Escherichia coli virulence factors iutA, an iron acquisition factor, and ibeA, an invasion factor, are related to severity of bacteremic acute biliary tract infections

Mahoko Ikeda (mhk-ikeda@umin.ac.jp)
University of Tokyo Hospital: Tokyo Daigaku Igakubu Fuzoku Byoin https://orcid.org/0000-0003-4732-3483

Tatsuya Kobayashi
University of Tokyo Hospital: Tokyo Daigaku Igakubu Fuzoku Byoin

Fumie Fujimoto
University of Tokyo Hospital: Tokyo Daigaku Igakubu Fuzoku Byoin

Yuta Okada
University of Tokyo Hospital: Tokyo Daigaku Igakubu Fuzoku Byoin

Yoshimi Higurashi
University of Tokyo Hospital: Tokyo Daigaku Igakubu Fuzoku Byoin

Keita Tatsuno
University of Tokyo Hospital: Tokyo Daigaku Igakubu Fuzoku Byoin

Shu Okugawa
University of Tokyo Hospital: Tokyo Daigaku Igakubu Fuzoku Byoin

Kyoji Moriya
University of Tokyo Hospital: Tokyo Daigaku Igakubu Fuzoku Byoin

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Abstract

Background

Although *Escherichia coli* is the most frequently isolated organism in acute biliary tract infections with bacteremia, data regarding its virulence are limited.

Results

Bacteremic acute biliary tract infection cases in a retrospective study were collected from 2013 to 2015 at a tertiary care hospital in Japan. Factors related to the severity of infection were investigated, including patient background, phylogenetic typing, and virulence factors of *E. coli*, such as adhesion, invasion, toxins, and iron acquisition.

In total, 72 *E. coli* strains were identified in 71 cases, most of which primarily belonged to the phylogenetic B2 group (68%). The presence of *IutA* (77.3% in the non-severe group, 46.4% in the severe group, $P = 0.011$) and *ibeA* (9.1% in the non-severe group, and 35.7% in the severe group, $P = 0.012$) were significantly associated with severity of infection, whereas patient characteristics did not relate to the severity of infection.

Conclusions

We showed that bacteremic *E. coli* strains from acute biliary tract infections belonged to a virulent (B2) group. The severity of biliary tract infection depended on *iutA* and *ibeA*.

Background

Escherichia coli is the most frequently isolated organism in acute biliary tract infections (1, 2), and the associated bacteremia is mainly caused by *E. coli* (3). Biliary tract infections normally start with the stasis of bile flow. Once intestinal bacteria flow into the bile duct, an acute biliary infection can develop. The causative organisms in bile cultures of acute biliary infections were shown to be polymicrobial (3, 4). These pathogens were considered to be commensal with the intestinal flora whose virulence were weak.

E. coli can be differentiated depending on pathogeny. These include commensals, which are considered avirulent, intestinal pathogenic groups, extraintestinal pathogenic groups, such as uropathogenic, neonate meningitis groups, and sepsis-associated groups, and avian pathogenic *E. coli* (5, 6).

Acute biliary infections, such as cholangitis and cholecystitis, can develop into a severe infection with bacteremia. In cases of severe infection, mortality can reach 10% (7). This begs the question, if the causative organisms were avirulent, why did the patients suffer severe disease and even death?

We hypothesized that the causative *E. coli* were virulent, and this influenced the severity of acute biliary infection. Uropathogenic *E. coli*, which cause urinary tract infections, are well-studied, and have been

shown to express several virulence factors, such as those involved in adhesion (*papC*, *papG2*, *sfaD/E*, *afaB/C*, *fimH*, *iha*, and *usp*), invasion (*ibeA*, *kpsMT2*, traT, *cvaC*, *ompT*), iron acquisition (*iutA*, *fyuA*, *ironEc*, *iucD*), toxins (*CNF1*, *hlyA*, *sat*), and the Toll/interleukin 1 receptor (TIR) domain-containing protein (*TcpC*). These virulence factors play important roles at each step of infection(8). Studies on *E. coli* strains from bacteremia in biliary tract infections (BEC) are limited. A previous study showed that different prevalence of 10 virulence factors in BEC compared in *E. coli* strains from blood cultures of patients with acute urinary tract infections (9).

Pathogenic *E. coli* express many common virulence factors even at different sites of infection, but an organ-specific strategy is needed to identify specific virulence factors. These common and/or different traits are now studied rigorously to understand pathogenesis and to cope with infection at different foci.

Our study aimed to analyze the relationship between the severity of biliary tract infections and virulence of BEC strains.

Results

A total of 71 cases of bacteremic acute biliary tract infections (72 BEC isolates) were identified. We compared the severe (Pitt's score 2 or greater) group to the non-severe (score less than 2) group. Patient background, such as age, gender, and comorbidity were the same in the two groups (Table 1). More than half of the patients in both groups had abnormalities of the biliary tract. Approximately half of the patients in both groups had experienced biliary tract infections in the past.

Table 1
Comparison of patient backgrounds and symptoms between severity of bacteremia

	Total	Pitt less than 2	Pitt 2 or more	*P* value
numbers	71	44	27	
age (median, range)	75 (31–94)	72.9 (34–93)	72 (31–94)	0.38
gender (male: female)	F28 M43	F16 M28	F12 M15	0.618
nursing home	4	2	2	0.632
antibiotic use within 3 months	29	19	10	0.479
Charlson index (median, range)	4 (0–11)	4 (0–11)	3 (0–8)	0.104
collagen diseases	4	4	0	0.29
diabetes mellitus without organ involvement	7	3	4	0.415
diabetes mellitus with organ involvement	20	17	3	0.015
malignancy	49	31	18	0.613
abnormality of biliary tract	43	29 (65.9%)	14 (52%)	0.318
abnormality of gastrointestinal tract	38	25	13	0.625
previous history of acute biliary infection	33	21	12	0.811
previous history of bacteremia	11	7	4	1
duration between onset of symptoms and admission (days)	0 (-24–10)	0 (-24–4)	0 (-10–10)	0.924
fever	39	23	16	0.629
immunosuppressant use	27	19	8	0.318
artificial device in biliary tract	19	11	8	0.784

Table 2 shows the comparison of laboratory data by severity. Liver enzymes and biliary markers were elevated to the same degree except for alkaline phosphatase. Median white blood cell counts were higher in the non-severe group than in the severe group.

Table 2
Comparison of laboratory data and severity of bacteremia

	total	Pitt less than 2	Pitt 2 or more	*P* value
white blood cell counts (per μL) median (min–max)	9800 (1200–25600)	10500(2400–25600)	8600 (1200–23200)	0.0492
platelets (× 10000/μL)	16.1 (7.0–38.1)	17.6 (7–38.1)	15.2 (7.0–35.1)	0.211
Albumin (g/dL)	3.2 (1.0–4.5)	3.15 (1.0–4.5)	3.5 (2.0–4.2)	0.136
total bilirubin (mg/dL)	2.0 (0.4–11.6)	2.0 (0.4–11.6)	2.2 (0.5–7.0)	0.148
AST (U/L)	100 (16–1591)	98 (16–1026)	133 (17–1591)	0.152
ALT (U/L)	91 (11–724)	77 (13–569)	100 (11–724)	0.4
g-GTP (U/L)	328 (11–2118)	346 (11–2118)	288 (14–817)	0.621
ALP (U/L)	733 (143–3520)	855 (143–3520)	594(232–3278)	0.026
CRP (mg/dL)	3.76 (0.08–22.32)	3.48 (0.08–18.53)	5.0 (0.11–22.32)	0.687

AST: aspartate aminotransferase, ALT: alanine aminotransferase, g-GTP: gamma-glutamyl transpeptidase, ALP: alkaline phosphatase, CRP: C-reactive protein,

Next, we compared the phylogenetic groups and virulence factors of BEC by severity (Table 3). BEC isolates mainly belonged to the phylogenetic group B2 (68%). ST 131 (22.2%) and ST 95 (19.4%) were detected frequently between four STs by multi-locus sequencing typing (MLST), but more than half belonged to other STs. The most detected phylogenetic group was B2 in both groups (70.5% in non-severe, 64.3% in severe), but the proportion of each phylogenetic group was similar.

Table 3
Comparison of patterns of phylogenetic groups and prevalence of virulence factors between non-severe and severe groups.

	Total	Pitt less than 2	Pitt 2 or more	*P* value
phylogenetic group	72	44	28	0.732
A	4	2	2	
B1	8	3	5	
B2	49	31	18	
D	1	1	0	
E	6	4	2	
F	4	3	1	
MLST				0.359
ST131	17	13	4	
ST95	14	8	6	
ST73	5	4	1	
ST69	1	1	0	
others	35	18	17	
virulence factors				
papC	21	15	6	0.296
sfaD/E	8	4	4	0.703
CNF1	9	5	4	0.728
iucD	24	18	6	0.124
afaB/C	2	1	1	1
hlyA	9	5	4	0.728
fyuA	52	35	17	0.108
cvaC	6	6	0	0.753
fimH	69	42	27	1
iutA	47	34	13	0.011
ibeA	14	4	10	0.012
iha	23	16	7	0.438

	Total	Pitt less than 2	Pitt 2 or more	*P* value
ompT	6	6	0	0.075
kpsMT2	51	33	18	0.427
papG2	10	8	2	0.297
usp	56	35	21	0.773
ironEc	16	10	6	1
sat	22	16	6	0.202
traT	49	33	16	0.128
TcpC	12	9	3	0.346

The presence of *iutA* (77.3% in the non-severe group, 46.4% in the severe group, $P = 0.011$) and *ibeA* (9.1% in non-severe group, and 35.7% in severe group, $P = 0.012$) were significantly associated with severity, whereas patient characteristics were not related to severity.

Discussion

In this study, BEC mainly belonged to group B2, which is known as a virulent group, and ST131. Although acute biliary tract infections are caused by obstruction/stasis of biliary flow and influx of commensal bacteria into the biliary tract, BEC detected from blood cultures belonged to a pathogenic group.

The human intestinal tract has been recognized as a reservoir of extraintestinal pathogenic *E. coli* strains, such as uropathogenic *E. coli* (10). BEC may also hide in the gut. Once BEC translocates into the biliary tract due to stasis/obstruction, acute biliary tract infections can occur and develop into bacteremia.

Commensal *E. coli* strains, once considered as avirulent, are now known to express many of the same virulence factors, such as adhesion factors, as pathogenic *E. coli* strains. This is because these factors are needed for persistent colonization in the gut. *E. coli* has resistance against the effects of bile (11). In response to bile stress, both commensal and pathogenic *E. coli* strains, and especially enteropathogenic *E. coli* strains, activate stress response pathways (12, 13), efflux pumps (14), and production of toxins (15) in the gut. As there are high concentrations of bile acid in the biliary tract, resistance against bile might play an important role in pathogenicity.

In our study, other virulence factors which are needed during infection, such as iron acquisition, adhesion, and invasion, were analyzed. Factors needed for colonization of the gut as compared to those needed during biliary tract infection must be analyzed to clarify specific virulence factors of the biliary tract-pathogenic *E. coli* strains. The differences between commensal and enteropathogenic *E. coli* strains in the gut have yet to be clarified.

In the present study, *iutA* was found at a lower level in the severe group than in the non-severe group. *IutA* codes for the aerobactin siderophore ferric receptor protein, which has a role in facilitating iron acquisition by mediating the uptake of siderophores (16). In a chicken infection model, *iutA* expression in extraintestinal pathogenic *E. coli* strains was at least 50-fold higher in organ tissues compared to in vitro grown bacteria during infection (17). In mammalian hosts, iron is tightly bound to various proteins, such as hemoproteins and ferritin (18), making free iron for use by pathogenic bacteria scarce. In biliary tract infections, bile is an iron-limiting environment (19). Bile stress also causes increased mRNA levels for virulence genes associated with iron scavenging in *E. coli* (20). Therefore, *E. coli* strains harboring the *iutA* gene may become competitive in bile, but those without the *iutA* gene might find it difficult to proliferate in bile and escape into the blood. An *iutA* vaccine protected mice in a sepsis challenge model (21) and UTI model (22). Because *iutA* as an antigen might be easily recognized by host immune systems, *E. coli* strains harboring the *iutA* gene may be easy to eliminate in bile.

In contrast, *E. coli* strains containing the *ibeA* gene were found in a higher proportion in the severe group. *IbeA* codes for a 50 kDa protein used for penetration of human brain microvascular endothelial cells to invade through the blood brain barrier, and is thought to have an important role in neonatal meningitis (23, 24). *IbeA* may also be essential for invasion into intestinal epithelial cells and macrophages (25). No information regarding the relationship between *ibeA* and biliary tract epithelium cells has been reported; therefore, the mechanism of *ibeA* on the severity of acute biliary infection is unknown. *ibeA* inhibitors have been discovered to prevent invasion of human brain microvascular endothelial cells in vitro (26). It would be worthwhile to investigate whether *E. coli* strains harboring *ibeA* can perform internalization, and whether *ibeA* is essential to invade from bile duct to vessels through *in vitro* experiments using *ibeA* inhibitors.

In a rat model with common bile duct ligation, clearance of *E. coli* from blood and trapping rates in the liver were decreased compared to controls, and also showed decreased phagocytic activity and superoxide production of Kupffer cells (27). In our results, no significant relation was observed between severity of infection and patient background, such as abnormalities in the biliary tract and laboratory data of jaundice markers, except ALP.

This study has several limitations. First, although cholangitis is a polymicrobial infection (3), we investigated only one *E. coli* strain from each patient without analyzing other *E. coli* strains found in the bile. This comparison might reveal whether *ibeA*-positive strains can more easily escape or invade into the blood compared to strains that remained in the bile. Second, this study was conducted at a single institution. A larger multi-center study is needed to assess potential bias in the epidemiology of phylogenetic patterns and virulence factors.

Conclusion

We showed that many BEC belonged to a virulent group (B2) with a high prevalence of ST131. The severity of biliary tract infections depended on the presence of *iutA* and *ibeA* in BEC.

Methods

Patients

This retrospective study was conducted at the University of Tokyo Hospital, a 1217-bed tertiary-care teaching hospital in Tokyo, Japan. Patients with acute biliary tract infection who also had *E. coli* isolates detected in their blood were included in the study from April 2013 to February 2015. Each patient in this study was included only once, even if the patients repeatedly suffered *E. coli* bacteremia with acute biliary tract infection. Patient data, including clinical symptoms and microbiological data were collected from the medical records.

Data collection and definitions

Patient data collected included age, sex, underlying disease (diabetes mellitus, malignancy with or without metastasis, lymphoma, and collagen disease), use of immunosuppressants, biliary tract abnormalities, such as insertion of an intrabiliary stent or surgery for biliary carcinoma, gastrointestinal tract abnormalities, and past history of acute biliary infection and bacteremia. History of residence in a nursing home and antibiotic use within three months before onset of bacteremia was also noted. Collected patient laboratory data included white blood cell counts, platelet counts, total bilirubin, aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, gamma glutamyltransferase, and C-reactive protein. Cases in which *E. coli* was detected from blood cultures obtained within 48 h after admission were defined as community acquired infection. Others were considered hospital acquired infections.

Biliary tract infection was defined according to the Tokyo guideline (28). Cholangitis was defined in cases where all the following sings were positive: 1) generalized inflammation sign such as fever (more than 38.0 °C), elevation of inflammation indicators in blood tests (white blood cell counts less than 4000/mL or more than 10,000/mL, and C-reactive protein 1 mg/dL or more), 2) signs of bile stasis such as jaundice (total bilirubin 2 mg/dL or more), elevation of liver function and biliary function tests (more than one-and-a-half times of the upper limit for normal values for alkaline phosphatase(ALP), gamma-glutamyltransferase(g-GTP), aspartate aminotransferase(AST), or alanine aminotransferase(ALT), normal range; ALP 106-322 U/L, g-GTP 13-64 U/L in men and 9-32 U/L in women, AST 13-30 U/L, ALT 10-42 U/L in men and 7-23 U/L in women, respectively), and 3) imaging of biliary tract abnormalities, such as dilatation of the biliary tract or the presence of a stent, and constriction. Cholecystitis was defined as: 1) localized clinical signs such as Murphy's sign and pain in right upper abdomen, 2) generalized inflammation such as fever and elevation of inflammation indicators in blood tests, and 3) typical findings, such as acute cholecystitis with echocardiography or CT scan. Severity was divided using the Pitt bacteremia score (29) as severe (score of 2 or more) and non-severe (score less than 2).

Microbiological procedures

All isolates were identified using the Walkaway system (Siemens, Berlin, Germany) or matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (using the MALDI Biotyper; Bruker Daltonik, Germany).

Assignment of *E. coli* isolates to phylogenetic groups, such as A, B1, B2, C, D, E, and F, was determined by the quadruplex polymerase chain reaction (PCR) method as described by Clermont et al (30). For rapid identification of *E. coli* sequence types (STs) 69, 73, 95, and 131, the multilocus sequence typing PCR method (31) was performed. The prevalence of 20 virulence factors (*papC, sfaD/E, CNF1, iucD, afaB/C*, *hlyA*, *tcpC*, *fyuA*, *cvaC*, *fimH*, *iutA*, *ibeA*, *iha*, *ompT*, *kpsMT2*, *papG2*, *usp*, *ironEc*, *sat*, and *traT*) were screened by multiplex PCR using extracted *E. coli* genomic DNA according to previous reports (32-38).

Statistical analysis

The two-tailed Fisher's exact test was used for analysis of categorical data. Non-parametric data were analyzed using the Mann-Whitney U test. Values of $P < 0.05$ were considered significant. All statistical analyses were performed using JMP Pro version 11 software (SAS Institute, Cary, NC, USA).

Ethical considerations

This study was approved by the research ethics committee at the University of Tokyo Hospital. Obtaining written informed consent from each patient was waived because it was an observational retrospective study. The data were analyzed anonymously.

Declarations

Ethics approval and consent to participate

This study was approved by the research ethics committee at the University of Tokyo Hospital. Obtaining written informed consent from each patient was waived because it was an observational retrospective study. The data were analyzed anonymously.

Consent for publication

Not applicable.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

MI designed this study, acquired and analyzed the patients' data, and was a major contributor in writing the manuscript. MI and TK performed PCR to analyze virulence of *E. coli*. FF and TH identified and collected the *E. coli* strains. KT made database of bacteremia, YO, SO and KM revised the manuscript. All authors read and approved the final manuscript.

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