

# Regional differences in phylogenetic group of *Escherichia coli* strains isolated from children with urinary tract infection in Korea

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**Purpose:** We phylogenetically analyzed the *Escherichia coli* strains isolated from children with urinary tract infection (UTI) in 2 regions of Korea. Virulence factors (VFs) and antibiotic resistance of the strains were also determined to compare the possible differences.

**Methods:** A total of 138 *E. coli* strains were collected from the 2 regions; Gyeongin (78 strains) and Gyeongnam (60 strains). The phylogenetic groups were determined using the triplex polymerase chain reaction (PCR) method and multiplex PCRs were used to detect 7 VFs genes (*fimH*, *papC*, *iutA*, *hlyA*, *sfa/focDE*, *afa/draBC*, and *kpsMT II*). We also tested for antibiotic resistance.

**Results:** Phylogenetic groups, B2 (61.6%) and D (26.8%), comprised the majority of all isolated strains. Regional comparisons revealed that more B2 strains and fewer non-B2 (A+B1+D) strains were found in Gyeongnam, than in the Gyeongin region ( $P=0.033$ ), and certain VFs were predominantly detected in Gyeongnam ( $P<0.05$ ). Neither regional nor phylogenetic differences, in antibiotic resistance of the strains, were significant.

**Conclusion:** We were able to confirm that the geographic location is an important determinant of the distribution of the phylogenetic groups and VFs among the *E. coli* strains that cause UTI in children.

**Key words:** Urinary tract infections, *Escherichia coli*, Phylogeny, Child

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## Introduction

*Escherichia coli* are the most frequent causative organisms of urinary tract infections (UTIs) in children<sup>1</sup>. Following the introduction of *Haemophilus influenzae* type b and pneumococcal vaccines, invasive bacterial infections that are caused by these pathogens have decreased<sup>2</sup>. In contrast, there have been some reports concerning the

increase of invasive infections caused by *E. coli* strains<sup>2-4</sup>.

Four main phylogenetic groups of *E. coli* have been described: A, B1, B2, and D<sup>5</sup>. Phylogenetic analysis has shown that the extraintestinal pathogenic *E. coli* (ExPEC) strains, which cause UTI, typically belong to the groups B2 or D, while commensal strains belong predominantly to group A<sup>6</sup>. Phylogenetic research on *E. coli* strains has been performed not only in the field of medical science, but also in

veterinary medicine and public health science<sup>7-9</sup>). However, there are a few phylogenetic studies on ExPEC strains conducted in children, especially in Korea<sup>10,11</sup>.

As our pilot study, we phylogenetically analyzed the *E. coli* strains isolated from children with UTI in two regions of Korea. Virulence profiles and antibiotic resistance of the strains were also determined to compare for the possible differences.

## Materials and methods

### 1. Subjects and strains

A total of 138 *E. coli* strains were collected, between January 2005 and December 2006, from two regions in Korea; 78 strains from Gyeongin (Incheon St. Mary Hospital and Seoul Adventist Hospital), and 60 strains from Gyeongnam (Changwon Fatima Hospital). These strains were isolated from subjects aged <15 years and clinically suspected of having UTI<sup>12</sup> on the basis of body temperature of >38.3°C and pyuria with >5 white blood cells/high-power field. Urine was collected with sterile urine bags for infants and midstream catch for older children. Strains with urine cultures of  $\geq 10^5$  colony-forming units/mL of *E. coli* were isolated to be stored at -70°C in tryptic-soy broth. De-identified samples of all strains, which were labeled only with the hospital of origin, were submitted to Incheon St. Mary Hospital for analysis. This study was approved by the Institutional Review Board (IRB) of the Catholic University of Korea (IRB number: OCMC080T29).

### 2. Methods

The phylogenetic groups, A, B1, B2, and D, were identified by amplifying the *chuA* and *yjaA* genes, and the DNA fragment TspE4.C2 using polymerase chain reaction (PCR), according to the triplex method that has been described by Clermont et al.<sup>13</sup>. Briefly, *chuA*-TspE4.C2- strains were classified as group A, *chuA*-TspE4.C2+ strains as group B1, *chuA*+ *yjaA*- strains as group D, and *chuA*+ *yjaA*+ strains as group B2. Two separate multiplex PCRs were used to detect genes for seven virulence factors (VFs), as previously reported by Le Bouguenec et al.<sup>14</sup>. The first PCR included primer pairs of type-1 fimbriae (*fimH*), P fimbriae (*papC*), aerobactin (*iutA*), and hemolysin (*hlyA*). The second multiplex PCR was used to detect genes for S fimbriae (*sfa/focDE*), Dr adhesions (*afa/draBC*), and K-antigen (*kpsMTII*).

Antimicrobial susceptibilities were determined, using the broth dilution method with Clinical and Laboratory Standards Institute criteria<sup>15</sup>. The following three antibiotics, which were used commonly in the treatment of children with UTI at outpatient clinics, were tested: amoxicillin/clavulanate, cefixime, and trimethoprim/sulfamethoxazole (TMP/SMX)<sup>1</sup>. Strains with intermediate susceptibility were considered as resistant.

### 3. Statistical analysis

Data were analyzed using SPSS ver. 12.0 (SPSS Inc., Chicago, IL, USA). The  $\chi^2$ -test or Fisher's exact test were conducted to compare the two groups. All tests were two-tailed, and *P* values of less than 0.05 were considered statistically significant.

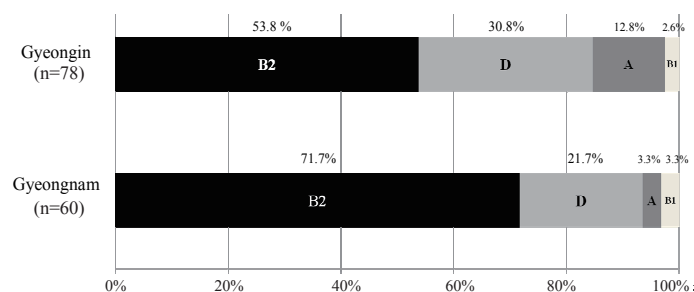
## Results

### 1. Phylogenetic groups

Of the 138 strains analyzed, those belonging to group B2 (61.6%) were the most prevalent, followed by groups D (26.8%), A (8.7%), and B1 (2.9%). Thus, groups B2 and D comprised the majority (88.4%) of all isolated strains. Regional comparisons revealed that more B2 strains and fewer non-B2 (A+B1+D) strains were observed in Gyeongnam than in Gyeongin (*P*=0.033) (Fig. 1).

### 2. Virulence profiles

Across all 138 strains, *fimH*, *papC*, *sfa/focDE*, *afa/draBC*, *kpsMTII*, *iutA*, and *hlyA* were present in 92.8%, 55.8%, 17.4%,



**Fig. 1.** Two regions of Korea are compared in the distribution of phylogenetic groups of *Escherichia coli* strains isolated from children with urinary tract infection. Regional comparisons revealed that more B2 strains and fewer non-B2 (A+B1+D) strains are found in Gyeongnam than in Gyeongin (*P*=0.033).

11.6%, 69.6%, 47.8%, and 21.7%, respectively. In the comparison of phylogenetic groups, genes for all VFs, except *afa/draBC*, were more frequently found in the B2 strains than in the non-B2 (A+B1+D) strains ( $P<0.05$ ). Regional comparisons showed that *sfa/focDE* ( $P=0.039$ ) and *kpsMTII* ( $P=0.007$ ) were predominantly detected in the Gyeongnam (Table 1).

### 3. Antibiotic resistance

Antimicrobial susceptibility testing revealed that 72.5% strains were resistant to amoxicillin/clavulanate; 47.1%, to TMP/SMX; and 22.5%, to cefixime. Regional comparisons showed that more strains of Gyeongin were resistant to amoxicillin/clavulanate ( $P=0.341$ ) and TMP/SMX ( $P=0.086$ ) than those of Gyeongnam (Table 1). In the comparison of phylogenetic groups, more of the non-B2 (A+B1+D) strains were resistant to cefixime ( $P=0.646$ ) and TMP/SMX ( $P=0.166$ ) than that of the B2 strains. However, neither regional nor phylogenetic differences in antibiotic resistance of the strains were statistically significant.

## Discussion

Pathogenic *E. coli* strains cause a wide variety of intestinal or extra-intestinal infections. In contrast to intestinal pathogenic *E. coli* strains, such as enteropathogenic *E. coli* or enterotoxigenic *E. coli*, the ExPEC strains do not cause disease within the intestinal tract, where they are adapted for peaceful coexistence with the host<sup>16</sup>. However, at the extraintestinal sites, these strains induce diverse infectious diseases, including UTI, bacteremia, neonatal meningitis, pneumonia, and

intraabdominal infections<sup>16,17</sup>. On the basis of phylogenetical analysis, ExPEC strains typically belong to the groups B2 or D, and are thus, distinct from both the commensal and the intestinal pathogenic strains<sup>5,18</sup>.

Group B2 strains are known to possess more VFs than strains of the other groups, and group D strains are clinically remarkable because of their antimicrobial resistance<sup>16,19</sup>. Phylogenetic groups and VFs are unique microbiological features of the *E. coli* strains, but these features are obviously influenced by the host genetic factors (immune status, specific blood group antigens, and dietary intake), and socio-environmental factors (climate, economic condition, and the extent of antibiotics usage)<sup>11,19,20</sup>.

The present study showed that a majority of the strains belonged to the group B2 or D, and that B2 strains and certain VFs were found predominantly in a particular region. The differences in the distribution of phylogenetic groups between nations have also been reported previously<sup>19,20</sup>. Among seven VFs detected, *fimH* was found to be present in nearly all the strains, which reflects an origin in a shared enterobacterial ancestor<sup>16</sup>. Most of the known extraintestinal VFs (*papC*, *sfa/focDE*, *kpsMTII*, and *hlyA* genes) were concentrated within groups B2 or D, whereas, the *afa/draBC* gene was more broadly distributed, which is consistent with the typically chromosomal versus plasmid location of these VFs<sup>21</sup>. With regard to the antibiotic resistance, neither regional nor phylogenetic differences were statistically significant. In general, antibiotic resistance of the strains is known to be associated negatively with virulence and positively with host compromise<sup>22</sup>. Our study, however, has certain limitations. A major limitation is that we could not include the host

**Table 1.** Regional and Phylogenetic comparison of Virulence Factors and Antibiotic Resistance for 138 *Escherichia coli* Strains Isolated from Korean Children with Urinary Tract Infections

Characteristic	Region			Phylogenetic group				
	Gyeongin (n=78)	Gyeongnam (n=60)	P value	A (n=12)	B1 (n=4)	D (n=37)	B2 (n=85)	P value*
Virulence factor (gene)								
Type 1 fimbriae ( <i>fimH</i> )	70 (89.7)	58 (96.7)	NS	7 (58.3)	4 (100.0)	35 (94.6)	82 (96.5)	0.033
P fimbriae ( <i>papC</i> )	40 (51.3)	37 (61.7)	NS	5 (41.7)	0 (0)	13 (35.1)	59 (69.4)	<0.001
S fimbriae ( <i>sfa/focDE</i> )	9 (11.5)	15 (25.0)	0.039	0 (0)	0 (0)	1 (2.7)	23 (27.1)	<0.001
Dr adhesins ( <i>afa/draBC</i> )	8 (10.3)	8 (13.3)	NS	1 (8.3)	0 (0)	5 (13.5)	10 (11.8)	NS
Capsule ( <i>kpsMT II</i> )	47 (60.3)	49 (81.7)	0.007	3 (25.0)	1 (25.0)	23 (62.2)	69 (81.2)	<0.001
Aerobactin ( <i>iutA</i> )	36 (46.2)	30 (50.0)	NS	1 (8.3)	1 (25.0)	15 (40.5)	49 (57.6)	0.003
Hemolysin ( <i>hlyA</i> )	13 (16.7)	17 (28.3)	NS	0 (0.0)	0 (0.0)	3 (8.1)	27 (31.8)	<0.001
Antibiotic resistance								
Amoxicillin/clavulanate	59 (75.6)	41 (68.3)	NS	7 (58.3)	1 (25.0)	28 (75.7)	64 (75.3)	NS
Cefixime	14 (17.9)	17 (28.3)	NS	2 (16.7)	0 (0)	11 (29.7)	18 (21.2)	NS
TMP/SMX	42 (53.8)	23 (38.3)	NS	4 (33.3)	2 (50.0)	23 (62.2)	36 (42.4)	NS

Values are presented as number (%).

NS, not significant; TMP/SMX, trimethoprim-sulfamethoxazole.

\*Statistical comparison between phylogenetic group B2 and non-B2 (A+B1+D) strains.

factors. Other limitations include the small sample size; the urine collection method; and the detection of genes, rather than gene expression.

In summary, we were able to confirm that the geographic location is an important determinant of the distribution of phylogenetic groups and VFs among these strains. Such regional differences should be considered, while conducting molecular epidemiological studies, on ExPEC strains in children.

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