Introduction

Extended spectrum β-lactamases (ESBLs) are enzymes produced by the members of the family Enterobacteriaceae that confer resistance to β-lactam antibiotics, including penicillins, cephalosporins, and monobactams. ESBL-producing *Escherichia coli* strains have been frequently identified as the causative agents of both community-onset and hospital-onset urinary tract infections (UTIs) [13–15, 19].

Recently, CTX-M-14 and CTX-M-15 ESBLs have been shown to have higher activity against cefotaxime than against other oxyimino-β-lactam substrates and to be expressed by most *E. coli* isolates worldwide [13–15, 19]. Several studies conducted in Korea have shown that 10–17% of *E. coli* strains produce ESBLs and that the prevalence of CTX-M-producing strains has increased to 17–92% since the first report about CTX-M in 2001 [8–11].

The *bla*<sub>CTX-M</sub> gene may vary according to the ST.
For characterization of the molecular epidemiological profiles of CTX-M-producing E. coli isolated from uropathogenic samples and their genetic relatedness, we used four major genotyping assays. Multi-locus sequence typing (MLST) is a molecular biology technique used for the typing of multiple loci. The method characterizes strains based on DNA sequences of multiple housekeeping genes; for each gene, the alleles at each locus define the allelic profile or sequence type (ST). Pulsed-field gel electrophoresis (PFGE) is a technique used for the separation of large DNA molecules by applying an electric field to the gel matrix so that the electric field periodically changes direction. PFGE is used for genotyping or genetic fingerprinting, and is considered a gold standard in epidemiological studies of pathogenic organisms. Rep-PCR is a simple but powerful molecular typing method that can differentiate between closely related strains, and can identify bacteria up to the strain level based on the presence of repeated elements within the genome. Phylogenetic analyses have become essential for characterizing the evolutionary relationship among different strains [3, 4, 12].

In this study, we aimed to determine genetic links among CTX-M-producing E. coli isolates obtained from urine in the four recent years, and to investigate their association with integrons and ISCRs, and origins.

**Materials and Methods**

**Bacterial Isolates and Infection Classification Criteria**

Non-duplicate ceftazidime- and/or cefotaxime-resistant clinical isolates of E. coli were obtained from urine samples collected at Chungnam National University Hospital from September 2011 to July 2014. All isolates were identified using the Vitek 2 automated ID system (bioMérieux Vitek Inc., USA). The MIC for ceftazidime and cefotaxime was determined by the E-test conducted in accordance with the Clinical and Laboratory Standards Institute guidelines [5]. E. coli ATCC 25923 and Pseudomonas aeruginosa ATCC 27853 were used as control strains.

The type of infection was defined according to the Centers for Disease Control and Prevention (CDC)/National Healthcare Safety Network criteria [6]. Community-onset and hospital-onset infections were determined as infections diagnosed within or after 48 h of hospitalization, respectively. In addition, patients admitted within 2 weeks prior to hospitalization or patients transferred from other hospitals were also considered as those with hospital-onset infections [6].

**Molecular Characterization of CTX-M-Producing E. coli Isolates**

All isolates were screened for the presence of bla\_{CTX-M} integrons, and ISCR using PCR and sequencing as described previously [10, 20]. Class 1, 2, and 3 integrons were amplified using the following primers: class 1, hep58 (5’-TCATGCTTGTATGACTGT-3’) and hep59 (5’-GTAGGGCTTATTATGCAGC-3’); class 2, hep51 (5’-GATGCCATCGAATGCAG-3’) and hep74 (5’-CGGGATCCCC GACGCCATGCGAGATTTGTA-3’); and class 3, Int3F (5’-GCC TCGCGACGGACTTTGC-3’) and Int3R (5’-ACGGATCTGCGCA AACCTGACT-3’). ISCRs were detected using primers CRF (5’-CACTWCCACATGTCGKTC-3’) and CRFF-r (5’-CGCTTG AGCCTGGCRYYCC-3’) [20].

**Molecular Typing for CTX-M-Producing E. coli Isolates**

Molecular typing of CTX-M-producing E. coli isolates was performed by MLST, phylogenetic analysis, rep-PCR, and PFGE.

**MLST**

To determine the ST, MLST was performed using Achtmann’s scheme. Seven housekeeping genes (adk, func, gyrB, icd, mdh, purA, and recA) were assessed, and the ST number was assigned by comparing the allele sequences of the isolates with those in the MLST site [http://mlst.warwick.ac.uk/mlst/].

**Phylogenetic Analysis**

The phylogenetic group of the strains was determined using the triplex PCR developed by Clermont et al. [4]. The assay involved the assessment of yjaA, chuA, and TspE4-C2 markers by PCR. The amplified products were separated on 1.5% agarose gels by electrophoresis, and strains were assigned to phylogenetic groups: A (yjaA-positive or all markers negative), B1 (TspE4-C2-positive), B2 (positive for chuA and yjaA or for all markers), and D (chuA-positive or chuA- and TspE4-C2-positive) [4].

**Rep-PCR**

Rep-PCR was conducted using primers REP1 (5’-IIIGGGCCGC ICATCAGGC-3’) and REP2 (5’-ACGTCATTACGGCCTAC-3’) as previously described [10].

**PFGE**

PFGE was used to analyze the genomic relatedness among the E. coli isolates according to the CDC PulseNet protocol [http://www.cdc.gov/pulsenet]. XbaI-digested chromosomal DNA fragments were separated using the CHEF-DR III system (Bio-Rad, Korea) at 6 V/cm for 23 h with pulse times ranging from 2.2-63.8 sec at 120° angle. Clustering analysis was performed by the Fingerprinting II Informatix software (Bio-Rad, USA) at 6 V/cm for 25 h with pulse times ranging from 2.2-63.8 sec at 120° angle. Clustering analysis was performed by the Fingerprinting II Informatix software (Bio-Rad, USA) using the Dice coefficient-unweighted pair group method with arithmetic averages of 1% tolerance and 0.5% optimizing setting value. Isolates were considered genetically related if the Dice coefficient correlation was ≥80%, which corresponds to the possibility-related criteria of Tenover et al. [24].

**Statistical Analysis**

The MIC data were analyzed by Student’s t test using SPSS ver. 12.0 (SPSS Inc., USA). The differences were considered statistically significant at p < 0.001.
**Results**

**Characteristics of *E. coli* Isolates from Urine**

During the study period, a total of 471 *E. coli* isolates were obtained from urine samples, and 84 of them (17.8%) showed resistance to cefotaxime and/or ceftazidime (Figs. 1 and 2). Among them, 80 (95.2%) were positive for bla_{CTX-M} by PCR: bla_{CTX-M-14} and bla_{CTX-M-15} were detected in 46 (57.5%) and 31 (38.8%) isolates, respectively, and 3 strains (3.8%) carried both genes. Other types of CTX-M ESBL were not observed.

Among the CTX-M-producing isolates, 50 and 30 were associated with hospital-onset and community-onset UTI episodes, respectively. In the 4-year study period, the proportion of hospital-onset UTIs was 56.3–78.9% and that of community-onset UTIs was 21.1–43.8% (Table 1).

**Prevalence of Integrons and ISCRs**

Among the CTX-M producers, 46 (57.5%) harbored class 1 integrons, whereas class 2 and 3 integrons were not detected. Four types of class 1 gene cassette arrays were observed: (1) type 1 amplicon in 38 isolates carrying dfrA17-aadA5 gene cassettes; (2) type 2 amplicon in six isolates carrying dfrA12-aadA2 gene cassettes; (3) type 3 amplicon in one isolate carrying arr3-dfrA27 gene cassettes; and (4) type 4 amplicon in one isolate carrying dfrA1-aadA1 gene cassettes.

ISCRs were detected in 10 isolates (12.5%): ISCR1 (n = 1), ISCR2 (n = 2), ISCR3 (n = 1), and ISCR14 (n = 6); among them, there were 8 CTX-M-14 and 2 CTX-M-15 producers (Figs. 1 and 2). Most ISCRs were identified as D-ST38 CTX-M-14 producers.

**Diversity and Comparison of Molecular Types**

**MLST.** MLST identified 11 unique STs; among them, the most prevalent was ST131 (n = 34, 42.5%), followed by ST38 (n = 22, 27.5%), ST405 (n = 8, 10.0%), ST69 (n = 6, 7.5%), ST95 (n = 3, 3.8%), and ST493 (n = 2, 2.53%). Each of the remaining five isolates was identified as a separate ST (Figs. 1 and 2, and Table 1).

**Phylogenetic Analysis**

Among the isolates of phylogenetic group B2 (n = 50, 50.0%), 21 were identified as CTX-M-15 producers and 17 as CTX-M-14 producers. Among the isolates of phylogenetic group D (n = 38, 47.5%), 9 produced CTX-M-15 and 28 produced CTX-M-14 (Figs. 1 and 2).

All ST131 (n = 34), ST95 (n = 3), and ST493 (n = 2) strains were phylogenetic group B2, and all ST38 (n = 22), ST405 (n = 8), and ST69 (n = 6) strains were phylogenetic group D.

**Rep-PCR**

Rep-PCR analysis indicated that 66 *E. coli* isolates mostly

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**Table 1.** Prevalence of bla_{CTX-M} genes and ST types in Daejeon in 2011–2014.

<table>
<thead>
<tr>
<th>Year (No. of isolates)</th>
<th>2011 (10)</th>
<th>2012 (16)</th>
<th>2013 (19)</th>
<th>2014 (35)</th>
<th>Total (80)</th>
</tr>
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<tbody>
<tr>
<td><strong>bla_{CTX-M} genes</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>CTX-M-14</td>
<td>7</td>
<td>7</td>
<td>12</td>
<td>20</td>
<td>46</td>
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<tr>
<td>CTX-M-15</td>
<td>2</td>
<td>9</td>
<td>6</td>
<td>14</td>
<td>31</td>
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<tr>
<td>Both</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>3</td>
<td></td>
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<tr>
<td><strong>STs</strong></td>
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<tr>
<td>(allele profile)</td>
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<td></td>
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<td></td>
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<tr>
<td>ST131 (53-40-47-13-36-28-29)</td>
<td>3</td>
<td>9</td>
<td>6</td>
<td>16</td>
<td>34</td>
</tr>
<tr>
<td>ST38 (4-26-2-25-5-5-19)</td>
<td>2</td>
<td>2</td>
<td>7</td>
<td>11</td>
<td>22</td>
</tr>
<tr>
<td>ST405 (35-37-29-25-4-5-73)</td>
<td>2</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>8</td>
</tr>
<tr>
<td>ST69 (21-35-27-6-5-5-4)</td>
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<td>1</td>
<td>1</td>
<td>6</td>
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<tr>
<td>ST95 (37-38-19-37-17-11-26)</td>
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<td>0</td>
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<td>3</td>
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<tr>
<td>ST493 (40-13-9-13-16-10-9)</td>
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<td>1</td>
<td>0</td>
<td>2</td>
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<tr>
<td>ST1488 (10-11-4-8-8-8-73)</td>
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<td>0</td>
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<tr>
<td>ST44 (10-11-4-8-8-8-7)</td>
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<td>0</td>
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<tr>
<td>ST354 (85-88-78-29-59-58-62)</td>
<td>0</td>
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<td>0</td>
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<tr>
<td>ST648 (92-4-87-96-70-58-2)</td>
<td>0</td>
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<tr>
<td>ST1193 (14-14-10-200-17-7-10)</td>
<td>0</td>
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<tr>
<td><strong>Type of infections</strong></td>
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<tr>
<td>HO</td>
<td>6</td>
<td>9</td>
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<td>20</td>
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<td>4</td>
<td>7</td>
<td>4</td>
<td>15</td>
<td>30</td>
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</tbody>
</table>

STs, sequence types; HO, hospital-onset infection; CO, community-onset infection.
showed four types of band patterns. Thus, 21 out of 22 A-type strains were ST38, and 34 B-type, 8 C-type, and 3 D-type strains were identified as ST131, ST405, and ST95, respectively (Figs. 1 and 2; D-type rep-PCR pattern is not shown). These four strain types were also found to be epidemiologically related. ST69 comprised diverse rep-
Fig. 2. A PFGE-based dendrogram and molecular characteristics of ST38 (n = 22), ST405 (n = 8), and ST69 (n = 6) E. coli isolates causing UTIs.
MIC, minimum inhibitory concentration; CTX, cefotaxime; CAZ, ceftazidime.
PCR patterns (Fig. 2).

PFGE

PFGE analysis was performed on ST131, ST38, ST405, and ST69 strains with a similarity cut-off > 80%. The results revealed 20 clusters in 34 ST131 strains, 19 clusters in 20 ST38 strains, 6 clusters in 8 ST405 strains, and 6 clusters in 6 ST69 strains (Figs. 1 and 2).

Discussion

ESBL-producing E. coli strains are most frequently isolated as UTI pathogens, and among them, CTX-M producers were predominantly observed in Korea. Although since 2001, the dominant CTX-M gene type among E. coli isolates has been blaCTX-M-14, the incidence of CTX-M-15-producing E. coli has increased after 2005. Recently, pandemic B2-ST131 E. coli strains expressing CTX-M-15 have also been detected in Korea [10, 18, 21].

Generally, most CTX-M β-lactamases are known to hydrolyze cefotaxime more efficiently than ceftazidime (MIC > 64 μg/ml, respectively) as in our earlier study [10]. Thus, we suggest that the CTX-M-15 enzyme is more specific for ceftazidime compared with the CTX-M-14 enzyme (p < 0.001).

Consistent with earlier observations, we identified B2-ST131 as the predominant type (42.5%); however, CTX-M-14 producers were more prevalent than CTX-M-15 producers (57.5% versus 38.8%, respectively), similar to our earlier data [10]. Furthermore, the ST38 incidence rate (27.5%) was considerably higher than that reported in previous studies (7.9%) [7, 21]. Therefore, in this study, we found that together with ST131, D-ST38 CTX-M producers were widely spread in Daejeon, although only B2-ST131 CTX-M-15 producers were predominant in other countries and different Korean cities [21, 25]. However, our results are in agreement with the findings of an earlier study conducted in the Daejeon area [18]. In addition, our present data indicate that all ST38 strains excluding three isolates harboring blaCTX-M-15 contained the blaCTX-M-14 gene, whereas about 68% of ST131 isolates had the blaCTX-M-15 gene. The results suggest variations in blaCTX-M genes according to the ST, which is consistent with previous reports [18, 21].

Although the ST distribution did not show any dramatic changes over the study period, our molecular epidemiological analysis revealed significant differences between hospital-onset and community-onset E. coli isolates. Among 12 clusters containing at least two strains, six included only hospital-onset isolates; moreover, ST131 and ST38 strains were associated with hospital-onset rather than community-onset infections. These findings suggest that ST131 and ST38 strains may be colonized in hospitals, which is in agreement with an earlier report on the outbreak in hospital-onset infections [18]. In addition, the phylogenetic groups and rep-PCR types were clustered according to the ST. All isolates of ST131, ST95, and ST493 belonged to phylogenetic group B2, whereas those of ST38, ST405, and ST69 belonged to phylogenetic group D. All ST131 and ST405 strains were of types B and C, respectively, whereas 19 out of 20 ST38 strains were of type A. However, ST69 strains demonstrated various band patterns. Together, the MLST and rep-PCR data indicated that strain types were mostly related to a single ST, although phylogenetic groups included more than one ST.

Unlike rep-PCR, the PFGE band patterns showed considerable variability within the same ST, indicating that the isolated strains were genetically diverse. These results were expected because urine samples were collected over a period of 4 years and the isolates may have undergone genetic changes during the time of the study, acquiring different types of mobile genetic elements such as plasmids, integrons, and ISCRs. In addition, PFGE analysis is more sensitive than rep-PCR and MLST to investigate genetic relatedness. Strain clustering based on PFGE data indicated genetic diversity within the same ST, suggesting that PFGE is a better approach to study genetic evolution among UTI-causing E. coli strains than rep-PCR and phylogenetic analysis.

In conclusion, we found that D-ST38 CTX-M-14 producers had relatively higher prevalence in Daejeon than in other countries or different Korean cities. CTX-M-producing E. coli isolates mostly belonged to ST131 and ST38 and were more associated with hospital-onset than with community-onset infections. In addition, we found ST-dependent variations in the blaCTX-M gene.

References

2. Bae IK, Lee YH, Jeong HJ, Hong SG, Lee SH, Jeong SH.


