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Molecular typing of uropathogenic *Escherichia coli* isolated from Korean children with urinary tract infection

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Purpose: We investigated the molecular types of uropathogenic *Escherichia coli* (UPEC) by using conventional phylogrouping, multilocus sequence typing (MLST), and *fimH* genotyping.

Methods: Samples of patients younger than 18 years of age were collected from the Chung-Ang University Hospital over 2 years. Conventional phylogenetic grouping for UPEC strains was performed by polymerase chain reaction (PCR). Bacterial strain sequence types (STs) were classified on the basis of the results of partial sequencing of seven housekeeping genes. In addition, we analyzed nucleotide variations in a 424-base pair fragment of *fimH*, a major virulence factor in UPEC.

Results: Sixty-four UPEC isolates were analyzed in this study. Phylogenetic grouping revealed that group B2 was the most common type (n=54, 84%). We identified 16 distinctive STs using MLST. The most common STs were ST95 (35.9%), ST73 (15.6%), ST131 (12.5%), ST69 (7.8%), and ST14 (6.3%). Fourteen *fimH* allele types were identified, of which 11 had been previously reported, and the remaining three were identified in this study. f1 (n=28, 45.2%) was found to be the most common allele type, followed by f6 and f9 (n=7, 11.3% each). Comparative analysis of the results from the three different molecular typing techniques revealed that both MLST and *fimH* typing generated more discriminatory UPEC types than did PCR-based phylogrouping.

Conclusion: We characterized UPEC molecular types isolated from Korean children by MLST and *fimH* genotyping. *fimH* genotyping might serve as a useful molecular test for large epidemiologic studies of UPEC isolates.

Key words: Uropathogenic Escherichia coli, Phylogeny, Multilocus sequence typing, fimH protein

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Introduction

Urinary tract infection (UTI) is the most common bacterial infection during childhood; many affected infants have severe symptoms and lobar nephronia. Kidney scarring related to UTI has been linked to long-term morbidity[1,2]. Uropathogenic *Escherichia coli* (UPEC) is the most common causative agent of UTI in children. Extended-spectrum beta-lactamase (ESBL)-producing UPEC strains have an appreciable deleterious impact on the clinical management of UTIs[3,4]. Virulence factors, including ESBL, seem to be correlated with certain UPEC strains. Knowledge of the distinguishing characteristics of various UPEC strains could conceivably lead to the development of strategies for the prevention and management of UTI in children; therefore, the molecular epidemiology of UPEC strains has been the subject of intensive research worldwide[5,6].

In 2000, Clermont et al.[7] described a triplex polymerase chain reaction (PCR) strategy for the rapid assignment of *Escherichia coli* isolates to one of four phylogroups: A, B1, B2, and D; this technique has been used extensively. Multilocus sequence typing (MLST)

is a standard molecular subtyping technique that may be used to determine the genetic relatedness between strains and identify a certain strain with high discriminatory power for various bacterial pathogens. This method has been standardized and is now widely used for the identification and classification of UPEC[8,9]. Furthermore, the digitalization of MLST data has facilitated the establishment of global, web-accessible databases for a variety of organisms and is rapidly contributing to our understanding of the clonal distribution of infectious disease agents. Single nucleotide polymorphisms (SNPs) occur in several different MLST loci and represent neutral genetic variation; they presumably exhibit minimal autocorrelation, and could potentially confound epidemiologic conclusions. On the other hand, a genotyping method for *fimH*, which encodes the P-fimbriae adhesion protein, was suggested as a simple screening test for epidemiological studies of UPEC and was used to evaluate a large population of *E. coli* isolates[10,11].

In this study, we aimed to identify the epidemiologic features of UPEC isolated from Korean children using the three different molecular typing methods that are widely used for *E. coli* identification.

Materials and methods

1. Subjects and UPEC isolates

Sixty-four UPEC isolates were obtained from children younger than 18 years of age, admitted to the Chung-Ang University Hospital between March 2011 and February 2013. All subjects were diagnosed with community-acquired UTI. Urine samples were collected from midstream urine in toilet-trained children and by catheterization in others. Clinical data were obtained from the medical records of the patients and entered into standardized case report forms. All UPEC isolates were identified by typical morphology, lactose fermentation, positive spot indole test, and by using VITEK 2-GN cards (bioMérieux, Hazelwood, MO, USA). Only one isolate per patient was examined. The isolates were stored at –80°C until further use.

2. 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 a VITEK 2 automated system (bioMérieux). In addition, each isolate was tested using a VITEK 2 system with the ESBL test panel (bioMérieux) contained in the NO45 card (bioMérieux). *In vitro* antimicrobial susceptibility testing was performed by the broth

microdilution method and the results were interpreted using the 2010 Clinical Laboratory Standard Institute breakpoints. Isolates resistant to antibiotics of three or more different classes were classified as multidrug-resistant (MDR).

3. Conventional phylogenetic grouping

Extraction and purification of bacterial whole-cell DNA from UPEC colonies were performed as described in the instruction manual of the QIAamp Kit (QIAGEN GmbH, Hilden, Germany). We modified the most recent version of the phylogenetic grouping method for UPEC[12], and performed multiple PCRs for the *gadA, chuA,* and *yjaA* genes and the TSPE4.C2 DNA fragment. All primers used in this study are listed in Table 1.

The PCRs were performed in 20-μL reaction volumes, with each reaction mixture containing 3.0 μL of 10× Tris-HCl buffer (100mM, pH 8.3, Mg^{2+}-free), 1.4 μL of 2.5mM dNTPs, 1.4 μL of MgCl$_2$, 0.2 μL of 5.0 U/μL Taq DNA polymerase (Takara Bio Inc., Otsu, Japan), 0.5 μL of each primer, and distilled water to a final volume of 17 μL. Finally, 3.0 μL of the DNA extract from the UPEC isolates was added to each reaction mixture. 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 95°C; 35 amplification cycles of 1 minute at 95°C, 1 minute at 58°C, and 2 minutes at 72°C; and a final extension of 10 minutes at 72°C. PCR products were electrophoresed on agarose gels and photographed under ultraviolet trans-illumination. We could identify the 373-, 281-, 216-, and 158-base pair (bp) bands for *gadA, chuA, yjaA,* and TSPE4.C2, respectively.

On the basis of the results of the PCRs, *chuA*⁻ TSPE4.C2⁻, *chuA*⁻ TSPE4.C2⁺, *chuA*⁺ *yjaA*⁻, and *chuA*⁺ *yjaA*⁺ strains were classified as belonging to groups A, B1, D, and B2, respectively. The glutamate decarboxylase-alpha *gadA* gene of *E. coli* was used as an internal amplification control.

4. Multilocus sequence typing

MLST was performed on all the UPEC isolates (n=64) using a previously standardized MLST protocol for *E. coli*[8]. The scheme used the following seven housekeeping genes: *adk* (adenylate kinase), *fumC* (fumarate hydratase), *gyrB* (DNA gyrase), *icd* (isocitrate dehydrogenase), *mdh* (malate dehydrogenase), *purA* (adenylosuccinate synthetase), and *recA* (adenosine triphosphate/guanosine triphosphate binding motif). PCR amplification for each gene was performed using the same reaction mixture and conditions described for the phylogenetic grouping in the previous section. The primer sequences used for the MLST are listed in Table 1.

The sequencing reactions were carried out using a BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems Inc., Foster City, CA, USA) with each forward primer. The

Table 1. Primer sequences used for genotyping of uropathogenic *Escherichia coli* isolates

Analysis	Marker	Direction	Sequence (5' → 3')	Product length (bp)
Conventional phylogenetic grouping	*gadA*	Forward	GATGAAATGGCGTTGGCGCAAG	373
		Reverse	GGCGGAAGTCCCAGACGATATCC	
	chuA	Forward	ATGATCATCGCGGCGTGCTG	281
		Reverse	AAACGCGCTCGCGCCTAAT	
	yjaA	Forward	TGTTCGCGATCTTGAAAGCAAACGT	216
		Reverse	ACCTGTGACAAACCGCCCTCA	
	TSPE4.C2	Forward	GCGGGTGAGACAGAAACGCG	152
		Reverse	TTGTCGTGAGTTGCGAACCCG	
MLST	*adk*	Forward	ATTCTGCTTGGCGCTCCGGG	583
		Reverse	CCGTCAACTTTCGCGTATTT	
	fumC	Forward	TCACAGGTCGCCAGCGCTTC	806
		Reverse	GTACGCAGCGAAAAAGATTC	
	gyrB	Forward	TCGGCGACACGGATGACGGC	911
		Reverse	ATCAGGCCTTCACGCGCATC	
	icd	Forward	ATGGAAAGTAAAGTAGTTGTTCCGGCACA	878
		Reverse	GGACGCAGCAGGATCTGTT	
	mdh	Forward	ATGAAAGTCGCAGTCCTCGGCGCTGCTGGCGG	932
		Reverse	TTAACGAACTCCTGCCCCAGAGCGATATCTTTCTT	
	purA	Forward	CGCGCTGATGAAAGAGATGA	816
		Reverse	CATACGGTAAGCCACGCAGA	
	recA	Forward	CGCATTCGCTTTACCCTGACC	780
		Reverse	TCGTCGAAATCTACGGACCGGA	
Genotyping	*fimH*	Forward	TGCAGAACGGATAAGCCGTGG	508
		Reverse	GCAGTCACCTGCCCTCCGGTA	

MLST, multilocus sequence typing.

reaction products were separated and detected using an Applied Biosystems 3730xl automated sequencer (Applied Biosystems Inc.), and the data were analyzed using the Sequencing analysis v3.3 software (Applied Biosystems Inc.). The allelic profile was summarized by assigning a sequence type (ST) via a web database (http://mlst.ucc.ie/mlst/dbs/Ecoli). The eBURST v3 software (available at http://www.mlst.net) was used to estimate the relationships between the isolates and to assign the strains to a clonal complex (CC)[13].

5. *fimH* genotyping

All isolates were tested for the presence of the *fimH* gene as described previously[14]. The primers were designed to amplify a 508-bp fragment of the 903-bp *fimH* gene (Table 1). The PCR and sequencing procedures were performed as described for the MLST analysis. Forward and reverse PCR primers were used for the sequencing of the *fimH* genes. Within the 508-bp amplicon, the region between nucleotides 401 and 824 (424-bp) of the *fimH* gene was selected for genotyping, as described previously [11]. The *fimH* sequence of the K12 strain (GenBank accession number GQ487190) was used as the reference sequence in this

study. These data were compared to *fimH* allele types determined previously, and the newly identified allele types were numbered in order. The *fimH* gene partial sequences were analyzed and deposited in the GenBank database under accession numbers KJ190185 to KJ190246.

For the phylogenetic analysis of the *fimH* gene, the sequences of all known *fimH* allele types and those identified in this study were aligned and subjected to neighbor-joining analysis. All evolutionary trees were drawn using MEGA5[15].

Results

1. Subject characteristics

The median age of the subjects was 5.3 months (range, 1.1–159.4 months) and the ratio of males to females was 1.7:1. Among the 64 subjects, 57 (89.1%) had a fever and 10 (15.6%) had at least one of the following urologic symptoms: dysuria, frequency, and flank pain. All subjects were tested with ultrasonography (US) and ^{99m}Tc-dimercaptosuccinic acid (DMSA) scans. Eight subjects

were further evaluated by voiding cystourethrography (VCUG). Radiologic abnormality was detected in 17 (26.6%), 16 (25.0%), and 3 (4.7%) subjects by US, DMSA scan, and VCUG testing, respectively.

2. Antimicrobial susceptibility

The susceptibility rates of UPEC isolates for all antimicrobial agents tested were as follows: piperacillin, 20.7%; ampicillin, 39.1%; trimethoprim-sulfamethoxazole, 64.1%; tobramycin, 65.5%; gentamycin, 73.4%; amoxicillin-clavulanate, 84.4%; ciprofloxacin, 85.7%; levofloxacin, 86.2%; aztreonam, 90.6%; cefepime, 90.6%; ceftazidime, 92.2%; cefotaxime, 92.2%; piperacillin-tazobactam, 97.1%; amikacin, 100%; ertapenem, 100%; meropenem, 100%; imipenem, 100%; and tigecycline, 100%. A total of 21 isolates (32.8%) were susceptible to all antibiotics, while 14 isolates (21.9%) were classified as MDR pathogens, which included 5 (7.8%) ESBL-producing isolates.

3. UPEC typing based on three different molecular methods

In the PCR-based phylogrouping method, the *gadA* gene was amplified in all the UPEC isolates. On the basis of the classification guidelines suggested previously[7], we assigned a phylogenetic group to each isolate by analyzing the PCR amplification patterns of the *chuA*, *yjaA*, and TSPE4.C2 genes (data not shown). Group B2 was the most common at 84% (n=54), followed by groups D (11%, n=7), A (3%, n=2), and B1 (2%, n=1). All isolates could be classified on the basis of this conventional phylogenetic grouping.

The MLST analysis identified 16 unique STs among the 64 UPEC isolates: ST95 (n=23), ST73 (n=10), ST131 (n=8), ST69 (n=5), ST14 (n=4), ST1193 (n=3), ST127 (n=2), ST12 (n=1), ST141 (n=1), ST349 (n=1), ST393 (n=1), ST550 (n=1), ST600 (n=1), ST697 (n=1), ST2253 (n=1), and ST2567 (n=1) (Fig. 1). ST550 and ST1193 were single-locus variants of ST14 (CC14).

The *fimH* gene was detected in 62 isolates (96.9%), and 14

allele types were identified by analyzing nucleotide differences in the targeted region (Table 2). The *fimH* sequences of 58 UPEC isolates corresponded to *fimH* allele types that had been reported previously; however, four *fimH* sequences were identified for the first time in this study and were assigned as f47 (n=2), f48 (n=1), and f49 (n=1). These three allele types were separated from each other in the phylogenetic tree (Fig. 2). Alleles f47 and f48 shared common ancestors with allele types identified in this study (f3 and f2, respectively). Although allele f49 had maximum sequence similarity with allele f7 (identified in this study), the two did not share a common ancestor. Among the *fimH* allele types identified in this study, the f1 type was most common (n=28, 45.2%), followed by the f6 and f9 types (n=7, 11.3%; each). Allele types f2 (n=1), f3 (n=2), f4 (n=2), f7 (n=1), f8 (n=4), f12 (n=3), f17 (n=2), and f28 (n=1) were also identified.

4. Comparison of UPEC strains classified on the basis of molecular typing

Phylogroups A, B1, and D were divided into two (ST697 and ST2253), one (ST600), and three (ST69, ST349, and ST393) STs, respectively (Fig. 2, Table 3). Each of the six STs (ST69, ST349, ST393, ST600, ST697, and ST2253) could be matched to a *fimH* type (f1, f48, f4, f7, f28, and f0, respectively). There were 10 STs and 12 *fimH* types in phylogroup B2. Among them, ST12, ST141, ST550, and ST2567 matched to a *fimH* type (f1, f12, f9, and f3, respectively). On the other hand, ST73 and ST95 were divided into five *fimH* types each, and ST131 was divided into four *fimH* types. The B2-ST95-f1 genotype was the most commonly identified genotype in this study (n=14, 21.9%).

The number of isolates with ESBL production and MDR pattern were low (0/23 [0%] and 2/23 [8.7%], respectively); the number of all-susceptible isolates was highest (11/23 [47.8%]) in ST95. However, a higher number of ST131 and ST73 isolates showed ESBL production (3/8 [37.5%] and 1/10 [10.0%], respectively) and MDR (5/8 [62.5%] and 3/10 [30.0%], respectively). The number of ST131 (12.5%, n=1) and ST73 (20%, n=2) isolates susceptible to all antibiotics tested was lower, relative to the total number of isolates (Table 3). Specifically, the ST131 strains were isolated mostly from infants younger than 12-months old (7/8, 87.5%), in whom the only symptom was fever, in the absence of any urologic symptoms. Among the subjects infected with the ST131 strains, radiologic abnormality was detected in four (50.0%), three (37.5%), and one subjects (12.5%) by US, DMSA scan, and VCUG testing, respectively. One subject had abnormalities in both US and DMSA scan and one subject had abnormalities in all three radiologic testing. Any radiologic abnormality was more frequently detected in UTI patients infected with ST131 strains (5/8, 62.5%) than in those infected with other strains (22/56, 39.3%), but statistically not significant (*P*=0.51).
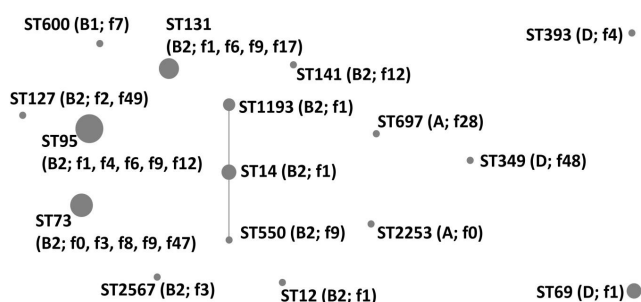

Fig. 1. eBURST analysis of 64 uropathogenic *Escherichia coli* isolates. Circle size correlates with the number of isolates for each sequence type (ST). The lengths and characteristics of the lines connecting the circles correspond to the relationship between the STs. Phylogroups and *fimH* allele types included in each ST are indicated in parentheses.
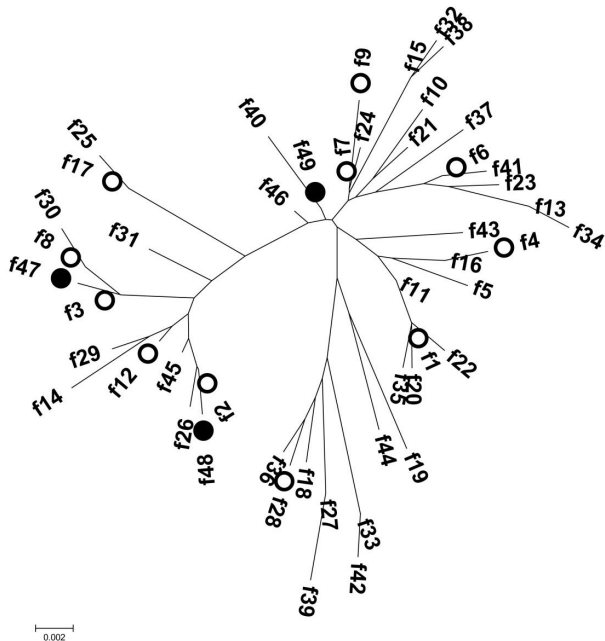
Table 2. Allele types and their corresponding variations in *fimH* nucleotide sequences

type	4 0 8	4 1 1	4 1 2	4 1 4	4 1 9	4 2 0	4 2 9	4 3 2	4 4 2	4 4 6	4 5 0	4 7 1	4 7 8	4 7 9	4 8 9	5 3 4	5 4 6	5 5 0	5 5 1	5 5 8	5 6 0	5 7 7	5 8 8	5 9 1	5 9 7	6 0 3	6 1 8	6 3 2	6 3 9	6 4 0	6 4 3	6 4 5	6 5 7	6 5 4	6 6 7	6 7 8	6 9 3	6 9 9	7 0 9	7 0 2	7 1 7	7 1 4	7 3 7	7 4 8	7 5 1	7 8 1	7 9 8	7 9 2	7 9 4	7 9 5	8 0 7
f1	G	C	G	G	C	G	T	C	G	T	T	C	A	G	C	T	C	G	T	T	G	C	C	T	A	A	T	G	C	C	G	G	A	C	C	C	G	C	C	G	T	T	A	G	G	A	C	A	G	G	G
f2		G		A												C	T					T					T					G											C	G							A
f3		G		A												C	T					T					T									A							C	G							A
f4				T												C											G																							A
f5					A											T											G																							A
f6																C	T																							A	G							A	A	A
f7																																								A	G									A
f8		G		A												C	T		C								G									A					A		C	G							A
f9		T																		A																				A	G									A
f10																T																A							C	A	G									A
f11																																																		A
f12		G		A		A										C											G													A			C	G							A
f13																C	T	A																						A	G			G				A	A	A
f14		G		A		A										C						T					G			T										A										A
f15													T																		T					T			A	G									A
f16																C											G													A	G									A
f17		G		A				A								T											G													A	G			T						A
f18																			T																		C			A	G	A								A
f19				T						T				T		T	C										G										C			C	G									A
f20			A																																																A
f21						A										T																								A	G									A
f22																				A																														A
f23												T			T																									A	G			G				A	A	A
f24																																				T			A	G									A
f25		G		A	T					A						T																							A	G				T					A
f26		G		A		A										C	T							T															A	G									A
f27									A							T							T															C		A										A
f28																T														T									A	G						A	A		A
f29		G		A		A										C					G			T															C	G									A
f30		G		A												C	T	C					G							A					C	C	G												A
f31		G		A												C					G							C										G											A
f32							A			T											T				T													A	G									A
f33				T								T	C							T C G G								T								A	G									A
f34																C	T	A							A													A	G			G				A	A	A
f35							T																																											A
f36																			T										T								C		A	G									A
f37															C																			A			A	G								A	A	A
f38												T																T				T		A					A	G									A
f39	T							A					T							T										C			A						G										A
f40									A																	T									A	G											A
f41										C	T															A											A	G								A	A		A
f42	T						T						T	C								T											T					A	A									A
f43																																		C		A				G								A
f44	T						T						T	C																				A	G	A											A
f45		G		A												C					T							A	G																A
f46																T														A	G											A
f47		G		A			A									C	T								G					A						C	G									A
f48		G		A												C	T			T				G	A							C	G							A
f49																G																				A	G								A

The *fimH* alleles observed in this study are indicated by tinted rows. Novel alleles are highlighted in dark grey, while the previously identified alleles are underscored in light grey.

Discussion

This study investigated the UPEC molecular types using three genotypic methods: PCR-based conventional phylogrouping, MLST, and *fimH* genotyping. The B2 phylogroup, ST95, and the f1 allele type were most commonly identified using the individual methods, respectively; three new *fimH* allele types were identified for the first time. To the best of our knowledge, this was the first study to determine the molecular epidemiologic data for UPEC clinical isolates of pediatric UTIs using MLST and *fimH* genotyping.

Approximately 7% of girls and 2% of boys have a UTI during

Fig. 2. Phylogenetic tree derived from all *fimH* sequence variants, including those established in the 62 clinical uropathogenic *Escherichia coli* isolates from this study (denoted by circles; ○ or ●). Three novel variants (f46, f47, and f48) were identified in this study (denoted by closed circles; ●).

Table 3. Comparison of results of the three different molecular typing techniques and antimicrobial susceptibility testing of uropathogenic *Escherichia coli* isolates

Type	ST	No.	*fimH*_SNP type	Antimicrobial susceptibility (n)		
				All-S	ESBL	MDR
A	ST697	1	f28	0	0	1
	ST2253	1	f0	0	0	1
B1	ST600	1	f7	0	0	0
B2	ST12	1	f1	0	0	0
	ST14	4	f1	2	1	1
	ST73	1	f0	0	0	0
		1	f3	1	0	0
		4	f8	1	0	1
		2	f9	0	0	0
		2	f47	0	1	2
	ST95	14	f1	4	0	2
		1	f4	1	0	0
		5	f6	4	0	0
		1	f9	1	0	0
		2	f12	1	0	0
	ST127	1	f49	1	0	0
		1	f2	0	0	0
	ST131	1	f1	0	0	1
		2	f6	0	1	2
		3	f9	0	2	2
		2	f17	1	0	0
	ST141	1	f12	1	0	0
	ST550	1	f9	0	0	0
	ST1193	3	f1	1	0	1
	ST2567	1	f3	1	0	0
D	ST69	5	f1	1	0	0
	ST349	1	f48	0	0	0
	ST393	1	f4	0	0	0
Total, n (%)		64 (100)		21 (32.8)	5 (7.8)	14 (21.9)

ST, sequence type; SNP, single nucleotide polymorphism; All-S, all susceptible; ESBL, extended spectrum beta-lactamase; MDR, multidrug resistant.

the first eight years of life. Febrile UTI occurs most frequently during the first year of life in both sexes, and is associated with a high probability of kidney involvement and consequent renal scarring. Antibiotic administration is the cornerstone of treatment for acute UTIs and is important for the prevention of parenchymal localization of the infection[1].

Certain bacteria have virulence factors that favor the establishment of infections like UTIs. In particular, UPEC is the most common pathogen causing UTIs in children; UPEC have various virulence factors that render them more competent for survival in the human urinary tract. These virulence factors are frequently confined to distinct UPEC strains, and are more likely to be changed or modified under selective pressure. Thus, genotypic characterization of pathogens and their virulence factors has become an important objective in the epidemiologic investigation of infectious agents. In addition, genotyping tests have enhanced our understanding of the epidemiology of UTIs caused by UPEC and have helped characterize the modes of transmission, sources, and risk factors for infection[5,6,11].

Sequence-based genotyping methods are becoming increasingly popular in epidemiological studies of infectious diseases such as UTIs. However, so far, only the conventional phylogrouping method have been used for analyzing clinical UPEC isolates from children with UTI in Korea[16,17].

Phylogenetic grouping analysis has shown that *E. coli* isolates

fall into four main phylogenetic groups—A, B1, B2, and D[7]. The phylogenetic strategy involved the use of three phylogenetic group markers, the *chuA* and *yjaA* genes that encode hypothetical proteins and the TSPE4.C2 DNA sequences that are situated within a gene that encodes a putative lipase esterase. The groups were assigned on the basis of different combinations of the presence and/or absence of these three amplicons. Recently, using data from whole-genome projects, an updated multiplex PCR strategy was developed for the rapid assignment of *E. coli* isolates to major phylogenetic groups[12].

This phylogrouping strategy has been used extensively worldwide. In many studies, phylogroup B2 has been shown to be

the most prevalent UPEC isolate in pediatric UTIs. For example, in a study involving Korean children, phylogroup B2 (68.1%) was found to be the most common, followed by phylogroups B1 and D (12.8% each)[17]. In addition, a recent study on Korean children with UTIs revealed that B2 (61.6%) and D (26.8%) comprised the majority of all isolated strains[17]. The B2 phylogroup was the most common (84%) type and the B2 and D phylogroups comprised the majority (95%) of the UPEC isolates in our study as well.

Nevertheless, we encountered inconsistent PCR amplification fidelities or low amplification efficiencies that led to some isolates being assigned to seemingly anomalous phylogroups. Another study has also reported unusual PCR profiles and incongruence between MLST results and the phylogenetic group assigned for some isolates[12]. Therefore, a more reliable, relevant, and consistent typing method using sequence analysis, such as MLST, is required for epidemiologic investigations.

MLST was developed as an attractive sequence-based ge-notyping technique because it provides reproducibility, compar-ability, and transferability between laboratories. Therefore, MLST is a powerful tool for the global and long-term surveillance of pathogenic bacteria[8]. In other studies on UPEC isolate using MLST data, ST14, ST69, ST73, ST95, and ST131 were the main STs identified[8,9,18]. We identified all these STs in our isolates as well; these were the five most common STs that comprised 78% of the isolates. In particular, ST131 is known to be a major ESBL-producing MDR pathogen[19,20]; we identified eight UPEC isolates of ST131 in this study. Also in this study, ST131 strains were more resistant to antimicrobials and, furthermore, related to radiologic abnormalities. However, further analysis with large amount of the strains will be needed to confirm these findings statistically.

Although MLST appears to be an excellent technique for se-quence-based genotyping, it is still impractical for large-scale epidemiological studies. In addition, as mentioned above, MLST is based on sequencing housekeeping genes, which are under stabilizing selection. Therefore, this system may not be optimal for distinguishing closely related strains[10]. On the other hand, virulence factors are frequently under selective pressure driven by host innate and acquired immunity. Therefore, genes encoding such factors are more likely to undergo mutations over a shorter period than the housekeeping genes[10]. In previous studies, *fimH* SNP analysis has been explored as a possible screening tool for the sequence-based typing of UPEC[10,11].

FimH is a mannose-binding subunit protein located at the tip of type 1 fimbriae. Phenotypic variants of *FimH* are pre-dominantly the products of SNPs in the *fimH* gene. FimH is a critical determinant of tropism in the urinary tract and vaginal epithelium for extraintestinal *E. coli*. The urovirulent phenotype is associated with genetic variants of this protein and, therefore, might be clinically relevant. A vast majority of both intestinal and extraintestinal *E. coli* strains express type 1 fimbriae. Previous

studies have shown that the *fimH* gene is the most commonly expressed virulence gene in UPEC[5,6,14]. Furthermore, many *fimH* sequences from a variety of *E. coli* isolates have been deposited in the nucleic acid sequence database for comparison. The *fimH* gene is known to be under strong selective pressure and is thus likely to show a high degree of sequence heterogeneity. It might be able to discriminate strains beyond those discriminated by the standardized MLST procedure. A previous study revealed that the two techniques (MLST and *fimH* SNP analysis) showed similar discriminatory powers, and in some of the strains, *fimH* SNP analysis provided more information than MLST[10].

In this study, analysis of the *fimH* gene identified 11 allele types of 46 known sequences and three additional allele types. Allele type f1, which corresponded to the sequence of the K12 reference strain, was most the commonly identified (45.2%) type and was distributed through the isolates with various STs (ST12, ST14, ST69, ST95, ST1313, and ST1193).

A comparison of the results of the three molecular typing methods revealed that MLST and *fimH* typing provided more discriminatory results than phylogenetic phenotyping. Isolates in ST73 and ST131 could be divided into several *fimH* allele types and, showed a more resistant antimicrobial susceptibility pattern. We postulated that they were under greater selective pressure in the host and in the environment. Although ST95 could be divided into five *fimH* types, it showed a less resistant antimicrobial susceptibility pattern; this observation could not be explained by the results obtained in this study.

This study was limited by including a relatively small number of isolates from a single tertiary-care hospital. Therefore, the results of this study are not representative of all UPEC isolates in Korean children. In addition, we analyzed only one virulence gene (*fimH*) and did not focus on the clinical aspects of UPEC strains. However, we identified new molecular epidemiologic data for UPEC that is especially valuable because this is the first example of the application of these methods for the analysis of clinical UPEC isolates from Korean children. We expect to conduct other molecular epidemiologic studies using these comprehensive and reliable methods in the future.

To the best of our knowledge, this was the first study that in-volved an MLST analysis of clinical UPEC isolates from pediatric UTIs in Korea; the results have improved upon the conventional phylogenetic groupings. This study was also the first to apply *fimH* genotyping to UPEC isolates in Korea, and led to the identi-fication of three new allele types. The *fimH* genotyping method might serve as a relatively simple, sequence-based screening test that could be applied to a large number of UPEC isolates for the characterization of recent epidemiological events.

Conflict of interest

Acknowledgments

References

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