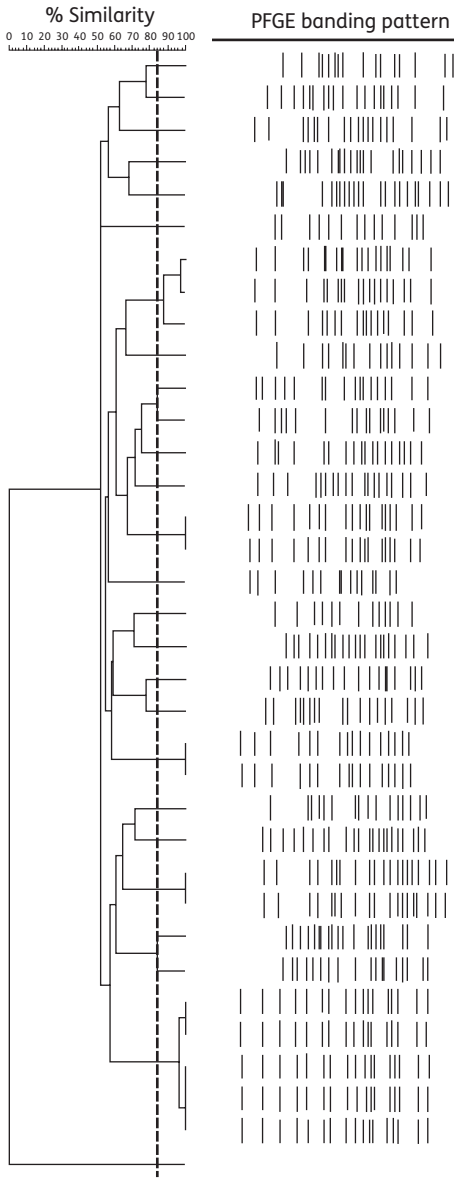
| Isolate | Specimen | DDS | Transfer | MIC (mg/L) | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | ATM | CAZ | CAZ/CLA | CTX | CTX/CLA | FEP | FEP/CLA |
| AJE0507 | urine | + | − | 32 | 4 | 1 | 64 | 4 | 64 | 4 |
| AJE0509 | urine | + | − | 8 | 1 | 0.5 | 64 | 4 | 16 | 1 |
| AJE0510 | urine | + | + | 2 | 0.5 | 0.25 | 32 | 1 | 4 | 0.5 |
| BDE0504 | urine | + | + | 8 | 1 | 0.5 | 32 | 2 | 16 | 1 |
| BDE0516 | fluid | + | − | 64 | 2 | 0.5 | 64 | 2 | 8 | 0.5 |
| HYE0515 | urine | + | + | >256 | >256 | 32 | >256 | 128 | 256 | 16 |
| JNE0502 | urine | − | + | 128 | 2 | 0.25 | 32 | 1 | 4 | 0.5 |
| JNE0507 | urine | + | + | 8 | 4 | 0.5 | 256 | 1 | 8 | 8 |
| JNE0510 | urine | + | + | 16 | 32 | 32 | 64 | 16 | 32 | 1 |
| KSE0510 | urine | + | + | 8 | 1 | 0.25 | 32 | 2 | 16 | 1 |
| KSE0512 | blood | + | − | 16 | 1 | 0.5 | 64 | 1 | 32 | 0.5 |
| KSE0519 | wound | + | + | 16 | 2 | 0.5 | 64 | 1 | 16 | 0.5 |
| SCE0502 | wound | + | − | 4 | 1 | 0.12 | 32 | 0.5 | 16 | 0.25 |
| SCE0503 | sputum | + | − | 16 | 4 | 0.5 | 32 | 2 | 32 | 0.5 |
| SCE0506 | sputum | + | + | 8 | 8 | 0.5 | 32 | 1 | 16 | 0.5 |
| SCE0507 | sputum | + | + | 8 | 8 | 0.5 | 32 | 2 | 16 | 1 |
| SCE0508 | wound | + | + | 16 | 16 | 0.5 | 32 | 4 | 16 | 2 |
| SCE0510 | wound | + | + | 64 | 64 | 0.5 | 64 | 4 | 16 | 2 |
| SCE0513 | urine | + | − | 128 | 16 | 4 | 256 | 32 | 64 | 1 |
| SCE0514 | urine | + | − | 8 | 8 | 0.25 | 32 | 0.5 | 16 | 0.5 |
| SCE0516 | urine | + | + | 16 | 16 | 0.5 | 32 | 4 | 16 | 1 |
| SME0507 | urine | + | − | 32 | 32 | 32 | 64 | 32 | 32 | 4 |
| SME0509 | urine | + | − | 8 | 2 | 1 | 32 | 2 | 8 | 1 |
| SME0511 | urine | + | − | 8 | 2 | 1 | 32 | 2 | 8 | 0.5 |
| SME0512 | urine | − | − | 32 | 16 | 16 | 16 | 16 | 8 | 0.5 |
| SME0513 | urine | + | + | 8 | 0.5 | 0.25 | 32 | 2 | 4 | 0.5 |
| SNE0506 | urine | + | + | 32 | 16 | 0.5 | 64 | 1 | 16 | 0.25 |
| SSE0501 | urine | + | − | 32 | 64 | 32 | 32 | 32 | 16 | 2 |
| SSE0511 | urine | + | − | 32 | 64 | 64 | 64 | 32 | 32 | 4 |
| SSE0514 | sputum | + | − | 4 | 1 | 0.5 | 16 | 0.25 | 4 | 0.25 |
| SSE0519 | urine | + | + | 32 | 64 | 64 | 32 | 32 | 16 | 2 |
| WKE0502 | urine | + | − | 4 | 0.5 | 0.5 | 16 | 0.5 | 4 | 0.5 |
| WKE0505 | urine | + | − | 64 | 16 | 16 | 128 | 128 | 64 | 32 |
| WKE0506 | urine | + | + | 32 | 8 | 4 | 128 | 32 | 32 | 16 |
| WKE0507 | wound | + | + | 32 | 2 | 0.5 | 128 | 4 | 32 | 1 |

DDS, double-disc synergy [in the DDS test, cefotaxime (30 μg), ceftazidime (30 μg) and cefepime (30 μg) discs were placed adjacent to an amoxicillin/clavulanic acid disc (20/10 μg) at inter-disc distances (centre to centre) of 20 mm on Mueller–Hinton agar]; ATM, aztreonam; CAZ, ceftazidime; CAZ/CLA, ceftazidime/clavulanic acid; CTX, cefotaxime; CTX/CLA, cefotaxime/clavulanic acid; FEP, cefepime; FEP/CLA, cefepime/clavulanic acid.

however, the downstream IS*903* element was absent. Type III also showed a structure similar to type I, but IS*10* disrupted the 3′ non-coding region of an IS*Ecp1*-like element upstream of the $bla_{CTX\text{-}M\text{-}14}$ gene (ΔIS*Ecp1*-IS*10*-$bla_{CTX\text{-}M\text{-}14}$-IS*903*). Finally, type IV also showed a structure similar to type I, but IS*26* disrupted a 5′ open reading frame region of the IS*Ecp1* upstream of the $bla_{CTX\text{-}M\text{-}14}$ gene (ΔIS*26*-ΔIS*Ecp1*-$bla_{CTX\text{-}M\text{-}14}$-IS*903*). Type I and type II possessed right-inverted repeats (IRRs; 5′-CACACGTGGAATTTAGG-3′, 17 bp) of IS*Ecp1* between the IS*Ecp1* and $bla_{CTX\text{-}M\text{-}14}$ gene, and a sequence of 42 bp common to all type I and II isolates was observed between the IRRs and the $bla_{CTX\text{-}M\text{-}14}$ start codon. This 42 bp sequence was also detected downstream of IS*10* in type III and ΔIS*Ecp1* in type IV isolates. Left-inverted repeats (5′-TTTGTTGAATAAATC-3′, 15 bp) of the IS*903* element were observed between the $bla_{CTX\text{-}M\text{-}14}$ gene and IS*903* element in types I, III and IV.

### *Location of the $bla_{CTX\text{-}M\text{-}14}$ gene*

Conjugation experiments for the $bla_{CTX\text{-}M\text{-}14}$ gene were successful in only 18/35 isolates, despite repeated attempts. A probe specific for the $bla_{CTX\text{-}M\text{-}14}$ gene hybridized with IncF plasmids of various sizes, ranging from 70–300 kb, in 26 isolates. The probe hybridized with a 150 kb IncA/C plasmid in one isolate.




| Isolate | Hospital | ST | Other β-lactamase | Genetic environment of $bla_{CTX-M-14}$ | The $bla_{CTX-M-14}$ probe hybridized with; | |
|---|---|---|---|---|---|---|
| | | | | | Plasmid (size, replicon type) | Chromosomal fragment (size) |
| JNE0507 | Jeonnam A | 167 | TEM-1 | IS*Ecp1*-14-IS*903* | 150-kbp, F31:A-:B7 | |
| SME0511 | Seoul C | 14 | TEM-1 | 14-IS*903* | 100-kbp, F-:A1:B- | |
| SCE0508 | Gyeongbuk A | 70 | TEM-1 | IS*Ecp1*-14 | 50-kbp, F-:A1:B- | 110-kbp |
| BDE0504 | Gyeonggi B | 354 | TEM-1 | IS*Ecp1*-14 | 75-kbp, F29:A1:B- | |
| SSE0514 | Seoul A | 46 | | IS*Ecp1*-14-IS*903* | | 800-kbp |
| SME0513 | Seoul C | 2026 | | IS*Ecp1*-14-IS*903* | 150-kbp, IncA/C | |
| KSE0510 | Busan A | 68 | TEM-1 | IS*Ecp1*-14 | 75-kbp, F2:A-:B- | |
| KSE0519 | Busan A | 69 | | IS*Ecp1*-14 | 75-kbp, F2:A-:B- | 110-kbp |
| KSE0512 | Busan A | 68 | | IS*Ecp1*-14 | 70-kbp, F-:A1:B- | |
| AJE0507 | Gyeonggi A | 38 | | IS*Ecp1*-14 | 70-kbp, F2:A-:B- | |
| JNE0510 | Jeonnam A | 1177 | TEM-1, CMY-2 | IS*Ecp1*-14-IS*903* | 200-kbp, F-:A-:B20 | |
| SCE0510 | Gyeongbuk A | 1177 | TEM-1, SHV-12 | IS*26*-ΔIS*Ecp1*-14-IS*903* | 150-kbp, F1:A2:B- | |
| WKE0505 | Gangwon A | 38 | TEM-1 | IS*Ecp1*-14-IS*903* | | 100-kbp |
| AJE0510 | Gyeonggi A | 38 | TEM-1 | IS*Ecp1*-14-IS*903* | 70-kbp, F2:A-:B- | |
| SME0509 | Seoul C | 405 | TEM-1 | IS*Ecp1*-14-IS*903* | | 100-kbp |
| SME0512 | Seoul C | 405 | TEM-1 | IS*Ecp1*-14-IS*903* | | 100-kbp |
| SSE0519 | Seoul A | 354 | TEM-1, CMY-2 | 14-IS*903* | 100-kbp, F44:A-:B- | |
| JNE0502 | Jeonnam A | 10 | TEM-1, CMY-2 | IS*Ecp1*-14 | 110-kbp, F31:A4:B1 | |
| WKE0506 | Gangwon A | 131 | | IS*Ecp1*-14 | 140-kbp, F-:A-:B20 | 150-kbp |
| AJE0509 | Gyeonggi A | 648 | TEM-1 | IS*Ecp1*-14-IS*903* | 170-kbp, F31:A-:B6 | |
| SNE0506 | Seoul B | 405 | TEM-1 | IS*Ecp1*-14-IS*903* | 200-kbp, F2:A-:B1 | 200-kbp |
| SSE0501 | Seoul A | 354 | TEM-1, CMY-2 | ΔIS*Ecp1*-IS*10*-14-IS*903* | 100-kbp, F44:A1:B1 | |
| SSE0511 | Seoul A | 354 | TEM-1, CMY-2 | 14-IS*903* | 100-kbp, F44:A1:B1 | |
| SME0507 | Seoul C | 617 | TEM-1 | IS*Ecp1*-14-IS*903* | | 70-kbp |
| WKE0507 | Gangwon A | 648 | | IS*Ecp1*-14 | 75-kbp, F31:A-:B1 | |
| SCE0502 | Gyeongbuk A | 69 | TEM-1 | IS*Ecp1*-14 | | 570-kbp |
| SCE0514 | Gyeongbuk A | 69 | TEM-1 | IS*Ecp1*-14-IS*903* | | 570-kbp |
| BDE0516 | Gyeonggi B | 131 | TEM-1 | IS*Ecp1*-14-IS*903* | | 70-kbp |
| HYE0515 | Seoul D | 131 | TEM-1, SHV-2a, CTX-M-15 | IS*Ecp1*-14-IS*903* | 120-kbp, F2:A1:B- | |
| SCE0513 | Gyeongbuk A | 405 | TEM-1, CMY-2 | IS*Ecp1*-14-IS*903* | 140-kbp, F31:A-:B6 | 600-kbp |
| SCE0516 | Gyeongbuk A | 405 | TEM-1 | IS*Ecp1*-14-IS*903* | 140-kbp, F45:A-:B6 | |
| SCE0503 | Gyeongbuk A | 405 | TEM-1 | IS*Ecp1*-14-IS*903* | 140-kbp, F31:A-:B6 | |
| SCE0506 | Gyeongbuk A | 405 | TEM-1 | IS*Ecp1*-14-IS*903* | 140-kbp, F31:A-:B6 | |
| SCE0507 | Gyeongbuk A | 405 | TEM-1 | IS*Ecp1*-14-IS*903* | 140-kbp, F31:A-:B6 | |
| WKE0502[a] | Gangwon A | 10 | TEM-1 | IS*Ecp1*-14-IS*903* | 180-kbp, F18A-:B1 | |

**Figure 1.** Dendrogram based on XbaI-macrorestriction patterns of *E. coli* isolates producing CTX-M-14. The broken line indicates 85% similarity. *E. coli* isolates showing similarities of <85% were considered to be unrelated. The location of the $bla_{CTX-M-14}$ gene is indicated in the far right-hand columns. [a]XbaI-macrorestriction analysis yielded no DNA banding patterns due to the degradation of the genomic DNA during preparation of the agarose plugs.

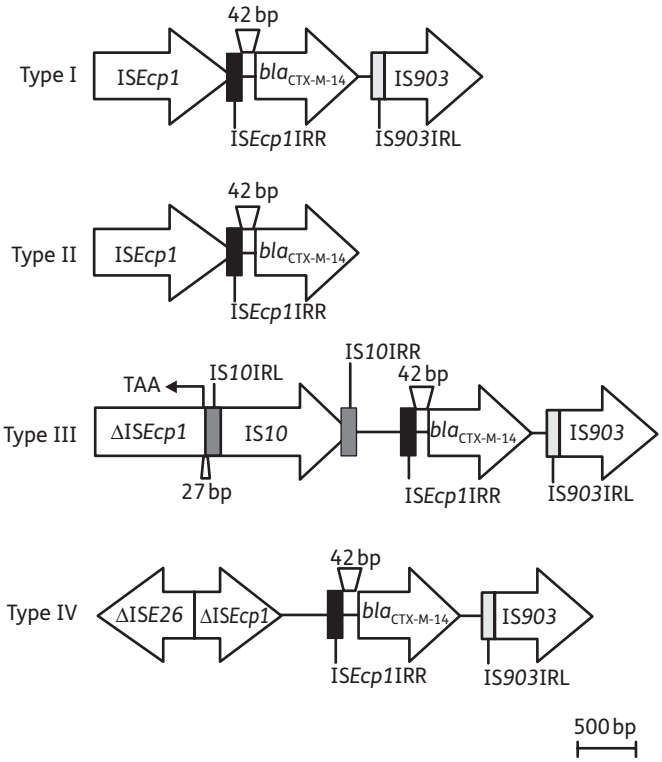



**Figure 2.** Schematic representation of the IS*Ecp1*-related $bla_{CTX-M-14}$ gene in *E. coli* clinical isolates. IRR, right-inverted repeat sequences; IRL, left-inverted repeat sequences.

The probe also hybridized with I-*Ceu*I-macrorestriction fragments ranging from 70–800 kb in 13 isolates. A probe specific for 16S rDNA also hybridized to I-*Ceu*I-macrorestriction fragments of identical sizes, indicating the presence of a $bla_{CTX-M-14}$ gene in the chromosome. In summary, the $bla_{CTX-M-14}$ gene was located on an IncF plasmid in 21 isolates, on an IncA/C plasmid in 1 isolate, in the chromosome in 8 isolates and on both an IncF plasmid and the chromosome in 5 isolates. Notably, the $bla_{TEM-1}$ gene was also present in the same IncF plasmid in 20 isolates.

### *Replicon sequence types of IncF plasmids*

A total of 16 replicon STs were identified in 26 IncF plasmids carrying the $bla_{CTX-M-14}$ gene. Four types of IncF plasmids were identified in multiple isolates from more than two hospitals: (i) the F31:A-;B6 plasmid in six isolates of two different STs (ST405 and ST648) from two hospitals; (ii) the F2:A-:B- plasmid in four isolates of three different STs (ST38, ST68 and ST69) from two hospitals; (iii) the F-:A1:B- plasmid in three isolates of three different STs (ST14, ST68 and ST70) from three hospitals; and (iv) the F-:A-:B20 plasmid in two isolates of two different STs (ST131 and ST1177) from two hospitals. Clinical isolates of ST38, ST69, ST131, ST354 and ST405 were recovered in multiple hospitals, but different types of IncF plasmids were found in isolates of the same ST from different hospitals. For example, isolates of ST354 were recovered in three hospitals, with the $bla_{CTX-M-14}$ gene located on three different plasmids (F44:A1:B1, F29:A1;B- and F44:A-:B-).

The spread of $bla_{CTX-M-14}$ in *E. coli* in Spain is reported to be conferred by IncK plasmids,[9,17] while in Korea and France, $bla_{CTX-M-14}$ is mostly carried on IncF plasmids.[7] During the last 40 years, the $bla_{TEM-1}$ gene carried by IncF plasmids, which are well adapted to *E. coli*, has successfully diffused into *E. coli* strains around the world. In fact, these plasmids, which were responsible for the $bla_{TEM-1}$ outbreak in the late 1980s, are now the vehicle for the successful dissemination of the $bla_{CTX-M-14}$ gene.[18] The results of our study, which showed that the $bla_{TEM-1}$ gene was present in the same IncF plasmids carrying the $bla_{CTX-M-14}$ gene in many isolates, support this model.

In this study, *E. coli* isolates of diverse STs carried the $bla_{CTX-M-14}$ gene, and the gene was located on IncF plasmids of diverse replicon sequence types. Four types of plasmids were identified in more than two isolates from different hospitals. Isolates of the same ST from different hospitals harboured different types of IncF plasmids carrying the $bla_{CTX-M-14}$ gene. These results suggest that horizontal transfer of some IncF plasmids, rather than clonal expansion, may play a role in dissemination of the $bla_{CTX-M-14}$ gene in *E. coli* isolates. Furthermore, our results show the usefulness of plasmid replicon typing in studying the epidemiology of ESBL-producing isolates.

The chromosomal location of genes encoding CTX-M ESBLs in Enterobacteriaceae other than *Proteus mirabilis* has rarely been described, although Coque *et al.*[19] recently reported eight *E. coli* isolates carrying the chromosome-borne $bla_{CTX-M-15}$ gene. Of note, the $bla_{CTX-M-14}$ gene was located in the chromosome in 13 *E. coli* clinical isolates in this study. The $bla_{CTX-M-14}$ gene in the chromosome was surrounded by genetic environments similar to those on plasmids, supporting the hypothesis that the $bla_{CTX-M-14}$ gene integrated into chromosome via transposable elements such as IS*Ecp1*-like elements in *E. coli* strains.[20]

In conclusion, the present data suggest that dissemination of the $bla_{CTX-M-14}$ gene in *E. coli* isolates in Korea is due to horizontal transfer of IncF plasmids rather than clonal expansion. Interestingly, the $bla_{CTX-M-14}$ gene was chromosomally located in many *E. coli* isolates. The clinical significance of this phenomenon needs to be elucidated.

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## Transparency declarations

None to declare.

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