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Original Article

Bloodstream infection with extended-spectrum beta-lactamase–producing *Escherichia coli*: The role of virulence genes

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Abstract *Background:* Extraintestinal pathogenic *Escherichia coli* (ExPEC) strains hold the responsibility for the majority of *E. coli* infections. Numerous extraintestinal virulence factors (VFs) were possessed by ExPEC which are involved in the pathogenesis of infection. However, the effect of comorbidities or infection syndrome in the association of VFs and mortality remains inconclusive.
Method: This study addressed whether specific sequence type (ST) and VFs of extended-spectrum beta-lactamase–producing *E. coli* (ESBL-EC) are associated with different outcomes in patients with bloodstream infection. 121 adults from southern Taiwan with ESBL-EC bloodstream infections were enrolled during a 6-year period. Demographic data, including infection

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syndromes, underlying disease and outcomes, were collected. The virulence factors in isolates were analyzed by PCR and multilocus sequence typing analyses were also performed.
Result: Positivity for the virulence genes *iha, hlyD, sat, iutA, fyuA, malX, ompT,* and *traT* was associated with ST131 positivity ($P < 0.05$). Some ESBL-EC virulence genes associated with urinary tract infection (UTI) were revealed. Positivity for ST405 and the virulence genes *iroN* and *iss* were significantly associated with increased 30-day mortality (death within 30 days) on univariate analysis ($P < 0.05$). Independent risk factors of 30-day mortality in bacteremic patients with UTI included underlying chronic liver disease and malignancy. ST131 was borderline associated with 30-day mortality. Independent risk factors associated with 30-day mortality among bacteremic patients without UTI included comorbidities and *iroN* positivity.
Conclusion: In bacteremic patients with UTI, and the ST131 clone was borderline associated with mortality. Positivity for the virulence gene *iroN* may be linked to mortality in bacteremic patients without UTI.

Introduction

Escherichia coli strains that induce extraintestinal diseases have been classified as extraintestinal pathogenic *E. coli* (ExPEC).[1]

Extraintestinal pathogenic *E. coli* (ExPEC) strains hold the responsibility for the majority of human *E. coli* infections, including urinary tract infection (UTI) syndromes, neonatal meningitis, intra-abdomen infection (IAI), skin and soft tissue infection (SSTI), biliary tree infection (BTI), pneumonia, and bacteremia.[2–4]

Numerous functional specialized extraintestinal virulence factors (VFs) (e.g., adhesins, toxins, Siderophores, polysaccharide Capsule, and invasins) were possessed by ExPEC that are uncommon among commensal or intestinal pathogenic strains.[1,5]

Various proteins have been studied as putative extraintestinal virulence factors and shown to be involved in adhesion to and invasion of host cells, iron availability, toxic effects on host cells, or protection against the host's immune system.

Some virulence factors are associated with urinary tract infection (UTI) and some are associated with blood invasion from the bowel. Previous study revealed *pap*, *malX*, *usp*, *fyuA* and phylogenetic group B genes positively associated with mortality.[6] The co-occurrence of multiple genes encoding *papC*, *sfa*, *usp* and *cnf1* virulence factors probably predisposes *E. coli* to translocation from the gastrointestinal tract to the vascular bed in patients with hematologic malignancies.[7] A recent study showed that differences in virulence genes prevalence between cystitis and pyelonephritis isolates could be limited to 8 genes.[8] However, the association of virulence genes and disease severity is still controversial.[9,10] Few studies have focused on the relationship of virulence factors and mortality in extended-spectrum beta-lactamase (ESBL) *E. coli* (ESBL-EC) bacteremia.[11,12] As well, these studies usually lack information on sequence type (ST) or comorbidities.

From 2000 to 2006, *E. coli* clone ST131 producing CTX-M-15 ESBL has been identified in 3 continents.[13,14] In most Asian countries outside India including Taiwan, the CTX-M-14 type was the most prevalent ESBL among *E. coli* isolates. In addition, ST131-producing CTX-M-15 has been found prevalent in Japan, with rare cases described in Indonesia and China.[15–18] Until this report, a detailed report of virulence data related to ST131 in Taiwan was lacking. In our previous study, we found ST131 in about one third of patients with bacteremia and ESBL-EC.[15] Our study cohort showed ST131 isolates in both community- and hospital-onset infections. The virulence genes distribution in CTX-M-14 ESBL-EC with ST131 is unknown. In addition, we do not know whether other STs (e.g., ST38, ST405, ST95, or ST69) are associated with mortality in ESBL-EC bacteremia.

This study aimed to understand the association between specific STs and virulence factors in patients with ESBL-EC bloodstream infection, in particular, any association with comorbidities and infection syndrome.

Methods

Patients and ethics statement

The study consisted of adults aged 16 years or older with ESBL-EC bloodstream infection in a teaching hospital in southern Taiwan during 2005–2010.

All patients were analyzed by using an organizing recording form. Each clinical course of infection was assessed and cataloged according to the information supplied by clinical physicians and medical records. The diagnosis of the infection focus of bacteremia was based on clinical, bacteriological, and radiological investigations. If no infection focus could be identified, the bacteremia was classified as primary infection. Only strains from the first bacteremic episode were included in the analysis.

Demographic data, including infection syndrome, underlying disease and outcome, were collected from medical charts. The following items were recorded for each patient: age; sex; underlying illness; severity of illness (classified by Charlson comorbidity score); history of hospitalization or outpatient department involvement; antibiotic use history for more than 7 days before the bacteremic episode; surgical history within the previous 3 months; existence of a nasogastric tube, central venous catheter, or urinary

catheter; initial empirical antimicrobial agents used; and outcome. If an *in vitro* active antimicrobial agent was administered before the final blood culture result, it was considered adequate empirical therapy. This study was approved by the institutional review board (IRB) at E-Da Hospital (no. EMRP-098-006). Written informed consents were waived by IRB due to no harms or minimal risk in the patients in this study.

Virulence genes detection

Unlike most commensal *E. coli* strains, ExPEC isolates typically are possess genes for various combinations of adhesins (e.g., P and S fimbriae), iron-acquisition systems (e.g., siderophore receptors, aerobactin iron transport system), host defense—avoidance mechanisms (e.g., capsule, increased serum survial), and toxins (e.g., hemolysin).[19,20]

The extraintestinal virulence factors for ESBL-EC were analyzed by PCR and multilocus sequence typing (MLST). Virulence genes of extraintestinal pathogenic *E. coli* (ExPEC) were examined, divided in five categories, which included adhesins: *papA* (P fimbriae structural subunit), *fimH* (type I Fimbriae), *papEF* (P fimbriae tip pilins*)*, *papG (*P fimbrial adhesin molecule, with variants II and III*)*, *iha (*putative adhesin-siderophore*);* Toxins*:* *hlyD* (hemolysin), *sat* (secreted autotransporter toxin), *vat* (vacuolating autotransporter toxin); Siderophores*:* *iroN (siderophore receptors)*, *iutA* (aerobactin iron transport system), *fyuA* (yersiniabactin receptor); capsule: *kpsMTII* (capsular polysaccharide), *kpsMTIIK1*, *kpsMTIIK5* (*K1*and *K5 kpsM II* variant); and Miscellaneous: *malX* (marker for pathogenicity-associated Island), *ompT* (outer membrane protein T), *iss (*increased serum survival), *usp* (uropathogen-specific protein), *traT* (serum-resistance—associated outer membrane protein).[21,22] PCR amplification involved a 25-μL reaction mixture containing template DNA (2 μL boiled lysate, 4 mM $MgCl_2$, 0.8 mM each of 4 dNTPs, 0.6 μM each primer [concentration 0.3 μM], and 2.5 units AmpliTaq Gold in 1 × PCR buffer [Perkin Elmer, Branchburg, NJ]). The primer use was as described.[23] MLST was conducted according to the website http://mlst.warwick.ac.uk/mlst/dbs/Ecoli.

Statistical analyses

Analyses involved use of SAS 9.3 (Cary, NC, USA) and all statistical tests were two-tailed. Continuous variables are described and were compared by Mann-Whitney U test. Categorical variables are described with percentages and were compared by Fisher exact test. Factors that may have a dose—response or trend relationship were tested by Cochran—Armitage test for trend. Previous studies suggested that the bacterial strain and site of infections may have important effects on the severity of diseases, so the following characteristics were compared first in the

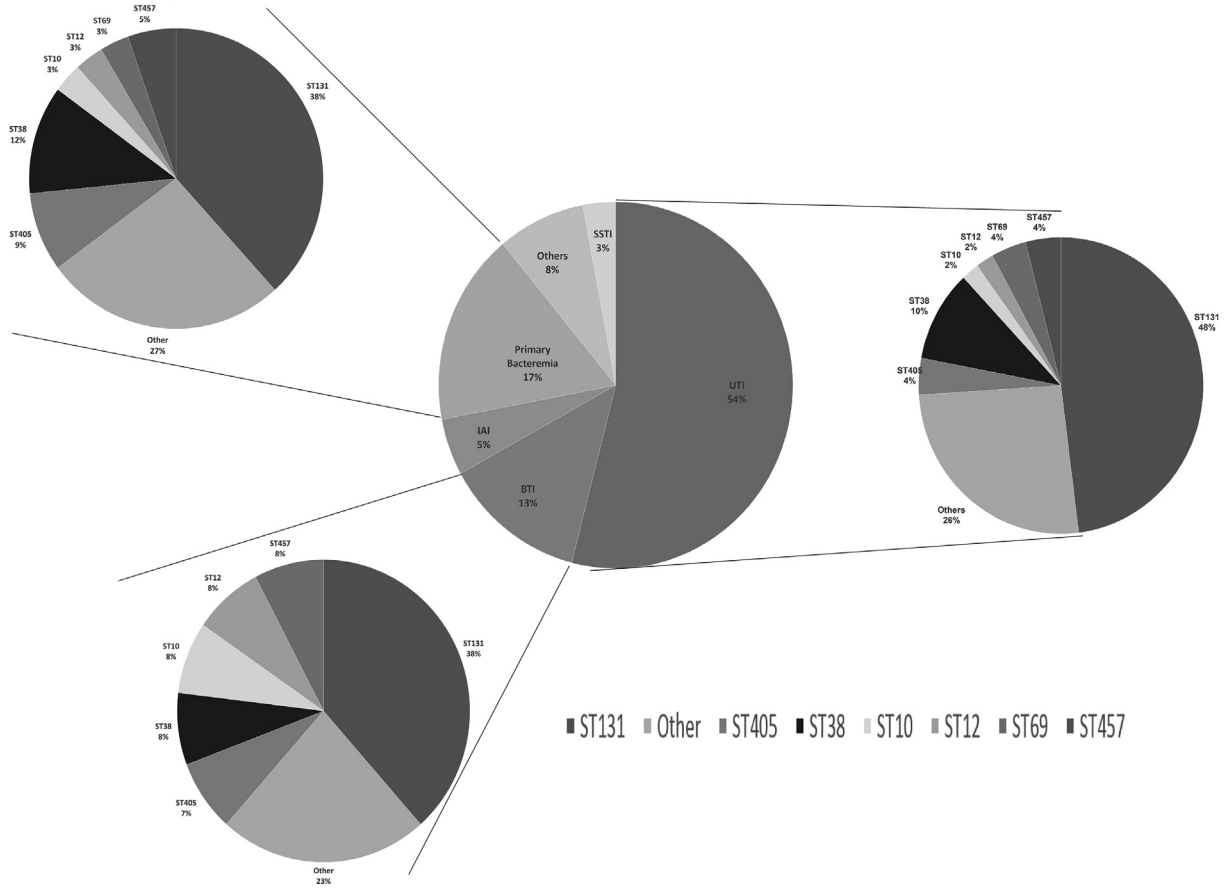

Figure 1. The percentage of infection focus and the association with sequence type. UTI: urinary tract infection. BTI: biliary tree infection. IAI: intra-abdomen infection. SSTI: skin and soft tissue infection. ST: sequence type

Table 1 The underlying disease in ESBL-EC and between UTI and non-UTI bacteremic patients.

	Total N = 121	ST131 N = 36	Non ST131 N = 85	P vale
Age (Mean ± SD)	64.4 ± 16.2	64 ± 15.2	64.6 ± 16.8	0.86
Gender				0.37
Male (%)	58 (47.9)	15 (41.7)	43 (50.6)	
Female (%)	63 (52.1)	21 (58.4)	42 (49.4)	
Comorbidity				
Charlson index (Mean ± SD)	6.82 (3.8)	6.64 (4.1)	6.89 (3.7)	0.74
Congestive heart failure (%)	59 (48.8)	19 (52.8)	40 (47.1)	0.57
Cerebral vascular disease (%)	33 (27.3)	10 (27.8)	23 (27.1)	0.94
Connective tissue disease (%)	4 (3.3)	0 (0)	4 (4.7)	0.05
Diabetes mellitus (%)	37 (30.6)	12 (33.3)	25 (29.4)	0.67
Chronic liver disease (%)	31 (25.6)	5 (13.9)	26 (30.6)	0.15
Malignancy (%)	34 (28.1)	10 (27.8)	24 (28.2)	0.96
ESRD with HD (%)	11 (9.1)	2 (5.6)	9 (10.6)	0.38

Data are no. (%).
ESBL-EC: extended-spectrum beta-lactamase—producing *Escherichia coli*.
UTI: urinary tract infection.
SD: standard deviation.
ESRD: end-stage renal disease.
HD: hemodialysis.

analyses: (1) *E. coli* O25b-ST131 versus non-O25b-ST131, (2) UTI versus non-UTI, and (3) death within 30 days versus survival > 30 days. Possible interactions or effect modification for these 3 characteristics with other patients or bacterial factors were also examined by stratified analyses with the Breslow-Day heterogeneity test, with significance set at $p < 0.1$. If a significant difference was found, subsequent analyses would be stratified by this factor. Factors showing significance at $p < 0.1$ on univariate analysis were all considered in the multivariate analysis. Stratified logistic regression was used to estimate independent odds ratios (ORs) and 95% confidence intervals (95% CIs) for bacterial strain, virulence factor or patient characteristics. $P < 0.05$ was considered statistically significant.

Results

The association between sequence type and focus of infection

Among 121 ESBL-EC bloodstream infection patients, the percentage of infection focus and the association with infection syndrome were revealed in Fig. 1.

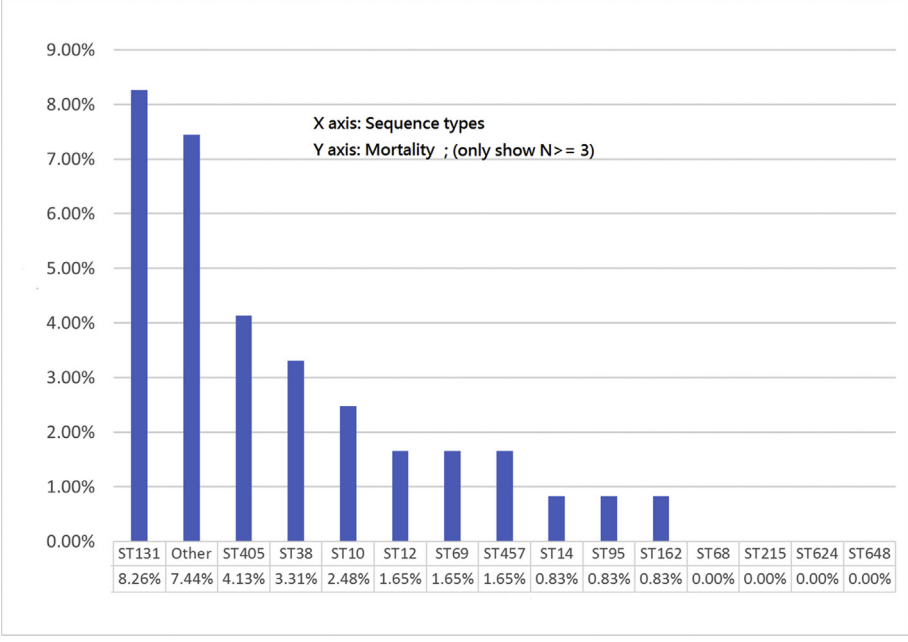

Figure 2. The mortality in different sequence type. ST: sequence type

UTI was the majority (54%), followed by ESBL-EC primary bacteremia (17%), the third place was biliary tract infection (13%), whereas others accounted for 8%.

Urinary tract infection (UTI)

Looking at in the data of UTI in more detail, ST131 contributed 48%, others and ST38 followed with 26% and 10% respectively.

ESBL-EC primary bacteremia

Thirty-eight percent of ESBL-EC primary bacteremia contributed by ST 131, others followed with over a quarter, 27%, ST38 came next with 12%.

Biliary tree infection (BTI)

Overall in BTI, ST131 made up the largest percentage with more than a third of the total (38%). ST405, ST38, ST10, ST12, ST457 were equally distributed around 7%～8%.

Virulence genes distribution in ESBL-EC and between UTI and non-UTI bacteremic patients

The underlying disease in this cohort was shown in Table 1 and the mortality in different ST was shown in Fig. 2. For the 121 adults included in the study, we analyzed the virulence genes of adhesins, toxins, siderophores, capsule and miscellaneous distribution with the ST131 and non-ST131 clone. Positivity for the genes *iha, hlyD, sat, iutA,*

Table 2 Virulence genes distribution for patients with ST131 and non-ST131 clone.

Virulence genes	Total n = 121	ST131 n = 36	Non-ST131 n = 85	*P*	OR (95% CI)
Adhesins					
papA	44 (36.4)	13 (36.1)	31 (36.5)	0.97	1.0 (0.43—2.21)
fimH	117 (96.7)	35 (97.2)	82 (96.5)	0.83	1.3 (0.12—12.7)
papEF	43 (35.5)	13 (36.1)	30 (35.3)	0.93	1.0 (0.46—2.33)
papG allele II	35 (28.9)	14 (38.9)	21 (24.7)	0.12	1.9 (0.84—4.45)
papG allele III	9 (7.4)	1 (2.8)	8 (9.4)	0.20	3.6 (0.43—30.20)
iha	61 (50.4)	31 (86.1)	30 (35.3)	**<0.01**	11.4 (4.00—32.29)
Toxins					
hlyD	25 (20.7)	12 (33.3)	13 (15.3)	**0.03**	2.8 (1.11—6.88)
sat	55 (45.5)	27 (75.0)	28 (32.9)	**<0.01**	6.1 (2.53—14.71)
vat	16 (13.2)	1 (2.8)	15 (17.6)	**0.02**	0.1 (0.01—1.05)
Siderophores					
iroN	23 (19.0)	3 (8.3)	20 (23.5)	0.05	0.3 (0.08—1.06)
iutA	97 (80.2)	34 (94.4)	63 (74.1)	**0.01**	5.9 (1.31—26.77)
fyuA	96 (79.3)	34 (94.4)	62 (72.9)	**0.01**	6.3 (1.40—28.38)
Capsule					
kpsMTII	70 (57.9)	15 (41.7)	55 (64.7)	**0.02**	0.4 (0.17—0.86)
kpsMTIIK1	22 (18.2)	1 (2.8)	21 (24.7)	**<0.01**	0.9 (0.11—0.67)
kpsMTIIK5	43 (35.5)	12 (33.3)	31 (36.5)	0.74	0.9 (0.38—1.98)
Miscellaneous					
malX	69 (57.0)	31 (86.1)	38 (44.7)	**<0.01**	7.7 (2.71—21.62)
ompT	67 (55.4)	32 (88.9)	35 (41.2)	**<0.01**	11.4 (3.70—35.22)
iss	14 (11.6)	3 (8.3)	11 (12.9)	0.47	0.6 (0.16—2.33)
traT	101 (83.5)	34 (94.4)	67 (78.8)	**0.03**	4.6 (1.00—20.84)

Data are no. (%).
ST: sequence type.
papA: P fimbriae structural subunit.
fimH: type I Fimbriae.
papEF: P fimbriae tip pilins.
papG:P fimbrial adhesin molecule, with variants II and III.
iha: putative adhesin-siderophore.
hlyD: hemolysin.
sat: secreted autotransporter toxin.
vat: vacuolating autotransporter toxin.
iroN: siderophore receptors.
iutA: aerobactin iron transport system.
fyuA: yersiniabactin receptor.
kpsMTII: capsular polysaccharide.
kpsMTIIK1, kpsMTIIK5: *K1*and *K5 kpsM II* variant.
malX: marker for pathogenicity-associated island.
ompT: outer membrane protein T.
iss: increased serum survival.
traT: serum-resistance—associated outer membrane protein.

fyuA, *malX*, *ompT*, and *traT* was more frequent with the ST131 than non-ST131 clone ($P < 0.05$) and that for *vat*, *kpsMTII* and *kpsMTIIK1* were more frequent with the non-ST131 clone ($P < 0.05$) (Table 2). Positivity for the virulence genes *pap*, *iha*, *hlyD*, *sat*, *iutA*, *fyuA*, *malX*, *ompT*, *usp*, and *traT* were more frequent with than without UTI (Table 3).

Virulence genes survey in patients with 30-day mortality and survival >30 days

Mortality within 30 days was associated more with ST131 than ST405 infection (28% vs. 15.6%). Positivity for most virulence genes was not associated with 30-day mortality, but 2 virulence genes, siderophores *iroN* and *iss*, were highly associated with 30-day mortality on univariate analysis (OR 3.36 [95% CI 1.30—8.68] and 3.23 [1.05—10.24], $P < 0.05$) (Table 4).

Factors associated with 30-day mortality with or without UTI

Independent risk factors associated with 30-day mortality among 65 bacteremia patients with UTI included underlying chronic liver disease and malignancy (OR 7.38 [95% CI 1.5—36.3], $p < 0.05$). ST131 was borderline associated with 30-day mortality in bacteremia patients with UTI (OR 10.8 [1—117.7], $P = 0.05$). Positivity for *malX* was a protective factor (OR 0.033 [0.03—0.357]). Independent risk factors associated with 30-day mortality among 56 bacteremic patients without UTI included underlying chronic liver disease and malignancy (OR 8.66 [1.7—42.63]) and positivity for *iroN* (OR 5.88 [1.39—24.9], $p < 0.05$) (Table 5).

Discussion

We performed a mortality analysis in a cohort of ESBL-EC bacteremic patients and considered host factors such as underlying disease and infection type, bacteria characteristics including ST, and virulence genes.

There was no difference in underlying disease between UTI and non-UTI bacteremic patients. For infection type, ExPEC induced disease in bodily sites outside of the gastrointestinal tract, the majority is UTI, followed by bacteremia and biliary tract infection in this study. ST131 was responsible heavily for these three-infection focus mentioned above. This result demonstrated ST131 was the major clone causing *E. coli* bacteremia in adult populations.[24]

Our virulence genes analysis indicated that clone ST131 strains were more like to be associated with *iha*, *hlyD*, *sat*,

Table 3 Virulence genes distribution for UTI and non-UTI bacteremic patients.

Type of infection	Total n = 121	UTI n = 65	Non-UTI n = 56	*P*	OR (95% CI)
Adhesins					
papA	44 (36.4)	33 (50.8)	11 (19.6)	<0.01	4.21 (1.86—9.57)
papEF	43 (35.5)	33 (50.8)	10 (17.9)	<0.01	4.74 (2.50—10.97)
papG allele II	35 (28.9)	29 (44.6)	6 (10.7)	<0.01	6.71 (2.53—17.84)
iha	61 (50.4)	44 (67.7)	17 (30.4)	<0.01	4.80 (2.22—10.39)
Toxins					
hlyD	25 (20.7)	20 (30.8)	5 (8.9)	<0.01	4.53 (1.57—13.06)
sat	55 (45.5)	40 (61.5)	15 (26.8)	<0.01	4.37 (2.01—9.48)
Siderophores					
iutA	97 (80.2)	60 (92.3)	37 (66.1)	<0.01	6.16 (2.12—17.91)
fyuA	96 (79.3)	38 (89.2)	38 (67.9)	0.004	3.92 (1.49—10.29)
Miscellaneous					
malX	69 (57.0)	45 (69.2)	24 (42.9)	0.003	3.00 (1.42—6.33)
ompT	67 (55.4)	46 (70.8)	21 (37.5)	<0.01	4.03 (1.88—8.63)
usp	56 (46.3)	36 (55.4)	20 (35.7)	0.03	2.23 (1.07—4.65)
traT	101 (83.5)	60 (92.3)	41 (73.2)	0.005	4.39 (1.48—13.02)

Data are no. (%).
UTI: urinary tract infection.
papA: P fimbriae structural subunit.
papEF: P fimbriae tip pilins.
papG:P fimbrial adhesin molecule, with variants II and III.
iha: putative adhesin-siderophore.
hlyD: hemolysin.
sat: secreted autotransporter toxin.
iutA: aerobactin iron transport system.
fyuA: yersiniabactin receptor.
malX: marker for pathogenicity-associated island.
ompT: outer membrane protein T.
usp: uropathogen-specific protein.
traT: serum-resistance—associated outer membrane protein.
Only those VFs yielding a significant UTI association are shown.

iutA, fyuA, malX, ompT, and traT genes than non-ST131 strains. Particularly, the virulence gene response for hemolysin and siderophores was predominant in ST131 strains. This finding is consistent with its high virulence potential in a mouse model of septicemia[25] and perhaps explains in part the higher epidemiological success of the ST131 clonal group.[26]

The prevalence of P fimbriation (*papA)* in isolates from patients with bacteremia with UTI (urosepsis and pyelonephritis) is high as in isolates from patients with bacteremia from other sources. P fimbriae may contribute to the ability of *E. coli* strains to cause UTI.[21]

The aerobactin system (*iutA*), responses for siderophores in *E. coli*, is encoded by a five-gene operon, which is commonly found together with P fimbriae in isolates from patients with UTI and urosepsis. An association of chromosomal aerobactin with hemolysin (*hlyD*, and *hlyA*) is found among urosepsis isolates. Hemolytic uropathogenic strains almost always also express P fimbriae (*papA*). Hemolysin production is associated with the human

Table 4 Virulence genes distribution for patients with 30-day mortality and survival beyond 30 days.

ST type	Total n = 121	Mortality ≤30 days n = 32	Survival >30 days n = 89	*P*	OR (95% CI)
ST131	36 (29.8)	9 (28.1)	27 (30.0)	0.81	0.90 (0.37–2.19)
ST405	8 (6.6)	5 (15.6)	3 (3.4)	**0.02**	5.30 (1.19–23.67)
Virulence genes					
Adhesins					
papA	44 (36.4)	4 (12.5)	40 (44.9)	**0.002**	0.17 (0.06–0.54)
papEF	43 (35.5)	4 (12.5)	39 (43.8)	**0.003**	0.18 (0.05–0.56)
papG allele II	35 (28.9)	3 (9.4)	32 (36.0)	**0.009**	0.18 (0.05–0.65)
papG allele III	9 (7.4)	3 (9.4)	6 (66.7)	0.63	0.69 (0.16–2.97)
iha	61 (50.4)	10 (31.3)	51 (57.3)	**0.01**	0.33 (0.14–0.79)
Toxins					
hlyD	25 (20.7)	2 (6.3)	23 (25.8)	**0.03**	0.19 (0.04–0.86)
sat	55 (45.5)	8 (25.0)	47 (52.8)	0.08	0.29 (0.12–0.74)
vat	16 (13.2)	3 (9.4)	13 (14.6)	0.45	0.60 (0.16–2.27)
Siderophores					
iroN	23 (19.0)	11 (34.4)	12 (13.5)	**0.01**	3.36 (1.30–8.68)
iutA	97 (80.2)	23 (71.9)	74 (83.1)	0.17	0.51 (0.20–1.33)
fyuA	96 (79.3)	25 (78.1)	71 (79.8)	0.84	0.90 (0.33–2.42)
Capsule					
kpsMTII	70 (57.9)	18 (56.3)	52 (58.4)	0.83	0.91 (0.40–2.06)
kpsMTIIK1	22 (18.2)	4 (18.2)	18 (20.2)	0.33	0.56 (0.17–1.81)
kpsMTIIK5	43 (35.5)	13 (40.6)	30 (33.7)	0.48	1.34 (0.58–3.09)
Miscellaneous					
malX	69 (57.0)	12 (37.5)	57 (64.0)	**0.01**	0.33 (0.14–0.77)
ompT	67 (55.4)	10 (31.3)	57 (64.0)	**0.002**	0.25 (0.10–0.60)
usp	56 (46.3)	13 (40.6)	43 (48.3)	0.45	0.73 (0.32–1.66)
iss	14 (11.6)	7 (21.9)	7 (7.9)	**0.03**	3.23 (1.05–10.24)
traT	101 (83.5)	28 (87.5)	73 (82)	0.47	1.53 (0.47–4.98)

Data are no. (%).
ST: sequence type.
papA: P fimbriae structural subunit.
papEF: P fimbriae tip pilins.
papG:P fimbrial adhesin molecule, with variants II and III.
iha: putative adhesin-siderophore.
hlyD: hemolysin.
sat: secreted autotransporter toxin.
vat: vacuolating autotransporter toxin.
iroN: siderophore receptors.
iutA: aerobactin iron transport system.
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kpsMTII: capsular polysaccharide.
kpsMTIIK1, kpsMTIIK5: *K1*and *K5 kpsM II* variant.
malX: marker for pathogenicity-associated island.
ompT: outer membrane protein T.
usp: uropathogen-specific protein.
iss: increased serum survival.
traT: serum-resistance–associated outer membrane protein.
p value <0.05 are defined in bold.

Table 5 Multivariate regression analysis of factors associated with 30-day mortality in patients with UTI (n = 65) and without UTI (n = 56).

	OR	95% CI	p-value
With UTI			
Patients with underlying disease[a]	7.38	1.5–36.3	0.014
ST131 strains	10.8	1–117.7	0.05
malX	0.033	0.03–0.357	0.005
Without UTI			
Patients with underlying disease[a]	8.66	1.7–42.63	0.008
iroN	5.88	1.39–24.9	0.016

[a] Including cancers and chronic liver disease (including liver cirrhosis).
UTI: urinary tract infection.
ST: sequence type.
malX: marker for pathogenicity-associated island.
iroN: siderophore receptors.

pathogenic strain of *E. coli*, especially those causing more clinically severe forms of UTI.[21] This finding is similar to our observation of the virulence genes response for adhesion genes (*papA, papEF* and *papG allele II*), toxins such as hemolysin (*hlyD*), and siderophores (*iutA,* and *fyuA*) associated with a specific infection syndrome such as UTI (Table 3).

A French cohort study revealed an association of positivity for *papGIII,* septic shock at baseline and a non-urinary tract origin of sepsis with a fatal outcome.[27] A cohort of cirrhotic patients with *E. coli* bacteremia and spontaneous bacterial peritonitis showed no significant association between specific clonal groups and patient characteristics, type of infection, or outcome.[28] Mora-Rillo et al. found positivity for *fyuA* associated with increased mortality, and that of P fimbriae genes had a protective role.[29] A study in Spain of ESBL *E coli* bacteremia showed *ibeA* associated with increased mortality, with *papG alleleII* having a protective effect.[11]

We found *iroN* and *iss* more frequently associated with 30-day mortality than other virulence genes on univariate analysis. However, we also found positivity for *papA, papEF, papG alleleII, iha, hlyD, malX* and *ompT* with a protective effect on mortality. *FyuA* and *papGIII* genes had no significant impact on 30-day mortality.

The virulence gene *iroN* is a novel *E. coli* gene, with 77% DNA homology to a catecholate siderophore receptor gene recently identified in Salmonella. It is linked to the P-pilus (*prs*) and F1C fimbrial (*foc*) gene clusters on a pathogenicity island and appears to have been acquired by IS1230-mediated horizontal transmission.[29] Johnson suggested that *iroN* of *E. coli* could be a good target for an anti-virulence factor (VF) intervention among immunocompromised and non-immunocompromised hosts because the prevalence of *iroN* of *E. coli* was increased in the presence of host compromise, and *iroN* of *E. coli* could also be an effective target for anti-VF intervention among multidrug-resistant *E. coli*.[30] Russo et al., in a mouse model of ascending UTI, showed that *iroN* positivity contributed significantly to the ability to colonize the mouse bladder, kidneys, and urine, evidence that *iroN* is a urovirulence factor.[31] Dozois et al. performed specific deletion of the aerobactin siderophore system and *E. coli iroN* locus and demonstrated that these pathogen-specific systems contribute to the virulence of avian strains.[32] Nègre et al. found that the *iroN* gene itself played a key role in the virulence of *E. coli* strain C5, possibly by contributing to the sustained high-level bacteremia that precedes meningitis.[33]

Our data showed *iroN* indeed associated with higher 30-day mortality on univariate analysis and in patients with cancers and chronic liver disease including liver cirrhosis, was associated with 30-day mortality without UTI.

There were several limitations in this study. First, the mortality analysis may be biased by selected isolates in single center in Taiwan. As well, the different strains may not be generalized to other parts of the world. Second, although we considered host factors in out multivariate analysis, we cannot exclude the possibility that some virulence genes were linked to some specific hosts. Third, after reviewing the virulence database on the website,[34] majorities of 5 functional categories, 19 VFs of ExPEC were analyzed. However, we did not include all the VFs mentioned in the references.

In conclusion, in adult ESBL *E.coli* bloodstream infection, some specific virulence genes were linked to specific ST and infection syndromes. Besides the host factors, the presence of virulence gene *iroN* may be linked to increased mortality in bacteremic patients without UTI.

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