Risk Factors and Microbiological Features of Recurrent *Escherichia coli* Bloodstream Infection

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Abstract

Background: Recurrent blood stream infection (BSI) is a significant complication associated with additional morbidity and mortality. We aim to understand the clinical risk factors and microbiological determinants in recurrent *E. coli* BSI.

Methods: A retrospective study was conducted involving adult patients with *E. coli* BSI and its recurrence from January 2017 to December 2018. Recurrence was subdivided into early onset (4–30 days after initial BSI) and late onset (31–270 days after initial BSI). We evaluated risk factors for recurrent BSI and microbiological determinants of *E. coli* isolated from patients with recurrent BSI.

Results: Of 808 patients with *E. coli* BSI, 57 (6.31%) had recurrence (29 of early onset and 28 of late onset) during study period. Data of 149 patients with single episode, whose samples were available for determining the sequence type 131 and extended-spectrum β -lactamase (ESBL) genotype, were selected for comparison. In recurrent BSI, liver cirrhosis, vascular catheter, and ESBL phenotype were more common, and the Charlson comorbidity index and sequential organ failure assessment score were higher than those of single episode BSI. Vascular catheter (odds ratio [OR], 4.588; 95% confidence interval [CI], 1.049–20.068), ESBL phenotype (OR, 2.037; 95% CI, 1.037–3.999) and sequential organ failure assessment score ≥ 9 (OR, 3.210; 95% CI, 1.359–7.581) were independent risk factors for recurrence. The proportion of the ESBL genotype was higher in early onset recurrent BSI (41.4%) than in late onset recurrent BSI (25%) or single episode BSI (16.8%); the *E. coli* isolated from the former was resistant to most antimicrobial agents. Whole genome sequencing performed on 27 of the available ESBL-producing *E. coli* samples (11 from single episode, 11 from early onset recurrence, and 5 from late onset recurrence) demonstrated that various virulence factors, resistant genes, and plasmid types existed in isolates from all types of BSI. Among 28 patients of late onset recurrence, 8 episodes were available for pulsed-field gel electrophoresis analysis and 3 (37.5%) were caused by an identical strain.

Conclusions: This study outlines risk factors contributing to the recurrence and microbiological features of *E. coli* causing recurrent BSI, which may be helpful for healthcare providers.

Introduction

Escherichia coli is a gram-negative bacteria that is a part of normal gut microbiota but also cause blood stream infections (BSI) in humans [1]. For the last decade, *E. coli* has been the leading causative organism resulting in BSI in both community and hospital settings [2–4]. The proportion of BSI episodes caused by *E. coli* was 27.1%, and the incidence rate was 48.0 per 100,000 person-year in high-income countries [5]. According a study conducted in England, BSI caused by *E. coli* has been increasing, and there was a 6% increase in incidence between 2012–13 and 2013–14 [2].

E. coli is one of the most common pathogens isolated from recurrent BSI [6–8], and the rates of recurrence varied from 3.1% [9] to 9.9% [10]. Several factors are associated with recurrence of *E. coli* BSI in patients [6, 7, 9–11], such as hematologic malignancy, non-urinary tract infection, inadequate antibiotic

therapy, and presence of vascular catheter. Antibiotic resistance in *E. coli* is also associated with an increase in recurrent BSI [12]. In particular, multidrug-resistant traits of extended-spectrum β -lactamase (ESBL)-producing *E. coli* hinder patients from receiving appropriate empirical antibiotic therapy and effective definite antibiotic therapy cause recurrent BSI [13]. Specific virulence factors of *E. coli* are associated with recurrence, because they prevent host-immune reactions resulting in delayed or failed clearance of pathogens [9].

Recurrence is a significant problem associated with *E. coli* BSI, as it may require re-admission to a hospital thereby adding to healthcare costs and can potentially increase the mortality rate. Therefore, it is important to investigate the prevalence, clinical characteristics, and microbiological features of recurrent BSI caused by *E. coli*. This information would be helpful for effectively planning patient management and follow-up after initial episode.

The aim of the current study was to determine risk factors for recurrent *E. coli* BSI in South Korea, where *E. coli* isolated from blood specimens exhibited high rates of resistance to several antimicrobial agents. In addition, we evaluated the molecular characteristics of *E. coli* causing recurrent BSI, since the molecular epidemiology of recurrent *E. coli* has not yet been determined in this setting.

Methods

Study design and population

A retrospective study was performed at the National Health Insurance Service Ilsan Hospital in South Korea from January 2017 to December 2018. Among patients admitted to the hospital, adult patients (age ≥ 18 years) who presented with positive blood culture for *E. coli* at least once were included in this study. Patients were followed up to 270 days after the initial episode of *E. coli* BSI. Patients who died or were transferred to another hospital within three days after initial detection of *E. coli* in the blood culture were excluded, as were patients who were expected to have recurrent bacteremia due to specific infection, such as infective endocarditis, mycotic aneurysm, and endovascular graft infection. All patients were included for analysis only once for each episode of BSI. Demographic and clinical variables were extracted through electronic medical records.

Definition and classification of recurrent BSI

Recurrent BSI was defined as a positive blood culture from a specimen collected 4 days or later after the first episode. Recurrence was subdivided into early onset (4–30 days after initial BSI) and late onset (31–270 days after initial BSI). Type of infection were classified as community- or hospital-acquired. Community-acquired BSI was defined as an infection occurred within 48 hours after admission. Infections developed more than 48 hours after admission were defined as hospital-acquired BSI. Comorbidities were defined using the International Classification of Diseases, 10th revision. Charlson Comorbidity Index, which predicts the 10-year mortality in patients who may have a range of comorbid

conditions [14], was used to quantify a patient's burden of disease. Prior antibiotics use was defined as use of antimicrobials for ≥ 3 days in the previous 30 days. The primary focus of BSI was classified according to the Centers for Disease Control and Prevention/National Healthcare Safety Network surveillance criteria [15]. Empirical antibiotics were regarded to be appropriate if administered within one calendar day of culture, and the pathogen was susceptible to any of antibiotics used. Appropriate definite antibiotic therapy was defined as the administration of antibiotics based on the susceptibility, within one calendar day of the susceptibility results becoming available. We classified recurrence as a relapse when a strain of the second episode had an identical Pulsed-field gel electrophoresis (PFGE) pattern to that of the initial episode. Recurrence was considered as a reinfection when the PFGE pattern was different between strains of the initial and subsequent episodes.

Microbiological analysis

Blood culture was performed using BacT/ALERT FA/NA (BioMerieux Inc., Durham, NC, USA) with the incubation of blood bottles at 37°C for five days. Species were identified with MALDI Biotyper MALDI-TOF MS (Bruker Daltonik, Bremen, Germany). The antimicrobial susceptibility test was conducted using MicroScan WalkAway plus system (Beckman Coulter, Inc., West Sacramento, CA, USA) and MicroScan Neg Breakpoint Combo Type 44 panel (Siemens Healthcare Diagnostics, Inc., West Sacramento, CA, USA). The susceptibility results were interpreted using the Clinical and Laboratory Standards Institute guidelines [16].

We performed polymerase chain reaction (PCR)-sequencing of O25b and O16 to determine the sequence type (ST) 131, and a DNA region corresponding to CTX-M, SHV, and TEM was sequenced to detect the genotype of ESBL. PFGE was performed on eight pairs of late onset recurrence to distinguish between relapse and reinfection. The patterns were analyzed using InfoQuest FP software (Bio-Rad) 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 [17].

We evaluated the ST, virulence factor, plasmid type, and antimicrobial resistance gene of ESBL-producing *E. coli* using the whole genome sequencing (WGS). WGS was performed on ESBL-producing *E. coli* isolated from patients with recurrent BSI and single episode BSI. The DNA of freshly subcultured isolates was extracted using GenElute™ Bacterial Genomic DNA Kit (Sigma-Aldrich, St. Louis, MO), and 8 µg of input genomic DNA was used. The entire genomes of ESBL-producing *E. coli* isolates were sequenced using NextSeq 550 instrument (Illumina, San Diego, CA, USA), and sequences were assembled by Spades (version 3.11.1) and annotated by Prokka (version 1.13.7). The data of multilocus sequence typing (MLST), plasmid typing, virulence genes, and resistance genes were obtained from the web site of center for genomic epidemiology (www.genomicepidemiology.org). The virulence score was calculated as the sum of all virulence factors, as described by Johnson et al [18].

Statistical analysis

The results were presented as numbers with percentages for categorical variables and median with interquartile range (IQR) for continuous variables. The continuous variables were compared using the MannWhitney U test. The chi-squared test was used to compare the categorical variables. Logistic regression analysis was performed to determine risk factors for recurrent BSI. Variables found to be significant in univariate analysis were included in a multivariate logistic regression analysis. Results were considered statistically significant at $P < 0.05$. All statistical analyses were performed using SPSS 23.0 software (SPSS, Chicago, IL, USA).

Results

Clinical characteristics of patients with recurrent and single episode BSI caused by *E. coli*

A total of 808 patients experienced *E. coli* BSI during study period, and of them, 57 (6.31%) patients had recurrent episodes. Clinical characteristics of 206 patients, 57 with recurrent episode and 149 whose samples were available for determining the ST 131 and ESBL genotype, with single episode were described in Table 1. Median age was 77 (IQR, 71–84) and 78 (IQR, 67–85) in the recurrent episode and single episode groups, respectively. Female biased sex ratio was observed (64.9% in recurrent episode group and 71.1% in single episode group). The proportion of liver cirrhosis was higher in the recurrent episode group (N = 6, 10.5%) than in the single episode group (N = 3, 2.0%). Higher Charlson comorbidity index was detected in patients with recurrent episode (2, IQR, 0–4) than in those with single episode (1, IQR, 0–2). Patients who had vascular catheter were more common in recurrent episode than in single episode (N = 6, 10.5% vs N = 3, 2.0%). *E. coli* with the ESBL phenotype was more frequently isolated from samples of recurrent episode than single episode (N = 24, 42.1% vs N = 37, 24.8%). Sequential organ failure assessment (SOFA) score was higher in recurrent episode group (4, IQR, 2–8) than in single episode group (3, IQR, 1–6). Mortality rates did not significantly differ between both groups ($P > 0.999$).

Table 1
Comparison of patients with recurrent *E. coli* BSI and single episode of *E. coli* BSI

Variables	Total (N = 206)	Single episode (N = 149)	Recurrent episode (N = 57)	P
Age, years	78 (70–84)	78 (67–85)	77 (71–84)	0.861
Gender, n (%)				0.385
Female	143 (69.42)	106 (71.1)	37 (64.9)	
Male	63 (30.6)	43 (28.9)	20 (35.1)	
Hospital acquired infection, n (%)	90 (43.7)	64 (43.0)	26 (45.6)	0.731
Transferred case, n (%)				0.894
Non-transferred	114 (55.3)	83 (55.7)	31 (54.4)	
Long-term care facility	17 (8.3)	13 (8.7)	4 (7.0)	
Acute care hospital	37 (18.0)	25 (16.8)	12 (21.1)	
Clinic	38 (18.5)	28 (18.8)	10 (17.5)	
Comorbidity, n (%)				
Diabetes mellitus	56 (27.2)	41 (27.5)	15 (26.3)	0.862
Heart failure	8 (3.9)	6 (4.0)	2 (3.5)	> .999
Pulmonary disease	4 (1.9)	3 (2.0)	1 (1.8)	> .999
Chronic kidney disease	23 (11.2)	15 (10.1)	8 (14.0)	0.419
Liver cirrhosis	9 (4.4)	3 (2.0)	6 (10.5)	0.015
Malignancy	40 (19.4)	25 (16.8)	15 (26.3)	0.122
Charlson comorbidity index	1 (0–3)	1 (0–2)	2 (0–4)	0.013
Primary focus of bacteremia, n (%)				0.114
Non-urinary tract infection	66 (32.0)	43 (28.9)	23 (40.4)	
Urinary tract infection	140 (68.0)	106 (71.1)	34 (59.7)	
Polymicrobial blood stream infection, n (%)	11 (5.3)	9 (6.0)	2 (3.5)	0.731

BSI, bloodstream infection; ESBL, extended spectrum β -lactamase; SOFA, sequential organ failure assessment

Variables	Total (N = 206)	Single episode (N = 149)	Recurrent episode (N = 57)	P
Prior antibiotic use, n (%)	180 (87.4)	132 (88.6)	48 (84.2)	0.397
Appropriate empirical antibiotic use, n (%)	152 (73.8)	112 (75.2)	40 (70.2)	0.466
Appropriate definite antibiotic use, n (%)	183 (88.8)	131 (87.9)	52 (91.2)	0.5
Duration of definite antibiotic use, days	14 (12–16)	14 (11–15)	14 (13–17)	0.126
Indwelling catheter in the previous 30 days, n (%)				
Vascular catheter	9 (4.4)	3 (2.0)	6 (10.7)	0.014
Urinary catheter	128 (62.1)	95 (63.8)	33 (57.9)	0.438
<i>E. coli</i> with ESBL phenotype, n (%)	61 (29.6)	37 (24.8)	24 (42.1)	0.015
SOFA score	3 (1–7)	3 (1–6)	4 (2–8)	0.035
30-day mortality, n (%)	13 (6.3)	10 (6.7)	3 (5.3)	> .999
BSI, bloodstream infection; ESBL, extended spectrum β -lactamase; SOFA, sequential organ failure assessment				

Fifty-seven recurrent *E. coli* BSI were subdivided into early onset recurrence (29) and late onset recurrence (28). Median time to recurrence since first episode was 6 days (IQR, 4–15.5) and 66.5 days (IQR, 44.3–134.3) in early onset recurrence and late onset recurrence, respectively. Comparison between two groups is demonstrated in Supplementary Table 1.

Risk factors for recurrent *E. coli* BSI

Univariate logistic regression analysis demonstrated that liver cirrhosis, Charlson comorbidity index ≥ 3 , vascular catheter, *E. coli* with ESBL phenotype, and SOFA score ≥ 9 were associated with recurrent *E. coli* BSI (Table 2). In multivariate analysis, independent risk factors for recurrent bacteremia were vascular catheter (odds ratio [OR], 4.588; 95% confidence interval [CI], 1.049–20.068), *E. coli* with ESBL phenotype (OR, 2.037; 95% CI, 1.037–3.999), and SOFA score ≥ 9 (OR, 3.210; 95% CI, 1.359–7.581).

Table 2
Risk factors for recurrent *E. coli* BSI

Variables	Univariate analysis		Multivariate analysis	
	OR (95% CI)	P	OR (95% CI)	P
Age $\geq$ 65	1.577 (0.703–3.538)	0.269	1.579 (0.663–3.763)	0.302
Gender				
Female	Reference		Reference	
Male	1.332 (0.696–2.550)	0.386	1.098 (0.533–2.265)	0.799
Hospital acquired infection	1.114 (0.603–2.058)	0.731		
Transferred case				
Non-transferred	Reference			
Long-term care facility	0.824 (0.25–2.719)	0.751		
Acute care hospital	1.285 (0.576–2.867)	0.54		
Clinic	0.956 (0.416–2.196)	0.916		
Comorbidity				
Diabetes mellitus	0.941 (0.472–1.877)	0.862		
Heart failure	0.867 (0.17–4.424)	0.863		
Pulmonary disease	0.869 (0.089–8.531)	0.904		
Chronic kidney disease	1.459 (0.582–3.654)	0.42		
Liver cirrhosis	5.725 (1.381–23.735)	0.016		
Cancer	1.772 (0.854–3.675)	0.124		
Charlson comorbidity index $\geq$ 3	1.973 (1.028–3.788)	0.041	1.591 (0.791–3.199)	0.193
Primary focus of bacteremia				

BSI, bloodstream infection; OR, odd ratio; CI, confidence interval, ESBL, extended spectrum β -lactamase; SOFA, sequential organ failure assessment

Variables	Univariate analysis	Multivariate analysis		
Non-urinary tract infection	Reference			
Urinary tract infection	0.6 (0.317–1.134)	0.116		
Polymicrobial bloodstream infection	0.566 (0.118–2.702)	0.475		
Antibiotic use $\leq$ 1 month before episode	1.456 (0.608–3.485)	0.399		
Appropriate empirical antibiotic use	0.777 (0.394–1.532)	0.467		
Appropriate definite antibiotic use	1.429 (0.504–4.049)	0.502		
Duration of definite antibiotic use	1.021 (0.995–1.047)	0.109		
Indwelling catheter in 30 days				
Vascular catheter	5.84 (1.408–24.222)	0.015	4.588 (1.049–20.068)	0.043
Urinary catheter	0.782 (0.419–1.457)	0.438		
Tracheal tube	0.867 (0.17–4.424)	0.863		
Nasogastric tube	1.456 (0.608–3.485)	0.399		
<i>E. coli</i> with ESBL phenotype	2.201 (1.156–4.191)	0.016	2.037 (1.037–3.999)	0.039
SOFA score $\geq$ 9	3.406 (1.487–7.804)	0.004	3.210 (1.359–7.581)	0.008
BSI, bloodstream infection; OR, odd ratio; CI, confidence interval, ESBL, extended spectrum β -lactamase; SOFA, sequential organ failure assessment				

Molecular epidemiology and antimicrobial resistance of *E. coli*

Among 206 isolates tested, *E. coli* ST131 clone was found in 53 (25.7%), comprising ST131–025 (N = 47) and ST131–016 (N = 6) (Table 3). ST131 strains were more frequently observed in early onset recurrent episode (N = 12, 41.4%) than in the late onset recurrent episode (N = 7, 25.0%) and single episode (N = 34, 22.8%). *E. coli* with ESBL genotype was more commonly observed in early onset recurrent episode (N = 12, 41.4%) than in late onset recurrent episode (N = 7, 25%) or in single episode BSI (N = 25, 16.8%). CTX-M-15 (N = 25, 56.8%) was the most common ESBL genotype, followed by CTX-M-14 (N = 11, 25%).

Table 3
The molecular epidemiology of *E. coli* isolated from patients with BSI

	Single episode blood stream infection (N = 149)	Early onset recurrent blood stream infection (N = 29)	Late onset recurrent blood stream infection (N = 28)
ST131, n (%)	34 (22.8)	12 (41.4)	7 (25)
O25	29 (19.5)	12 (41.4)	6 (21.4)
O16	5 (3.4)	0	1 (3.6)
ESBL genotype, n (%)	25 (16.8)	12 (41.4)	7 (25)
CTX-M-3	0	0	1 (3.6)
CTX-M-14	5 (3.4)	3 (10.3)	3 (10.7)
CTX-M-15	17 (11.4)	5 (17.2)	3 (10.7)
CTX-M-27	2 (1.3)	4 (13.8)	0
CTX-M-55	1 (0.7)	0	0
BSI, bloodstream infection; ST, sequence type; ESBL, Extended spectrum β -lactamase			

E. coli isolated from early onset recurrent BSI had higher resistance rates to most antimicrobial agents than that isolated from late onset recurrent BSI or single episode BSI (Fig. 1). Resistance rates to piperacillin/tazobactam, cefoxitin, minocycline, chloramphenicol, fosfomycin, and tigecycline were very low in all the groups. A strain resistant to amikacin, colistin, and carbapenem was not identified in this study.

WGS of ESBL-producing *E. coli*

Twenty-seven of ESBL-producing *E. coli* were available for WGS; 11 from single episodes, 11 from early onset recurrent episodes, and 5 from late onset recurrent episodes. Various antimicrobial resistance genes, plasmid types, and virulence factors were detected isolated from all type of BSI (Table 4). There were resistance genes to β -lactam (*bla*), streptomycin (*str*), aminoglycoside (*aac*, *aad*), macrolide (*mph*), quinolone (*aac(6')lb-cr*), trimethoprim-sulfamethoxazole (*dfr*, *su*), tetracycline (*tet*), and chloramphenicol (*cat*). IncFIA, IncFIB, and IncFII were commonly detected plasmid types, but there was no dominant plasmid type. Variable virulence genes were detected, and *gad* was the most common virulent gene, followed by *iss*, *iha*, and *sat*. The median scores of virulence were 5 (IQR, 3–7), 4.5 (IQR, 1–6), and 5 (IQR, 2–8) for early onset recurrent, late onset recurrent, and single episode BSI, respectively.

Table 4
Whole genome sequencing data of ESBL-producing *E. coli* isolated from patients with BSI

No	Episode	ST	Resistance gene	Plasmid	Virulence gene
EC 0049	Single	131	<i>aac(3)-IIa, catB3, aac(6')Ib-cr, bla_{OXA-1}, bla_{CTX-M-15}</i>	Not detected	<i>nfaE, iha, gad, cnf1, iss, sat</i>
EC 0075	Single	131	<i>bla_{CTX-M-27}</i>	IncFIA, IncFIB(AP001918), IncFIB(pLF82-PhagePlasmid), IncFII	<i>sat, iha, iss, gad</i>
EC 0131	Single	131	<i>aacA4, catB3, aac(6')Ib-cr, bla_{CTX-M-15}, bla_{OXA-1}</i>	Col(BS512), IncFIA, IncFIB(AP001918)	<i>iss, gad</i>
EC 0306	Single	131	<i>aadA5, mph(A), sul1, dfrA17, catB3, bla_{CTX-M-15}, bla_{OXA-1}, aac(6')Ib-cr</i>	Col(BS512), Col156, IncFIA, IncFIB(AP001918)	<i>sat, senB, iha, iss, gad</i>
EC 0364	Single	131	<i>aac(3)-IIa, aadA5, mph(A), sul1, dfrA17, tet(A), catB3, aac(6')Ib-cr, bla_{OXA-1}, bla_{CTX-M-15}</i>	Col156, IncFIA, IncFIB(AP001918)	<i>cnf1, iha, gad, nfaE, iss, celb, senB, sat</i>
EC 0436	Single	131	<i>aadA5, aac(3)-IIa, sul1, dfrA17, catB3, bla_{CTX-M-15}, mph(A), bla_{OXA-1}, aac(6')Ib-cr</i>	Col(BS512), IncFIA, IncFIB(AP001918)	<i>sat, iha, iss, gad</i>
EC 0500	Single	131	<i>aadA5, aac(3)-IIa, sul1, dfrA17, tet(A), catB3, bla_{CTX-M-15}, mph(A), bla_{OXA-1}, aac(6')Ib-cr</i>	IncFIA, IncFIB(AP001918)	<i>cnf1, iha, gad, nfaE, iss, senB, sat</i>
EC 0530	Single	131	<i>strA, strB, aac(3)-IIa, aadA5, mph(A), sul1, sul2, dfrA17, tet(A), catB3, bla_{CTX-M-15}, bla_{OXA-1}, aac(6')Ib-cr</i>	Col156, IncFIA, IncFIB(AP001918), IncFII(pRSB107)	<i>senB, astA, iss, gad</i>
EC 0745	Single	131	<i>aac(3)-IIa, catB3, bla_{CTX-M-15}, bla_{OXA-1}, aac(6')Ib-cr</i>	IncFIA, IncFIB(AP001918)	<i>nfaE, iha, gad, cnf1, iss, sat</i>
EC 1064	Single	744	<i>tet(A), tet(B), catA2, dfrA14, bla_{CTX-M-55}, aac(3)-IIa</i>	IncFIB(AP001918), IncFIC(FII), IncX4	<i>cma, gad</i>

ESBL, extended spectrum β-lactamase; BSI, bloodstream infection; ST, sequence type

No	Episode	ST	Resistance gene	Plasmid	Virulence gene
EC 1346	Single	1193	<i>strA, strB, aadA5, mph(A), sul1, sul2, dfrA17, tet(A), bla_{CTX-M-27}</i>	Col(BS512), Col156, IncFIA, IncFIB(AP001918)	<i>cnf1, ireA, iha, gad, vat, senB, sat</i>
EC 0295	Early onset recurrent	131	<i>aac(3)-Ild, bla_{TEM-1B}, bla_{CTX-M-14}</i>	IncFIA, IncFIB(AP001918)	<i>sat, cnf1, iha, iss, gad</i>
EC 0355	Early onset recurrent	95	<i>aadA5, aac(3)-Ild, sul1, dfrA17, bla_{CTX-M-14}, mph(A)</i>	Col156, IncB/O/K/Z, IncFIB(AP001918), IncFII(29)	<i>vat, ireA, gad, iss, senB</i>
EC 0511	Early onset recurrent	131	<i>strA, strB, aadA5, mph(A), sul1, sul2, dfrA17, tet(A), bla_{CTX-M-27}</i>	Col(KPHS6), IncFIA, IncFIB(AP001918), IncFII(pRSB107)	<i>sat, iss, gad</i>
EC 0578	Early onset recurrent	131	<i>aac(3)-Ild, strA, strB, aadA5, mph(A), sul1, sul2, dfrA17, tet(A), bla_{CTX-M-14}, bla_{TEM-1B}</i>	Col156, IncFIA, IncFIB(AP001918), IncFII(pRSB107)	<i>nfaE, iha, gad, cnf1, iss, senB, sat</i>
EC 0632	Early onset recurrent	131	<i>bla_{CTX-M-27}</i>	Col156, IncFIA, IncFIB(AP001918), IncFII(pRSB107), IncI1-I(Gamma)	<i>senB, iss, gad</i>
EC 1132	Early onset recurrent	1193	<i>mph(A), bla_{CTX-M-15}</i>	Col(BS512), IncFIA, IncFIB(AP001918), IncFII(pHN7A8)	<i>sat, vat, iha, senB, gad</i>
EC 1286	Early onset recurrent	131	<i>aac(3)-IIa, aadA5, mph(A), sul1, dfrA17, tet(A), catB3, aac(6')Ib-cr, bla_{OXA-1}, bla_{CTX-M-15}</i>	IncFIA, IncFIB(AP001918), IncI1-I(Gamma)	<i>nfaE, iha, gad, cnf1, iss, senB, sat</i>
EC 1317	Early onset recurrent	131	<i>aac(3)-IIa, tet(A), catB3, aac(6')Ib-cr, bla_{OXA-1}, bla_{CTX-M-15}</i>	IncB/O/K/Z, IncFIA, IncFIB(AP001918)	<i>sat, cnf1, iha, iss, gad</i>
EC 1398	Early onset recurrent	131	<i>strA, strB, aac(3)-Ild, aadA5, mph(A), sul1, sul2, dfrA17, tet(A), bla_{CTX-M-14}, bla_{TEM-1B}</i>	Col156, IncFIA, IncFIB(AP001918), IncFII(pRSB107)	<i>nfaE, iha, cnf1, gad, iss, senB, sat</i>
EC 1508	Early onset recurrent	131	<i>aadA5, aac(3)-IIa, mph(A), sul1, dfrA17, tet(A), catB3, bla_{CTX-M-15}, bla_{OXA-1}, aac(6')Ib-cr</i>	IncFIA, IncFIB(AP001918)	<i>cnf1, iha, gad, nfaE, iss, senB, sat</i>

ESBL, extended spectrum β-lactamase; BSI, bloodstream infection; ST, sequence type

No	Episode	ST	Resistance gene	Plasmid	Virulence gene
EC 1631	Early onset recurrent	131	<i>strA, strB, aadA5, mph(A), sul1, sul2, dfrA17, tet(A), bla_{CTX-M-27}</i>	IncFIA, IncFIB(AP001918), IncFII(pRSB107)	<i>senB, ireA, iha, iss, gad</i>
EC 0151	Late onset recurrent	131	<i>aac(3)-IIa, catB3, aac(6')Ib-cr, bla_{OXA-1}, bla_{CTX-M-15}, tet(A)</i>	Col156, IncFIA, IncFIB(AP001918)	<i>cnf1, iha, nfaE, gad, iss, sat</i>
EC 1254	Late onset recurrent	648	<i>aac(3)-IIa, aadA5, mph(A), sul1, dfrA17, tet(B), bla_{CTX-M-14}</i>	Col(MG828), IncFIA, IncFIB(AP001918), IncFII(pRSB107)	<i>eilA, lpfA, air</i>
EC 1256	Late onset recurrent	131	<i>aac(3)-IIa, aadA5, mph(A), sul1, dfrA17, tet(A), catB3, aac(6')Ib-cr, bla_{OXA-1}, bla_{CTX-M-15}</i>	Col156, ColpVC, IncFIA, IncFIB(AP001918)	<i>cnf1, iha, gad, iss, senB, sat</i>
EC 1448	Late onset recurrent	131	<i>aac(3)-IId, strA, strB, aadA5, mph(A), sul1, sul2, dfrA17, tet(A), bla_{CTX-M-3}, bla_{TEM-1B}</i>	IncFIB(AP001918), IncFII	<i>iha</i>
EC 1563	Late onset recurrent	131	<i>aac(3)-IIa, aadA5, mph(A), sul1, dfrA17, tet(A), catB3, aac(6')Ib-cr, bla_{OXA-1}, bla_{CTX-M-15}</i>	Col156, IncFIA, IncFIB(AP001918)	<i>cnf1, iha, gad, nfaE, iss, senB, sat</i>
ESBL, extended spectrum β -lactamase; BSI, bloodstream infection; ST, sequence type					

Relapses versus reinfections in late onset recurrent blood stream infection

PFGE was performed on eight paired isolates of the initial and subsequent episodes in late onset recurrent BSI (Fig. 2). Three pairs (28/28 – 2, 36/36 – 2, and 48/48 – 2) of eight paired isolates had identical PFGE patterns, ST type, and ESBL genotype, which suggested relapse rather than reinfection.

Discussion

This study demonstrated that the recurrent *E. coli* BSI was not uncommon, and the rate of early onset recurrence was similar to that of late onset recurrence. The risk factors for recurrent *E. coli* BSI were vascular catheter, ESBL phenotype, and SOFA score ≥ 9 . Multidrug-resistant *E. coli* of ST131 clone producing ESBL were more common in early onset recurrent BSI than in late onset recurrent BSI or single episode BSI. PFGE analysis suggested that a few cases of late onset recurrent BSI were attributed to relapse rather than reinfection. WGS demonstrated that various resistant genes and plasmid types were present in ESBL-producing *E. coli* causing recurrent BSI.

Recurrence is a serious complication associated with BSI, and patients may have recurrent BSI. The rate of recurrent BSI varies and depends on the causative pathogens, definition of recurrence, and follow-up

duration, but it is estimated to be 5–12% for *Staphylococcus aureus* [19–21], 2.3% for *Streptococcus pneumonia* [22], 5.6% for *Acinetobacter baumannii* [23], and 15.7% for *Salmonella* species [24]. In this study, we found that in 6.3% of patients with *E. coli* BSI, the infection recurred. Our study also showed additional data for recurrence rate subdivided into early onset (3.6%) and late onset (3.5%) groups. To date, there is limited information on the recurrence rate changing with regard to time after the first episode, which may be clinically relevant and can help plan an appropriate follow-up for *E. coli* BSI patients.

We identified that ESBL–producing *E. coli* was an important risk factor for recurrent BSI. Maslow et al. showed that impaired host defense system was associated with recurrent episodes [9]. According to study by Sanz-García et al., male sex, presence of hematologic malignancy, inadequate antibiotic use, and extra-urinary source of BSI were independent risk factors for recurrence [11]. As these studies were conducted before the widespread infection with ESBL–producing *E. coli* [25–27], they could not evaluate the association of ESBL–producing *E. coli* with recurrence. Patients with BSI caused by ESBL–producing *E. coli* may have a greater chance of subsequent recurrent BSI due to multidrug-resistant traits of ESBL–producing *E. coli*. There is some evidence to support our results that antibiotic resistance of *E. coli* isolated from initial episode increases the risk of recurrent BSI [12, 13].

Recurrent BSI can be caused by infection with identical (relapse) or different (reinfection) isolates which is usually determined by molecular typing. PFGE analysis was performed on available *E. coli* isolated from late onset recurrence. Relapse was less common than reinfection but was observed at a substantial rate. Reinfection is associated with a higher degree of severity of the BSI-induced illness that may be difficult to modify and/or prevent [6]. In contrast, relapse may occur from the remnant foci of infection, undertreated during the treatment regimen of the prior episodes. This implies that appropriate therapy, such as elimination of residual infection foci, may reduce the rate of relapse.

The prevalence of ESBL–producing *E. coli* BSI has increased over the past decade owing to the widespread infection with the ST 131 clone containing CTX-M-15 [25–27]. To date, limited data are available for the rate of ESBL–producing *E. coli* in recurrent BSI. We showed the rate of ESBL–producing *E. coli* (33.3%) and the most common ESBL genotype (CTX-M-15) in recurrent BSI. Multiple virulence factors may be involved in the survival of ESBL–producing *E. coli* resulting in recurrence. In our study, *gad* that is associated with protection of cells in acidic environment was the most prevalent virulence gene. Another virulence genes observed in ESBL–producing *E. coli* causing recurrence were *iss*, *iha*, and *sat*, which are involved in immune evasion, adhesion, and protease production, respectively [28, 29].

This study has some limitations. First, not all isolates of *E. coli* BSI were included in the analysis; therefore, there may have been a selection bias. Second, we could not perform PFGE on *E. coli* isolated from the patients with early onset recurrent BSI. However, we postulated that most of early onset recurrent BSI were infected by identical strains. *E. coli* isolated from early onset recurrent BSI was highly resistant to third-generation cephalosporin or ciprofloxacin, which are most commonly used as initial therapy after identification of gram-negative BSI. Since initial empirical antibiotic therapy was not be effective, early

onset recurrence might be caused by remnants of *E. coli* that had not been properly eliminated during the first episode of infection. Third, we showed that vascular catheter was associated with recurrent *E. coli* BSI, as described in previous studies [13, 30]. However, the number of patients with a vascular catheter was too low in our study. Therefore, the findings related to those few patients will need to be interpreted cautiously.

Conclusion

We found risk factors for recurrent *E. coli* BSI, which may be helpful to healthcare providers in planning the patient follow-up after initial *E. coli* BSI episode. The detection of variable molecular feature in *E. coli* causing recurrent BSI suggests the need for an in-depth study to identify microbiological determinants associated with recurrent *E. coli* BSI.

Abbreviations

BSI: bloodstream infection; ESBL: extended spectrum β -lactamase; PFGE: pulsed-field gel electrophoresis; ST 131: sequence type 131; WGS: whole genome sequencing

Declarations

Ethics approval and consent to participate:

The study protocol was approved by the Institutional Review Board (NHIMC-2019-01-018) of National Health Insurance Service Ilsan Hospital.

Consent for publication:

Not applicable.

Availability of data and materials:

All data generated or analyzed during this study are included in this published article (and its supplementary information files).

Competing interests:

The authors declare that they have no competing interests.

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Authors' contributions:

Conception and design of the study, YAK and YSP; acquisition of data, HC, YAK and YHS; analysis and interpretation of data, YCK and YAK; drafting the article, YCK; revising the article, HC, HL and KL; final approval, YAK and YSP

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Figures

Figure 1

Antimicrobial resistance rates of *Escherichia coli* isolated from patients with bloodstream infection (N=206). Abbreviation: BSI, bloodstream infection; Amp, ampicillin; Sam, ampicillin/sulbactam; Pip, piperacillin; Tzp, piperacillin/tazobactam; Amc, amoxicillin/clavulanic acid; Cxm, cefuroxime; Ctx, cefotaxime; Caz, ceftazidime; Fep, cefepime; Fox, ceftazidime; Atm, aztreonam; Dor, doripenem; Mem, meropenem; Imp, imipenem; Lev, levofloxacin; Cip, ciprofloxacin; Gen, gentamicin; Tob, tobramycin; Amk, amikacin; Mno, minocycline; Tet, tetracycline; Sxt, cotrimoxazole; Chlor, chloramphenicol; Fos, fosfomycin; Tgc, tigecycline; Col, colistin; ESBL, extended spectrum β -lactamase

Figure 2

Dendrogram of *Xba*I-restricted DNA of *Escherichia coli* isolated from late onset recurrent bloodstream infection (N=107). Pulsed-field gel electrophoresis was performed with size marker, Lambda Ladders (Promega, Fitchburg, WI, USA). Abbreviation: ST, sequence type; ESBL, Extended spectrum β -lactamase

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