Molecular Epidemiology of Ciprofloxacin-Resistant Escherichia coli Isolated from Community-Acquired Urinary Tract Infections in Korea

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ABSTRACT

Background: Escherichia coli is the predominant causative pathogen for community-acquired urinary tract infections (UTIs), and the increase in fluoroquinolone-resistant E. coli is of great concern in Korea. The objectives of this study were to investigate the genotypic characteristics and molecular epidemiology of ciprofloxacin-resistant (CIP-R) E. coli isolated from community-acquired UTIs in Korea.

Materials and Methods: E. coli samples isolated from the blood or urine were collected from patients with community-acquired acute pyelonephritis aged 15 years and more who were admitted to 12 Korean hospitals from 1st April 2010 to 29th February 2012. Phylogenetic typing, multilocus sequence typing, and molecular characterization of β-lactamase and plasmid-mediated quinolone resistance determinants were performed for CIP-R E. coli isolates.

Results: A total of 569 E. coli isolates were collected, and 122 (21.4%) isolates were CIP-R isolates. The most prevalent sequence type (ST) was ST131 (28.7%, 35/122), followed by ST38 (14.7%, 18/122), ST1193 (13.1%, 16/122), ST38 (9.0%, 11/122), and ST405 (8.2%, 10/122). The antimicrobial resistance rates of ST131 to cefepime (22.9%, 8/35), ST38 to gentamicin (100%, 11/11), and ST405 to cefotaxime (66.7%, 6/9) were significantly higher than the resistance rates of all other STs combined. Notably, 40% (4/10) of ST405 clones produced extended-spectrum β-lactamases and were co-resistant to trimethoprim/sulfamethoxazole. aac(6')-Ib-cr (20%, 7/35) and CTX-M-14 (40%, 4/10) were more frequently observed in ST131 and ST405 compared with other clones, respectively.
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Conclusions: Among the CIP-R uropathogenic E. coli isolates in this study, ST131, ST38, and ST405 were specifically associated with antimicrobial resistance.

Keywords: Urinary tract infection; Escherichia coli; Antimicrobial resistance; Multilocus sequence typing; Korea

INTRODUCTION
Urinary tract infection (UTI) is one of the most common community-acquired bacterial infections, and Escherichia coli is the predominant causative pathogen for community-acquired UTIs. The current Infectious Diseases Society of America (IDSA) and European Society for Microbiology and Infectious Diseases (ESMID) guidelines as well as guidelines in Korea recommend fluoroquinolones (FQs) as first-line empirical antibiotics for the treatment of UTIs if the local resistance of community uropathogens to FQs is less than 10% [1, 2]. Unfortunately, the resistance to FQs has been increasing among uropathogenic E. coli strains worldwide [3]. Considering that UTIs caused by FQ-resistant E. coli can lead to unfavorable clinical outcomes, the increase in FQ-resistant uropathogenic E. coli is of great concern [3]. In Korea, the resistance rate of E. coli to FQs in community-acquired UTIs has increased from 15.2% in 2002 to 24.8% in 2008 – 2009 [4, 5].

There are several possible explanations for the increased prevalence of FQ-resistant uropathogenic E. coli isolates in the community. Horizontal gene transfer is an important mechanism for acquiring resistance; E. coli becomes resistant to certain antibiotics when resistance genes are transferred via a plasmid [6]. In addition, the increased prevalence may be attributed to the dissemination of clonal organisms containing resistance genes; the spread of ST131 is closely linked to an increase in FQ resistance [6]. Indeed, changes in the molecular epidemiology of E. coli have been found to affect the resistance pattern [7, 8].

The objectives of this study were to investigate the genotypic characteristics and molecular epidemiology of ciprofloxacin-resistant (CIP-R) E. coli isolated from community-acquired UTIs in Korea.

MATERIALS AND METHODS

1. Study setting
E. coli samples isolated from the blood or urine were collected from patients aged 15 years or more who were admitted to 12 Korean hospitals (3 in Seoul, 4 in Gyeonggi-do, 2 in Incheon, 2 in Daegu, and 1 in Busan) from 1st April 2010 to 29th February 2012. The hospitals had 582 – 1,250 beds, and 11 of the 12 hospitals were university teaching hospitals. All subjects were diagnosed with community-acquired acute pyelonephritis and recruited prospectively [9]. Only the first isolate from each patient was included in the study. One isolate was collected from each patient.

The study was approved by the Institutional Review Board of Hanyang University Hospital (IRB number: HYUH 2010-007), and the requirement for written informed consent from patients was waived.
2. Antimicrobial susceptibility testing

The species and susceptibility to ampicillin (AMP), cefotaxime (CTX), ceftazidime (CAZ), gentamicin (GEN), tobramycin (TOB), and amikacin (AMK) were determined using a semi-automated system [(VITEK; bioMerieux, Hazelwood, MO, USA) or (MicroScan; DADE Behring, Sacramento, CA, USA)] in each hospital. Extended-spectrum β-lactamase (ESBL)-producing isolates were defined as Enterobacteriaceae when identified as positive by the ESBL test in the semi-automated system and/or double disk diffusion test according to the criteria of the Clinical and Laboratory Standards Institute (CLSI) [10]. The minimal inhibitory concentration (MIC) was determined for trimethoprim-sulfamethoxazole (TMP/SMX) by the agar dilution method. Tryptic soy agar (TSA; Difco Laboratories, Detroit, MI, USA) was used with an inoculum of 0.5 McFarland, and quality control was performed using the strain E. coli ATCC 25922. Antimicrobial susceptibility to CIP, fosfomycin (FOF), cefepime (FEP), temocillin (TMO), and nitrofurantoin (NIT) was determined by the E-test method (bioMérieux, Marcy-l’Étoile, France) according to the criteria of the CLSI [10].

3. Characterization of CIP-R E. coli isolates

1) Phylogenetic typing and multilocus sequence typing (MLST)

Phylogenetic groups were determined for CIP-R E. coli isolates according to the presence of the chuA and yjaA genes and TspE4.C2 by polymerase chain reaction (PCR) [11]. MLST was conducted as previously described based on the 7 housekeeping genes [12]. Clonal complexes were defined according to the E. coli MLST website (http://mlst.ucc.ie/mlst/dbs/Ecoli/).

2) Molecular characterization of β-lactamase and plasmid-mediated quinolone resistance (PMQR) determinants

PCR and DNA sequence analyses were conducted to determine the genes responsible for ESBL or plasmid-mediated AmpC β-lactamase (PABL) production and the presence of PMQR determinants [13, 14].

The presence of the aac(6′)-Ib gene was investigated by PCR using primers and conditions as previously described [15]. The aac(6′)-Ib-positive PCR products were digested with BciI (Promega, Madison, WI, USA) to identify aac(6′)-Ib-cr, which lacks the BciI restriction site [15]. The wild-type aac(6′)-Ib PCR product yielded 233 bp, 209 bp, and 40 bp fragments after restriction digestion.

4. Statistical analysis

SPSS version 24.0 for Windows (IBM Corporation, Armonk, NY, USA) was used for statistical analysis. Categorical variables were analyzed by the Chi-square test or Fisher’s exact test. A P value of <0.05 (two-tailed test) was considered to be significant.

RESULTS

During the study period, 569 E. coli isolates were collected. The number of E. coli isolates per hospital ranged from 6 (1.0%) to 144 (25.3%). Among them, 122 (21.4%) isolates were CIP-R isolates.

Of 122 isolates, 25 (20.5%) and 97 (79.5%) were isolated from blood and urine, respectively. The number of isolates from each region was as follows: 84 (68.9%) from Gyeonggi-do, 22 (18.0%) from Daegu, 6 (4.9%) from Busan, 3 (2.5%) from Seoul, and 7 (5.7%) from Incheon.
1. Phylogenetic grouping and MLST analysis

Table 1 shows the distribution of the phylogenetic groups of CIP-R *E. coli* isolates. The phylogenetic group B2 was the most common (48.4%, 59/122), followed by D (41.8%, 51/122), B1 (6.5%, 8/122), and A (3.3%, 4/122).

We identified 22 different sequence types (STs) among 122 CIP-R *E. coli* isolates. The most prevalent ST was ST131 (28.7%, 35/122), followed by ST393 (14.7%, 18/122), ST1193 (13.1%, 16/122), ST38 (9.0%, 11/122), and ST405 (8.2%, 10/122).

All of the ST131 and ST1193 isolates belonged to the phylogenetic group B2, and most of the ST393, ST38, and ST405 isolates belonged to the phylogenetic group D (Table 1).

2. Antimicrobial resistance

Table 2 and Supplementary Table 1 shows the antibiotic resistance of CIP-R *E. coli* isolates. Among 122 CIP-R *E. coli* isolates, 60.7% (74/122), 79.5% (93/117), 29.4% (35/119), and 53.8% (64/119) of isolates were resistant to TMP/SMX, AMP, CAZ, and GEN, respectively. On the other hand, less than 5% of isolates were resistant to FOF, NIT, and AMK. A total of 35 (28.7%, 35/122) isolates were ESBL-producing pathogens, and 20 (16.4%, 20/122) isolates showed TMP/SMX resistance concomitantly.

In the analysis of resistance according to the ST, the FEP resistance rate of ST131 was significantly higher than that of all other STs combined (22.9% vs. 8.0%, *P* = 0.034). The antimicrobial resistance rates of ST393 to CTX (0% vs. 33.7%, *P* = 0.004) and CAZ (5.6% vs. 33.7%, *P* = 0.016) and its ESBL production rate (5.6% vs. 36.2%, *P* = 0.019) were lower than

### Table 1. Distribution of phylogenetic groups of ciprofloxacin-resistant *Escherichia coli* isolates

| Phylogenetic group (N) | Sequence type (N) |
|------------------------|------------------|
| A (4)                  | ST393 (1), ST93 (1), ST102 (1), ST106 (1) |
| B1 (6)                 | ST69 (1), ST10 (5), ST93 (1), ST707 (1), ST744 (1), ST3337 (1) |
| B2 (59)                | ST131 (35), ST1193 (16), ST38 (1), ST73 (2), ST85 (2), ST130 (1), ST638 (1), unknown (2) |
| D (31)                 | ST393 (17), ST38 (10), ST405 (10), ST69 (5), ST117 (2), ST457 (2), ST648 (2), ST68 (1), ST3901 (1), unknown (1) |

### Table 2. Antimicrobial resistance of ciprofloxacin-resistant *Escherichia coli* isolates for five major clones and each phylogenetic group

| Antibiotics | Major clones | Phenylogenetic groups | Total (%) |
|-------------|--------------|-----------------------|-----------|
|             | ST131 (%)    | ST393 (%)             | ST38 (%)  | ST405 (%) | A (%) | B1 (%) | B2 (%) | D (%) | ST131 (100) |
| FOF         | 0/35 (0.0)   | 0/16 (0.0)            | 0/16 (0.0) | 0/10 (0.0) | 0/4 (0.0) | 0/4 (0.0) | 0/5 (0.0) | 0/5 (0.0) | 0/122 (0.0) |
| FEP         | 8/35 (22.9)% | 0/16 (0.0)            | 1/6 (6.3)  | 3/11 (27.3) | 1/10 (10.0) | 0/4 (0.0) | 0/8 (0.0) | 9/59 (15.3) | 6/51 (11.8) | 15/122 (12.3) |
| TMO         | 3/35 (8.6)   | 0/16 (0.0)            | 1/6 (6.3)  | 2/11 (18.2) | 1/10 (10.0) | 0/4 (0.0) | 0/6 (0.0) | 5/59 (8.5)  | 5/51 (9.8)  | 10/122 (8.2) |
| NIT         | 0/35 (0.0)   | 0/16 (0.0)            | 0/16 (0.0) | 0/10 (0.0) | 0/4 (0.0)  | 0/4 (0.0) | 0/8 (0.0) | 1/59 (1.7)  | 0/51 (0.0) | 1/122 (0.1) |
| TMP/SMX     | 17/35 (48.6)| 13/16 (81.2)          | 10/16 (62.5)| 9/11 (81.8)| 8/10 (80.0)| 2/4 (50.0)| 5/8 (62.5)| 30/59 (50.8%)| 37/51 (72.5%)| 74/122 (60.7) |
| AMP         | 28/32 (87.5)| 11/16 (68.8)| 10/16 (62.5)| 9/10 (90.0)| 10/10 (100.0)| 4/4 (100.0)| 5/7 (71.4)| 45/56 (80.4)| 39/50 (78.0)| 93/117 (79.5) |
| CTX         | 12/30 (40.0)| 0/16 (0.0)           | 0/16 (0.0) | 3/10 (30.0) | 6/9 (66.7) | 1/4 (25.0)| 1/6 (16.7)| 15/56 (26.8)| 15/47 (31.9)| 32/113 (28.3) |
| CAZ         | 12/30 (40.0)| 1/18 (5.6)           | 0/16 (0.0)| 3/11 (27.3)| 6/10 (60.0)| 3/4 (75.0)| 1/7 (14.3)| 15/57 (26.3)| 16/55 (31.4)| 35/119 (29.4) |
| GEN         | 20/33 (60.6)| 9/10 (90.0)          | 3/16 (18.8)| 11/11 (100.0)| 4/10 (40.0)| 2/4 (50.0)| 2/7 (28.6)| 29/57 (50.9)| 31/51 (60.8)| 64/119 (55.0) |
| TOB         | 21/32 (65.6)| 9/16 (56.3)          | 3/16 (18.8)| 8/10 (80.0)| 4/9 (44.4)| 2/4 (50.0)| 2/7 (28.6)| 30/56 (53.6)| 28/46 (60.9)| 62/113 (54.8) |
| AMK         | 1/3 (33.3)   | 0/12 (0.0)           | 0/15 (0.0)| 0/10 (0.0)| 1/8 (12.5)| 0/4 (0.0)| 1/3 (33.3)| 1/5 (20.0)| 3/8 (37.5)| 1/4 (100.0) |
| ESBL production | 12/35 (34.3)| 3/18 (16.7)         | 0/16 (0.0)| 3/11 (27.3)| 6/10 (60.0)| 3/4 (75.0)| 1/8 (12.5)| 15/59 (25.4)| 16/55 (31.4)| 35/122 (28.7) |
| ESBL production + TMP/SMX resistance | 6/35 (17.1)| 1/18 (5.6)           | 0/16 (0.0)| 3/11 (27.3)| 4/10 (40.0)| 2/4 (50.0)| 1/8 (12.5)| 7/59 (11.9)| 10/51 (19.6)| 20/122 (16.4) |

FOF, fosfomycin; FEP, cefepime; TMO, temocillin; NIT, nitrofurantoin; TMP/SMX, trimethoprim-sulfamethoxazole; AMP, ampicillin; CTX, cefotaxime; CAZ, ceftazidime; GEN, gentamicin; TOB, tobramycin; AMK, amikacin; ESBL, extended-spectrum beta-lactamase.

*P* < 0.05 and these relate to differences found when susceptibility profiles for isolates of each ST were compared with those all other STs combined.

*P* < 0.05 and these relate to differences found when susceptibility profiles for isolates of each phylogenetic group were compared with those all other phylogenetic groups combined.
those of all other STs combined. In addition, the AMP resistance rate of ST393 was lower than that of all other STs with marginal significance (61.1% vs. 82.8%, P = 0.054). The resistance rates of ST1193 were significantly lower than those of all other STs for CTX (0% vs. 32.7%, P = 0.006), CAZ (0% vs. 34.0%, P = 0.003), GEN (18.8% vs. 59.2%, P = 0.003), and TOB (18.8% vs. 60.8%, P = 0.002). The ESBL production rate of ST1193 was also lower than that of all other STs (0% vs. 33.0%, P = 0.004). ST38 showed a significantly higher resistance rate to GEN compared with the resistance rate of all other STs combined (100.0% vs. 49.1%, P = 0.001). The CTX resistance rate (66.7% vs. 25.0%, P = 0.015) and ESBL production rate (60.0% vs. 25.9%, P = 0.032) of ST405 were significantly higher than those of all other STs. The prevalence of SXT resistance with ESBL production was higher in ST405 than in all other STs combined with marginal significance (40.0% vs. 14.3%, P = 0.058).

There were no significant differences in the resistance rates to most antibiotics for each phylogenetic group compared with all others combined. The TMP/SMX resistance rate was lower in the phylogenetic group B2 (50.8% vs. 69.8%, P = 0.032) and higher in the phylogenetic group D (72.5% vs. 52.1%, P = 0.023) compared with all other phylogenetic groups combined.

3. Distribution of ESBL/PABL and PMQR determinants

Table 3 shows the distribution of ESBL/PABL and PMQR determinants among CIP-R E. coli isolates. Among 122 CIP-R E. coli isolates, ESBL/PABL determinants were found in 35 (28.7%, 35/122) isolates, which belonged to 12 different STs. The most prevalent ESBL/ PABL determinant was CTX-M-15 (45.7%, 16/35), followed by CTX-M-14 (40.0%, 14/35). CTX-M-15 was present in 6 different STs (ST131, ST38, ST405, ST117, ST648, and ST130), and CTX-M-14 was also present in 6 different STs (ST131, ST405, ST69, ST10, ST93, and ST457). The prevalence of CTX-M-14 was significantly higher in ST405 than in all other STs combined (40.0% vs. 8.9%, P = 0.016). In addition, 3 isolates produced both CTX-M-14 and CTX-M-15; 2 of them belonged to ST38. CMY-2 was present in 2 isolates, which belonged to ST393 and ST602 (1.6%, 2/122).

PMQR determinants were found in 13 (10.7%, 13/122) CIP-R E. coli isolates, which belonged to 5 different STs. aac(6′)-Ib-cr was the most prevalent PMQR determinant (84.6%, 11/13), and most of the isolates belonged to ST131 (63.6%, 7/11). One ST131 isolate carried aac(6′)-Ib-cr and qnrB concomitantly. Another isolate, which belonged to ST117, carried aac(6′)-Ib-cr and qnrS concomitantly (Supplementary Table 2).

Table 3. Distribution of Extended-spectrum beta-lactamase (ESBL) / Plasmid-mediated AmpC beta-lactamase (PABL) and Plasmid-mediated quinolone resistance (PMQR) determinants of ciprofloxacin-resistant Escherichia coli isolates according to multilocus sequence typing (MLST) analysis

| Major clones | Phylogenetic groups | Total (%) |
|--------------|---------------------|-----------|
|               | ST131 (%) | ST393 (%) | ST1193 (%) | ST38 (%) | ST405 (%) | A (%) | B1 (%) | B2 (%) | D (%) |
| **ESBL/PABL** |
| CMY-2        | 0/35 (0.0) | 1/18 (5.6) | 0/16 (0.0) | 0/11 (0.0) | 0/10 (0.0) | 2/4 (50.0) | 0/8 (0.0) | 0/59 (0.0) | 0/51 (0.0) | 2/122 (1.6) |
| CTX-M-14     | 4/35 (11.4) | 0/18 (0.0) | 0/16 (0.0) | 1/11 (9.1) | 4/10 (40.0) | 0/4 (0.0) | 0/8 (0.0) | 5/59 (8.5) | 9/51 (17.6) | 14/122 (11.5) |
| CTX-M-15     | 8/35 (22.9) | 0/18 (0.0) | 0/16 (0.0) | 0/11 (0.0) | 2/10 (20.0) | 1/4 (25.0) | 1/8 (12.5) | 9/59 (15.3) | 5/51 (9.8) | 16/122 (13.1) |
| CTX-M-14 + CTX-M-15 | 0/35 (0.0) | 0/18 (0.0) | 0/16 (0.0) | 2/11 (18.2) | 0/10 (0.0) | 0/4 (0.0) | 0/8 (0.0) | 1/59 (1.7) | 2/51 (3.9) | 3/122 (2.5) |
| **PMQR** |
| aac(6′)-Ib-cr | 7/35 (20.0)$^a$ | 0/18 (0.0) | 0/16 (0.0) | 1/11 (9.1) | 0/10 (0.0) | 1/4 (25.0) | 0/8 (0.0) | 9/59 (15.3) | 1/51 (2.0) | 11/122 (9.0) |
| qnrB + aac(6′)-Ib-cr | 1/35 (2.9) | 0/18 (0.0) | 0/16 (0.0) | 0/11 (0.0) | 0/10 (0.0) | 0/4 (0.0) | 0/8 (0.0) | 1/59 (1.7) | 0/51 (0.0) | 1/122 (0.0)$^b$ |
| qnrS + aac(6′)-Ib-cr | 0/35 (0.0) | 0/18 (0.0) | 0/16 (0.0) | 0/11 (0.0) | 0/10 (0.0) | 0/4 (0.0) | 0/8 (0.0) | 0/59 (0.0) | 1/51 (2.0) | 1/122 (0.0)$^b$ |

$^a$P < 0.05 and these relate to differences found when the distribution of ESBL/PABL or PMQR determinants for isolates of each ST were compared with those all other STs combined.

$^b$P < 0.05 and these relate to differences found when the distribution of ESBL/PABL or PMQR determinants for isolates of each phylogenetic group were compared with those all other phylogenetic groups combined.
The proportion of major clones was significantly higher among isolates from urine specimens (52.0% vs. 79.4%, \( P = 0.006 \)). In comparison, there was no difference in the proportion of each ST. The proportion of isolates that presented ESBL/PABL or PMQR determinants was not significantly different by specimen type.

There was no regional difference in the proportion of major STs between the Metropolitan area (Seoul, Gyeonggi-do, and Incheon) and the Gyeongsang area (Daegu and Busan). CTX-M-15 was more commonly found in the Gyeongsang area than in the Metropolitan area (28.6% vs. 7.4%, \( P = 0.006 \)) (Table 4).

### DISCUSSION

In the present study, we found that 21.4% of E. coli isolates associated with community-acquired UTIs in Korea were CIP-R isolates, and ST131, ST393, ST1193, ST38, and ST405 were the major CIP-R uropathogenic E. coli clones. The clonal distribution was consistent with the findings of previous studies in Korea. A multicenter study found that ST131 and ST393 were the main clones (24.8% and 17.8% of CIP-R E. coli, respectively) in 2006 – 2007 [16]. Another study demonstrated that ST131 and ST1193 were the major CIP-R E. coli clones in 2013 – 2014 [17].

ST131 is largely responsible for the global dissemination of multidrug-resistant (MDR) E. coli strains [18]. The pandemic ST131 has been identified among MDR E. coli isolates in several European countries and Asian countries [18]. In Korea, ST131 has emerged as the predominant clone among ESBL-producing or CIP-R E. coli isolates from the community and healthcare settings [9, 16]. The distinctive genetic characteristics of ST131 clones might give them a more competitive edge over other clones, allowing them to rapidly disseminate worldwide [9]. ST131 belongs to the phylogenetic group B2, which is the most virulent group [18]. In addition, ST131 harbors various resistance determinants/genes such as CTX-M-15, TEM-1, OXA-1, and aac(6′)-1b-cr [19].
Consistent with the reported genetic characteristics of ST131, we found that ST131 contained several resistance determinants/genes including CTX-M-15, CTX-M-14, and aac(6′)-Ib-cr. CTX-M-15 and CTX-M-14 are the most widely distributed CTX-M-type ESBL enzymes and are responsible for resistance to penicillins, cephalosporins, and monobactams [9]. aac(6′)-Ib-cr is a plasmid-mediated gene that encodes a variant of aminoglycoside acetyltransferase, which confers reduced susceptibility to FQs [20]. Several previous studies have demonstrated the close relationship between ST131 and these resistance determinants/genes [9, 16, 21-23].

ST393 and ST1193 are also major clones identified among CIP-R E. coli isolates in Korea [16, 17]. Unlike ST131, ST393 is less virulent, which belongs to the phylogenetic group D [16]. They are predominant clonal groups in some regions. In Spain, ST393 was identified as one of the most prevalent MDR E. coli clones in extraintestinal infections [24]. In China, ST1193 was reported as the second most abundant FQ-resistant E. coli clone [25]. We found that the overall antimicrobial resistance rates of ST393 and ST1193 were significantly lower than the resistance rates of other clones. Furthermore, ESBL or PMQR determinants were largely absent in ST393 and ST1193; only a ST393 clone produced CMY-2. Similar to our findings, previous studies have demonstrated the lower antimicrobial resistance rate and ESBL production activity of ST393 and ST1193 clones [16, 17]. ST393 was not higher than that of other clones in the present study.

In addition to ST131, ST38 and ST405 are dominant MDR E. coli strains [26]. A single-center study in Saudi Arabia revealed the strong association of ST405 with FQ resistance [26]. Another single-center study in Korea found that ST38 and ST405 accounted for 27.5% and 10.0% of CTX-M-producing uropathogenic E. coli, respectively [27]. Furthermore, a study in the United Kingdom revealed that most E. coli strains carrying the OXA-48 carbapenemase gene belonged to ST38 [28]. Our study found that 60% of ST405 clones were ESBL-producing E. coli strains, which was a significantly higher rate compared with the rate for other clones. Notably, we found that 40% (4/10) of ST405 clones produced ESBLs and were co-resistant to TMP/SMX. The MDR characteristic of ST405 is of great concern considering that this will lead to limited treatment options for E. coli infection.

Although it is meaningful work to provide basic molecular characteristics of a common causative pathogen for a community-based bacterial infection, the major drawback of this study is the studied isolates were collected 2010 – 2012 and does not reflect the current situation well. In a prospective observational multi-center study which was conducted in 4 university hospitals in Korea, significantly higher antimicrobial resistance against fluoroquinolone (33.5% vs. 21.0%, P = 0.001), cefotaxime (34.8% vs. 7.6%, P < 0.001), and TMP/SMX (37.7% vs. 28.4%, P = 0.040) were observed for E. coli isolates in 2017 – 2018 compared with those in 2010 – 2011 [29]. There are possibilities that major clones of E. coli strains might have changed in Korea in the last decade. In the France, an increase in ST131, ST38, and ST1193 clones were observed among E. coli isolated from UTIs in children from 2014 to 2017 [30]. Further studies on changes in microbiological characteristics in Korea are necessary to clarify this issue.

In the present study, we identified 5 major clones among CIP-R E. coli isolates causing community-acquired UTIs in Korea (ST131, ST393, ST1193, ST38, and ST405). ST131, ST38, and ST405 were specifically associated with MDR pathogens. Continuous monitoring of the molecular characteristics of E. coli strains and further investigation of the transmission dynamics are necessary.
SUPPLEMENTARY MATERIALS

Supplementary Table 1
Antimicrobial resistance of ciprofloxacin-resistant Escherichia coli isolates for minor clones

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Supplementary Table 2
Distribution of Extended-spectrum beta-lactamases (ESBL) / Plasmid-mediated AmpC beta-lactamases (PABL) and Plasmid-mediated quinolone resistance (PMQR) determinants of ciprofloxacin-resistant Escherichia coli isolates for minor clones

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REFERENCES

1. Gupta K, Hooton TM, Naber KG, Wullt B, Colgan R, Miller LG, Moran GJ, Nicolle LE, Raz R, Schaeffer AI, Soper DE; Infectious Diseases Society of America; European Society for Microbiology and Infectious Diseases. International clinical practice guidelines for the treatment of acute uncomplicated cystitis and pyelonephritis in women: A 2010 update by the Infectious Diseases Society of America and the European Society for Microbiology and Infectious Diseases. Clin Infect Dis 2011;52:e103-20.

PUBMED | CROSSREF

2. Kang CI, Kim J, Park DW, Kim BN, Ha US, Lee SJ, Yeo JK, Min SK, Lee H, Wie SH. Clinical practice guidelines for the antibiotic treatment of community-acquired urinary tract infections. Infect Chemother 2018;50:67-400.

PUBMED | CROSSREF

3. Bouchillon S, Hoban DJ, Badal R, Hawser S. Fluoroquinolone resistance among gram-negative urinary tract pathogens: global smart program results, 2009-2010. Open Microbiol J 2012;6:74-8.

PUBMED | CROSSREF

4. Lee SJ, Lee SD, Cho IR, Sim BS, Lee JG, Kim CS, Kim ME, Cho YH, Woo YN. Antimicrobial susceptibility of uropathogens causing acute uncomplicated cystitis in female outpatients in South Korea: a multicentre study in 2002. Int J Antimicrob Agents 2004;24(Suppl 1):S61-4.

PUBMED | CROSSREF

5. Lee SJ, Lee DS, Choe HS, Shim BS, Kim CS, Kim ME, Cho YH. Antimicrobial resistance in community-acquired urinary tract infections: results from the Korean antimicrobial resistance monitoring system. J Infect Chemother 2011;17:440-6.

PUBMED | CROSSREF

6. Nicolas-Chanoine MH, Bertrand X, Madec JV. Escherichia coli ST131, an intriguing clonal group. Clin Microbiol Rev 2014;27:543-74.

PUBMED | CROSSREF

7. Day MJ, Douniith M, Abernethy J, Hope R, Reynolds R, Wain J, Livermore DM, Woodford N. Population structure of Escherichia coli causing bacteremia in the UK and Ireland between 2001 and 2010. J Antimicrob Chemother 2016;71:2139-42.

PUBMED | CROSSREF

8. Park SH, Byun JH, Choi SM, Lee DG, Kim SH, Kwon JC, Park C, Choi JH, Yoo JH. Molecular epidemiology of extended-spectrum /lactamase-producing Escherichia coli in the community and hospital in Korea: emergence of ST131 producing CTX-M-45. BMC Infect Dis 2012;12:149.

PUBMED | CROSSREF

9. Kim Y, Wie SH, Chang UI, Kim J, Ki M, Cho YK, Lim SK, Lee JS, Kwon KT, Lee H, Cheong HI, Park DW, Ryu SY, Chung MH, Pah H. Comparison of the clinical characteristics of diabetic and non-diabetic women with community-acquired acute pyelonephritis: a multicenter study. J Infect 2014;69:244-51.

PUBMED | CROSSREF

10. Clinical and Laboratory Standards Institute (CLSI). Performance standards for antimicrobial susceptibility testing; 15th informational supplement. CLSI document M100-S15. Wayne: CLSI; 2007.
11. Clermont O, Bonacorsi S, Bingen E. Rapid and simple determination of the *Escherichia coli* phylogenetic group. Appl Environ Microbiol 2000;66:4555-8.

12. Wirth T, Falush D, Lan R, Colles F, Mensa P, Wieler LH, Reeves PR, Maiden MCI, Ochman H, Achtman M. Sex and virulence in *Escherichia coli*: an evolutionary perspective. Mol Microbiol 2006;60:136-51.

13. Woodford N, Fagan EJ, Ellington MJ. Multiplex PCR for rapid detection of genes encoding CTX-M extended-spectrum (beta)-lactamases. J Antimicrob Chemother 2006;57:154-5.

14. Kim HB, Park CH, Kim CJ, Kim EC, Jacoby GA, Hooper DC. Prevalence of plasmid-mediated quinolone resistance determinants over a 9-year period. Antimicrob Agents Chemother 2009;53:639-45.

15. Park CH, Robicsek A, Jacoby GA, Sahm D, Hooper DC. Prevalence in the United States of aac(6′)-Ib-cr encoding a ciprofloxacin-modifying enzyme. Antimicrob Agents Chemother 2006;50:3953-5.

16. Lee MY, Choi HJ, Choi JY, Song M, Song Y, Kim SW, Chang HH, Jung SI, Kim YS, Kim JS, Kwon KT, Heo ST, Yeom JS, Shin SY, Chung DR, Peck KR, Song JH, Ko KS. Dissemination of ST131 and ST393 community-onset, ciprofloxacin-resistant *Escherichia coli* clones causing urinary tract infections in Korea. J Infect 2010;60:146-53.

17. Kim Y, Oh T, Nam YS, Cho SY, Lee HJ. Prevalence of ST131 and ST1193 among bloodstream isolates of *Escherichia coli* not susceptible to ciprofloxacin in a tertiary care university hospital in Korea, 2013-2014. Clin Lab 2017;63:1541-3.

18. Picard B, Garcia JS, Gouriou S, Duriez P, Brahimi N, Bingen E, Elion J, Denamur E. The link between phylogeny and virulence in *Escherichia coli* extraintestinal infection. Infect Immun 1999;67:546-53.

19. Rogers BA, Sidjabat HE, Paterson DL. *Escherichia coli* O25b-ST131: a pandemic, multiresistant, community-associated strain. J Antimicrob Chemother 2011;66:i14.

20. Kim ES, Jeong JY, Jun JB, Choi SH, Lee SO, Kim MN, Woo JS, Kim YS. Prevalence of aac(6′)-Ib-cr encoding a ciprofloxacin-modifying enzyme among Enterobacteriaceae blood isolates in Korea. Antimicrob Agents Chemother 2009;53:2643-5.

21. Nicolas-Chanoine MH, Blanco J, Leflon-Guibout V, Demarty R, Alonso MP, Caniça MM, Park YJ, Lavigne JP, Pitout J, Johnson JR. Intercontinental emergence of *Escherichia coli* clone O25:H4-ST131 producing CTX-M-15. J Antimicrob Chemother 2008;61:273-81.

22. Guillard T, Bertrand X, de Champs C, Cholley P, Bajolet O, Ghaguidi-Haore H. aac(6′)-Ib-cr is the major plasmid-mediated quinolone resistance determinant in extended-spectrum beta-lactamase-producing *Escherichia coli* in eastern France. J Glob Antimicrob Resist 2014;2:111-3.

23. Musumeci R, Rausa M, Giovannoni R, Cialdella A, Bramati S, Sibra B, Giltri G, Viganò F, Cocuzza CE. Prevalence of plasmid-mediated quinolone resistance genes in uropathogenic *Escherichia coli* isolated in a teaching hospital of northern Italy. Microb Drug Resist 2012;18:33-41.

24. Blanco I, Morá A, Mamani R, López C, Blanco M, Dahbi G, Herrera A, Blanco JE, Alonso MP, Garcia-Garrote F, Chaves F, Orellana MA, Martínez-Martínez L, Calvo J, Prats G, Larrosa MN, González-López JJ, López-Cerero I, Rodríguez-Baño J, Pascual A. National survey of *Escherichia coli* causing extraintestinal infections reveals the spread of drug-resistant clonal groups O25b:H4-B2-ST131, O15:H1-D-ST393 and CGA-D-ST69 with high virulence gene content in Spain. J Antimicrob Chemother 2011;66:2011-21.

25. Zhao L, Zhang J, Zheng B, Wei Z, Shen P, Li S, Li L, Xiao Y. Molecular epidemiology and genetic diversity of fluoroquinolone-resistant *Escherichia coli* isolates from patients with community-onset infections in 30 Chinese county hospitals. J Clin Microbiol 2015;53:766-70.

26. Alghoribi MF, Gibreel TM, Farnham G, Al Johani SM, Balkhy HH, Upton M. Antibiotic-resistant ST38, ST131 and ST405 strains are the leading uropathogenic *Escherichia coli* clones in Riyadh, Saudi Arabia. J Antimicrob Chemother 2015;70:2752-62.
27. Kim S, Sung JY, Cho HH, Kwon KC, Koo SH. Characteristics of the molecular epidemiology of CTX-M-producing *Escherichia coli* isolated from a tertiary hospital in Daejeon, Korea. J Microbiol Biotechnol 2016;26:1643-9.

28. Turton JF, Doumith M, Hopkins KL, Perry C, Meunier D, Woodford N. Clonal expansion of *Escherichia coli* ST38 carrying a chromosomally integrated OXA-48 carbapenemase gene. J Med Microbiol 2016;65:538-46.

29. Kwon KT, Ryu SY, Wie SH, Jo HU, Park SY, Hong KW, Kim HI, Kim HA, Kim MH, Bae MH, Sohn YH, Kim I, Lee Y, Kim B, Pai H. 1451. Change in clinical characteristics of community-acquired acute pyelonephritis in South Korea: comparison between 2010-2011 and 2017-2018. Open Forum Infect Dis 2019;6(Suppl 2):S530.

30. Birgy A, Madhi F, Jung C, Levy C, Cointe A, Bidet P, Hobson CA, Bechet S, Sobral E, Vuthien H, Ferroni A, Abetranne S, Cuzon G, Beraud L, Gajdos V, Launay E, Pinquier D, Haas H, Desmarest M, Dommergues MA, Cohen R, Bonacorsi S; Group of the National Observatory of Urinary tract Infection due to ESBL-producing Enterobacteriaceae in children. Diversity and trends in population structure of ESBL-producing Enterobacteriaceae in febrile urinary tract infections in children in France from 2014 to 2017. J Antimicrob Chemother 2020;75:96-105.