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Original Article

Changes in the characteristics of community-onset fluoroquinolone-resistant *Escherichia coli* isolates causing community-acquired acute pyelonephritis in South Korea

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KEYWORDS
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Escherichia coli;
Antimicrobial resistance;
ST131;
Korea

Abstract *Purpose:* This study aimed to examine the changes in the characteristics of community-onset fluoroquinolone-resistant (FQ-R) *Escherichia coli* isolates causing community-acquired acute pyelonephritis (APN) in South Korea.
Methods: Blood or urine samples were prospectively collected from patients aged ≥15 years with community-acquired APN who were admitted to one of the eight Korean hospitals included in this study between September 2017 and August 2018. Phylogenetic typing, multilocus sequence typing, and molecular characterization of β-lactamase resistance and plasmid-mediated quinolone resistance (PMQR) determinants were performed. The data were compared with those from a previous study with the same design conducted in 2010–2011.
Results: A total of 300 and 346 isolates were identified in 2010–2011 and 2017–2018, respectively. Among them, 76 (22.0%) and 77 (25.7%) FQ-R isolates were identified in 2010–2011 and 2017–2018, respectively. A significantly higher antimicrobial resistance against third-to fourth-generation cephalosporins, including cefotaxime (23.9% vs. 77.9%, $P < 0.001$), were observed among FQ-R isolates in 2017–2018 than among those in 2010–2011. A higher proportion of ST131 isolates (27.6% vs. 66.2%, $P < 0.001$), as well as isolates that had extended-spectrum β-lactamase (ESBL)/plasmid-mediated AmpC β-lactamase (PABL) (23.7% vs. 79.2%, $P < 0.001$), was observed in 2017–2018 than in 2010–2011. Further, more PMQR determinants (11.8% vs. 40.8%, $P < 0.001$) were observed in 2017–2018 than in 2010–2011.
Conclusions: Among uropathogenic FQ-R *E. coli* isolates in South Korea, the prevalence of ST131 and the proportion of isolates containing ESBL and/or PMQR determinants have increased.

Introduction

Urinary tract infection (UTI) is one of the most common community-based bacterial infections, affecting half of all women during their adult life, and *Escherichia coli* is the common causative pathogen in >80% of UTI cases.[1]

In previous studies, we found that the epidemiological and clinical characteristics of acute pyelonephritis (APN), such as incidence, antibiotic prescription pattern, and clinical outcomes, have changed in South Korea.[2] In addition to the changes in population structure and healthcare system, the evolution of the microbiological characteristics of *E. coli* may have affected the change. The widespread ST131 strain, which is closely linked to antimicrobial resistance, has led to an increase in the resistance of *E. coli* to various antibiotic classes in worldwide.[3]

Fluoroquinolone (FQ) is commonly prescribed as a first-line empirical antibiotic treatment for UTI, including APN, as recommended by the current clinical guidelines based on the overall antimicrobial resistance of *E. coli*.[4] However, the increase in the prevalence of FQ-resistant (FQ-R) pathogens is of great concern as it is an unfavorable outcome of the empirical use of FQ.[4–6] In fact, the resistance rate of uropathogenic *E. coli* against FQ increased from 21.6% in 2010–2011 to 32.0% in 2017–2018 among patients admitted to large hospitals in South Korea.[7] Furthermore, the resistance rate of uropathogenic *E. coli* against almost all antibiotics, including extended-spectrum cephalosporin, which is another commonly prescribed antibiotic for the treatment of UTI, increased significantly during the same period.[7]

However, the evolution of the microbiological characteristics of uropathogenic *E. coli*, including sequence type (ST) and resistance genes, has not yet been evaluated in South Korea. This study aimed to examine the changes in the characteristics of community-onset FQ-R *E. coli* isolates causing community-acquired APN in South Korea.

Methods

Study setting and sample collection

E. coli isolates that cause community-acquired APN were obtained prospectively from blood or urine samples of patients admitted to one of the eight hospitals (580–915 beds), included in this study, between September 1, 2017, and August 31, 2018, in South Korea and were compared with those from a previous study with the same design conducted between April 1,2010, and February 29,2011, in which 12 hospitals (582–1250 beds) in South Korea participated. Of them, four hospitals participated in both cohorts.[7,8] All participants in both cohorts were aged ≥15 years and were hospitalized for the treatment of community-acquired APN. Only the first identified isolate from each patient was included in this study.

The study protocol was approved by the Institutional Review Board (IRB) of Hanyang University Seoul Hospital (IRB number: 2017-07-009) and the IRB of each hospital. Written informed consent was obtained from the patients by researchers at each hospital.

Antimicrobial susceptibility test

The causative pathogen of community-acquired APN was determined when organisms at concentrations

$\geq 10^5$ CFU/mL were identified in urine culture, and/or the pathogens were isolated from the blood culture of patients diagnosed with community-acquired APN. The bacterial species and their susceptibilities to antibiotics were determined using a semi-automated system (VITEK, bioMèrieux, Hazelwood, MO, USA, or Microscan, Dade Behring, West Sacramento, CA, USA). The results were interpreted using the 2020 Clinical Laboratory Standards Institute breakpoints and resistance or intermediate resistance was considered resistance.[9] FQ-R isolates were defined as isolates that showed resistance to ciprofloxacin or levofloxacin.

For the isolates identified in 2017–2018, additional *in vitro* antimicrobial susceptibility testing for determining the MIC_{50} and MIC_{90} was performed using the agar dilution method with ampicillin, amikacin, gentamicin, tobramycin, trimethoprim/sulfamethoxazole, cefepime, cefotaxime, ceftazidime, ciprofloxacin, and levofloxacin.

DNA extractions

The bacterial lysates were prepared by suspending 5–10 *E. coli* colonies in 500 μL purified water, which was heated for 10 min at 100 °C and centrifuged for 5 min at 13,000×*g*. The harvested supernatant was stored at −20 °C until the polymerase chain reaction (PCR) test was performed.

Phylogenetic typing

For phylogenetic classification of the isolated *E. coli*, the *chuA*, *yjaA*, *arpA*, and TspE4.C2 genes were amplified using a multiplex PCR test and classified as previously described.[10]

Multilocus sequence typing and sequencing analysis

Seven housekeeping genes (*adk*, *fumC*, *gyrB*, *icd*, *mdh*, *purA*, and *recA*) were amplified using specific primers, sequence analysis was performed, and ST was obtained (https://pubmlst.org/). PCR was performed using the AccuPower® Taq master mix (Bioneer, Daejeon, Korea) in a 20-μL total reaction volume, using the following steps: denaturation for 2 min at 95 °C, 30 cycles for 60 s at 95 °C, 60 s at 54°C-60 °C, 2 min at 72 °C, and a final extension for 5 min at 72 °C.

The purified PCR products were sequenced in both directions at Bioneer Co. Multiple sequence alignments were performed using the BLAST program of the National Center for Biotechnology Information and concatenated using MEGA 6.0.[11] Phylogenetic trees were constructed using the maximum likelihood method and 1000 bootstrap replicates.

Detection of resistance genes

Detection of the Extended-spectrum β-lactamase (ESBL) ($bla_{CTX\text{-}M}$ groups), plasmid-mediated AmpC β-lactamase (PABL) (bla_{CMY}), and plasmid-mediated quinolone resistance (PMQR) encoding genes (*qnr* and *aac(6′)-lb-cr*) was performed using AccuPower PCR premix (Bioneer Daejeon, Korea). PCR was performed in a 20-μL reaction mixture containing 2 μL of DNA template, 0.5 μL of each forward and reverse primers, and sterile distilled water, 17 μL; $bla_{CTX\text{-}M}$, bla_{CMY}, *qnr*, and *aac(6′)-lb* genes were analyzed after amplification using specific primers.[12,13] The PCR condition were initial denaturation at 95 °C for 3 min, followed by 30 cycles of 95 °C for 45 s, annealing at a specific temperatures (58 °C for CTX-M, 52 °C for CMY-2, 53 °C for *qnr*, and 55 °C for *aac(6′)-lb*) for 45 s with extension steps at 72 °C for 50 s and a final extension at 72 °C for 5 min.

Detection of virulence factor genes

Multiplex PCR was performed to screen 11 virulence factor genes: *fimH*, *papA*, *papEF*, *sfa/foc*, *ompT*, *hlyA*, *sat*, *fyu*, *iutA*, *kpsMTII*, and *usp*.[14,15] The PCR primers and conditions for each gene are described in Supplementary Table 1.

Statistical analysis

SPSS version 24.0 (IBM Corporation, Armonk, NY, USA) was used for performing all statistical analyses. Categorical variables were analyzed using the chi-square test or Fisher's exact test. A P-value < 0.05 (two-tailed test) was considered significant.

Results

A total of 300 and 346 isolates were identified in 2010–2011 and 2017–2018, respectively. Among them, 76 (22.0%) and 77 (25.7%) FQ-R isolates were identified in 2010–2011 and 2017–2018, respectively.

Antimicrobial resistance

Table 1 shows the changes in antimicrobial resistance, and Supplementary Table 2 shows MIC_{50} and MIC_{90} of FQ-R *E. coli* isolates identified in 2017–2018. Among these isolates, the resistance rate to ampicillin was 75.7% in 2010–2011 and increased to 100% in 2017–2018 ($P < 0.001$). Similarly, the resistance rates to third-to fourth-generation cephalosporins, such as cefepime, ceftazidime, and cefotaxime, increased from 25.0% to 77.9% ($P < 0.001$), 25.3%–41.6% ($P = 0.034$), and 23.9%–77.9% ($P < 0.001$), respectively. In contrast, the resistance rate to amikacin was 8.1% in 2010–2011 and decreased to 0% in 2017–2018 ($P = 0.016$). The resistance rates to gentamicin (52.6% vs. 61.0%, $P = 0.329$), tobramycin (57.7% vs. 61.0%, $P = 0.684$), and trimethoprim/sulfamethoxazole (55.3% vs. 62.3%, $P = 0.329$) did not change significantly.

Molecular epidemiology

Table 2 shows the changes in the molecular epidemiology. In the phylogenetic groups, B2 (44.7%) and D (44.7%) were the two predominant groups in 2010–2011; in 2017–2018, B2 was the most predominant group (79.2%), followed by D (19.5%). The distribution of the phylogenetic groups of FQ-R *E. coli* isolates is shown in Supplementary Table 3.

In 2010–2011, the predominant clone identified using multilocus sequence typing (MLST) was ST131 (21/76, 27.6%), followed by ST393 (14/76, 18.4%), ST1193 (8/76, 10.5%), ST38 (6/76, 7.9%), ST405 (6/76, 7.9%), and ST69 (4/

Table 1 Change in antimicrobial resistances of fluoroquinolone-resistant *E. coli* isolated from community-acquired acute pyelonephritis.

	Isolates from 2010 to 2011 (n = 76)	Isolates from 2017 to 2018 (n = 77)	*P*-value
Ciprofloxacin	74 (97.4)	71 (92.2)	0.276
Levofloxacin	72 (94.7)	77 (100)	0.058
Ampicillin	56/74 (75.7)	77 (100)	<0.001
Cefepime	18/72 (25.0)	60 (77.9)	<0.001
Ceftazidime	19/75 (25.3)	32 (41.6)	0.034
Cefotaxime	17/71 (23.9)	60 (77.9)	<0.001
Amikacin	5/62 (8.1)	0 (0)	0.016
Gentamicin	40 (52.6)	47 (61.0)	0.329
Tobramycin	41/71 (57.7)	47 (61.0)	0.684
Trimethoprim/sulfamethoxazole	42 (55.3)	48 (62.3)	0.414

Table 2 Change in molecular epidemiology of fluoroquinolone-resistant *E. coli* isolated from community-acquired acute pyelonephritis.

	Isolates from 2010 to 2011 (n = 76)	Isolates from 2017 to 2018 (n = 77)	*P*-value
Phylogenic groups			
A	4 (5.3)	0 (0)	<0.001
B1	4 (5.3)	1 (1.3)	–
B2	34 (44.7)	61 (79.2)	–
D	44 (44.7)	15 (19.5)	–
Major clones by MLST			
ST131	21 (27.6)	51 (66.2)	<0.001
ST393	14 (18.4)	3 (3.9)	0.004
ST1193	8 (10.5)	10 (13.0)	0.637
ST38	6 (7.9)	7 (9.1)	0.791
ESBL/PABL			
Total	18 (23.7)	61 (79.2)	<0.001
CTX-M-15	10 (13.2)	30 (39.0)	<0.001
CTX-M-14	8 (10.5)	30 (39.0)	<0.001
CMY-2	2 (2.6)	1 (1.3)	0.620
PMQR determinants			
Total	9 (11.8)	31 (40.3)	<0.001
aac(6′)-1b-cr	7 (9.2)	28 (36.4)	<0.001
qnrB	1 (1.3)	0 (0)	0.497
qnrS	0 (0)	3 (3.9)	0.245

Abbreviations; MLST, multilocus sequence typing; ESBL, Extended-spectrum beta-lactamase; PABL, Plasmid-mediated AmpC beta-lactamase; PMQR, Plasmid-mediated quinolone resistance.

76, 5.3%). Fifteen other types of ST were detected among the rest of the *E. coli* isolates: ST73 and ST648 were detected in two isolates, while ST68, ST93, ST95, ST117, ST130, ST162, ST457, ST602, ST638, ST707, ST744, ST3210, and ST3337 were detected in one isolate. In 2017–2018, the predominant clone identified using MLST was ST131 (51/77, 55.2%), followed by ST1193 (10/77, 13.0%), ST38 (7/77, 9.1%), and ST393 (3/77, 3.9%). Five other types of ST were detected among the remaining *E. coli* isolates: ST648 was detected in two isolates, while ST68, ST95, ST101, and ST9213 were detected in one isolate. The proportion of ST131 (27.6% vs. 66.2%, $P < 0.001$) increased, while that of ST393 (18.4% vs. 3.9%, $P = 0.004$) decreased significantly (Figs. 1 and 2).

ESBL and PABL were detected in 23.7% (18/76) of *E. coli* isolates in 2010–2011, and the proportion increased to 79.2% (61/77) in 2017–2018 ($P < 0.001$). The predominant ESBL/PABL determinants were CTX-M-15 (13.2% vs. 89.0%, $P < 0.001$) and CTX-M-14 (10.5% vs. 39.0%, $P < 0.001$) in both periods, and their prevalence was higher in 2017–2018 than in 2010–2011. Similar to ESBL/PABL determinants, a higher proportion of *E. coli* carrying PMQR determinants was observed in 2017–2018 than in 2010–2011 (11.8% vs. 40.3%, $P < 0.001$). The most prevalent PMQR determinant was *aac(6')-1b-cr* in both periods, and its prevalence increased from 9.2% (7/76) to 36.4% (28/77) ($P < 0.001$).

Virulence factors

Table 3 shows the changes in the distribution of virulence factors. Of the *E. coli* isolates in 2010–2011, the most common virulence factor was *sat* (47/76, 70.2%), followed

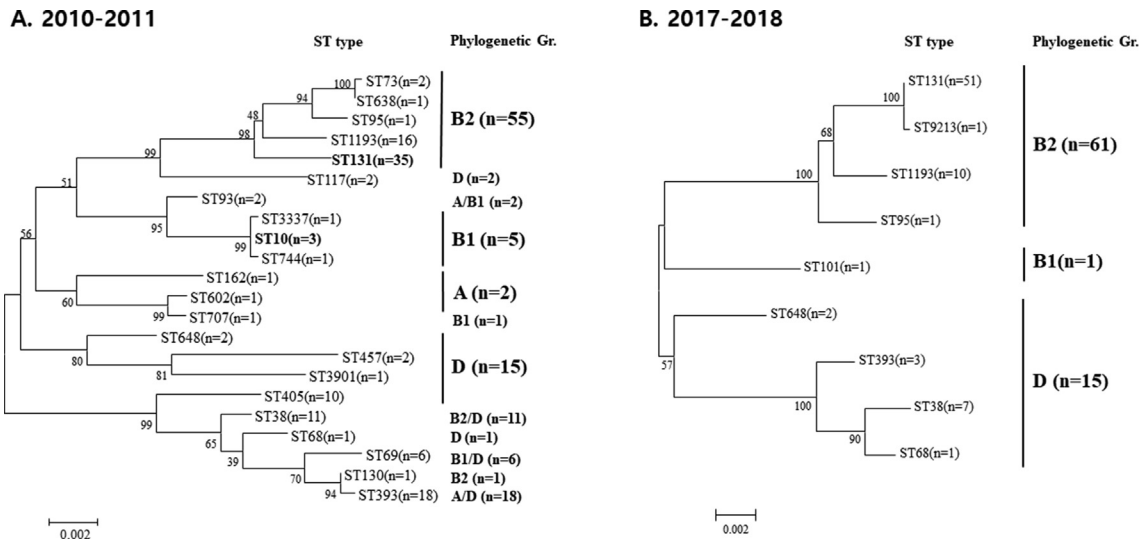

Figure 1. Phylogenetic trees for fluoroquinolone-resistant *Escherichia coli* isolates.

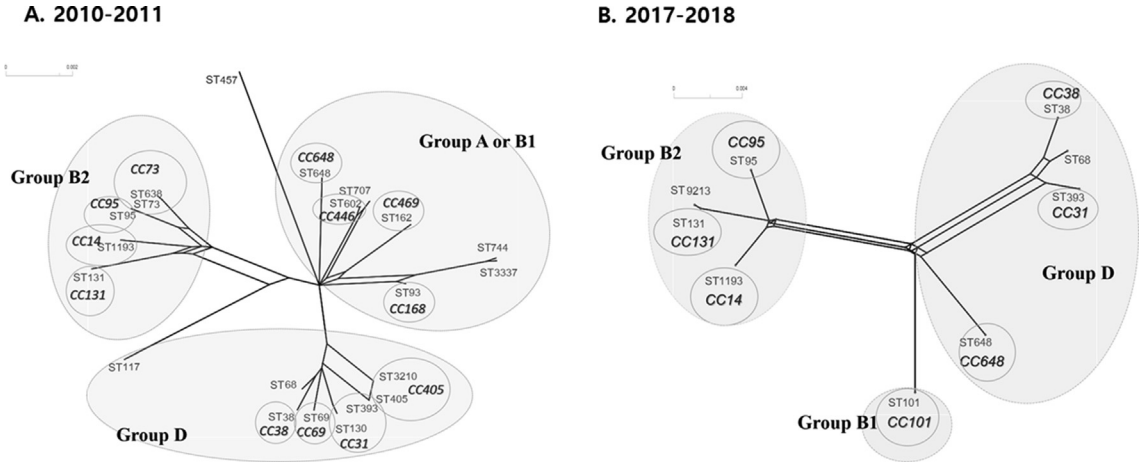

Figure 2. Clonal clusters for fluoroquinolone-resistant *Escherichia coli* isolates.

Table 3 Change in distribution of virulence factors of fluoroquinolone-resistant *E. coli* isolated from community-acquired acute pyelonephritis.

		Isolates from 2010 to 2011 (n = 76)	Isolates from 2017 to 2018 (n = 77)	*P*-value
Adhesins	*sfa/foc*	43 (64.2)	35 (45.5)	0.169
	fimH	35 (52.2)	59 (76.6)	<0.001
	papEF	14 (20.9)	20 (26.0)	0.261
	papA	10 (14.9)	10 (13.0)	0.975
	ompT	10 (14.9)	15 (19.5)	0.290
Toxins	*sat*	47 (70.2)	46 (59.7)	0.790
	hlyA	1 (1.5)	7 (9.1)	0.063
Siderophores	*iutA*	33 (49.3)	42 (54.6)	0.169
	fyuA	4 (6.0)	8 (10.4)	0.238
Capsules	*kpsMTII*	41 (61.2)	57 (74.0)	0.010
	usp	10 (14.9)	19 (24.7)	0.069

by *sfa/foc* (43/76, 64.2%), *kpsMTII* (41/76, 61.2%), and *fimH* (35/76, 52.2%). In 2017–2018, the most common virulence factor was *fimH* (59/77, 76.6%), followed by *kpsMTII* (57/77, 74.0%), *sat* (46/77, 59.7%), and *iutA* (42/77, 54.6%). The proportion of *E. coli* isolates that contained *fimH* (52.2% vs. 76.6%, $P < 0.001$) and *kpsMTII* (61.2% vs. 74.0%, $P = 0.010$) increased significantly.

Microbiological characteristics of *E. coli* ST131

Table 4 shows the changes in the microbiological characteristics of *E. coli* ST131. The resistance rate to ampicillin was 85.0% in 2010–2011 and increased to 100% in 2017–2018 ($P = 0.020$). In addition, the resistance rate to extended-spectrum cephalosporins, such as cefepime (36.8% vs. 96.1%, $P < 0.001$) and cefotaxime (33.3% vs. 96.1%, $P < 0.001$), increased significantly. The resistance rate to aminoglycosides, including amikacin, gentamicin, and tobramycin, remained unchanged. For trimethoprim/sulfamethoxazole, the resistance rate was 28.6% in 2010–2011 and increased to 60.8% in 2017–2018 ($P = 0.013$).

Compared with that of *E. coli* ST131 isolates in 2010–2011, a higher proportion of *E. coli* ST131 isolates in 2017–2018 contained resistance genes such as CTX-M-15 (23.8% vs. 51.0%, $P = 0.034$), CTX-M-14 (9.5% vs. 45.1%, $P = 0.004$), and *aac(6′)-1b-cr* (23.8% vs. 51.0%, $P = 0.034$). Most *E. coli* ST131 isolates that had CTX-M-15 carried *aac(6′)-1b-cr* simultaneously in both periods (80%, 4/5 in 2010–2011 and 88.5%, 23/26 in 2017–2018); the proportion of isolates that carried CTX-M-15 and *aac(6')-1b-cr* simultaneously increased significantly (19.0% vs. 45.1%, $P = 0.038$).

Discussion

In 2017–2018, 25.7% of *E. coli* isolates obtained from patients with community-acquired APN in South Korea were FQ-R isolates, and the resistance rates of those isolates against third-to fourth-generation cephalosporins had increased when compared with those in 2010–2011. Given that ST131 isolates have become more prevalent among FQ-R *E. coli* isolates, the change in resistance may be associated with the change in the molecular epidemiology of *E. coli*.

ST131 is one of the most commonly isolated lineages among uropathogenic *E. coli* strains, along with ST69, ST73, and ST95.[16] Most ST131 strains are strongly associated with multidrug resistance, including resistance to FQ and extended-spectrum cephalosporins.[17] Furthermore, ST131 strains more prevalent worldwide, and the global dissemination of ST131 is a major contributor to community-acquired UTI and bloodstream infections caused by multidrug-resistant *E. coli* strains in several countries.[18] Similar to other regions, ST131 has become a predominant clone among ESBL-producing or FQ-R *E. coli* isolates in the community and healthcare-associated settings in South Korea.[19,20] Interestingly, this study shows that the predominance of the ST131 strain has become more significant, accounting for 66.2% of FQ-R *E. coli* isolates obtained from patients with community-acquired APN in 2017–2018. Other previous studies have also indicated that the proportion of ST131 strains has increased among ESBL-producing *E. coli* isolates causing clinical infectious diseases such as UTI and bacteremia.[21] According to a nationwide study in Taiwan, the proportion of ST131 strains among uropathogenic *E. coli* isolates resistant to both ciprofloxacin and cefotaxime was 33.3% in 2002–2004 and increased to 72.1% in 2014–2016.[22] Given that the ST131 strain is rarely isolated from the environment or animals, the success of this clone might be attributable to its virulence and distinctive genetic characteristics that could allow adaptation in humans.[21] A recent study in Taiwan found that ST131 strains were found in about 2.3% of all healthy dogs and FimH41 and H22 were major subtypes.[23]

ST131 is known for harboring various resistance determinants or genes such as CTX-M-15, TEM-1, OXA-1, and

Table 4 Change in microbiological characteristics of *E. coli* ST131.

	Isolates from 2010 to 2011 (n = 21)	Isolates from 2017 to 2018 (n = 51)	*P*-value
Antimicrobial resistance			
Ciprofloxacin	21 (100.0)	40 (78.4)	0.027
Levofloxacin	20 (95.2)	51 (100.0)	0.292
Ampicillin	17/20 (85.0)	51 (100.0)	0.020
Cefepime	7/19 (36.8)	49 (96.1)	<0.001
Ceftazidime	7 (33.3)	27 (52.9)	0.130
Cefotaxime	7 (33.3)	49 (96.1)	<0.001
Amikacin	1/19 (5.3)	0 (0)	0.271
Gentamicin	12 (57.1)	34 (66.7)	0.444
Tobramycin	14 (66.7)	38 (74.5)	0.499
Trimethoprim/sulfamethoxazole	6 (28.6)	31 (60.8)	0.013
Resistance gene			
CTX-M-15	5 (23.8)	26 (51.0)	0.034
CTX-M-14	2 (9.5)	23 (45.1)	0.004
aac(6′)-1b-cr	5 (23.8)	26 (51.0)	0.034
CTX-M-15 + *aac(6′)-1b-cr*	4 (19.0)	23 (45.1)	0.038
CTX-M-14 + *aac(6′)-1b-cr*	0 (0)	3 (5.9)	0.551

aac(6′)-1b-cr.[17] Therefore, the increase in the proportion of ST131 strains might be associated with the increase in the prevalence of FQ-R *E. coli* isolates containing CTX-M-15, CTX-M-14, and *aac(6′)-1b-cr*. Because CTX-M-15 and CTX-M-14 are responsible for inducing resistance to beta-lactam antibiotics such as penicillin, monobactam, and cephalosporins, a significant increase in the resistance rate to ampicillin and third-to fourth-generation cephalosporins was observed in the present study.[20] Most *E. coli* ST131 strains that had CTX-M-15 carried *aac(6′)-1b-cr* simultaneously. Similar to our findings, *aac(6')-1b-cr,* which is a gene associated with resistance to both aminoglycosides and ciprofloxacin, has frequently been detected in association with CTX-M-15 in previous studies.[21] Interestingly, an increase in the proportion of isolates that harbor resistance determinants or genes was also observed among ST131 isolates. The emergence of CTX-M-15 and CTX-M-14 genes within *E. coli* ST131 was likely associated with the evolution of the genetic group. After becoming predominant in the H30-R group, which is nearly always a part of the FQ-R ST131 strain, a subgroup named H30-Rx that contains bla_{CTX-M} has emerged subsequentially since the 2000s.[24] Unlike the resistance determinants of FQ, the type of β-lactamase varies and is lost and recovered frequently.[24] Thus, the subgroup H30-Rx might have been formed inadvertently from exposure to antibiotics.[24]

With the increase in the prevalence of the ST131 strain, the phylogenetic group B2, to which ST131 belongs, became more predominant in 2017—2018. The phylogenetic group B2 strains are known to harbor more virulence factors than other groups, and the change in the proportion of virulence factor-encoding genes in this study seems to be associated with the increase in the prevalence of the ST131 strain and the proportion of phylogenetic group B2 strains as well.[25] In fact, virulence factor-encoding genes that have been frequently found in *E. coli* ST131 isolates were *sat, fimH, fyuA, kpsMII, usp, malX, iha, ompT, iucD, iutA,* and *tratT*.[21] Though we found that the proportion of uropathogenic *E. coli* isolates that contained *fimH* and *kpsMTII* had increased significantly, it could not be clearly determined whether these virulence factors are associated with the changes in the clinical characteristics of community-acquired APN in South Korea. However, further studies that control several confounders that might influence the change in clinical characteristics, including changes in population structure, are necessary to clarify this issue.

There are some potential limitations in the present study. First, this study was conducted mainly in large hospitals and only hospitalized patients were recruited. Therefore, the findings might not be generalized to the whole population in South Korea. Second, the antimicrobial susceptibility tests for the study isolates in 2010—2011 were not performed in the central lab with the standard broth microdilution method. Therefore, some other factors such as the quality of the laboratory in each hospital might affect the results. Finally, the major resistance mechanisms of FQ-R *E. coli* including the mutation in the quinolone resistance determining regions in chromosomal *gyrA* and *parC* genes were not analyzed. In fact, the analysis of resistance genes in this study focused on the genes that can be carried by mobile genetic elements, which are associated with the horizontal transmission of antimicrobial resistance.

In conclusion, among uropathogenic FQ-R *E. coli* isolates in South Korea, the prevalence of ST131 and the proportion of ESBL and/or PMQR determinants have increased.

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Declaration of competing interest

None declared.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jmii.2022.01.001.