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ORIGINAL ARTICLE

Virulence factors of uropathogenic *Escherichia coli* of urinary tract infections and asymptomatic bacteriuria in children

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Background/Purpose: The clinical aspects of virulence genes of uropathogenic *Escherichia coli* (UPEC) are not fully understood. This study compared the presence of virulence genes in UPEC isolated from urinary tract infections (UTIs) and asymptomatic bacteriuria (ABU) in children.
Methods: The study included children with UTI ($n = 15$) or ABU ($n = 49$) treated at Chung-Ang University Yongsan Hospital between 2010 and 2011. The strains were acquired from each urine sample collected, and 18 major virulence genes were detected by polymerase chain reaction. Antimicrobial susceptibility of all UPEC isolates was determined.
Results: Sixty-four *E. coli* strains were isolated from the urine samples. The most commonly identified virulence gene in both groups was *fimH* (100.0% in the UTI group and 95.9% in the ABU group). The UTI isolates showed a higher prevalence of *papEF* and *fyuA*, and a lower prevalence of *feoB* than ABU isolates ($p < 0.01$ for all). The profile of virulence gene, $fimH^{+}kpsMTII^{+}feoB^{+}$ also showed a significant difference between the two groups ($p < 0.01$). Isolates from ABU were more resistant to most antimicrobials tested. The presence of *papEF, feoB,* and *fyuA* also correlated with the antimicrobial susceptibility of UPEC.
Conclusion: The virulence gene repertoire was different in the UPEC of UTI and ABU. The *papEF*, *feoB*, and *fyuA* genes showed meaningful differences between the two groups and may have an important role in the pathogenesis of overt UTI.

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Introduction

Urinary tract infection (UTI) refers to an infection with microbial pathogens at any site in the urinary tract, which includes the urethra, bladder, ureter, and kidneys. UTI is the most common bacterial infection in children; many affected children, particularly infants, have severe symptoms and lobar nephronia. Kidney scarring related to UTI has been linked to long-term morbidity.[1,2] By contrast, asymptomatic bacteriuria (ABU) is a condition in which bacteria subclinically colonize the urinary tract, closely resembling commensalism at other mucosal sites. ABU strains are well adapted for growth in the human urinary tract, without causing any clinical symptoms. Therefore, ABU strains are an interesting model for the study of mechanisms of commensalism and the driving forces between the pathogen and the host.[3,4]

Uropathogenic *Escherichia coli* (UPEC) are the most common bacterial pathogens that cause UTI and ABU in children. Extended-spectrum beta-lactamases-producing UPEC strains, which are increasing in prevalence worldwide, have an appreciable deleterious impact on the clinical management of UTI.[5,6] The UPEC strains harbor many genes that encode various virulence factors, which contribute to enhanced pathogenicity. The molecular characteristics and functions of these virulence genes have been well established.[7–12] The severity of UTI reflects the virulence profile or phenotype of the infecting strain, with many virulence factors being expressed fully at a higher frequency by strains causing UTI than by strains causing ABU.[13–15]

In this study, we sought to identify the prevalence and expression patterns of virulence genes in UPEC isolated from children in South Korea. We also compared the virulence gene repertoire in UPEC strains isolated from UTIs and ABU, with the aim of clarifying important virulence factors for the development of clinical diseases of the urinary tract in children.

Materials and methods

Participants

This study was performed at Chung-Ang University Yongsan Hospital between March 2010 and February 2011. All participants were children $<$18 years of age who lived in Seoul, Korea, during the study period. Inclusion criteria were fever with an axillary temperature of $\geq$38°C, positive urine culture ($>10^5$ colony-forming units/mL), and pyuria ($>$5 white blood cells/high-power field). Children who did not have any urinary symptoms (i.e., urgency, frequency, and dysuria) but had significant growth of *E. coli* ($>10^5$ colony-forming units/mL) were considered to have ABU. The urine samples were collected through midstream urine in toilet-trained children and by a sterile urine-collecting bag in others. The study protocol was reviewed and approved by the Institutional Review Board (No. 10-013-02-03) at Chung-Ang University Hospital, and written informed consent was obtained from the parents/guardians of all participants.

Detection of virulence genes

All *E. coli* isolates were identified by typical morphology, lactose fermentation, positive spot indole test, and VITEK 2-GN card (bioMérieux, Hazelwood, MO, USA). Only one isolate per patient was examined. Extraction and purification of DNA from all UPEC isolates were performed as described in the QIAamp Kit (Qiagen GmbH, Hilden, Germany). The presence of the following virulence genes was assessed by 18 simplex polymerase chain reaction (PCR):adhesion proteins (*papA, papC, papEF, papGI, papGII, papGIII, sfa, fimH, afa, bmaE*), toxins (*hlyA, cdtB*), siderophores (*fyuA, iutA, feoB*), capsule synthesis proteins (*kpsMTII, kpsMTIII*), and uropathogenic-specific protein (*usp*). Primer sequences for amplification of these individual virulence genes have been published in previous studies[16–19] and are listed in Table 1. PCR was consistently performed in a 20-μL reaction volume, with each reaction mixture containing 1.0 μL of DNA template, 10 μM of each primer, 2.0 μL of 10× *Taq* buffer, 2.5 mM of deoxynucleotide triphosphates, and 2.5 mM of *Taq* polymerase (iNtRON, Seoul, Korea). Thermal cycling was performed in a PTC-200 Peltier thermal cycler DNA engine (MJ Research, Watertown, MA, USA) under the following conditions: denaturation for 5 minutes at 94°C; 35 1-minute amplification cycles at 94°C, and additional amplification cycles for 1 minute at 55°C, 2 minutes at 72°C, and a final extension cycle for 10 minutes at 72°C. The PCR products were electrophoresed on agarose gels, stained with ethidium bromide, and photographed using a UV transillumination imaging system.

Antimicrobial susceptibility test

Antimicrobial susceptibility testing of all isolates to ampicillin, gentamicin, piperacillin, trimethoprim/sulfamethoxazole, tetracycline, amoxicillin/clavulanic acid, cefazolin, aztreonam, ciprofloxacin, cefepime, cefotaxime, tobramycin, piperacillin/tazobactam, and levofloxacin was performed using the VITEK 2 automated system (bioMérieux). In addition, *in vitro* antimicrobial susceptibility testing was performed by the broth microdilution method and the results were interpreted using the 2010 Clinical and Laboratory Standards Institute breakpoints.

Statistical analyses

All statistical analyses were performed using SPSS version 18.0 (SPSS, Chicago, IL, USA). The prevalence of virulence genes and antibiotic resistance patterns were compared between the two groups using Pearson Chi-square test and Fisher exact test. Continuous variables were compared with the Student *t* test and Mann–Whitney *U* test. A *p* value <0.05 was considered statistically significant.

Results

Clinical characteristics

A total of 64 patients were included in this study, of which 15 were diagnosed with UTI and 49 with ABU. In patients with

Table 1 Sequence of primers used in polymerase chain reaction for virulence genes

Gene	Primer sequence (5′–3′)	Size of product (bp)	Primer coordinates	Source of primer
papA	F: ATGGCAGTGGTGTTTTGGTG	720	1796–1817	16
	R: CGTCCCACCATACGTGCTCTTC		2495–2516	
papC	F: GTGGCAGTATGAGTAATGACCGTTA	200	4774–4798	16
	R: ATATCCTTTCTGCAGGGATGCAATA		4952–4976	17
papE/F	F: GCAACAGCAACGCTGGTTGCATCAT	336	8025–8049	17
	R: AGAGAGAGCCACTCTTATACGGACA		8336–8360	
papGI	F: TCGTGCTCAGGTCCGGAATTT	461	8871–8891	17
	R: TGGCATCCCCCAACATTATCG		9311–9331	
papGII	F: GGGATGAGCGGGCCTTTGAT	190	1604–1623	17
	R: CGGGCCCCCAAGTAACTCG		1775–1793	
papGIII	F: GGCCTGCAATGGATTTACCTGG	258	1420–1441	17
	R: CCACCAAATGACCATGCCAGAC		1656–1677	
sfa/foc	F: CTCCGGAGAACTGGGTGCATCTTAC	410	NA	17
	R: CGGAGGAGTAATTACAAACCTGGCA			
fimH	F: TGCAGAACGGATAAGCCGTGG	508	1814–1839	16
	R: GCAGTCACCTGCCCTCCGGTA		2256–2278	
Afa	F: GGCAGAGGGCCGGCAACAGGC	559	4589–4609	16
	R: CCCGTAACGCGCCAGCATCTC		5160–5180	
bmaE	F: ATGGCGCTAACTTGCCATGCTG	507	79–100	16
	R: AGGGGGACATATAGCCCCCTTC		562–583	
hlyA	F: AACAAGGATAAGCACTGTTCTGGCT	1177	2420–2449	17
	R: ACCATATAAGCGGTCATTCCCGTCA		3572–3596	
cdtB	F: AAATCACCAAGAATCATCCAGTTA	430	1735–1758	16
	R: AAATCTCCTGCAATCATCCAGTTTA		1463–1485	
iutA	F: GGCTGGACATCATGGGAACTGG	300	851–872	17
	R: CGTCGGGAACGGGTAGAATCG		1132–1152	
feoB	F: AATTGGCGTGCATGAAGATAACTG	470	NA	17
	R: AGCTGGCGACCTGATAGAACAATG			
fyuA	F: TGATTAACCCCGCGACGGGAA	880	775–795	16
	R: CGCAGTAGGCACGATGTTGTA		1539–1559	18
kpsMTII	F: GCGCATTTGCTGATACTGTTG	272	297–317	16
	R: CATCCAGACGATAAGCATGAGCA		544–566	
kpsMTIII	F: TCCTCTTGCTATTATTCCCCCT	392	4050–4071	16
	R: AGGCGTATCCATCCCTCCTAAC		4418–4439	
Usp	F: ATGCTACTGTTTCCGGGTAGTGTGT	1000	NA	19
	R: CATCATGTAGTCGGGGCGTAACAAT			

bp = base pairs; F = forward; NA = not available; R = reverse.

ABU, 21 were healthy children and 28 were children with other diseases; upper respiratory tract infection ($n = 9$), bronchiolitis ($n = 8$), functional gastrointestinal disorder ($n = 5$), and acute gastroenteritis ($n = 6$). Each UTI and ABU group contained both outpatients and inpatients. The median age was 7.5 months (range: 1.6–98.8 months) in the UTI group and 15.4 months (range: 0.5–167.9 months) in the ABU group (Table 2). The ratio of males to females was 2:1 in the UTI group and 1.7:1 in the ABU group. There were no statistically significant differences in age and sex of participants ($p > 0.05$, respectively). By contrast, higher levels of C-reactive protein, increased erythrocyte sedimentation rate, and a proportion of neutrophil were identified among patients diagnosed with UTI ($p < 0.05$, for all).

Virulence gene repertoire in UPEC

Eighteen virulence genes were explored to determine the virulence repertoire in the UPEC isolates. The frequently identified virulence genes were *fimH* ($n = 62$, 96.9%), *kpsMTII* ($n = 54$, 84.4%), *feoB* ($n = 43$, 67.2%), *iutA* ($n = 34$, 53.1%), *fyuA* ($n = 29$, 45.3%), and *papA* ($n = 29$, 45.3%). The *papGI*, *cdtB*, and *kpsMTIII* genes were not detected (Table 3). Isolates from the ABU group expressed fewer virulence factors than those from the UTI group (4.98 ± 1.81 vs. 5.93 ± 1.44, respectively, $p < 0.05$, data not shown). The prevalence of virulence genes in patients over 6 months of age did not differ from those in patients <6 months of age ($p > 0.05$, data not shown). The components of the virulence genes were similar in both sexes, except for *papEF*, which was found more frequently in females than in males ($p < 0.05$, data not shown).

In patients with UTI and ABU, the virulence gene most frequently detected was *fimH* (UTI group: $n = 15$, 100.0%; ABU group: $n = 47$, 95.9%). In the UTI group, *kpsMTII* ($n = 14$, 93.3%), *fyuA* ($n = 12$, 80.0%), *iutA* ($n = 9$, 60.0%), and *papA* ($n = 9$, 60.0%) were commonly identified in that order. The detected genes in the ABU group, in descending

Table 2 Patient characteristics of the urinary tract infection and asymptomatic bacteriuria groups

	UTI group ($n = 15$)	ABU group ($n = 49$)	p
Median age (mo)	7.5	15.4	0.117
Male:female	2:1	1.7:1	0.810
WBC (/mm^3)	13,732 ± 7448	10,384 ± 4304	0.076
Neutrophil (%)	55 ± 22	41 ± 19	0.013*
CRP (mg/dL)	4 ± 4.2	0.5 ± 0.6	0.001**
ESR (mm)	30.8 ± 25.9	17.2 ± 18.5	0.027*
BUN (mg/dL)	8.5 ± 4.1	9.7 ± 4.4	0.326
Creatinine (mg/dL)	0.5 ± 0.1	0.4 ± 0.1	0.093

*$p < 0.05$.
**$p < 0.01$.
ABU = asymptomatic bacteriuria; BUN = blood urea nitrogen; CRP = C-reactive protein; ESR = erythrocyte sedimentation rate; UTI = urinary tract infection; WBC = white blood cell.

order of prevalence, were *feoB* ($n = 40$, 81.6%), *kpsMTII* ($n = 40$, 81.6%), *iutA* ($n = 25$, 51.0%), and *papA* ($n = 20$, 40.8%). Only three virulence genes showed a meaningful difference of distribution between the groups; UTI isolates showed a higher prevalence of *papEF* and *fyuA*, and a lower prevalence of *feoB* than ABU isolates ($p < 0.01$ for all, Table 3). There were no significant differences in the number of detected virulence genes according to sex and age group (<6 months vs. ≥6 months of age) in the UTI and ABU groups (data not shown).

Prevalence of the specific profiles of virulence genes was analyzed. On the basis of *fimH* and *kpsMTII*, we considered the combinations of three common virulence genes, namely, *iutA*, *feoB*, and *papA*. The virulence profiles *fimH*$^+$*kpsMTII*$^+$*iutA*$^+$ and *fimH*$^+$*kpsMTII*$^+$*papA*$^+$ ($n = 8$, 53.3%, in both) were most common in the UTI group and *fimH*$^+$*kpsMTII*$^+$*feoB*$^+$ ($n = 33$, 67.3%) was most common in the ABU group. There was a significant difference between the two groups concerning the prevalence of the *fimH*$^+$*kpsMTII*$^+$*feoB*$^+$ profile (20.0% in the UTI group vs. 67.3% in the ABU group; $p < 0.01$, Table 3).

Antimicrobial susceptibility

Antimicrobial susceptibility patterns were different according to the origin of UPEC. Isolates from ABU were more resistant to all tested antimicrobials, except cefazolin and tetracycline. Among them isolates of ABU were significantly more resistant to piperacillin and cefotaxime than UTI (67% vs. 27%; 100% vs. 74%, $p < 0.05$, respectively, Table 4). However, there were no significant differences in

Table 3 Prevalence of virulence factors detected and its selected profiles from the urinary tract infection and asymptomatic bacteriuria groups

Function	Virulence genes	Total ($n = 64$), % (n)	UTI ($n = 15$), % (n)	ABU ($n = 49$), % (n)	p
Adhesion	*afa*	9.4 (6)	13.3 (2)	8.2 (4)	0.618
	bmaE	1.6 (1)	6.7 (1)	0.0 (0)	0.234
	fimH	96.9 (62)	100.0 (15)	95.9 (47)	> 0.99
	papA	45.3 (29)	60.0 (9)	40.8 (20)	0.192
	papC	3.1 (2)	0.0 (0)	4.1 (2)	> 0.99
	papEF	17.2 (11)	46.7 (7)	8.2 (4)	0.002*
	papGI	0.0 (0)	0.0 (0)	0.0 (0)	NA
	papGII	28.1 (18)	46.7 (7)	22.4 (11)	0.068
	papGIII	10.9 (7)	6.7 (1)	12.2 (6)	> 0.99
	sfa/foc	15.6 (10)	13.3 (2)	16.3 (8)	> 0.99
Toxins	*cdtB*	0.0 (0)	0.0 (0)	0.0 (0)	NA
	hlyA	20.3 (13)	20.0 (3)	20.4 (10)	> 0.99
Siderophores	*feoB*	67.2 (43)	20.0 (3)	81.6 (40)	< 0.001*
	fyuA	45.3 (29)	80.0 (12)	34.7 (17)	0.003*
	iutA	53.1 (34)	60.0 (9)	51.0 (25)	0.542
Capsule synthesis	*kpsMTII*	84.4 (54)	93.3 (14)	81.6 (40)	0.429
	kpsMTIII	0.0 (0)	0.0 (0)	0.0 (0)	NA
Uropathogen specific	*usp*	21.9 (14)	26.7 (4)	20.4 (10)	0.723
Profiles of virulence genes	*fimH*$^+$*kpsMTII*$^+$*iutA*$^+$	43.8 (28)	53.3 (8)	40.8 (20)	0.393
	fimH$^+$*kpsMTII*$^+$*papA*$^+$	42.2 (27)	53.3 (8)	38.8 (19)	0.318
	fimH$^+$*kpsMTII*$^+$*feoB*$^+$	56.3 (36)	20.0 (3)	67.3 (33)	0.002*
	fimH$^+$*kpsMTII*$^+$*iutA*$^+$*papA*$^+$	25.0 (16)	26.7 (4)	24.5 (12)	> 0.99
	fimH$^+$*kpsMTII*$^+$*iutA*$^+$*feoB*$^+$	25.0 (16)	6.7 (1)	30.6 (15)	0.089
	fimH$^+$*kpsMTII*$^+$*papA*$^+$*feoB*$^+$	25.0 (16)	6.7 (1)	30.6 (15)	0.089
	fimH$^+$*kpsMTII*$^+$*papA*$^+$*iutA*$^+$*feoB*$^+$	12.5 (8)	0.0 (0)	16.3 (8)	0.181

*$p < 0.01$.
ABU = asymptomatic bacteriuria; NA = not available; UTI = urinary tract infection.

antimicrobial susceptibility according to sex and age group (<6 months vs. ≥6 months of age) in the UTI and ABU groups (data not shown).

By contrast, there was no relationship between the presence of virulence genes and antimicrobial susceptibility of UPEC, except for *papEF, feoB,* and *fyuA*. The *papEF* and *fyuA* genes were more prevalent in tetracycline-resistant isolates than tetracycline-susceptible isolates (60% vs. 14%; 100% vs. 41%, $p < 0.05$, respectively, Fig. 1A). However, *feoB* was exclusively identified in tetracycline- and cefazolin-susceptible isolates, and was more frequently identified in cefotaxime-resistant isolates than susceptible isolates (92% vs. 61%, $p < 0.05$, Fig. 1B).

Discussion

ABU is a condition closely resembling bacterial commensalism at the mucosa of urinary tract, but is also considered as the most common form or risk factor of symptomatic UTI.[3,20–22] A recent study revealed that ABU might protect against symptomatic recurrence of UTI in young women.[23] The UPEC strains causing ABU may outcompete strains causing UTI in the urinary tract of humans.[24] These characteristics of ABU differentiate the virulence of the bacterial strains causing ABU from those causing UTI. The ABU strains reportedly express fewer virulence factors or harbor attenuated virulence genes than strains causing symptomatic UTI.[13–15] However, these studies were mostly performed in adults rather than in children, particularly infants, having more severe symptoms and complications. In the present study, we reported that ABU strains also expressed fewer virulence factors than UTI strains in children.

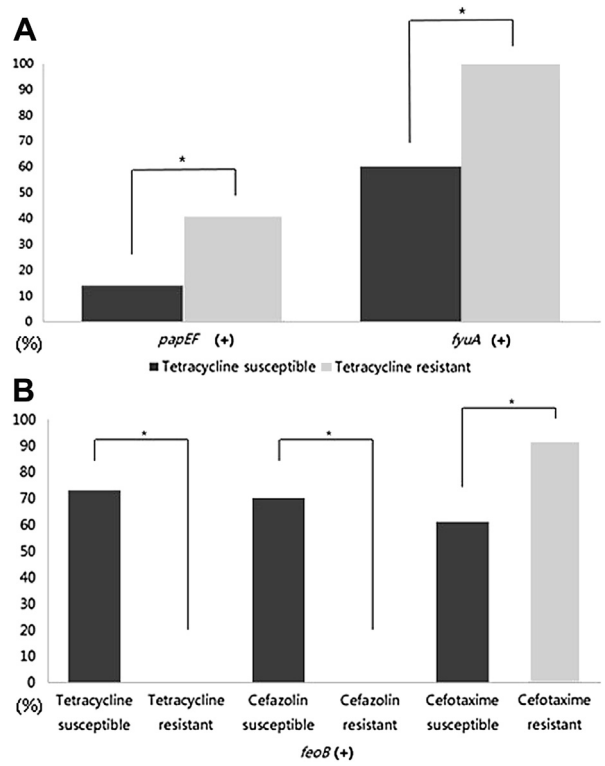

Figure 1. Relationship between virulence factors and antimicrobial susceptibility: (A) tetracycline susceptibility was related to the prevalence of *papEF* and *fyuA* genes. (B) Strains with *feoB* gene were exclusively susceptible to tetracycline and cefazolin, but more resistant to cefotaxime. *$p < 0.05$.

Table 4 Antimicrobial resistance of uropathogenic *Escherichia coli* isolated from the urinary tract infection and asymptomatic bacteriuria groups

Antimicrobials	Resistance, % (*n*)		*p*
	UTI (*n* = 15)	ABU (*n* = 49)	
Ampicillin	53.3 (8)	75.5 (37)	0.100
Amoxicillin/clavulanate	6.7 (1)	26.5 (13)	0.157
Piperacillin	33.3 (5)	73.5 (36)	0.012*
Piperacillin/tazobactam	0.0 (0)	14.3 (7)	0.185
Cefazolin	6.7 (1)	4.1 (2)	0.558
Cefotaxime	0.0 (0)	26.5 (13)	0.028*
Ceftriaxone	0.0 (0)	20.4 (10)	0.100
Cefepime	0.0 (0)	20.4 (10)	0.100
Aztreonam	0.0 (0)	20.4 (10)	0.100
Gentamicin	26.7 (4)	40.8 (20)	0.313
Tobramycin	0.0 (0)	12.2 (6)	0.322
Tetracycline	13.3 (2)	6.1 (3)	0.583
Trimethoprim/sulfamethoxazole	20.0 (3)	46.9 (23)	0.078
Ciprofloxacin	0.0 (0)	4.1 (2)	> 0.99
Levofloxacin	0.0 (0)	8.2 (4)	0.565

*$p < 0.05$.
ABU = asymptomatic bacteriuria; UTI = urinary tract infection.

Virulence factors of UPEC are associated with colonization and survival in the normal urinary tract, and influence the pathogenicity of symptomatic or complicated UTIs.[9,25–27] These virulence factors are adhesion proteins (e.g., P fimbriae, S and F1C fimbriae, and type 1 fimbriae), iron-acquisition systems (e.g., aerobactin), toxins (e.g., hemolysin), and host defense-avoidance mechanisms (e.g., capsule or O-specific antigen).[28] Bacterial adherence to urinary epithelia is a crucial step in the development of UTI, allowing the bacteria to persist in the urinary tract against flushing by urine flow and activation of host signaling pathways.[11,29] In mammalian hosts, free iron concentrations are very low for the growth of pathogenic bacteria. Thus, many bacteria, including UPEC, have multiple ways of getting iron from the host through the expression of iron-acquisition systems.[30] The utilization of various toxins secreted by UPEC strains is well recognized. Among these toxins, α-hemolysin encoded by *hlyA* is an extracellular cytolytic protein toxin that is produced by up to 50% of UPEC isolates. α-Hemolysin has been associated with clinical severity in UTI patients.[31] In our study, *hlyA* was detected in 20.0% of UTI strains and in 20.4% of ABU strains.

In this study, the *fimH* adhesion gene was the most common virulence gene in both UTI and ABU isolates (frequencies of 100% and 96%, respectively). Previous studies

have established that *fimH* is most frequent in isolates from a variety of forms of UTI.[15,32–34] The *pap* gene family of adherence virulence genes (*papA, papC, papEF, papGII*, and *papGIII* in this study), which are expressed as P fimbriae, was also prevalent in the UTI and ABU isolates. Among the *pap* genes, *papA* was the second most prevalent adhesion gene in the UTI group (60.0%, $n = 9$) and ABU group (40.8%, $n = 49$). Furthermore, *papEF* was more frequently identified in the UTI group than in the ABU group (46.7% vs. 8.2%, respectively, $p < 0.05$). In some UPEC strains, it is conceivable that *papEF* is active in the progression to symptomatic UTI from ABU. One study described the presence of *papEF* in over half of the isolates obtained from severe invasive urinary tract diseases.[33]

The third most common gene in the UTI isolates was *fyuA* ($n = 12$, 80.0%), which is one of the bacterial iron-acquisition systems. However, ABU isolates showed a much lower prevalence of *fyuA* ($n = 17$, 34.7%, $p < 0.01$). The finding supports the importance of *fyuA* in virulence, particularly in the establishment of symptomatic UTI. The relationship between *fyuA* and invasive UTI had been suspected in previous studies.[16,18,33] By contrast, a recent study reported that *feoB* and *fimH* were the most prevalent virulence genes of UPEC isolated from patients with community-acquired UTI in China.[34] In the present study, the prevalence of *feoB* in UTI isolates was much lower (20.0%) than those (>92%) reported in China. By contrast, *feoB* was identified in ABU strains at a higher frequency (81.6%) in our isolates. These discrepancies might be due to the difference in epidemiology of strains or lower UTI sample size in this study. The *feoB* gene might have a role in iron acquisition in both UTI and ABU, although it is unclear whether *feoB* works differently or has an additional individual mechanism in UTI or ABU strains.

Virulence factors may have distinctive and complex associations with one another.[16] To identify these associations and the phenotypes of the present isolates, the prevalence of profiles of the virulence genes was ascertained. The *fimH*$^+$*kpsMTII*$^+$*feoB*$^+$ profile was significantly more prevalent in the ABU group ($p < 0.01$). This finding might be due to a much larger discrepancy of *feoB* prevalence than *fimH* and *kpsMTII* between the two groups. In addition, it could be possible that *fimH* and *kpsMTII* make the UPEC strain more suitable for commensalism in the human urinary tract when expressed with *feoB*.

Patients with UTI need early diagnosis and prompt antibacterial treatment to minimize renal scarring and progressive kidney damage. Empirical antibiotic treatment is usually started in patients with suspected UTI. However, antibiotic resistance has increased and is currently a major problem in many countries.[6,28] In this study, isolates from ABU were found to be more resistant to most antimicrobials tested, as shown in a previous study.[15] It might be that long-term exposure to various antimicrobials for ABU strains induced antimicrobial resistances. By contrast, several studies reported the relationship between antimicrobial resistance and virulence factors of UPEC. Nalidixic acid resistance has been associated with a lower prevalence of *sfa* and a lower prevalence of *pap*, *hly*, and *cnf1* has been reported in fluoroquinolone-resistant strains than their susceptible counterparts.[35,36] Another study described that all *pap* elements except for *papG* allele III, *sfa/foc*, *kpsMTII*, *fyuA*, and *hlyA* were associated with decreased antibiotic resistance.[16] In this study, tetracycline-resistant strains more often harbored *papEF* and *fyuA* ($p < 0.05$) and *feoB* was associated with increased cefotaxime resistance and tetracycline susceptibility ($p < 0.05$). The presence and prevalence of virulence genes might depend on the antibiotic's mode of action or an unknown interaction between virulence factors and antibiotics.

This study had several limitations. One was a relatively small sample size, which requires further confirmation of the findings. In addition, there was a possibility of contamination of the urine samples derived from a urine bag. Nevertheless, the possibility of selection bias might be low because the inclusion criteria were strictly applied to all participants. Finally, we could not match the UTI and ABU groups for other conditions, such as host immunity, socioeconomic status, and history of antibiotic use. Despite these limitations, our study could be valuable for understanding molecular characteristics of UPEC, especially strains isolated from Korean children. The findings support the importance of some identified virulence genes as important effectors in the development of symptomatic UTI.

In conclusion, UPEC strains from Korean children with UTI and ABU had different molecular epidemiologic features concerning their major virulence genes. The *papEF* and *fyuA* genes were implicated as important virulence genes for development of UTI in children. Further investigations about *feoB* are needed to understand its significance in UTI or ABU.

Conflicts of interest

All contributing authors declare no conflicts of interest.

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