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ORIGINAL RESEARCH

Molecular Characterization of High and Low Virulent *Escherichia coli* Clinical Strains Isolated from Patients with Urinary Tract Infections with or without Bacteremia in Southern Taiwan

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Objective: The most common extraintestinal pathogen and infection site is uropathogenic *Escherichia coli* (UPEC), which causes urinary tract infections (UTIs). UPEC is also a common pathogen in bloodstream infections; in severe cases, it can lead to death. Although host and bacterial virulence factors have been demonstrated to be associated with UTI pathogenesis, the role of the related contributing factors in UTI and urinary source bacteremia is not yet fully understood. This study aimed to compare and analyze the factors contributing to urinary bacteremia in patients with UTI.

Methods: A total of 171 *E. coli* strains collected from patients with UTI and urinary source bacteremia at Chiayi Christian Hospital were used. Phylogenetic groups and virulence factors were determined using PCR. Drug resistance patterns were determined using the disk diffusion assay.

Results: Previous studies have demonstrated that fimbriae and papGII may be associated with first-step infections and severe UTIs, respectively. As expected, highly virulent *E. coli* strains (belonging to the phylogenetic B2 and D groups) were dominant in the bacteremic UTI (90%) and UTI (86.27%) groups. However, our results showed that the UTI group had a significantly higher prevalence of *sfa/focDE* (belonging to the S and FIC fimbriae) than the bacteremic UTI group (29.4% vs 12.5%; p=0.008). In the bacteremic group, we found that *sfa/focDE* was only detected in highly virulent strains. The bacteremic UTI group had a significantly higher prevalence of *pap*GII (belonging to P fimbriae) than the UTI group (55.8% vs 37.3%; p=0.026). In addition, the P fimbriae gene cluster, including *papC, papEF*, and *papGII*, was predominant in highly virulent strains. Notably, our results show that multidrug-resistant (MDR) strains were significantly less virulent than non MDR strains.

Conclusion: Taken together, our results provide insights into the contributing factors in patients with UTI and urinary bacteremia.

Keywords: *Escherichia coli*, urinary tract infection, urinary source bacteremia, virulence factors, multidrug resistance

Introduction

Urinary tract infections (UTI) are among the most common extra-intestinal infections caused by several pathogens.[1] Epidemiological studies have reported that various gram-positive and gram-negative bacteria, including *Escherichia coli, Klebsiella pneumonia, Enterococcus faecalis, Proteus mirabilis, Pseudomonas aeruginosa*, and *Staphylococcus saprophyticus* can cause UTIs.[2–4] The leading UTI-causing pathogen, *E. coli*, can cause severe invasive diseases such as acute kidney injury, bacteremia, and sepsis.[2,5–7] Approximately 30% of sepsis cases originating from urinary sites are caused

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by malignancies of the urinary tract.[6] Notably, the all-cause mortality rate of sepsis ranges from 28–56%.[6] Therefore, an investigation of the factors that contribute to the pathogenesis of sepsis is urgently needed.

Several factors, including virulence and antibiotic resistance genes in uropathogenic *E. coli* (UPEC), could affect the severity of UTIs.[8,9] Virulence genes are key factors that assist UPEC in host invasion, colonization, and survival in the host.[9,10] In addition, UPEC can be further divided into different phylogenetic groups that differ in their gene content, pathogenicity islands, virulence factors, and genomic islands, resulting in great diversity in the pathogenicity and antibiotic resistance rates in bacteria.[9,11,12] The adherence of pathogens to host cells is a key step in the pathogenesis.[13,14] Several adhesins, including type I, type II, S, and FIC fimbriae, are involved in host cell invasion.[15] Type I fimbriae are regulated by *fim* gene clusters and are related to biofilm formation on abiotic surfaces and binding to kidney cells.[16] Type II fimbriae are encoded by *pap* genes required for P-fimbrial synthesis.[17,18] Previous studies reported that the *papGII* gene plays a vital role in developing bacteremia in patients who have upper UTI.[17] The expression of type I and P fimbriae is controlled by phase variations in *E. coli*.[19,20] Type I fimbriae are repressed during P fimbrial expression and vice versa.[8] Although multiple virulence factors have been reported in the pathogenesis of UTIs, the physiological role of virulence factors associated with invasiveness remain incompletely defined.[21]

The aim of this study was two-fold. First, we estimated the prevalence of virulence factors in UTI and urinary-source bacteremia isolates associated with bacterial pathogenesis and patient progression. Second, we determined the prevalence of drug resistance patterns in the same sample. In conclusion, this study provides information concerning the factors contributing to urinary bacteremia in pathogens and patients with UTI.

Material and Methods

Study Setting

Patients hospitalized with a diagnosis of UTI or urinary bacteremia between 2016 and 2018 were enrolled in this study. Bacterial strains were isolated for phylogenetic classification and virulence factor determination. Patients who received antibiotic therapy before bacterial culture were excluded. Moreover, patients with UTI were excluded from the study because they had no blood cultures (Figure 1). Demographic characteristics and clinical features of the patients were also studied.

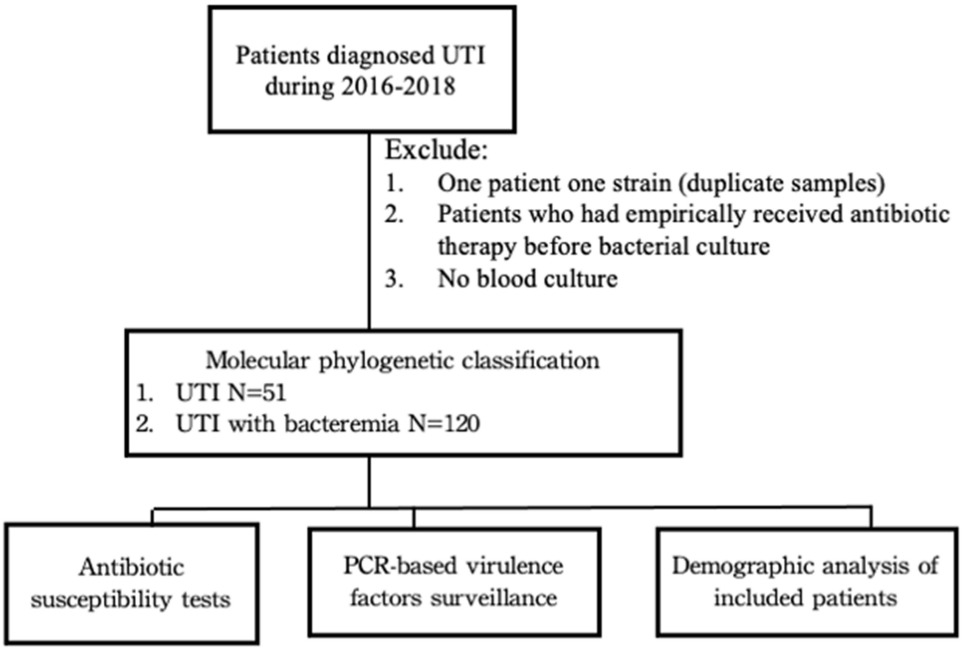

Figure 1 Flow chart of study design.

Molecular Phylogenetic Classification

Bacterial genomic DNA was extracted according to the manufacturer's instructions (QIAGEN). *Escherichia coli* phylogenetic groups were divided into four groups (A, B1, B2, and D) based on specific genetic markers (*chuA, yjaA*, and *TSPE4.C2*) using PCR, as described by Cermont et al.[22]

Virulence Factors Determination

Extracted bacterial DNA was used to detect 26 virulence genes. Virulence genes, including adhesins (1. P fimbriae: *papAH, papC, papEF, papGI, papGII*; 2. M fimbriae: *bmaE*; 3. S and FIC fimbriae: *sfa/focDE, focG, sfaS*; 4. Type I fimbriae: *fimH;* 5. Dr binding adhesin: *afa/draBC*; 6.N-acyl D-glucosamine specific fimbriae: *gafD*; 6. Non-fimbriae adhesin:*nfaE*), capsules (*kpsMTII, kpsMTIII*), iron acquisition system (*fyuA, iuTA*), toxins (*hylA, cnf1, cdtB-A, cdtB-S*), and vasin and protein genes (*cvaT, ibeA,traT, rfc)* were detected using PCR using specific primer pairs as described in previous studies.[12,23,24] Positive and negative controls were used for each PCR assay.

Antibiotic Susceptibility Test

The antibiotic susceptibility was performed using disc diffusion assay against 13 antibiotics (beta-lactam: ampicillin/sulbactam, piperacillin/tazobactam; aminoglycosides: amikacin, gentamicin; 1^{st}–2^{nd} cephalosporins: cefazolin, cefuroxime; 3^{rd}–4^{th} generation cephalosporin: cefotaxime, cefepime; Carbapenem: ertapenem, meropenem; fluoroquinolone: levofloxacin; folate pathway/sulfamethoxazole: trimethoprim/sulfamethoxazole [TMP/SMX]), according to the recommendations of Clinical and Laboratory Standards Institute (CLSI) guidelines (CLSI, 2020). Quality control strains, *E. coli* ATCC25922 and *Pseudomonas aeruginosa* ATCC2785, were used for the tests.

Statistical Analysis

All statistical analyses were performed using SPSS statistical software for Windows version 23 (IBM Corporation, Armonk, NY, USA). Pearson's chi-square, Fisher's exact, and Mann–Whitney *U*-tests were used to compare variables between the different groups. Virulence scores were calculated as the sum of the virulence factors in each isolate. Statistical significance was set at $p < 0.05$.

Results

Characteristics of Patients and Clinical Data

A total of 171 patients with UTI were enrolled in the study, of whom 120 (70.18%) were diagnosed with UTI with bacteremia and 51 (29.82%) without bacteremia. The most common underlying disease was hypertension (54.4%), followed by upper UTI (46.2%), and diabetes mellitus (45.6%) (Table 1). As shown in Table 1, no significant differences were observed in the clinical characteristics between the two groups, except for hypertension and septic shock, which showed that patients with bacteremia had a higher prevalence of hypertension (60% vs 41.2%; p=0.024) and septic shock (30.8% vs 9.8%; p=0.003) than patients without bacteremia. Notably, patients with UTI with bacteremia had a longer average length of hospital stay than patients with UTI without bacteremia (9.52 ± 4.14 vs 7.73 ± 3.46; p=0.007) (Table 1).

In this study, phylogenetic groups were grouped based on the presence or absence *chuA*, y*jaA*, and *TSPE4-2*. Of the 171 isolates, 118 (69%) belonged to group B2, followed by groups D (19.9%), A (5.8%), and B1 (5.3%). Phylogenetic groups B2 and D dominated in the two patient groups. The phylogenetic groups did not differ significantly between patients with UTI with and without bacteremia (Table 1).

Prevalence of Virulence Factors in UTI and Bacteremic UTI Groups

To determine the pathogenicity and association between virulence factors and phylogenetic groups (high vs low virulence) in patients with UTI and bacteremia, 26 virulence genes classified into 5 groups were examined and analyzed. The prevalence of virulence factors detected in isolated strains has been 93.6% for *fimH*, 85.4% for *fyuA*, 68.4% for *iutA*, 67.8% for *traT* and *kpsMTII*, 62.6% for *papAH*, 61.4% for *papC*,60.2% for *papEF* and 50.3% for *papGIII* (Table 2).

Table 1 Demographic Characteristics of the Study Patients and Isolated Strains

	All (n = 171)	Isolation site		*P*-value
		Blood (n=120)	Urine (n=51)	
Gender				<0.001¥
Male	36 (21.1%)	16 (13.3%)	20 (39.2%)	
Age				0.490¥
≧65 yr	104 (60.8%)	75 (62.5%)	29 (56.9%)	
BMI				0.957¥
<24	76 (44.4%)	54 (45.0%)	22 (43.1%)	
≧24 and <27	44 (25.7%)	31 (25.8%)	13 (25.5%)	
≧27	51 (29.8%)	35 (29.2%)	16 (31.4%)	
Mean white blood cell (10^3/uL)	12.54±5.74	12.42±6.03	12.83±5.05	0.675*
Mean white blood cell group				0.907¥
≦12,000	85 (49.7%)	60 (50.0%)	25 (49.0%)	
>12,000	86 (50.3%)	60 (50.0%)	26 (51.0%)	
Urine glucose				0.749¥
Normal	129 (75.4%)	89 (74.2%)	40 (78.4%)	
Trace (±)	14 (8.2%)	11 (9.2%)	3 (5.9%)	
1+, 2+, 3+	28 (16.4%)	20 (16.7%)	8 (15.7%)	
Hospital stay length (days)	8.98±4.03	9.52±4.14	7.73±3.46	0.007*
Charlson comorbidity index	4.53±3.06	4.76±3.12	4.00±2.88	0.139*
History of UTI				0.232¥
Over one time	44 (25.7%)	34 (28.3%)	10 (19.6%)	
Underlying disease				
Diabetes mellitus	78 (45.6%)	57 (47.5%)	21 (41.2%)	0.448¥
Hypertension	93 (54.4%)	72 (60%)	21 (41.2%)	0.024¥
Acute kidney injury	23 (13.5%)	19 (15.8%)	4 (7.8%)	0.161¥
Septic shock	42 (24.6%)	37 (30.8%)	5 (9.8%)	0.003¥
Chronic kidney disease	37 (21.6%)	27 (22.5%)	10 (19.6%)	0.674¥
Upper UTI	79 (46.2%)	60 (50.0%)	19 (37.3%)	0.126¥
MDR	67 (39.2%)	42 (35.0%)	25 (49.0%)	0.086¥
Phylogenetic group (bacteria)				
A	10 (5.8%)	4 (3.3%)	6 (11.8%)	0.067¥
B1	9 (5.3%)	8 (6.7%)	1 (2.0%)	0.283¥
B2	118 (69.0%)	82 (68.3%)	36 (70.6%)	0.771¥
D	34 (19.9%)	26 (21.7%)	8 (15.7%)	0.370¥
B2+D	152 (88.9%)	108 (90.0%)	44 (86.3%)	0.478¥
A+B1	19 (11.1%)	12 (10.0%)	7 (13.7%)	0.478¥

Notes: *:*T*-test; ¥:Chi-Square test or Fisher's exact test.

Table 2 Prevalence of Virulent Factors Identified from Pathogenic Strains in UTI Infected Patients with or Without Bacteremia

	All (n = 171)	Isolation site		*P*-value
		Blood (n=120)	Urine (n=51)	
Virulence genes				
traT	116 (67.8%)	82 (68.3%)	34 (66.7%)	0.831¥
cvaC	9 (5.3%)	5 (4.2%)	4 (7.8%)	0.454¥
kpsMTII	116 (67.8%)	83 (69.2%)	33 (64.7%)	0.568¥
kpsMTIII	1 (0.6%)	1 (0.8%)	0 (0%)	1.000¥
rfc	17 (9.9%)	13 (10.8%)	4 (7.8%)	0.550¥
fyuA	146 (85.4%)	99 (82.5%)	47 (92.2%)	0.102¥

(*Continued*)

Table 2 (Continued).

	All (n = 171)	Isolation site		*P*-value
		Blood (n=120)	Urine (n=51)	
iutA	117 (68.4%)	81 (67.5%)	36 (70.6%)	0.691¥
hlyA	38 (22.2%)	28 (23.3%)	10 (19.6%)	0.592¥
cnf1	8 (4.7%)	4 (3.3%)	4 (7.8%)	0.241¥
cdtB-A	N.D	N.D	N.D	
cdtB-S	N.D	N.D	N.D	
fimH	160 (93.6%)	111 (92.5%)	49 (96.1%)	0.509¥
focG	15 (8.8%)	11 (9.2%)	4 (7.8%)	1.000¥
sfaS	30 (17.5%)	19 (15.8%)	11 (21.6%)	0.367¥
papAH	107 (62.6%)	77 (64.2%)	30 (58.8%)	0.509¥
papC	105 (61.4%)	79 (65.8%)	26 (51.0%)	0.068¥
papEF	103 (60.2%)	76 (63.3%)	27 (52.9%)	0.204¥
papGI	N.D	N.D	N.D	
papGII	86 (50.3%)	67 (55.8%)	19 (37.3%)	0.026¥
papGIII	3 (1.8%)	1 (0.8%)	2 (3.9%)	0.212¥
sfa/focDE	30 (17.5%)	15 (12.5%)	15 (29.4%)	0.008¥
bmaE	N.D	N.D	N.D	
afa/draBC	7 (4.1%)	4 (3.3%)	3 (5.9%)	0.427¥
gafD	N.D	N.D	N.D	
nfaE	N.D	N.D	N.D	
ibeA	7 (4.5%)	5 (4.8%)	2 (3.9%)	1.000¥
5 major groups				
Adhesins	169 (98.8%)	118 (98.3%)	51 (100.0%)	1.000¥
P fimbriae	123 (71.9%)	90 (75.0%)	33 (64.7%)	0.171¥
M fimbriae	N.D	N.D	N.D	
S and FIC fimbriae	51 (29.8%)	29 (24.2%)	22 (43.1%)	0.013¥
Type I fimbriae	160 (93.6%)	111 (92.5%)	49 (96.1%)	0.509¥
Dr-binding adhesin	7 (4.1%)	4 (3.3%)	3 (5.9%)	0.427¥
N-acetyl D-glucosamine specific fimbriae	N.D	N.D	N.D	
Non-fimbriae adhesin	N.D	N.D	N.D	
Capsule	117 (68.4%)	84 (70.0%)	33 (64.7%)	0.496¥
Iron acquisition system	157 (91.8%)	109 (90.8%)	48 (94.1%)	0.559¥
Toxins	40 (23.4%)	28 (23.3%)	12 (23.5%)	0.978¥
Invasins and protectin	123 (71.9%)	88 (73.3%)	35 (68.6%)	0.531¥

Note: ¥:Chi-Square test or Fisher's exact test.
Abbreviations: N.D, Not-detected.

Interestingly, the bacteremic UTI group had a significantly higher prevalence of *pap*GII (P fimbriae) than the UTI group (55.8% vs 37.3%; p=0.026) (Table 2). However, the bacteremic UTI group had a significantly lower prevalence of sfa/focDE (S and FIC fimbriae) than the UTI group (12.5% vs 29.4%; p=0.008) (Table 2). The UTI group had a significantly higher prevalence of S and FIC fimbriae adhesins than the bacteremic UTI group (43.1% vs 24.2%; p=0.013) (Table 2).

Association among strain virulence, virulence factors, and demographic factors in patients with UTI with or without bacteremia

The prevalence of virulence factors in high (B2 and D strains) and low-virulence strains (A and B1 strains) isolated from patients with bacteremic UTI and UTI is presented in Table 3. As expected, highly virulent *E. coli* strains were dominant in patients with bacteremic UTI (90%) and UTI (86.27%) (Table 3). In contrast to low virulence group, high virulence group had significantly high prevalence of pap gene cluster including *papC* (bacteremic UTI: 70.4% vs 25.0%; p=0.03; UTI: 59.1% vs 0%; p=0.04), *papEF* (bacteremic UTI: 66.7% vs 33.3%; p=0.03; UTI: 59.1% vs 14.3%; p=0.042) and *papGII* (bacteremic UTI: 59.3% vs 25.0%; p=0.023) in both bacteremic UTI and UTI patient groups (Table 3).

Table 3 Prevalence of Virulent Factors Identified from High/Low Pathogenic Strains in UTI Infected Patients with or without Bacteremia

All (n = 171)	Blood (n=120)		*P*-value	Urine (n=51)		*P*-value
	B2+D (n=108)	A+B1 (n=12)		B2+D (n=44)	A+B1 (n=7)	
Virulence gene						
Trat	75 (69.4%)	7 (58.3%)	0.516¥	32 (72.7%)	2 (28.6%)	0.034¥
Cvac	5 (4.6%)	0 (0%)	1.000¥	2 (4.5%)	2 (28.6%)	0.086¥
Kpsmtii	75 (69.4%)	7 (58.3%)	0.185¥	31 (70.5%)	2 (28.6%)	0.082¥
Kpsmtiii	1 (0.9%)	0 (0%)	1.000¥	N.D.	N.D.	
Rfc	8 (7.4%)	5 (41.7%)	0.003¥	4 (9.1%)	0 (0%)	1.000¥
Fyua	91 (84.3%)	8 (66.7%)	0.220¥	43 (97.7%)	4 (57.1%)	0.006¥
Iuta	76 (70.4%)	5 (41.7%)	0.056¥	33 (75%)	3 (42.9%)	0.174¥
Hlya	27 (25%)	1 (8.3%)	0.291¥	10 (22.7%)	0 (0%)	0.320¥
Cnf1	4 (3.7%)	0 (0%)	1.000¥	4 (9.1%)	0 (0%)	1.000¥
Cdtb-A	N.D.	N.D.		N.D.	N.D.	
Cdtb-S	N.D.	N.D.		N.D.	N.D.	
Fimh	100 (92.6%)	11 (91.7%)	1.000¥	43 (97.7%)	6 (85.7%)	0.258¥
Focg	11 (10.2%)	0 (0%)	0.600¥	4 (9.1%)	0 (0%)	1.000¥
Sfas	15 (13.9%)	4 (33.3%)	0.097¥	10 (22.7%)	1 (14.3%)	1.000¥
Papah	72 (66.7%)	5 (41.7%)	0.114¥	27 (61.4%)	3 (42.9%)	0.427¥
Papc	76 (70.4%)	3 (25%)	0.003¥	26 (59.1%)	0 (0%)	0.004¥
Papef	72 (66.7%)	4 (33.3%)	0.030¥	26 (59.1%)	1 (14.3%)	0.042¥
Papgi	N.D.	N.D.		N.D.	N.D.	
papGII	64 (59.3%)	3 (25%)	0.023¥	18 (40.9%)	1 (14.3%)	0.236¥
papGIII	1 (0.9%)	0 (0%)	1.000¥	2 (4.5%)	0 (0%)	1.000¥
sfa/focDE	15 (13.9%)	0 (0%)	0.358¥	11 (25%)	4 (57.1%)	0.174¥
bmaE	N.D.	N.D.		N.D.	N.D.	
afa/draBC	4 (3.7%)	0 (0%)	1.000¥	3 (6.8%)	0 (0%)	1.000¥
gafD	N.D.	N.D.		N.D.	N.D.	
nfaE	N.D.	N.D.		N.D.	N.D.	
ibeA	5 (5.1%)	0 (0%)	1.000¥	2 (4.5%)	0 (0%)	1.000¥
5 Major groups						
Adhesins	106 (98.1%)	12 (100%)	1.000¥	44 (100%)	7 (100%)	
P fimbriae	85 (78.7%)	5 (41.7%)	0.010¥	29 (65.9%)	4 (57.1%)	0.686¥
M fimbriae	N.D.	N.D.		N.D.	N.D.	
S and FIC fimbriae	25 (23.1%)	4 (33.3%)	0.481¥	17 (38.6%)	5 (71.4%)	0.216¥
Type I fimbriae	100 (92.6%)	11 (91.7%)	1.000¥	43 (97.7%)	6 (85.7%)	0.258¥
Dr-binding adhesin	4 (3.7%)	0 (0%)	1.000¥	3 (6.8%)	0 (0%)	1.000¥
N-acetyl D-glucosamine specific fimbriae	N.D.	N.D.		N.D.	N.D.	
Non-fimbriae adhesin	N.D.	N.D.		N.D.	N.D.	
Capsule	78 (72.2%)	6 (50%)	0.180¥	31 (70.5%)	2 (28.6%)	0.082¥
Iron acquisition system	100 (92.6%)	9 (75%)	0.080¥	43 (97.7%)	5 (71.4%)	0.046¥
Toxins	27 (25%)	1 (8.3%)	0.291¥	12 (27.3%)	0 (0%)	0.177¥
Invasins and protectin	80 (74.1%)	8 (66.7%)	0.731¥	33 (75%)	2 (28.6%)	0.025¥
Hospital stay length (days)	9.66±4.28	8.25±2.45	0.266*	7.45±2.91	9.43±5.94	0.481**
Charlson comorbidity index	4.79±3.1	4.50±3.42	0.762*	3.64±2.76	6.29±2.69	0.022*
Underlying Diseases (patients)						
Diabetes mellitus	55 (50.9%)	2 (16.7%)	0.024¥	17 (38.6%)	4 (57.1%)	0.427¥
Hypertension	64 (59.3%)	8 (66.7%)	0.761¥	15 (34.1%)	6 (85.7%)	0.015¥
Acute kidney injury	18 (16.7%)	1 (8.3%)	0.688¥	2 (4.5%)	2 (28.6%)	0.086¥
Septic shock	36 (33.3%)	1 (8.3%)	0.102¥	5 (11.4%)	0 (0%)	1.000¥
Chronic kidney disease	25 (23.1%)	2 (16.7%)	1.000¥	6 (13.6%)	4 (57.1%)	0.021¥
Upper UTI	54 (50%)	6 (50%)	1.000¥	19 (43.2%)	0 (0%)	0.037¥
MDR	38 (35.2%)	4 (33.3%)	1.000¥	22 (50%)	3 (42.9%)	1.000¥

Notes: *:*T*-test; **:Mann–Whitney *U*-test; ¥:Chi-Square test or Fisher's exact test;
Abbreviation: ND, Not detected.

According to the virulence gene classification, the highly virulent bacteremic UTI group had a significantly higher prevalence of P fimbriae adhesin than the low-virulence bacteremic UTI group (78.7% vs 41.7%; p=0.01). In iron acquisition system related genes, high prevalence related genes were found in high virulent UTI group (bacteremic UTI: 92.6% vs 75.0%; p=0.08; UTI: 75.0 vs 28.6%; p=0.025) (Table 3). Notably, the highly virulent UTI group had a significantly higher prevalence of invasin and protectin genes than the low-virulence group (75% vs 28.6%; p=0.025), whereas no significant difference was observed in the bacteremic UTI group (74.1% vs 66.7%; p=0.731) (Table 3).

To determine the association between strain virulence and demographic factors, the Charlson Comorbidity Index (CCI), length of hospital stay, and related diseases were analyzed (Table 3). Patients with a high CCI were more likely to be in low virulent UTI strain group than in high virulent strain group (6.29 ± 2.69 vs 3.64 ± 2.76; p=0.022) (Table 3). Notably, the highly virulent UTI group had a significantly higher prevalence of upper UTI than the low-virulence UTI group (43.2% vs 0%, p=0.037) (Table 3). However, the upper UTI rate did not differ significantly between the high- and low-virulence bacteremic UTI patient groups (50.0% vs 50.0%, p=1.000) (Table 3). In addition, multidrug resistance (MDR) rates did not differ significantly between the bacteremic UTI and UTI patient groups (bacteremic UTI: 35.2% vs 33.3%; p=1.000; UTI: 50.0% vs 42.9%; p=1.000) (Table 3).

Virulence scores differed significantly between high- and low-virulence strains (Table 4). Highly virulent strains recovered from both blood and urine samples had significantly higher virulence scores, especially adhesin and iron acquisition system gene scores (Table 4). Notably, the highly virulent UTI group had significantly higher capsule gene scores than the low-virulence UTI strain group (Table 4).

Association among MDR, virulence scores, and demographic factors in patients with UTI with or without bacteremia

To analyze MDR and the association between virulence scores and demographic factors in patients, antibiotic susceptibility tests were performed. The results showed that all isolates were susceptible to ertapenem and meropenem. Notably, the isolated strains were more susceptible to amikacin (98.8%) and piperacillin/tazobactam (96.5%) but showed high rates of resistance to cefazolin (69.6%) and TMP/SMX (49.7%). Notably, no significant differences in drug resistance patterns were found between high and low-virulence strains.

Of the 171 isolates, 39.18% (67/171) were MDR strains. In contrast to the bacteremic UTI group, the UTI group had a higher prevalence of MDR (25/26, 49.02% vs 42/120, 35%) (Table 5). As shown in Table 5, the MDR rates in the different phylogenetic groups did not differ significantly between the bacteremic UTI and UTI patient groups (Table 5).

Table 4 Comparison of Virulence Scores Calculated from High/Low Pathogenic Strains in UTI Infected Patients with or Without Bacteremia

All (n = 171)	**Isolation site**					**P value**
	Blood(n=120)			**Urine (n=51)**		
Virulence gene score	7.12±2.82			6.94±2.75		0.708*
Adhesins gene score	3.83±1.93			3.65±1.84		0.559*
Capsule gene score	0.70±0.46			0.65±0.48		0.499*
Iron acquisition system gene score	1.50±0.66			1.63±0.60		0.238*
Toxins score	0.27±0.51			0.27±0.53		0.928*
Invasins and protectin gene score	0.88±0.64			0.86±0.69		0.911*
High/Low virulent strains	**B2+D (n=108)**	**A+B1 (n=12)**	**P value**	**B2+D (n=44)**	**A+B1 (n=7)**	**P value**
Virulence gene score	7.33±2.70	5.17±3.24	0.011*	7.43±2.56	3.86±1.68	0.001*
Adhesin gene score	3.98±1.87	2.50±1.98	0.011*	3.86±1.88	2.29±0.76	0.035**
Capsule gene score	0.72±0.45	0.50±0.52	0.113*	0.70±0.46	0.29±0.49	0.031*
Iron acquisition system gene score	1.55±0.63	1.08±0.79	0.021*	1.73±0.50	1.00±0.82	0.002*
Toxins score	0.29±0.53	0.08±0.29	0.191**	0.32±0.56	0±0	0.120**
Invasins and protectin gene score	0.86±0.62	1.00±0.85	0.480*	0.91±0.64	0.57±0.98	0.235*

Notes: *:*T*-test; **:Mann–Whitney *U*-test.

Table 5 Characteristics of MDR and Non-MDR Strains Isolated from UTI Infected Patients with or Without Bacteremia

All (n = 171)	Drug resistance pattern					P value
	Non-MDR (n=104)			MDR (n=67)		
Phylogenetic groups						
A	7 (6.7%)			3 (4.5%)		0.742¥
B1	5 (4.8%)			4 (6%)		0.739¥
B2	70 (67.3%)			48 (71.6%)		0.550¥
D	22 (21.2%)			12 (17.9%)		0.604¥
B2+D	92 (88.5%)			60 (89.6%)		0.825¥
A+B1	12 (11.5%)			7 (10.4%)		0.825¥
Diseases						
Diabetes mellitus	51 (49.0%)			27 (40.3%)		0.263¥
Hypertension	57 (54.8%)			36 (53.7%)		1.000¥
Acute kidney injury	7 (6.7%)			16 (23.9%)		0.001¥
Septic shock	23 (22.1%)			19 (28.4%)		0.355¥
Chronic kidney disease	25 (24.0%)			12 (17.9%)		0.342¥
Upper UTI	44 (42.3%)			35 (52.2%)		0.204¥
Bacteremia	78 (75.0%)			42 (62.7%)		0.086¥
Hospital stay length (days)	8.35±3.58			9.97±4.49		0.010*
Charlson comorbidity index	4.75±3.19			4.19±2.85		0.248*
Virulence scores						
Virulence gene score	7.34±2.68			6.64±2.93		0.112*
Adhesin gene score	3.97±1.86			3.48±1.93		0.097*
Capsule gene score	0.74±0.44			0.60±0.49		0.050**
Iron acquisition system gene score	1.55±0.64			1.52±0.66		0.800*
Toxins score	0.21±0.43			0.36±0.62		0.159**
Invasins and protectin gene score	0.97±0.69			0.72±0.57		0.013*
	Blood (n=120)			Urine (n=51)		
	Non-MDR (n=78)	MDR (n=42)	P value	Non-MDR (n=26)	MDR (n=25)	P value
Phylogenetic group						
A	4 (5.1%)	0 (0%)	0.296¥	3 (11.5%)	3 (12.0%)	1.000¥
B1	4 (5.1%)	4 (9.5%)	0.448¥	1 (3.8%)	0 (0%)	1.000¥
B2	54 (69.2%)	28 (66.7%)	0.773¥	16 (61.5%)	20 (80.0%)	0.148¥
D	16 (20.5%)	10 (23.8%)	0.676¥	6 (23.1%)	2 (8.0%)	0.248¥
B2+D	70 (89.7%)	38 (90.5%)	1.000¥	22 (84.6%)	22 (88.0%)	1.000¥
A+B1	8 (10.3%)	4 (9.5%)	1.000¥	4 (15.4%)	3 (12.0%)	1.000¥
Diseases						
Diabetes mellitus	38 (48.7%)	19 (45.2%)	0.716¥	13 (50%)	8 (32.0%)	0.192¥
Hypertension	48 (61.5%)	24 (57.1%)	0.639¥	9 (34.6%)	12 (48.0%)	0.332¥
Acute kidney injury	5 (6.4%)	14 (33.3%)	<0.001¥	2 (7.7%)	2 (8.0%)	1.000¥
Septic shock	20 (25.6%)	17 (40.5%)	0.093¥	3 (11.5%)	2 (8.0%)	1.000¥
Chronic kidney disease	20 (25.6%)	7 (16.7%)	0.261¥	5 (19.2%)	5 (20.0%)	1.000¥
Upper UTI	34 (43.6%)	26 (61.9%)	0.056¥	10 (38.5%)	9 (36.0%)	0.856¥
Hospital stay length (days)	8.88±3.76	10.69±4.59	0.022*	6.73±2.39	8.76±4.11	0.035*
Charlson comorbidity index	4.85±3.23	4.60±2.96	0.670*	4.46±3.11	3.52±2.58	0.247*
Virulence scores						
Virulence gene score	7.38±2.74	6.62±2.93	0.157*	7.19±2.53	6.68±2.98	0.511*
Adhesins gene score	4.01±1.92	3.5±1.92	0.166*	3.85±1.69	3.44±2	0.437*
Capsule gene score	0.76±0.43	0.6±0.5	0.067**	0.69±0.47	0.6±0.5	0.500*
Iron acquisition system gene score	1.55±0.64	1.4±0.7	0.248*	1.54±0.65	1.72±0.54	0.283*
Toxins score	0.22±0.45	0.36±0.62	0.262**	0.19±0.4	0.36±0.64	0.393*
Invasins and protectin gene score	0.94±0.67	0.76±0.58	0.158*	1.08±0.74	0.64±0.57	0.023*

Notes: *:*T*-test; **:Mann–Whitney *U*-test; ¥:Chi-Square test or Fisher's exact test.

To analyze the association between MDR and virulence scores, patients infected with non-MDR strains had significantly higher invasin and protectin gene score than those infected with MDR strains (0.97 ± 0.69 vs 0.72 ± 0.57; p=0.013) (Table 5).

Notably, MDR strains isolated from patients with bacteremia had a significantly higher prevalence of acute kidney injury than non-MDR strains (AKD: 33.3% vs 6.4%, p<0.001) (Table 5). A similar trend was found in patients with upper UTI (61.9% vs 43.6%, p=0.056) (Table 5). Expectedly, patients with bacteremic UTI infected with MDR strains had significantly long average length of hospital stay days than those infected with non-MDR strains (bacteremic UTI: 10.69 ± 4.59 vs 8.88 ± 3.76; p=0.022; UTI: 8.76 ± 4.11 vs 6.73 ± 2.39; p=0.035) (Table 5).

Discussion

In this molecular epidemiological analysis of pathogenic *E. coli* clinical strains from patients with UTI and urinary source bacteremia, we found that virulence factor is associated with strain virulence and UTI disease progression. The crucially important first step during infection is adhesion of *E. coli* strain to urothelial cell.[25] Alternatively, pathogen's ability to colonize and invade host cells strictly depend on adhesins such as fimbriae.[25,26] Compared to bacteremic UTI group, *E. coli* isolated from UTI group with high prevalence of *sfa/focDE* gene (belonged to S and FIC fimbriae) was observed (29.4% vs 12.5%; p=0.008). Besides, we also found that UTI group had significantly higher prevalence of S and FIC fimbriae adhesins than bacteremic UTI group (43.1% vs 24.2%; p=0.013). In this study, our results suggested S and FIC fimbriae might play an important role in the first step of infection. Previous studies also demonstrated that S and FIC fimbriae are correlated with UPEC pathogenicity and mediate binding to kidney cell.[27–29]

The role of adhesins in UTI to bacteremic UTI is not fully understood. In the bacteremic UTI group, we found that *sfa/focDE* gene was only detected from high virulent bacteremic group. Naziri et al showed that almost all (99%) of UPEC isolated had biofilm formation ability, whereas only presence of *sfa/focDE* gene was significantly association with moderate and strong biofilm formation.[30] The role of *sfa/focDE* gene in pathogenic strains associated with disease progression (UTI to bacteremic UTI) remains to be investigate experimentally. In addition, high virulent strain had a significantly higher prevalence of P fimbriae including *papC, papEF* and *papGII* (Table 2, Table 3). Besides, our results showed that high virulent bacteremic UTI groups had significantly higher prevalence of *papGII* gene than low virulent group (Table 3). Recently, Biggel et al using genome-wide association approach to analyze 385 invasive UPEC isolates and 337 non-invasive UPEC isolates.[17] They reported *papGII* locus as the key features specifically associated with invasiveness and severe UTI.[17]

Interestingly, we found no significant difference in virulence scores and prevalence of MDR between unclassified strains isolated from blood and urine (Table 1, Table 4). Kim et al showed the similar trends by using whole genome sequencing technique to analyze 80 clinical strains.[31] However, virulence scores were significantly different between high virulent and low virulent groups. In general, high virulent strains had significantly high virulence scores than low virulent strains (Table 4). Besides, our results showed that both bacteremic and MDR strain groups has directly impacted extended hospital stays. The disease progression and severity associated with virulence factors needs to be confirmed experimentally.

Diabetes has been reported to be associated with UTI infection.[7,32] In our study, high virulent strains were dominant at bacteremic UTI with diabetes (50.9%; 55/108). Over all, 96.5% (55/57) of bacteremic UTI patients with diabetes were infected with high virulent *E. coli* strains (B2:36, 63.2%; D:19, 33.3%). The similar trend was observed in patient with upper UTI. All upper UTI patients were infected with high virulent strains. In this study, high virulent strains have been found to be associated with pathogenesis of UTI in patients with diabetes or upper UTI.

In the past decade, an increase and spread of MDR UPEC strains has been observed and become a public health concern, particularly in women with recurrent UTIs.[21,33] In the study, 39.18% MDR UPEC strains were isolated. In addition, no significant difference in the MDR UPEC isolation rates between high virulent and low virulent or bacteremic UTI and UTI groups (Table 1, Table 3). Interestingly, MDR strains were significantly less virulent than non MDR strains (Table 5). However, large multiple-center cohort studies must be initiated to confirm the relationship between drug resistance and bacteria virulence. To adapt and survival in the host, the pathogen modulates its virulence potential and fitness.[34] Some studies reported that bacterial virulence and fitness are directly or indirectly affected by drug

resistance.[35–37] Hwang et al reported that MDR strains were significantly less virulent than drug sensitive strains.[37] Pathogens occur in the presence of various selective pressures promote its evolution in a direction to increase the survival fitness.[38]

This study has several limitations. First limitation of the present study was small sample size in certain subgroup, which reduced statistical power for detecting population differences. We hope to initiate a large-scale collection in multiple centers to further clarify association between virulence factors and host diseases. Second, it will be worth comparing the characteristics of isolated strains including prevalence of virulence factors and drug resistant pattern to those collect from the different region of hospital.

In summary, this study provided insight into the contributing factors in patients with UTI to urinary source bacteremia. Our results revealed that P fimbriae (including *papC, papEF* and *papGII*) and *sfa/focDE* might be associated with invasiveness and disease progression, respectively. These findings have implications for the pathogenesis and control measures in patients with UTI infection.

Ethical Statement

This study was approved by the Institutional Review Board of Ditmanson Medical Foundation Chia-Yi Christian Hospital (IRB NO: CYCH-106040). Informed consent was obtained from all study participants prior to study commencement. This study complied with the Declaration of Helsinki.

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Disclosure

The authors declare they have no conflicts of interest.

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