

Original article

Risk Factors Associated with Recurrent Urinary Tract Infection Caused by *Escherichia coli*

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*Escherichia coli*에 의한 반복적 요로감염과 관련된 위험인자 분석

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ABSTRACT

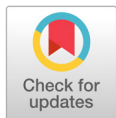
Background: The purpose of this study was to analyze the clinical and microbiological characteristics of recurrent urinary tract infection (UTI) caused by *Escherichia coli*— the most common etiological agent.

Methods: Cases of recurrent and single episodes of UTI caused by *E. coli* were evaluated retrospectively for a period of 6 months (January-June 2019) to analyze the clinical and molecular characteristics of this disease.

Results: Healthcare-associated UTI, *E. coli* bacteremia, and poor microbial clearance 7 days post infection were associated more with the recurrent episodes of infection. *E. coli* isolates from subjects with recurrent UTIs showed higher rates of antimicrobial resistance and extended-spectrum β -lactamase (ESBL) production. The *E. coli* clone— sequence type 131 was detected in similar proportions in isolates, recovered from subjects in both groups— recurrent episodes and single episode of UTI.

Conclusion: The control of antimicrobial-resistant ESBL-producing *E. coli* strains may be difficult using antimicrobial therapy and subsequently delay the clearance of the etiologic agent. This could play a major role in the development of recurrent UTIs.

Keywords: *Escherichia coli*, Extended-spectrum β -lactamases, Recurrent urinary tract infection, Risk factors, Sequence type 131



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INTRODUCTION

Urinary tract infections (UTIs), which are defined microbiologically as the inflammatory response of the urothelium to microbial pathogens, are common bacterial infections. Approximately half of all women will experience symptomatic acute bacterial cystitis in their lifetime [1]. When the first infection is caused by

Escherichia coli, women appear to be more likely to develop a second cystitis episode within six months than those with a first infection due to another organism [2]. In a Finnish study of women ages 17 to 82 who had *E. coli* cystitis, 44% had a recurrence within one year [3]. Repeated UTIs degrade the quality of life and can be accompanied by mood disturbances, such as anxiety and depression. Data on the current prevalence and factors influencing recurrent UTIs are scarce in Korea. The purpose of this study was to analyze the clinical and microbiological characteristics of recurrent UTIs by *E. coli* to identify the risk factors. The findings could help establish a management strategy to reduce recurrent UTIs and improve patient prognosis.

MATERIALS AND METHODS

Study population and definition

The study population was selected from non-duplicated patients admitted to the National Health Insurance Service Ilsan Hospital between January and June in 2019 and diagnosed with *E. coli* urinary tract infections based on culture results ($n = 1,060$). Patients with *E. coli* isolates from urine culture with 10^4 colony forming units (CFU)/mL or higher were included in this study. If two species were isolated from the urine culture (polymicrobial), we included only patients with *E. coli* at 10^5 CFU/mL or higher. We excluded cases with more than three species in the urine culture. For the risk factor analysis, we randomly selected cases ($n=64$) and controls ($n=124$) in which there were 213 recurrent episodes (cases) and 847 single episodes (controls). Molecular analysis was performed in 62 randomly selected *E. coli* isolates from subjects with recurrent episodes (two isolates were excluded because of repeated failure of cultures) and 62 *E. coli* isolates with single episodes.

Recurrent UTIs were defined when *E. coli* was isolated from urine cultures more than twice in six months. Healthcare-associated UTI was defined if the patient was transferred from a long-term care facility or diagnosed with an *E. coli* UTI after 48 hours of hospital admission [4]. Community-associated UTI was defined if *E. coli* was isolated within 48 hours of admission. The disease severity was classified by the Charlson comorbidity index [5]. Urinary tract anomalies included obstruction, hydronephrosis, renal tract calculi, and colovesical fistulas. Immunosuppressive therapy was defined as treatment with prednisolone or an equivalent drug at a dosage of at least 10 mg/day for 15 days, and chemotherapy or radiotherapy within six months before bacteremia [6]. Microbiological clearance was determined based on the results of follow-up urine cultures. Adequate empirical antimicrobial therapy was the administration of at least one dose of susceptible antibiotics within one calendar day of culture collection. Adequate definite antimicrobial therapy was the administration of at least one dose of susceptible antibiotics within one calendar day of culture results or within 72 hours of culture collection.

Microbiological analysis

Urine cultures were performed by inoculation of clean midstream urine on blood agar and MacConkey

agar and incubation at 37°C for two days. Species identification was made with a MALDI Biotyper MALDI-TOF MS (Bruker Daltonik, Bremen, Germany). Antimicrobial susceptibility testing was conducted using a MicroScan WalkAway Plus system (Beckman Coulter, Inc., West Sacramento, CA, USA) and a MicroScan Neg Breakpoint Combo Type 44 panel (Siemens Healthcare Diagnostics, Inc., West Sacramento, CA, USA) according to Clinical and Laboratory Standards Institute guidelines [7].

Molecular analysis

PCR (polymerase chain reaction) of serogroup O25b and O16 was done to determine the sequence type (ST) 131 [4]. The ESBL genotype was verified with PCR-sequencing of CTX-M, SHV, and TEM-type genes [6]. For a carbapenem-non-susceptible isolate, carbapenemase genes were identified by PCR and sequenced using primers to detect IMP, KPC, NDM, VIM, and OXA-48 [8]. Pulsed-field gel electrophoresis (PFGE) was performed as described in our previous study [6]. A total of 124 isolates were included from recurrent episodes ($n = 62$) and single episodes of UTIs ($n = 62$). A total of 122 *E. coli* isolates were finally analyzed by PFGE because of repeated fails in two *E. coli* isolates. The patterns were analyzed using InfoQuest FP software (Bio-Rad, Hercules, CA, USA) to generate a dendrogram based on the unweighted pair group method, with an arithmetic average (UPGMA) from the Dice coefficient with 1% band position tolerance and 0.5% optimization settings.

Clinical features and risk factors

The first episode was included in the analysis. Clinical data were retrospectively obtained from a review of the electronic medical record. The included variables were age, sex, underlying disease, Charlson comorbidity index (CCI), urinary tract anomaly, acquisition site, transfer from another healthcare facility, appropriate antimicrobial treatment (empirical and final), microbiological clearance, 30-day mortality, and ESBL *E. coli* phenotype at the first episode. Infection evidence, such as fever, laboratory findings (C-reactive protein, albumin, white blood cell count), and blood cultures were also reviewed. Up to 90 days from the first episode, previous surgery, use of Foley catheter, immunosuppressive therapy, and total parenteral nutrition, and admission to an intensive care unit were recorded.

Statistical analysis

Continuous variables, such as age, were analyzed by the Mann-Whitney U test. The chi-squared test was used for comparative analysis of the categorical variables to determine the independent risk factors. Odds ratio (OR) and 95% confidence interval (CI) values were calculated for the binomial variables. Variables with P -values less than 0.1 in univariate analyses were included in a multivariate logistic regression analysis model to determine the independent risk factors. Statistical significance was defined as $P < 0.05$. SPSS 23.0 software (SPSS, Chicago, IL, USA) was used for the univariate and multivariate analyses. This study was approved by the Institutional Review Boards as required by the hospital policy (IRB No. NHIMC 2020-03-002).

RESULTS

Microbiological analysis

The *E. coli* isolates from patients with recurrent UTIs were much less sensitive to antimicrobial agents than those isolated from patients with single episodes, except for piperacillin-tazobactam, amoxicillin-clavulanic acid, cefoxitin, and minocycline (Fig. 1). Only one *E. coli* isolate was resistant to doripenem, meropenem, and imipenem, which was from a subject with recurrent UTIs. Neither colistin nor tigecycline-resistant *E. coli* were isolated from patients in either group.

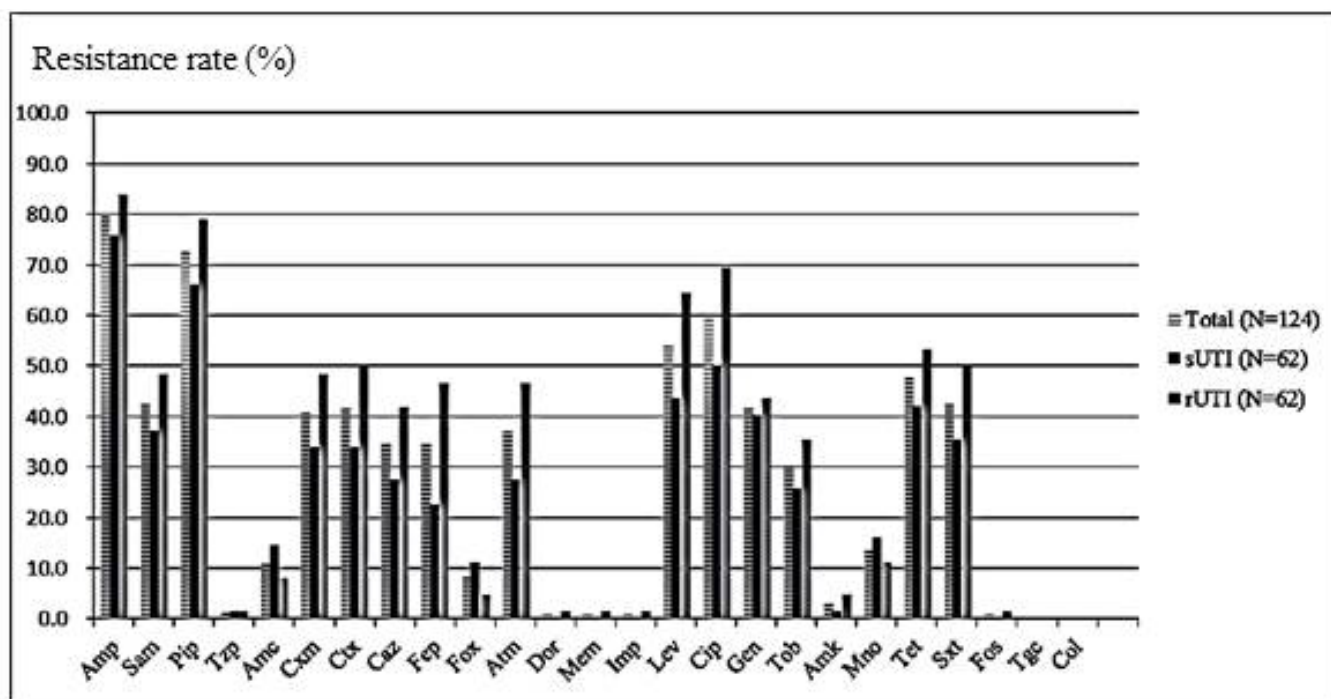


Fig 1. Antimicrobial resistance rates of *Escherichia coli*.

Total UTI (n = 124) included both recurrent episodes (n = 62) and single episodes (n = 62).

Abbreviations: rUTI, recurrent urinary tract infection; sUTI, single episode of urinary tract infection; N, number, Amp, ampicillin; Sam, ampicillin-sulbactam; Pip, piperacillin; Tzp, piperacillin-tazobactam; Amc, amoxicillin-clavulanic acid; Cxm, cefuroxime; Ctx, cefotaxime; Caz, ceftazidime; Fep, cefepime; Fox, cefoxitin; Atm, aztreonam; Dor, doripenem; Mem, meropenem; Imp, imipenem; Lev, levofloxacin; Cip, ciprofloxacin; Gen, gentamicin; Tob, tobramycin; Amk, amikacin; Mno, minocycline; Tet, tetracycline; Sxt, trimethoprim-sulfamethoxazole; Fos, fosfomycin; Tgc, tigecycline; Col, colistin.

Molecular analysis

The ST 131 *E. coli* clone was frequently detected but the proportions were similar in both groups (35.5% in recurrent episodes and 30.7% in single episodes). The rate of ESBL producers in the recurrent episode group was higher than that of the single episode group (45.2% versus 21.0%, $P = 0.0042$) but genotypes CTX-M-15 and CTX-M-14 were common, i.e., more than 20% in both groups (Table 1). KPC-2 carbapenemase was detected in a carbapenem-resistant *E. coli*. There was no dominant clone in the PFGE patterns using an 80% cut-off (Fig. 2).

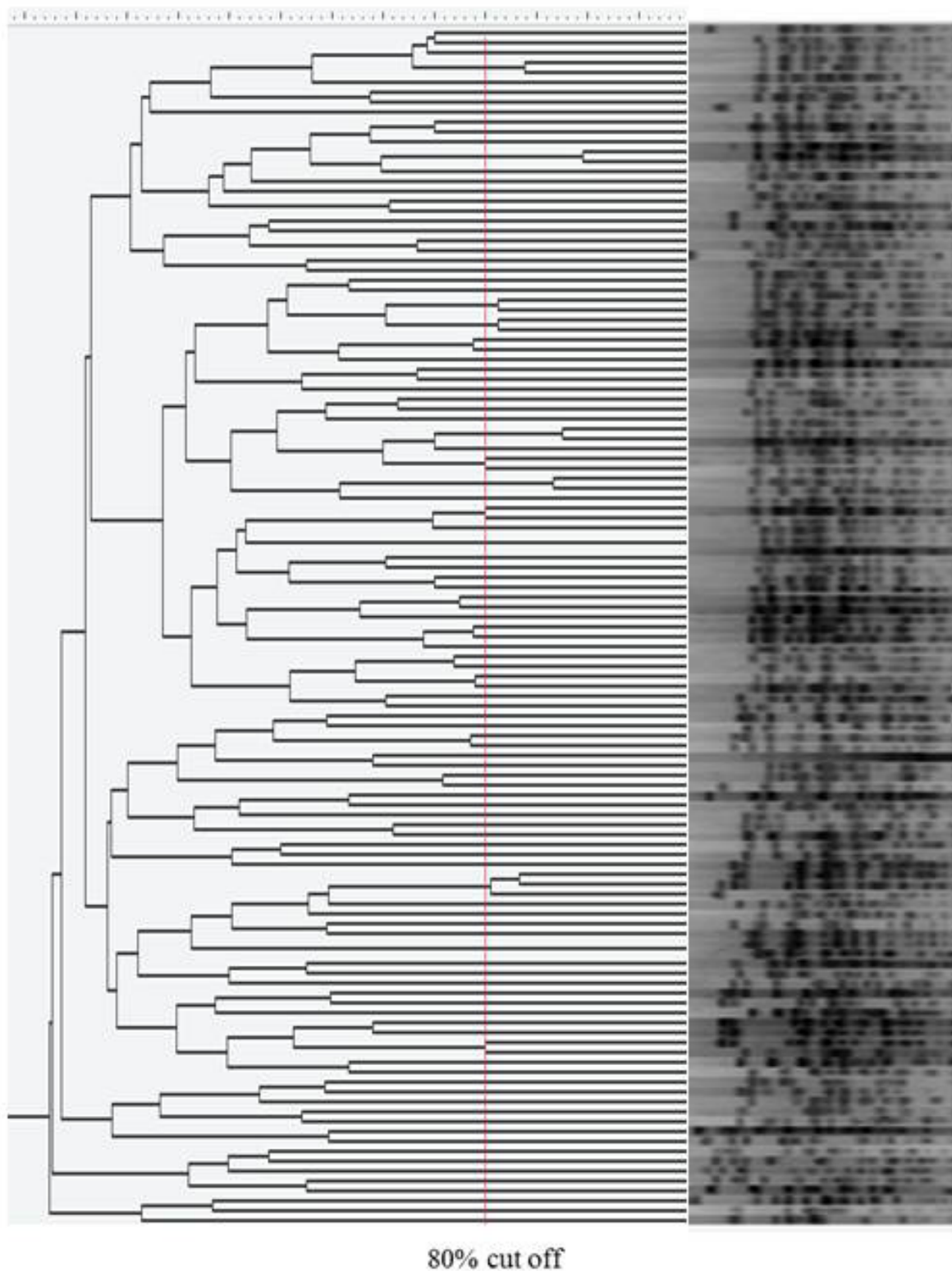


Fig. 2. Dendrogram of *XbaI*-restricted DNA of *Escherichia coli* isolated from urinary tract infection (n=122). PFGE was performed with size marker, Lambda Ladders (Promega, Fitchburg, WI, USA).

Abbreviations: ESBL, extended-spectrum β -lactamase; ST, sequence type; rUTI, recurrent urinary tract infection; sUTI, single episode of *E. coli* urinary tract infection.

Table 1. The molecular epidemiology of *E. coli* isolated from urinary tract infections

Characteristic	No. (%) in Recurrent episodes	No. (%) in Single episodes	P-value
ST131	22/62 (35.5)	19/62 (30.6)	0.5669
O25	20/22 (90.9)	19/19 (100)	
O16	2/22 (9.1)	0/19 (0)	
ESBL genotype	28/62 (45.2)	13/62 (30.8)	0.0042*
CTX-M-14-type	6/28 (21.4)	4/13 (46.2)	0.0619†
CTX-M-15-type	15/28 (53.6)	6/13 (30.0)	
CTX-M-27-type	3/28 (10.7)	2/13 (15.4)	
CTX-M-55-type	4/28 (14.4)	1/13 (7.7)	

The molecular analysis was performed in 62 cases and 62 controls at a 1:1 ratio.

*The difference of ESBL production rates in both groups.

†The difference of ESBL genotypes in both groups.

Abbreviations: ST, sequence type; ESBL, extended-spectrum β -lactamase.

Clinical features and risk factors

When the two groups of patients with *E. coli* UTIs were compared, hemiplegia (12.5% in recurrent episodes versus 2.4% in single episodes, $P = 0.0053$), healthcare-associated acquisition (21.9% in recurrent episodes versus 5.7% in single episodes, $P = 0.0008$), and the isolation of *E. coli* in follow-up urine cultures after seven days (28.1% in recurrent episodes versus 1.6% in single episodes, $P < 0.0001$) were found more in patients with recurrent episodes than in single episodes (Table 2). However, adequate antimicrobial treatment was more common in patients with single episodes, regardless of empirical treatment (34.4% in recurrent episodes versus 56.5% in single episodes, $P = 0.0041$) or definite treatment (45.3% in recurrent episodes versus 64.5% in single episodes, $P = 0.0115$).

Healthcare-associated UTIs (OR = 3.731, $P = 0.0239$), *E. coli* bacteremia (OR = 2.580, $P = 0.0344$), and no microbiological clearance after seven days (OR = 13.792, $P = 0.0020$) were more associated with recurrent episodes in multivariate analysis (Table 3).

DISCUSSION

E. coli is the most common etiology of UTIs and 6,394 (11.1%) *E. coli* isolates were recovered from 57,477 urine cultures according to the first one-year report from Korea Global Antimicrobial Resistance Surveillance System (2016-2017) [4]. For the treatment of UTI, oral fluoroquinolone and oral cephalosporin are usually recommended for non-complicated cystitis and ceftriaxone and fluoroquinolone are strongly recommended according to the results of susceptibility testing for adult simple acute pyelonephritis [9]. However, the resistance rates in the *E. coli* urine isolates to cefotaxime, ceftazidime, and ciprofloxacin were high [4] because extended-spectrum- β -lactamase-producing *E. coli* (ESBL-EC) has increased in both community and hospital settings in Korea [10,11]. ESBL-EC breaks down extended-spectrum cephalosporin and aztreonam, showing wide-ranging resistance to cephalosporins except for carbapenem. Specifically, ST 131 *E. coli*, a high-risk clone with extensive antibacterial resistances, high-virulent traits, and increased propagation power, has contributed to the worldwide dissemination. ESBL-EC had multidrug

Table 2. Comparison of Recurrent and single episodes of urinary tract infections caused by *E. coli*

Variable	No. (%) in Recurrent episodes (n = 64)	No. (%) in Single episodes (n = 124)	P-value
Age in years	71.8 ± 18.8	71.6 ± 16.8	0.9362
Male sex	15 (23.4)	17 (13.7)	0.0926
Acquisition site			
Community-associated*	50 (78.1)	117 (94.4)	0.0008
Healthcare-associated	14 (21.9)	7 (5.7)	
Transfer from			
Long-term care facility	2 (3.1)	4 (3.2)	0.9618
Acute care facility	16 (25.0)	27 (21.8)	
Clinics	16 (25.0)	34 (27.4)	
Associated disease			
Diabetes mellitus	24 (37.5)	35 (28.2)	0.1941
Chronic renal disease	11 (17.2)	16 (12.9)	0.4274
Liver cirrhosis	1 (1.6)	2 (1.6)	0.9792
Kidney transplantation	1 (1.6)	1 (0.8)	0.6321
Cancer	9 (9.4)	16 (12.9)	0.4758
BPH (male only)	4 (26.7)	5 (29.4)	0.8632
Hemiplegia	8 (12.5)	3 (2.4)	0.0053
Cerebrovascular disease	8 (12.5)	18 (14.5)	0.7044
Dementia	4 (6.3)	15 (12.1)	0.2076
Charlson comorbidity index	2.6 ± 1.5	2.5 ± 1.9	0.6923
Urinary tract anomaly	5 (7.8)	7 (5.7)	0.5646
Surgical history	2 (3.1)	6 (4.8)	0.5812
Urinary catheter use	38 (59.4)	70 (56.5)	0.7009
Immunosuppressive therapy	1 (1.6)	1 (0.8)	0.6321
TPN use	7 (10.9)	16 (12.9)	0.6967
ICU admission	1 (1.6)	9 (7.3)	0.0992
Fever (> 37.5°C)	8 (12.5)	17 (13.7)	0.8170
Laboratory findings			
C-reactive protein (mg/dL, n=132)	6.8 ± 8.8	8.9 ± 8.3	0.1182
WBC (10 ³ /μL, n=152)	12.0 ± 7.5	10.9 ± 6.1	0.3580
Albumin (g/dL, n=128)	3.4 ± 0.7	3.4 ± 0.7	0.3657
Microbiologic al clearance			
Clearance within 7 days	19 (29.7)	35 (28.2)	< 0.0001
Persistence after 7 days	18 (28.1)	2 (1.6)	
<i>E. coli</i> bacteremia	17 (26.6)	19 (15.3)	0.0635
Adequate empirical antibiotic use	22 (34.4)	70 (56.5)	0.0041
Adequate definite antibiotic use	29 (45.3)	80 (64.5)	0.0115

Data are numbers (%), except for age, Charlson comorbidity index, and laboratory findings, which are mean ± standard deviation.

*Bold formatting indicates statistical significance.

Abbreviations: rUTI, recurrent urinary tract infection; sUTI, single episode of urinary tract infection; BPH, benign prostate hyperplasia; TPN, total parenteral nutrition; ICU, intensive care unit; WBC, white blood cell.

Table 3. Risk factors for recurrent urinary tract infections by *E. coli* by multivariate analysis

Risk factor*	OR (95% CI)	P-value
Healthcare-associated	3.731 (1.190-11.698)	0.0239
<i>E. coli</i> bacteremia	2.580 (1.072-6.209)	0.0344
Persistence after 7 days	13.792 (2.603-73.063)	0.0020

*Statistical significances were maintained after the adjustment for sex, underlying disease (hemiplegia), acquisition sites, intensive care unit admission, *E. coli* bacteremia, microbiological clearance, and adequate antibiotic treatment (empirical and definite). Abbreviations: OR, odds ratio; CI, confidence interval.

resistance traits, which shows resistance to other classes, such as fluoroquinolone and aminoglycoside [12].

Old age, female, catheterization, and sexual activity are well-known risk factors of UTI [13] but the molecular epidemiology and risk factors of recurrent UTI have not been fully evaluated in Korea. Both bacterial factors and deficiencies in host defense could contribute to the pathogenesis of recurrent UTIs [14]. Bacterial survival in the urinary bladder after antibiotic treatment and progression to forming intracellular bacterial communities might be the most important bacterial factors. Immunodeficiency and urogenital tract anatomical abnormalities have been considered essential risk factors for recurrent UTIs.

In this study, the rates of ESBL production and antimicrobial resistance were different between the two groups of patients with recurrent episodes and single episodes. The differences in microbial clearance and the proportions of adequate antimicrobial treatment were also noticeable in the two groups. However, the proportions of associated disease, disease severity, urinary tract anomalies, and immunosuppressive therapy were similar between the two groups. We inferred that the multidrug-resistant traits of ESBL-EC may hinder adequate antimicrobial therapy and consequently delay the clearance of the etiologic agents, which could play a major part in the development of recurrent UTIs. Risk factors, such as healthcare-associated UTIs, *E. coli* bacteremia, and no microbiological clearance after seven days, were more associated with recurrent episodes, which could be used as predictors.

The catheter-associated UTIs were more associated with older age, diabetes mellitus, chronic renal disease, cancer, cerebrovascular accidents, dementia, higher CCI, surgical history, total parenteral nutrition use, intensive care unit admission, concomitant *E. coli* bacteremia, higher 30 day mortality and more ESBL phenotypes (data was not shown).

This study could provide recent data, including microbiology and molecular features, in addition to clinical features and antimicrobial treatments. The limitation of this study was that we defined UTIs based on urine culture results without consideration of UTI clinical features. As a result, asymptomatic bacteriuria could be included, even though we set the criteria to rule out contaminated urine cultures. Another limitation of this study, the used breakpoints are intended for the bloodstream infection. Because the definition of adequate antimicrobial therapy was based on this result, the resistance to specific antimicrobials did not definitely indicate UTI treatment failure due to a high concentration of antimicrobials in the urine. These two limitations possibly affected the low rates of empirical and definitive antimicrobial treatment.

In this study, we could not discriminate between reinfections or relapses in the recurrent episodes. Most recurrences are thought to represent reinfections rather than relapses (even recurrences caused by the same uropathogenic strain), although occasionally, a persistent focus can produce a relapsing infection [15]. However, others have said that it is because phenotypically based typing methods or less specific DNA-based typing methods were used [15]. It was reported that most recurrent UTIs were caused by relapse with the primary infecting *E. coli* according to PFGE analysis and may support the recent observation that *E. coli* could be an intracellular reservoir for recurrent UTIs [16]. Further studies are needed to evaluate the microbiological nature of recurrent episodes.

요약

배경: 본 연구의 목적은 요로감염의 가장 흔한 원인균인 *Escherichia coli*에 의한 반복적 요로감염의 임상 및 미생물학적 특성을 분석하여 위험인자를 파악하는 것이다.

방법: 2019년 1월부터 6개월동안 *E. coli*에 의한 반복적 요로감염 환자와 단일 에피소드를 후향적으로 비교하였다.

결과: 반복적 요로감염 환자에서 의료기관 관련 요로감염, 대장균 균혈증 동반, 소변 추적배양에서 음전되지 않은 경우가 많았다. 반복적 요로감염 환자에서 분리된 *E. coli*는 extended-spectrum β -lactamase (ESBL) 생성이 빈번하고 항균제 내성률이 높았다. 자주 검출되는 ST131 *E. coli*의 비율은 두 군이 유사하였다.

결론: ESBL을 생성하는 *E. coli*는 흔히 다제내성을 보이므로 적절한 항균제 치료의 가능성을 낮추어 요로 내 *E. coli*의 제거를 지연시켜 반복적인 요로감염과 관련성이 큰 것으로 생각된다.

CONFLICTS OF INTEREST

No potential conflicts of interest relevant to this article were reported.

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