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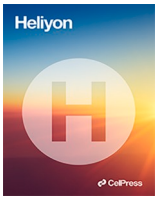

Comparison of phylogenetic and virulence factors between *Escherichia coli* isolated from biliary tract infections and uropathogenic *Escherichia coli*

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A B S T R A C T

Escherichia coli is a gram-negative intestinal commensal that can also cause various infections, including urinary tract infections, biliary tract infections, neonatal meningitis, and septicemia. Although the characteristics of uropathogenic *E. coli* and the mechanisms of urinary tract infection have been well studied, the genetic distinctions among *E. coli* isolates from different types of infections have not yet been determined. This study compared the phylogenetic and virulence factors of *E. coli* isolates from bacteremic biliary tract infections with those from bacteremic urinary tract infections. The phylogenetic B2 group was the most prevalent in both pathogenic groups (68 % in biliary pathogenic isolates and 85 % in uropathogenic isolates), but the frequency pattern of the phylogenetic group was different. Half of the uropathogenic isolates belonged to ST95 and ST131 (51 %). Among the biliary pathogenic isolates, ST131 was the most prevalent, while the remaining half belonged to other STs outside the four major STs. The frequency of some virulence factors, such as *papC*, *papG2*, *hlyA*, *tcpC*, *fyuA*, *kpsMT2*, *sat*, and *traT,* was lower in the biliary pathogenic isolates than in the uropathogenic isolates. The frequency of phylogenetic groups and STs in MLST differed between *E. coli* isolates from bacteremic biliary tract infections and urinary tract infections. Additionally, some virulence factors, including adhesion and toxin gene groups, showed lower frequencies in the biliary pathogenic group than in the uropathogenic group. Studying the differences in *E. coli* pathovars from different infection sites is important for developing pathovar-specific targeted therapies such as vaccine therapy.

1. Introduction

Escherichia coli (*E. coli*) is both a commensal and a pathogen responsible for various intestinal and extraintestinal diseases such as urinary tract infections and meningitis [1]. With advances in molecular microbiology, *E. coli* have been classified according to their pathogenicity and genomic characteristics [2].

Recent studies have extensively examined various pathovars in addition to those responsible for uropathogenic and meningitis-related infections. These studies include *E. coli* isolates from skin and soft tissue infections [3] and *E. coli* isolates that cause pneumonia [4]. *E. coli* is also a major causative pathogen for biliary tract infections and is often accompanied by bacteremia [5]. Previous studies have reported that the frequency of some virulence factors was different in uropathogenic extraintestinal *E. coli* (UPEC) and fecal isolates [6]. Additionally, major sequence types (STs) in multi-locus sequencing typing (MLST) detected in bloodstream infections, such as ST131, ST95, ST73, and ST69, were detected less frequently in isolates from acute biliary tract infections compared to

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isolates from non-acute biliary tract infections [7]. These studies had small sample sizes (n = 24 [6] and n = 15 [7], respectively); therefore, their characteristics, including genomic information and virulence, remain unclear.

This study compared the phylogenetic groups and virulence patterns of bacteremic *E. coli* isolates with those of biliary tract infections and UPEC isolates. This study will enhance our understanding of virulence gene expression in diverse environments, including the urinary and biliary tracts.

2. Materials and methods

We conducted a retrospective study of *E. coli* bloodstream infections at the University of Tokyo Hospital from April 2013 to February 2015 and analyzed bacteremic *E. coli* isolates from biliary tract infections, as published previously [8]. In this study, we collected UPEC isolates from bacteremic *E. coli* isolates during the same period and compared them with biliary pathogenic *E. coli* isolates analyzed in a previous study. Data on *E. coli* isolates from biliary tract infections were obtained from a previous study [8]. We considered UPEC isolates as detected in both blood and urine cultures before starting antimicrobial treatment in the same patient with symptoms suspected of urinary tract infection, such as costovertebral knock pain, frequent urination, and fever. In this study, we compared the phylogenetic groups, MLST, and the frequency of virulence factors between UPEC and *E. coli* isolates from patients with biliary tract infections.

2.1. Microbiological procedures

E. coli isolates were classified into phylogenetic groups, such as A, B1, B2, C, D, E, and F, using the quadruplex polymerase chain reaction (PCR) method as described previously [9]. The frequency of 20 virulence factors (*afaB*, *afaC*, *cnf1*, *cvaC*, *fimH*, *fyuA*, *hlyA*, *ibeA*, *iha*, *iroN*, *iucD*, *iutA*, *kpsMT2*, *ompT*, *papC*, *papG2*, *sat*, *sfaD*, *sfaE*, *tcpC*, *traT*, and *usp*) was screened by multiplex PCR using extracted *E. coli* genomic DNA as previously reported [10–16]. For the identification of ST131, ST95, ST73, and ST69, which are major STs in bloodstream infections [17], MLST was typed using multiplex PCR [18].

Table 1
Comparison of the phylogenetic pattern and frequency of virulence factors between *Escherichia coli* isolates from bacteremia with biliary tract infection and bacteremic uropathogenic *E. coli* isolates.

Variables	Total	**Biliary pathogenic** Escherichia coli	**Uropathogenic** Escherichia coli	*p*-value
	202	72	130	
Phylogenetic group				0.007
A	6 (3.0 %)	4 (5.6 %)	2 (1.5 %)	
B1	9 (4.5 %)	8 (11 %)	1 (0.8 %)	
B2	159 (79 %)	49 (68 %)	110 (85 %)	
C	2 (1.0 %)	0	2 (1.5 %)	
D	8 (4.0 %)	2 (2.8 %)	6 (4.6 %)	
E	10 (5.0 %)	5 (6.9 %)	5 (3.8 %)	
F	7 (3.5 %)	4 (5.6 %)	3 (2.3 %)	
Un-typable	1 (0.5 %)	0	1 (0.8 %)	
Virulence factors				
Adhesion				
papC	93 (46 %)	21 (29 %)	72 (55 %)	<0.001
papG2	70 (35 %)	10 (14 %)	60 (46 %)	<0.001
sfaD/E	36 (18 %)	8 (11 %)	28 (22 %)	0.084
fmH	197 (98 %)	69 (96 %)	128 (99 %)	0.350
afaB/C	6 (3.0 %)	2 (2.8 %)	4 (3.1 %)	1
iha	84 (42 %)	23 (32 %)	61 (47 %)	0.052
Toxin				
usp	164 (81 %)	56 (78 %)	108 (83 %)	0.355
cnf1	29 (14 %)	9 (13 %)	20 (15 %)	0.678
hlyA	45 (22 %)	9 (13 %)	36 (28 %)	0.014
sat	83 (41 %)	22 (31 %)	61 (47 %)	0.026
Iron uptake				
fyuA	178 (88 %)	52 (72 %)	126 (97 %)	<0.001
ironN	50 (25 %)	16 (22 %)	34 (26 %)	0.611
iucD	77 (38 %)	24 (33 %)	53 (41 %)	0.364
iutA	119 (59 %)	47 (65 %)	72 (55 %)	0.182
Capsule				
kpsMT2	160 (79 %)	51 (71 %)	109 (84 %)	0.045
Miscellaneous				
ibeA	41 (20 %)	14 (19 %)	27 (21 %)	0.857
traT	160 (79 %)	49 (68 %)	111 (85 %)	0.006
cvaC	16 (7.9 %)	6 (8.3 %)	10 (7.7 %)	1
ompT	18 (8.9 %)	6 (8.3 %)	12 (9.2 %)	1
TcpC	56 (28 %)	12 (17 %)	44 (34 %)	0.009

2.2. Statistical analysis

A two-tailed Fisher's exact test was used to analyze the categorical data. A p-value of <0.05 was considered statistically significant. The Bonferroni correction method for post hoc multiple comparisons was applied using the Easy R (EZR) statistical software application for R version 4.2.2 [19]. All statistical analyses, excluding the Bonferroni correction, were performed using the JMP Pro version 17 software (SAS Institute, Cary, NC, USA).

3. Results

Seventy-two isolates caused bacteremia with biliary tract infections (biliary pathogenic), and 130 isolates caused bacteremia with urinary tract infections. We compared the proportions of phylogenetic groups, positivity rates for various virulence factors (Table 1), and ST frequencies (Fig. 1A and B) between the two groups.

Biliary pathogenic *E. coli* isolates exhibited a wider variety of phylogenetic patterns than uropathogenic isolates ($p = 0.007$). In post hoc multiple comparison analysis, there was a significant difference between B1 and B2 (Bonferroni-corrected $p = 0.023$). As a result, the frequency of the biliary pathogenic isolates was higher than the uropathogenic isolates in the B1 group, and in the B2 group, the uropathogenic isolates occupied more than the biliary pathogenic isolates. However, the phylogenetic B2 group was the most prevalent in both groups (68 % in the biliary pathogenic group, 85 % in the uropathogenic group).

ST frequency also differed between the two groups (Fig. 1A and B; $p = 0.001$). In post hoc multiple comparison analysis, there was a significant difference between the others and the ST95 (Bonferroni-corrected $p = 0.036$). The biliary pathogenic isolates in the others and the uropathogenic isolates in ST95 showed higher frequencies, respectively. The frequencies of MLST in UPEC were as follows: ST131 (19 %), ST95 (32 %), ST73 (16 %), ST69 (6.2 %), and others (27 %). Among the UPEC isolates, ST95 and ST 131 accounted for 51 %, and the four major pathogenic STs encompassed 73 %. In contrast, in the biliary pathogenic *E. coli* group, 50 % of the isolates belonged to STs other than the four major STs, and ST131 (24 %), ST95 (20 %), and ST73 (6.9 %) were detected, respectively. ST69 was not detected in biliary pathogenic *E. coli* isolates.

Some virulence genes in biliary pathogenic isolates, such as *papC* (29 %), *papG2* (14 %), *hlyA* (13 %), *tcpC* (17 %), *fyuA* (72 %), *kpsMT2* (71 %), *sat* (31 %), and *traT* (68 %), were detected less frequently compared to UPEC ($p < 0.05$), although others showed positivity similar to UPEC. The *iutA* (65 %) and *cvaC* (8.3 %) genes in the biliary pathogenic group were more frequent than in the uropathogenic group, but these differences did not reach statistical significance.

4. Discussion

This study compared the frequency of phylogenetic groups and the patterns of virulence factors in biliary pathogenic *E. coli* with bacteremia and UPEC isolates. The frequency of phylogenetic groups and STs in MLST was different in the two groups. Moreover, the frequency of virulence factors was lower in the biliary pathogenic group than in the UPEC group, particularly in the adhesion and toxin gene groups.

Although isolation from blood cultures was common in both groups, the variations observed in phylogenetic groups and STs might be attributed to different environmental stresses within the biliary or urinary tracts. The differences in the frequency of virulence factors was similar to that of previous reports on biliary pathogenic isolates comparing biliary pathogenic isolates to UPEC, fecal isolates, or bacteremic isolates from sources other than biliary tract infections [6,7]. The *iutA* gene in the iron uptake system and the *cvaC* gene encoding colicin V [20], which is now classified in microcin as an antimicrobial peptide [21,22], were more highly positive in biliary pathogenic isolates than in UPEC isolates. The frequency of *iutA* in previous reports [6,7] was over half of the biliary pathogenic isolates.

Virulence factors required at different infection steps vary, with some being site-specific. UPEC causes urinary tract infections by proceeding into the bladder, adhering to the epithelium, forming a biofilm, invading, forming intracellular bacterial communities, and disseminating into the kidney and blood [23]. On the other hand, biliary pathogenic *E. coli* could come from the intestinal tract with

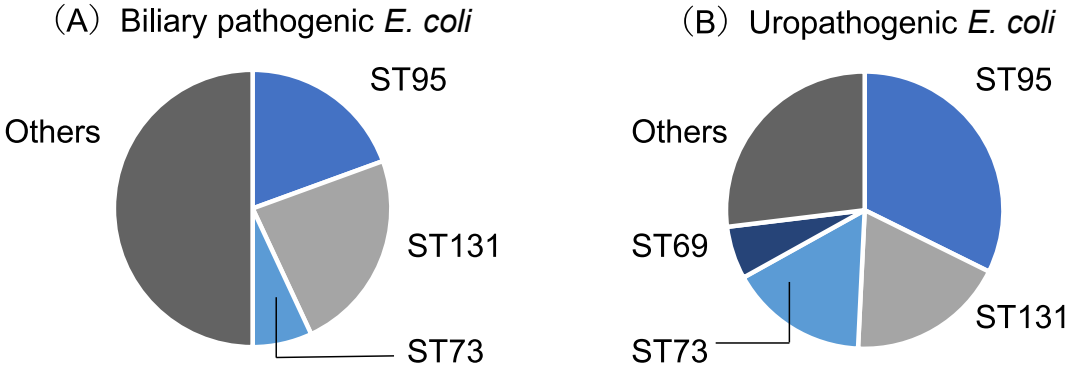

Fig. 1. Distribution of sequence types of *E. coli* isolates. (A) Biliary pathogenic *E. coli* (B) Uropathogenic *E. coli*.

various other bacterial species and develop an infection. Indeed, surviving in strongly acidic and iron-limited bile, which is required for bacterial proliferation, is challenging [24]. The mechanism of competition with other bacteria in the bile environment has not been well studied. Biliary pathogenic *E. coli* may use known virulence genes, such as *iutA* and *cvaC*, but could also possess as-yet-undiscovered specific virulence factors.

Identifying differences between various pathotypes will contribute to the exploration of new treatment strategies, such as vaccine development [25]. Four vaccine candidates for the clinical development of extraintestinal pathogenic *E. coli* are currently being studied [26]. However, *E. coli* also belongs to commensals, making it difficult to find detailed differences between commensals and pathovars. Because virulence factors and antimicrobial resistance have expanded in commensal strains in the past 30 years [27], regular modification of pathogenic strain-targeted vaccines might be required. The intestinal tract is also the reservoir of extra-intestinal pathogenic *E. coli* and antimicrobial resistance determinants [28]. If genetic and phenotypic differences between pathovars and commensals are indistinguishable, new treatment strategies, such as altered relationships of pathogens between each environment of infection focused on weakening virulence, might be the next candidate.

This study had several limitations. First, this was a targeted analysis of known virulence factors. There may be unknown virulence factors functioning in bile environments. Second, the isolates used in this study were collected between 2013 and 2015. Thus, the epidemiology of the isolates from the blood cultures may have changed.

5. Conclusion

The frequency of phylogenetic groups and STs in MLST was different in *E. coli* isolates between bacteremic biliary tract infections and urinary tract infections. Some virulence factors, such as adhesion and toxin gene groups, had lower frequencies in the biliary pathogenic group than those in the uropathogenic group. Further clinical studies, such as ours, that consider differences in infectious disease lesions using molecular microbiology will provide more information for the classification of *E. coli* pathovars with clinical implications and help to develop new treatment strategies, such as bacterial vaccines, in the era of antimicrobial resistance.

Ethical statement

This study was approved by the Research Ethics Committee of the University of Tokyo Hospital (No. 10799). The requirement for written informed consent was waived because this was an observational, retrospective study.

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Data availability statement

Data included in article/supplementary material/referenced in the article.

CRediT authorship contribution statement

Mahoko Ikeda: Writing – original draft, Visualization, Validation, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Tatsuya Kobayashi:** Resources, Methodology, Investigation. **Shu Okugawa:** Writing – review & editing, Supervision, Resources, Methodology, Investigation. **Fumie Fujimoto:** Resources. **Yuta Okada:** Investigation. **Keita Tatsuno:** Resources. **Yoshimi Higurashi:** Resources. **Takeya Tsutsumi:** Writing – review & editing, Supervision, Resources. **Kyoji Moriya:** Writing – review & editing, Supervision, Resources.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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