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Original Article

Escherichia coli urinary tract infections: Host age-related differences in bacterial virulence factors and antimicrobial susceptibility

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KEYWORDS
Age-related differences;
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Abstract *Background:* Urinary tract infections (UTIs) caused by uropathogenic *Escherichia coli* (UPEC) are one of the most common outpatient bacterial infections. Although bacterial and host factors are reported to be associated with UTI pathogenesis, little is known about the host age-related differences in bacterial virulence factors and antimicrobial susceptibility.
Methods: PCRs were carried out to detect K1 capsule antigen, 15 virulence factors, and phylogenetic groups in *E. coli* isolates. Antimicrobial susceptibility of selected agents was determined by the disk diffusion method. Isolates were divided into 6 groups based on their host age.

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Urinary tract infection;
Virulence factors

Results: The results showed that virulence factors PapGII, PapGIII, Cnf1, Aer, Usp, Iha, OmpT, HlyA, and Sat, had highest frequencies in the host age group 0—3. Phylogenetic group B2 dominated in our isolates (59.6%) followed by group D (20.7%). In addition, 77.4% of strains isolated from 0 to 3 age group belonged to phylogenetic group B2. Antimicrobial susceptibility tests showed that *E. coli* strains isolated were significantly more resistant to antimicrobial agents as host age increased. Phylogenetic group B2 isolates were more susceptible to antimicrobial agents, compared to A, B1, and D isolates.
Conclusion: We found *E. coli* isolated from elders were more resistant to antimicrobial agents and had less virulence factors.

Introduction

Urinary tract infections (UTIs) are one of the most common outpatient bacterial infections with a lifetime incidence of 50—60% in adult women.[1] Lower UTIs affect the urethra and bladder. In contrast, upper UTIs affect the function of kidneys and could be potentially life threatening when bacteria invade into the bloodstream from infected kidneys, a condition called urosepsis.[2] Host risk factors associated with UTIs include host immunodeficiency, urinary tract abnormality, bladder dysfunction in type 2 diabetes, behavioral factors, and estrogen deficiency.[3]

Bacterial factors are also reported to be associated with UTI pathogenesis and progression.[1,4—7] Uropathogenic *Escherichia coli* (UPEC) is the dominant infectious pathogen in both uncomplicated and complicated UTIs.[1] In addition, UPEC strains show great diversity in gene content, virulence factors, genomic islands, and pathogenicity islands.[8] Previous studies showed that regarding the bacterial characteristics in diabetic patients with *E. coli* causing UTIs, the isolated *E. coli* strains had more *neuA*, *papG*II, *afa* and *hlyA* virulence genes.[9] In patients with upper UTIs, the *papG* class II gene plays a critical role in the development of *E. coli* bacteremia.[7] Moreover, FimH adhesin plays a role not only in lower UTI, but also in kidney infection by acting synergistically with PapGII adhesin.[5] Carriage of putative urovirulence factors is thought to enhance *E. coli* uropathogenicity and is used to measure and categorize clinical UPEC strains isolated from different patient populations.[10—12]

Although the association of host factors and bacterial virulence genes with the pathogenesis of UTIs caused by *E. coli* has been reported.[13,14] The host age-related differences in bacterial virulence factors and antimicrobial susceptibility are still unknown. Therefore, in this study, we aimed to compare the characteristics of *E. coli* strains isolated from UTI patients in the different age groups.

Materials and methods

Sampling and isolation of UTI *E. coli* isolates

UTI *E. coli* isolates were recovered from National Cheng Kung University hospital, 2009 to 2010. The Ethics Committee approved that no formal ethical approval was needed to use these clinically obtained materials because the isolates were remnants from patient samples, and the data were analyzed anonymously. *E. coli* isolates were identified in the clinical laboratory by colony morphology, Gram stain, biochemical tests, and the Vitek system (bioMérieux, Marcy l'Etoile, France) according to the manufacturer's recommendations. A total of 1512 non-duplicate clinical isolates were collected and stored at −80 °C in lysogeny broth (LB) containing 20% glycerol (v/v) until used.

Virulence factors identification

K1 capsule antigen and 15 virulence factor genes of UPEC were detected by PCR. Primer pairs specific for K1 capsule gene (*neuA*), 3 PapG adhesion genes of P-fimbriae (*papG* class I to III), type 1 fimbrial adhesins (*fimH*), S-/F1C-fimbriae (*sfa*/*foc*), afimbrial adhesins (*afa*), iron regulated gene A homologue adhesins (*iha*), hemolysin (*hlyA*), cytotoxic necrotizing factor 1 (*cnf1*), catecholate siderophore receptor (*iroN*), aerobactin receptor (*iutA*), outer membrane protease T (*ompT*), and uropathogenic specific protein (*usp*), were described in our previous studies.[13,15] PCRs were carried out in duplicate, and positive and negative control strains for the traits of interest were included in each assay.

Phylogenetic grouping

Based on PCR amplification patterns of specific genetic markers (*chuA*, *yjaA*, and TSPE4.C2), *E. coli* strains were divided into four phylogenetic groups: A, B1, B2, and D according to previous study.[16]

Antimicrobial susceptibility testing

The antimicrobial susceptibility was experimentally determined through disc diffusion assay against 17 antibiotics (amikacin, amoxiclav, ampicillin, cefazolin, cefixime, cefmetazole, cefpirome, ceftriaxone, cefuroxime, ciprofloxacin, cotrimoxazole, ertapenem, gentamicin, imipenem, meropenem, levofloxacin, and piptazobactam) in accordance with the Clinical and Laboratory Standards Institute (CLSI) guidelines.[17] *E. coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853 were used as quality control strains. The interpretation of resistance to these antimicrobial

Table 1 The association between bacterial virulence factors, host age groups, and host gender in 907 *E. coli* isolates.

Virulence factors[a]	Age groups (years old) (No. (%) of *E. coli* isolates)						total (n = 907)	*p* value[c]	Gender (No. (%) of *E. coli* isolates)		*p* value[d]
	0-3 (n = 62)	4-20 (n = 45)	21-40 (n = 110)	41-60 (n = 198)	61-80 (n = 346)	81-100 (n = 146)			Male (n = 213)	Female (n = 694)	
PapGII	36 (58.1)	14 (31.1)[b]	45 (40.9)[b]	52 (26.3)[b]	86 (24.9)[b]	29 (19.9)[b]	262 (28.9)	**<0.001**	68 (31.9)	194 (28.0)	0.263
PapGIII	13 (21.0)	4 (8.9)	21 (19.1)	33 (16.7)	39 (11.3)[b]	15 (10.3)[b]	125 (13.8)	**0.018**	35 (16.4)	90 (13.0)	0.200
Sfa	4 (6.5)	0 (0)	10 (9.1)	19 (9.6)	19 (5.5)	13 (8.9)	65 (7.2)	0.643	15 (7.0)	50 (7.2)	0.936
Foc	7 (11.3)	13 (28.9)[b]	16 (14.5)	25 (12.6)	20 (5.8)	9 (6.2)	90 (9.9)	**<0.001**	25 (11.7)	65 (9.4)	0.311
Cnf1	21 (33.9)	14 (31.1)	31 (28.2)	52 (26.3)	44 (12.7)[b]	21 (14.4)[b]	183 (20.2)	**<0.001**	65 (30.5)	118 (17.0)	**<0.001**
Aer	45 (72.6)	27 (60.0)	62 (56.4)[b]	113 (57.1)[b]	220 (63.6)	103 (70.5)	570 (62.8)	0.405	131 (61.5)	439 (63.3)	0.643
Usp	48 (77.4)	34 (75.6)	80 (72.7)	129 (65.2)	190 (54.9)[b]	85 (58.2)[b]	566 (62.4)	**<0.001**	139 (65.3)	427 (61.5)	0.325
Iha	30 (48.4)	17 (37.8)	32 (29.1)[b]	67 (33.8)[b]	123 (35.5)	57 (39.0)	326 (35.9)	0.652	87 (40.8)	239 (34.4)	0.080
OmpT	54 (87.1)	38 (84.4)	95 (86.4)	156 (78.8)	254 (73.4)[b]	106 (72.6)[b]	703 (77.5)	**<0.001**	167 (78.4)	536 (77.2)	0.720
Afa	20 (32.3)	18 (40.0)	43 (39.1)	111 (56.1)[b]	210 (60.7)[b]	79 (54.1)[b]	481 (53.0)	**<0.001**	96 (45.1)	385 (55.5)	**0.008**
IroN	23 (37.1)	16 (35.6)	53 (48.2)	83 (41.9)	126 (36.4)	45 (30.8)	346 (38.1)	0.089	73 (34.3)	273 (39.3)	0.183
FimH	61 (98.4)	43 (95.6)	109 (99.1)	195 (98.5)	322 (93.1)	131 (89.7)[b]	861 (94.9)	**<0.001**	198 (93.0)	663 (95.5)	0.134
HlyA	25 (40.3)	12 (26.7)[b]	31 (28.2)	53 (26.8)[b]	64 (18.5)[b]	24 (16.4)[b]	209 (23.0)	**<0.001**	68 (31.9)	141 (20.3)	**<0.001**
Sat	29 (46.8)	17 (37.8)	29 (26.4)[b]	62 (31.3)[b]	113 (32.7)[b]	56 (38.4)	306 (33.7)	0.608	82 (38.5)	224 (32.3)	0.093
K1 antigen	15 (24.2)	14 (31.1)	41 (37.3)	50 (25.3)	83 (24.0)	32 (21.9)	235 (25.9)	0.075	44 (20.7)	191 (27.5)	**0.045**

Bold font indicates statistical significance.

[a] Virulence factor PapGI was not detected in all 907 isolates.

[b] Comparison of virulence factors in the tested age group to age group 0—3. Statistically significant at $p < 0.05$.

[c] The association of bacterial virulence factors with host age groups was determined by Cochran—Armitage test.

[d] The association of bacterial virulence factors with host gender was determined by Pearson's Chi-square test.

agents was determined according to the recommendations of the CLSI.[17]

Statistical analysis

Pearson's Chi-square test or Fisher exact test was used for comparing categorical variables. Cochran–Armitage test for trend was used to determine significant trends in virulence factors and bacterial antibiotic susceptibility by age. All statistical analyses were performed using IBM SPSS statistics version 24.0 (IBM Corporation, Armonk, NY, USA). A p value < 0.05 was taken as significant.

Results

The prevalence of bacterial virulence factors is associated with host age and gender

To characterize the UTI *E. coli* isolated from different host age groups and gender, we determined the presence of K1 capsule antigen and 15 virulence factors in 907 randomly selected isolates (Table 1). Isolates were divided into 6 groups based on their host age to ensure the appropriate sample size for detecting a statistical difference between groups (Table 1). Strains isolated from 0 to 3 age group are relative rare to other age groups, and thus we had one group less than 3 years of age as a single cohort (Table 1). Overall, virulence factors Aer, Usp, OmpT, and FimH, were found in more than 60% of 907 isolates (Table 1). The prevalence of PapGII ($p < 0.001$), PapGIII ($p = 0.018$), Foc ($p < 0.001$), CnfI ($p < 0.001$), Usp ($p < 0.001$), OmpT ($p < 0.001$), FimH ($p < 0.001$), and HlyA ($p < 0.001$), was significantly decreased following increasing age but Afa ($p < 0.001$) increased by p for trend (Table 1). When the strains isolated from 6 host age groups were compared, we found strains isolated from elderly age groups (>60 years old) had less virulence factors such as PapGIII, Cnf1, Usp, OmpT, and HlyA, compared to other 4 groups (Table 1). Afa showed significantly higher frequency in the >40 years old age groups (Table 1). Interestingly, PapGII, PapGIII, Cnf1, Aer, Usp, Iha, OmpT, HlyA, and Sat, showed highest frequencies in the 0–3 age group (Table 1). We further compared the distribution of virulence factors based on host gender, and the results showed that strains isolated from male UTI patients had more Cnf1 (30.5% vs 17.0%, $p < 0.001$) and HlyA (31.9% vs 20.3%, $p < 0.001$), compared to strains isolated from female UTI patients (Table 1). In contrast, strains isolated from female UTI patients had more Afa (55.5% vs 45.1%, $p = 0.008$) and K1 capsule antigen (27.5% vs 20.7%, $p = 0.045$) (Table 1).

Bacterial phylogenetic groups are associated with host age groups and gender

Phylogenetic analysis has shown that *E. coli* strains fall into four main groups (A, B1, B2, and D).[17] We next examined the distribution of phylogenetic groups among 907 isolates by host ages and gender (Table 2). The results showed that the distribution of phylogenetic groups in UTI *E. coli* isolates was associated with host age ($p = 0.001$) (Table 2). In addition, phylogenetic group B2 dominated in our isolates (541 isolates, 59.6%), followed by group D (188 isolates, 20.7%) (Table 2). Interestingly, no phylogenetic group B1 strains were isolated from 0 to 3 age group, and only 2 (3.2%) group A strains were isolated from this age group (Table 2). Moreover, 77.4% of strains isolated from 0 to 3 age group belonged to phylogenetic group B2 (Table 2). The distribution of phylogenetic groups among 907 isolates was not associated with host gender ($p = 0.084$) (Table 2).

We next determined whether the prevalence of virulence factors in group B2 strains isolated from 6 age groups is different (Table 3). The results showed that virulence factors Usp (96.3%), OmpT (98.2%), and FimH (99.3%), were found in more than 96% of 541 B2 isolates (Table 3). PapGII, HlyA, and Sat, showed highest frequencies in the 0–3 age group, compared to other age groups (Table 3). Afa showed significantly higher frequency in the >40 years old age groups (Table 3). However, the characteristics and virulence of B2 strains isolated from different age groups remain to be investigated.

Antimicrobial susceptibility of UTI isolates are associated with host age groups and gender

Previous studies showed the association between phylogenetic group distributions, virulence factors and antimicrobial resistance properties of *E. coli* strains isolated from patients with UTIs.[4,18] Therefore, we next aimed to determine the

Table 2 The association between bacterial phylogenetic grouping, host age groups, and host gender.

Phylogenetic Group	Age groups (years old) (No. (%) of *E. coli* isolates)						total (n = 907)	*p* value[a]	Gender (No. (%) of *E. coli* isolates)		*p* value[a]
	0-3 (n = 62)	4-20 (n = 45)	21-40 (n = 110)	41-60 (n = 198)	61-80 (n = 346)[a]	81-100 (n = 146)[a]			Male (n = 213)	Female (n = 634)	
A	2 (3.2)	2 (4.4)	4 (3.6)	15 (7.6)	51 (14.7)	21 (14.4)	95 (10.5)	**0.001**	23 (10.8)	72 (11.4)	0.084
B1	0 (0)	4 (8.9)	10 (9.1)	16 (8.1)	40 (11.6)	13 (8.9)	83 (9.2)		12 (5.6)	71 (11.2)	
B2	48 (77.4)	33 (73.3)	74 (67.3)	121 (61.1)	182 (52.6)	83 (61.0)	541 (59.6)		140 (65.7)	401 (63.2)	
D	12 (19.4)	6 (13.3)	22 (20.0)	46 (23.2)	73 (21.2)	29 (19.9)	188 (20.7)		38 (17.8)	150 (23.7)	

Bold font indicates statistical significance.

[a] The association of bacterial phylogenetic grouping with host age and gender was determined by Pearson's Chi-square test.

Table 3 The association between bacterial virulence factors and host age groups in 541 *E. coli* B2 isolates.

Virulence factors[a]	Age groups (years old) (No. (%) of *E. coli* isolates)						total (n = 541)	*p* value[b]
	0-3 (n = 48)	4-20 (n = 33)	21-40 (n = 74)	41-60 (n = 121)	61-80 (n = 182)	81-100 (n = 83)		
PapGII	32 (66.7)	12 (36.4)	34 (45.9)	40 (33.1)	63 (34.6)	24 (28.9)	205 (37.9)	**<0.001**
PapGIII	11 (22.9)	3 (9.1)	19 (25.7)	28 (23.1)	28 (15.4)	13 (15.7)	102 (18.9)	0.225
Sfa	4 (8.3)	0 (0)	9 (12.2)	18 (14.9)	19 (10.4)	13 (15.7)	63 (11.6)	0.128
Foc	7 (14.6)	13 (39.4)	15 (20.3)	21 (17.4)	19 (10.4)	7 (8.4)	82 (15.2)	**0.001**
Cnf1	20 (41.7)	14 (42.4)	28 (37.8)	46 (38.0)	41 (22.5)	19 (22.9)	168 (31.1)	**<0.001**
Aer	36 (75.0)	20 (60.6)	41 (55.4)	71 (58.7)	130 (71.4)	65 (78.3)	363 (67.1)	0.078
Usp	47 (97.9)	32 (97.0)	74 (100)	115 (95.0)	173 (95.1)	80 (96.4)	521 (96.3)	0.232
Iha	27 (56.3)	14 (42.4)	20 (27.0)	49 (40.5)	97 (53.5)	46 (55.4)	253 (46.8)	0.067
OmpT	48 (100)	33 (100)	73 (98.6)	119 (98.3)	177 (97.3)	81 (97.6)	531 (98.2)	0.135
Afa	11 (22.9)	10 (30.3)	13 (17.6)	49 (40.5)	88 (48.4)	36 (43.4)	207 (38.3)	**<0.001**
IroN	21 (43.8)	15 (45.5)	45 (60.8)	62 (51.2)	76 (41.8)	31 (37.3)	250 (46.2)	0.080
FimH	48 (100)	33 (100)	74 (100)	121 (100)	179 (98.4)	82 (98.8)	537 (99.3)	0.119
HlyA	24 (50.0)	12 (36.4)	28 (37.8)	47 (38.8)	53 (29.1)	21 (25.3)	185 (34.2)	**0.002**
Sat	26 (54.2)	14 (42.4)	20 (27.0)	45 (37.2)	89 (48.9)	46 (15.3)	240 (44.4)	0.114
K1 antigen	15 (31.3)	12 (36.4)	36 (48.6)	39 (32.2)	73 (40.1)	29 (34.9)	204 (37.7)	0.909

Bold font indicates statistical significance.
[a] Virulence factor PapGI was not detected in all 541 isolates.
[b] The association of bacterial virulence factors with host age groups was determined by Cochran—Armitage test.

Table 4 The association between bacterial antibiotic susceptibility, host age groups, and host gender.

Antimicrobial agent[a]	Age groups (years old) (% of antibiotic non-susceptible isolates)						total	*p* value[b]	Gender (% of antibiotic non-susceptible isolates)		*p* value[c]
	0—3	4—20	21—40	41—60	61—80	81—100			Male	Female	
amikacin (n = 1381)	2.0	0	0	0	2.6	1.4	1.3	0.133	1.8	1.1	0.514
amoxiclav (n = 988)	24.3	26.1	18.6	31.5	38.5	47.7	34.4	**<0.001**	40.9	32.5	**0.036**
ampicillin (n = 1391)	80.4	71.2	56.0	72.7	81.6	80.3	75.6	**<0.001**	78.2	74.7	0.331
cefazolin (n = 1390)	20.6	19.7	10.9	29.5	38.0	43.1	31.3	**<0.001**	41.2	28.1	**<0.001**
cefixime (n = 1389)	15.7	13.6	6.3	27.0	33.4	40.4	27.4	**<0.001**	36.5	24.4	**<0.001**
cefmetazole (n = 980)	8.6	6.7	3.4	15.9	23.2	29.1	18.3	**<0.001**	24.4	16.4	**0.004**
cefpirome (n = 1389)	7.8	4.5	2.3	10.7	12.2	15.1	10.4	**<0.001**	18	7.9	**<0.001**
ceftriaxone (n = 981)	18.6	13.3	6.7	25.3	28.4	38.6	25.3	**<0.001**	22.7	28.1	**<0.001**
cefuroxime (n = 1363)	15.0	12.5	6.4	28.0	32.3	38.3	26.8	**<0.001**	35.7	23.9	**<0.001**
piptazobactam (n = 307)	0	16.7	0	18.3	34.6	29.6	26.7	**0.001**	29.0	25.5	0.713
ertapenem (n = 1361)	0	0	0	0.3	0.2	1.4	0.4	0.060	0.9	0.2	0.063
cotrimoxazole (n = 1364)	50.0	42.4	36.8	46.3	54.6	59.7	50.3	**<0.001**	50.5	50.2	0.116
ciprofloxacin (n = 307)	57.1	66.7	66.7	76.1	79.4	90.1	79.5	**0.001**	82.2	78.0	0.509
levofloxacin (1,388)	15.7	16.7	10.9	29.2	41.3	47.7	32.6	**<0.001**	38.5	30.7	**0.017**
gentamicin (n = 1388)	32.4	18.8	14.3	27.0	32.9	33.9	28.7	**0.002**	33.8	27.0	0.054

Bold font indicates statistical significance.
[a] All isolates were susceptible to imipenem (n = 307) and meropenem (n = 307).
[b] The association of bacterial antibiotic susceptibility with host age groups was determined by Cochran—Armitage test.
[c] The association of bacterial antibiotic susceptibility with host gender was determined by Pearson's Chi-square test.

antimicrobial susceptibility of UTI strains isolated from different host age groups and gender (Table 4). Disk diffusion method is the routine laboratory test for microorganisms' antimicrobial susceptibility test in our hospital. The results of this test are reported as susceptible, intermediate resistant, or resistant. Although there were 1512 strains collected in this study, some antimicrobial susceptibility test (AST) results were missing due to the change of selected antibiotic disk for testing and thus the total strain numbers of each antibiotic group were different (Table 4). Therefore, we showed the percentage of non-susceptible isolates (resistant and intermediate resistant) in Table 4.

We found all UTI isolates were susceptible to imipenem (n = 307) and meropenem (n = 307). UTI strains were more

Table 5 The association between bacterial antibiotic susceptibility and phylogenetic groups in UTI *E. coli* isolates.

Antimicrobial agent[a]	Phylogenetic groups (S/NS (%))[b]				*p* value
	A	B1	B2	D	
amikacin (n = 780)	82/0 (0)	69/2 (2.8)	465/3 (0.6)	156/3 (1.9)	0.499
amoxiclav (n = 607)	33/41 (55.4)[c]	31/34 (52.3)[c]	274/75 (21.5)	72/47 (39.5)[c]	**<0.001**
ampicillin (n = 786)	12/71 (85.5)[c]	12/60 (83.3)[c]	151/320 (67.9)	26/134 (83.8)[c]	**<0.001**
cefazolin (n = 785)	46/37 (44.6)[c]	39/33 (45.8)[c]	379/91 (19.4)	91/69 (43.1)[c]	**<0.001**
cefixime (n = 784)	46/36 (43.9)[c]	40/32 (44.4)[c]	396/74 (15.7)	95/65 (40.6)[c]	**<0.001**
cefmetazole (n = 600)	48/24 (33.3)[c]	42/22 (34.4)[c]	311/34 (9.9)	92/27 (22.7)[c]	**<0.001**
cefpirome (n = 785)	77/6 (7.2)	68/4 (5.6)	435/35 (7.4)	126/34 (21.2)[c]	**<0.001**
ceftriaxone (n = 601)	42/32 (43.2)[c]	37/28 (43.1)[c]	291/53 (15.4)	77/41 (34.7)[c]	**<0.001**
cefuroxime (n = 759)	47/33 (41.3)[c]	37/31 (45.6)[c]	386/70 (15.4)	92/63 (40.6)[c]	**<0.001**
piptazobactam (n = 168)	17/8 (32.0)	17/6 (26.1)	51/16 (23.9)	47/6 (11.3)	**0.306**
ertapenem (n = 785)	82/0 (0)	72/0 (0)	470/1 (0.2)	160/0 (0)	0.881
cotrimoxazole (n = 769)	26/55 (67.9)[c]	26/44 (62.9)[c]	273/189 (40.9)	52/104 (66.7)[c]	**<0.001**
ciprofloxacin (n = 168)	10/15 (60.0)[c]	8/15 (65.2)[c]	6/61 (91.0)	13/40 (75.5)[c]	**0.023**
levofloxacin (n = 785)	37/46 (55.4)[c]	38/33 (46.5)[c]	345/126 (26.8)	105/55 (34.4)	**<0.001**
gentamicin (n = 785)	55/28 (33.7)[c]	52/20 (27.8)	369/102 (21.7)	95/64 (40.3)[c]	**<0.001**

Bold font indicates statistical significance.

[a] All isolates were susceptible to imipenem (n = 307) and meropenem (n = 307).

[b] S, number of susceptible isolates; NS, number of non-susceptible isolates; %, percentage of non-susceptible isolates.

[c] The resistant rate of antimicrobial agent in phylogenetic B2 group was statistically different with other groups ($p < 0.05$).

susceptible to amikacin (resistant rate, 1.3%), cefpirome (resistant rate, 10.4%), and ertapenem (resistant rate, 0.4%), but UTI strains showed high resistance to ampicillin (75.6%), ciprofloxacin (79.5%), and cotrimoxazole (50.3%) (Table 4). In addition, *E. coli* strains isolated from 81 to 100 age group were more resistant to amoxiclav (47.7% vs 34.4%), cefazolin (43.1% vs 31.3%), cefixime (40.4% vs 27.4%), cefmetazole (29.1% vs 18.3%), ceftriaxone (38.6% vs 25.3), cefuroxime (38.3% vs 26.8%), ciprofloxacin (90.1% vs 79.5%), levofloxacin (47.7% vs 32.6%), than the average resistant rate (Table 4). In contrast, *E. coli* strains isolated from age group 0—3 were more susceptible to amoxiclav (24.3% vs 34.4%), cefixime (15.7% vs 27.4%), cefuroxime (15.0% vs 26.8%), ciprofloxacin (57.1% vs 79.5%), levofloxacin (15.7% vs 32.6%), and piptazobactam (0% vs 26.7%), than the average resistant rate (Table 4). All the antibiotics except amikacin and ertapenem had the trend of significant increasing resistance following higher age groups (Table 4). Our results also showed strains isolated from males were more resistant to amoxiclav ($p = 0.036$), cefazolin ($p < 0.001$), cefixime ($p < 0.001$), cefmetazole ($p = 0.004$), cefpirome ($p < 0.001$), cefuroxime ($p < 0.001$), and levofloxacin ($p = 0.017$), compared to strains isolated from females with UTIs (Table 4). In contrast, strains isolated from females were more resistant to ceftriaxone, compared to strains isolated from males with UTIs (<0.001) (Table 4).

In addition to host age groups, we determined whether bacterial antimicrobial susceptibility was associated with phylogenetic groups (Table 5). Compared to UTI isolates belonging to group A, B1, and D, B2 isolates were more susceptible to amoxiclav, ampicillin, cefazolin, cefixime, cefmetazole, ceftriaxone, cefuroxime, cotrimoxazole, gentamicin, and levofloxacin (Table 5). Interestingly, B2 isolates were only more resistant to ciprofloxacin (resistant rate in group A, 60%; B1, 65.2%; B2, 91.0%; D, 75.5%) (Table 5). Moreover, group D isolates were more resistant to cefpirome (resistant rate in group A, 7.2%; B1, 5.6%; B2, 7.4%; D, 21.2%) (Table 5).

Discussion

To our knowledge, this is the first report to investigate the association between host age, bacterial virulence factors and antimicrobial susceptibility. Overall, we found that the prevalence of virulence factors and antibiotic susceptibility of UTI *E. coli* are associated with host age (Tables 1 and 4). *E. coli* isolated from the elders were more resistant to antimicrobial agents and had fewer virulence factors (Tables 1 and 4). The role(s) of specific virulence factors in *E. coli* causing UTI in different host age groups remains to be studied experimentally. In addition, increases in host risk factors, such as urinary tract abnormality, bladder dysfunction in type 2 diabetes, and estrogen deficiency in female, have been reported to be associated with the pathogenesis of UTI.[3] However, direct evidence demonstrating that the elders are more vulnerable to low virulent UPEC due to the decline of host immune protection or increase in host risk factors is still lacking.

Lee et al. showed that among the phylogenetic groups in UTI *E. coli* isolates, most of the virulence genes were found significantly high in groups B2 and D compared to other groups.[4] Our findings are consistent with Lee's report, most strains isolated from the 0—3 age group belonged to phylogenetic group B2 (Table 2) and had more virulence factors (Table 1). Nowrouzian et al. defined commensal *E. coli* strains that were repeatedly isolated from intestinal microflora of 70 infants over a period of 3 weeks as resident in their study, whereas strains colonizing for shorter periods were defined as transient.[19] They reported the majority

(60%) of the resident strains in infant gut microflora belonged to phylogenetic group B2, compared with only 21% of the transient strains ($p = 0.004$).[19] Most UTIs are caused by *E. coli* that live harmlessly in the gut. However, when shed in the feces, the bacteria can spread to the opening of the urinary tract and thus cause UTIs. Consistent with Nowrouzian's finding, more B2 *E. coli* were identified in the 0—3 age group, compared to other age groups in this study (Table 2). However, factors contribute to the dominancy of B2 *E. coli* in infant's gut remain to be investigated.

Zhanel et al. reported the resistance of UTI isolates to fluoroquinolones, ciprofloxacin and levofloxacin was increased with age in the United States and Canada.[20] Banerjee et al. also showed ST131 was a dominant and antimicrobial-resistant clonal group associated with healthcare settings, elderly hosts, and persistent or recurrent symptoms.[21] In this study, we showed that *E. coli* strains isolated from age group 81—100 were more resistant to most commonly used antimicrobial agents than strains isolated from other age groups. However, whether ST131 clone dominate in our older age groups is unclear and worth investigating. Moreover, the factors associated with the high resistance rate of *E. coli* isolated from elders remains unclear.

In summary, we found *E. coli* isolated from elders were more resistant to antimicrobial agents and had less virulence factors. However, UTI *E. coli* enrolled in this study were isolated during the years 2009 and 2010. Therefore, it will be worth comparing the characteristics of UTI *E. coli* isolated recently to those collected 11 years ago from the same hospital to determine the evolution of *E. coli* regionally.

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Authors' contributions

MCW contributed to isolates collection. WHL, PYL, PSC, LLW, and CHT carried out the experiments and interpreted of results of bacterial identification, antibiotic susceptibility tests, and virulence factors identification. WHL, MCW and CYK were responsible for manuscript preparation. CYK conceived the study and were in charge of overall direction and planning. All authors read and approved the final manuscript.

Declaration of competing interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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