Differences of Virulence Factors, And Antimicrobial Susceptibility According To Phylogenetic Group In Uropathogenic *Escherichia Coli* Strains Isolated From Korean Patients

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Research

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Abstract

Background: *Escherichia coli* is among the most common uropathogens. Increased antibiotic resistance to gram negative bacilli is global concern. Alternative therapeutic options including vaccines against uropathogenic *E. coli* (UPEC) have been developed. In this study, we compared the genotypic characteristics and antimicrobial susceptibility of UPEC according to phylogenetic groups.

Methods: We retrospectively reviewed the medical records of pyelonephritis patients with UPEC between February 2015 and June 2018. We compared the clinical and genotypic characteristics of UPEC according to phylogenetic groups. The phylogenetic groups and 29 virulence factors were identified using multiplex polymerase chain reaction.

Results: Phylogenetic group analysis revealed that most uropathogenic *E. coli* belonged to groups B2 and D: B2 (276, 77.7%), D (62, 17.5%), B1 (12, 3.4%), and A (5, 1.4%). Among the virulence factors, *fyuA*, *fimH*, *traT*, *iutA*, *papG allele II*, and *papC* were the most frequently observed. Phylogenetic group B2 was more closely related to virulence factors, including *fimH, sfa/focED, focG, hlyA, cnf1, fyuA*, and *PAI*, than group D. Groups B2 and D showed similar clinical presentations and complications. Group B2 had mostly healthcare-associated infections and antimicrobial resistance. Group D mostly had community-acquired infections. The K1 serotype was prevalent in group B2, and K5 was the most prevalent in group D.

Conclusions: Phylogenetic group B2 had more proportions and types of virulence factors than group D. Group B2 showed a high presentation of virulence factors related to adhesions and toxins. An increased presentation of antimicrobial resistance and healthcare-associated infections was also noted. Considering the genetic characteristics of UPEC, alternative therapeutic options targeting frequent virulence factors might be considered in addition to antibiotics.

Background

Urinary tract infection (UTI) is one of the most common bacterial infections worldwide [1]. Among the uropathogens, uropathogenic *Escherichia coli* is the most predominant, causing up to 95% of community-acquired UTIs and 50% of healthcare-associated UTIs [2–4]. The clinical spectrum of UTI ranges from asymptomatic bacteriuria to cystitis, pyelonephritis, and prostatitis, and septic shock [5]. Clinical manifestations of UTI may differ depending on the underlying disease, preceding factors, and infecting bacteria [6–7]. These manifestations can be influenced by bacterial pathogenicity [8]. The in-hospital mortality is more dependent on pathogens and females than the comorbidity index and age [4]. *E. coli* can be categorized into four major groups, A, B1, B2, and D, using four phylogenetic group markers, *gadA, chuA, yjaA*, and TSPE4.C2 [9]. Most uropathogenic *E. coli* belong to the phylogenetic group B2 or D [10–12]. Uropathogenic *E. coli* have many virulence factors, including adhesins, toxins, iron acquisition, and immune evasion that enable them to invade, colonize, and survive in the urinary tract [13]. However, studies on the differences in virulence factors and clinical characteristics of *E. coli* according to the phylogenetic group remain limited. Increasing multidrug-resistant gram-negative bacilli are a global
concern [14–15]. In addition to antibiotics, vaccines, immunomodulating agents, and probiotics have been proposed as alternative therapeutic options for urinary tract infections [16–17]. Vaccines for uropathogenic *E. coli* are mainly targeting adhesion molecules [18]. As virulence factors related to iron metabolism, vaccines targeting *iutA* and *fyuA* are also being developed [18–19]. Therefore, in this study, we compared the virulence factors and antimicrobial susceptibility according to the phylogenetic groups B2 and D, which accounted for the majority of uropathogeni *E. coli*, and determined whether differences exist in clinical manifestations between the two groups. Additionally, the clinical characteristics and predisposing factors between the two groups were examined.

**Methods**

**Study subjects**

Patients who visited Keimyung University Dongsan Medical Center and had uropathogenic *E. coli* UTI from February 2015 to June 2018 were divided into two groups according to phylogenetic group B2 or D. Patients aged <18 years or with polymicrobial infections were excluded, along with patients transferred to other hospitals during the treatment. *E. coli* isolates from the blood, urine, or pus were collected, and only one isolate per patient was examined. The categories of infection were further divided into community-acquired, healthcare-associated, and nosocomial infections. Community-acquired infections were defined as those in which symptoms occurred within 48 hours after visiting the hospital. However, patients with community-acquired infections and healthcare-associated risk factors were categorized under healthcare-associated infections. Healthcare-associated risk factors included hospitalization within 90 days, hemodialysis, intravenous medication in outpatient clinics, or residency in long-term care facilities. Nosocomial infections were defined as those in which symptoms occurred 48 h after hospital admission. This study was approved by the Institutional Review Board of Keimyung University Dongsan Medical Center (File No. 2020-02-003). The requirement for written informed consent was waived by the committee because of the retrospective nature of the study and the use of identifiable specimens. Medical records were reviewed retrospectively. Inclusion criteria for UTI were defined: 1) a quantitative culture of $\geq 10^5$ CFU/mL for *E. coli* isolated from midstream urine or catheter, and 2) the presence of urinary symptoms such as urgency, high frequency of urination, and dysuria. Diagnostic criteria for upper UTI included fever, flank pain, urinary symptoms, and/or tenderness of the costovertebral angle. We relied on medical records for this information.

**Study design**

1. Data collection

Medical records, including underlying diseases, predisposing factors, antibiotics used within last 3 months, previous hospitalization, antimicrobial susceptibility, clinical features, current antibiotics being administered, and treatment outcomes, were retrospectively analyzed. Obstructive UTI was defined as UTI due to urinary tract obstruction such as one of the following: benign prostate hyperplasia, uterine
prolapse, or malignancy. Urinary tract stones were not regarded as obstructive UTI and were classified as predisposing factors. Severe UTI was defined as UTI combined with multiorgan failure or hypotension and complicated UTI as UTI with predisposing factors for persistent and relapsing infections, such as urinary tract stones, foreign bodies (for example, indwelling urinary catheters or other drainage devices), or obstructions. The short-term treatment outcome was determined after 72 hours of empirical antibiotic treatment based on persistent fever and acute kidney injury. Persistent fever was defined as fever persisting over 72 hours. Acute kidney injury was defined as an increase in serum creatinine level by > 0.3 mg/dL within 48 h or increase in serum creatinine level to > 1.5 times baseline, which would have occurred within the prior 7 days or urine volume < 0.5 mL/kg/h for 6 hours. The long-term outcome was determined by infection-related 30-day mortality and relapsed UTI within 3 months. Infection-related 30-day mortality was defined as death due to uropathogenic *E. coli* UTI or complications of infection within 30 days.

2. Phylogenetic groups

Phylogenetic groups of the *E. coli* isolates were determined using the polymerase chain reaction (PCR)-based method developed by Doumith et al. [1]. *E. coli* were categorized into one of the four main phylogenetic groups—A, B1, B2, and D—using four phylogenetic group markers — *gadA*, *chuA*, *yjaA*, and TSPE4.C2. The groups were determined according to the different combinations of the four amplicons. Crude DNA was prepared by lysis of colonies in 500 μL of sterile distilled water at 100 °C for 15 min, followed by centrifugation. The lysis supernatant was used for the polymerase chain reaction. The polymerase chain reaction conditions were as follows: an initial activation at 94 °C for 4 min; then, 30 cycles at 94 °C for 30 s, 65 °C for 30 s, 72 °C for 30 s; and finally, extension at 72 °C for 5 min [9]. The primers used in this study are listed in Table 1.

Table 1. Primers used for phylogenetic groups in this study

| Marker   | Primer direction | Primer sequence (5′-3′)                     | Product length (bp) |
|----------|------------------|---------------------------------------------|---------------------|
| *gadA*   | Forward          | GATGAAATGGCGTTGGGCAGAAG                    | 373                 |
|          | Reverse          | GGCAGAAGTCCAGACGATATCC                      |                     |
| *ChuA*   | Forward          | ATGATCATCGCGCGCTTGCTG                      | 281                 |
|          | Reverse          | AAACCGCCTCGCGCCTAAT                        |                     |
| *yjaA*   | Forward          | TGTTCGCGATTTGAAGACATCGT                    | 216                 |
|          | Reverse          | ACCTGTGACAAACCGGCCTCA                      |                     |
| TSPE4.C2 | Forward          | GCGGGTGAGACAGAAACCGCG                     | 152                 |
|          | Reverse          | TTGTCTGAGTTGCGAACC                   |                     |

3. Virulence genes
Virulence genes were detected using a multiplex polymerase chain reaction assay developed by Johnson and Stell [2]. This involved five primer pools, with 29 primers listed in order of decreasing amplicon size (bp) within each pool as follows: pool 1: PAI, papA, fimH, kpsMT III, papEF, and ibeA; pool 2: fyuA, bmaE, sfa/focDE, iutA, papG allele III, and K1; pool 3: hlyA, rfc, nfaE, papG allele I, kpsMT II, and papC; pool 4: gafD, cvaC, cdtB, focG, traT, and papG allele II; and pool 5: papG allele I, papG alleles II and III, afa/draBC, cnf1, sfas, and K5. The reaction was conducted with an initial activation at 95 °C for 12 min; followed by 25 cycles of denaturation (94 °C, 30 s), annealing (63 °C, 30 s), and extension (68 °C, 3 min); and a final extension at 72 °C for 10 min. The amplicons were electrophoresed in 2% agarose gels, stained with ethidium bromide, and destained with distilled water [10]. The primers used in this study are listed in Table 2.

Table 2. Primers used for virulence factors used in this study
| Marker         | Primer direction | Primer sequence (5′-3′)                                      | Product length (bp) |
|---------------|------------------|-------------------------------------------------------------|---------------------|
| **papA**      | Forward          | ATGGCAGTGGTGTCCTTTTGGT                                     | 720                 |
|               | Reverse          | CGTCCCCCATACGTGCTCTTC                                       |                     |
| **papC**      | Forward          | GTGGCAGTATGAGTAATGACCGTTA                                   | 200                 |
|               | Reverse          | ATATCCTTTCTGCAGGGATGCAATA                                    |                     |
| **papEF**     | Forward          | GCAACAGCAACGCTGGTGTCATCAT                                   | 336                 |
|               | Reverse          | AGAGAGAGCCACTCTTATACGGAC                                     |                     |
| **papG allele I** | Forward          | TCGTGCTCAGGTCCCGGAATT                                       | 461                 |
|               | Reverse          | TGGCATCCCCCAACATTATCG                                       |                     |
| **papG allele II** | Forward      | GGGATGAGCGGGGCTTGTGAT                                       | 190                 |
|               | Reverse          | CGGGCCCCCAAGTAACCTCG                                        |                     |
| **papG allele III** | Forward    | GGCCTGCAATGGATTACCTG                                       | 258                 |
|               | Reverse          | CCACCAAATGACCATGGCCAGAC                                     |                     |
| **sfa/focDE** | Forward          | CTCCGGAGAACTGGTGTCATTTAC                                    | 410                 |
|               | Reverse          | CGGAGGAGTAATTACAAACCTGGA                                    |                     |
| **sfaS**      | Forward          | GTGGATAACGACGATTACTGTG                                     | 240                 |
|               | Reverse          | CCGCCAGCATTCCCTGTATTC                                       |                     |
| **focG**      | Forward          | CAGCACAGGCGAATGGTACGAG                                      | 360                 |
|               | Reverse          | GAATGTCGCCTGCCCATTG                                        |                     |
| **afa/draBC** | Forward          | GGCAGAGGCGCGGCAACAGGC                                       | 559                 |
|               | Reverse          | CCCGTAACGCGCCAGCATCTC                                       |                     |
| **bmaE**      | Forward          | ATGGCGCTAATTTGCCGACTG                                       | 507                 |
|               | Reverse          | AGGGGGACATAGGCCCCCTTC                                       |                     |
| **gafD**      | Forward          | TGTGGACCGTCTCAGGGGTGCT                                       | 952                 |
|               | Reverse          | CTCCGGAAACTCGCTTTACT                                        |                     |
| **nfaE**      | Forward          | GCTTACTGATTTCTGGGTGGA                                       | 559                 |
|               | Reverse          | CGGTGGCCAGTCTGATGCAA                                       |                     |
| **fimH**      | Forward          | TGCAGGAACGTAAGCCCGTGG                                       | 508                 |
|               | Reverse          | GCAGTCACCTGCCCCTCGGA                                       |                     |
| **hlyA**      | Forward          | AACAAGGATAAGCAGACTGTTCTGGCT                                  | 1177                |
Reverse | ACCATATAAAGCGGTACTTCCCGTCA
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cnf1 | Forward | AAGATGGAGTTTCTATCGAGGAG | 498
Reverse | CATTCAAGTGTCCTGCCCCTCATTATT

fyuA | Forward | TGATTAATCCCGGCACGGGAA | 880
Reverse | CGCAGTAGCCAGCATGGTTGTA

iutA | Forward | GGCTGGACATCATGGGAACCTGG | 300
Reverse | CGTCGGGAACGGGTAGAATCG

kpsMT II | Forward | GCGCATTTGCTGATACTGTTG | 272
Reverse | CATCCAGACGATAAGCATGAGCA

kpsMT III | Forward | TCCTCTTGCTACTATTCCCTCTC | 392
Reverse | AGGCCTATCCATCCCTCCTAAC

rfc | Forward | ATCCATCAGGAGGGGACTGGA | 788
Reverse | AACCATAACCAACCATGCGGAG

ibeA | Forward | AGGCAGGTGTGCSCGCCTCTAC | 170
Reverse | TGGTGCTCCGGAACACCATGC

cvaC | Forward | CACACACAAACGGGAGCTGTTT | 680
Reverse | TTTCCGCAGCATAGTTCCAT

traT | Forward | GGTGTGGTGCGATGAGCACAG | 290
Reverse | CACGGTCAGCCATCCCTGAG

PAI | Forward | GGACATCCTGTTACATCGCGCA | 930
Reverse | TCGCCACCAATCACAGCCGAAC

PAI: pathogenicity island

4. Antibiotic resistance and extended spectrum beta-lactamase (ESBL)-disk diffusion test

Clinical specimens, such as blood, urine, and pus, were collected for microbial identification. *E. coli* was isolated using a Vitek system (BioMerieux, Lyon, France). Antimicrobial susceptibility profiles were determined by interpreting the breakpoints recommended by the Clinical and Laboratory Standards Institute (CLSI). ESBL production was measured using Phoenix GN Combo Panels 448541, which were inoculated and incubated according to the manufacturer's recommendations [20]. Disk diffusion tests were performed in cases of resistance to cefotaxime or ceftazidime, twice for each specimen, and interpreted according to the CLSI guidelines, using Mueller-Hinton agar. Thirty microgram disks containing ceftazidime and ceftriaxone and 30/10 μg disks containing cefotaxime/clavulanate or
ceftazidime/clavulanate were used (BD BBLå”™ Sensi-Discå”™ Antimicrobial Susceptibility Test Discs, BD Diagnostic Systems, Sparks, Maryland, U.S.A) [21].

Statistical analysis

Statistical analysis was performed using the Statistical Package for the Social Sciences software (version 21.0; SPSS Inc., IBM Corp., Armonk, NY, USA). Categories were compared using the chi-square test or Fisher’s exact test. For continuous variables, the normal distribution was calculated using the Kolmogorov-Smirnov test. The Mann–Whitney U test and independent t-test were performed for data that followed non-normal and normal distributions, respectively. Statistical significance was defined as $P < 0.05$.

Results

Basic characteristics of the study group

Phylogenetic group analysis revealed that most uropathogenic E. coli belonged to groups B2 and D: B2 (276, 77.75%), D (62, 17.46%), B1 (12, 3.38%), and A (5, 1.41%). In B2, 276 patients were included; 57 (20.7%) were men, and the mean age was 69.43 years. In group D, 62 patients were included; 4 (6.5%) were men, and the mean age was 69.16 years. The proportion of male patients was significantly higher in the group B2 than in other groups ($P = 0.009$). For the underlying diseases, diabetes mellitus (DM) was more frequently observed in group D (51.6%) than in group B2 (36.6%) ($P = 0.029$). No significant differences between the two groups occurred, except for DM. The McCabe classification showed no significant differences between the two groups. Obstructive uropathy and previous use of urinary catheters were more frequently observed in group B2 than in other groups, but without significant difference. Complicated UTI was more frequently observed in group B2 than in other groups ($P = 0.009$). Bacteremic UTI and severe UTIs did not differ significantly between the two groups; besides, analysis of UTI categories revealed no significant difference in the proportions of renal abscess, acute prostatitis, and prostatic abscess. However, analysis of infection categories revealed that the proportion of community-acquired and healthcare-associated infections were significantly higher in groups D and B2, respectively, than in other groups (Table 3).
Table 3
Baseline characteristics and clinical manifestations of uropathogenic *Escherichia coli* infection according to phylogenetic group

|                         | Phylogenetic group B2 (n = 276) | Phylogenetic group D (n = 62) | p value |
|-------------------------|---------------------------------|-------------------------------|---------|
| Age, years              | 69.43 ± 14.59                   | 69.16 ± 14.13                 | 0.893   |
| Male sex                | 57 (20.7%)                      | 4 (6.5%)                      | 0.009   |
| Category of UTI         |                                 |                               |         |
| Acute pyelonephritis    | 269 (97.5%)                     | 61 (98.4%)                    | 0.999*  |
| Acute prostatitis       | 8 (2.9%)                        | 2 (3.2%)                      | 0.999*  |
| Renal abscess           | 20 (7.2%)                       | 8 (12.9%)                     | 0.144   |
| Prostatic abscess       | 4 (1.4%)                        | 0 (0.0%)                      | 0.999*  |
| Category of infection   |                                 |                               |         |
| Community-acquired      | 192 (69.6%)                     | 52 (83.9%)                    | 0.023   |
| Healthcare-associated   | 71 (25.7%)                      | 7 (11.3%)                     | 0.015   |
| Nosocomial              | 13 (4.7%)                       | 3 (4.8%)                      | 0.999*  |
| Underlying diseases     |                                 |                               |         |
| Solid tumor             | 39 (14.1%)                      | 9 (14.5%)                     | 0.937   |
| Hematologic malignancy  | 0                               | 0                             |         |
| Chronic liver disease   | 43 (15.6%)                      | 7 (11.3%)                     | 0.390   |
| Liver cirrhosis         | 14 (5.1%)                       | 0 (0.0%)                      | 0.082*  |
| Cardiovascular disease  | 75 (27.2%)                      | 16 (25.8%)                    | 0.826   |
| Hypertension            | 143 (51.8%)                     | 39 (62.9%)                    | 0.113   |
| Neurologic disease      | 98 (35.5%)                      | 19 (30.6%)                    | 0.467   |
| Chronic renal disease   | 14 (5.1%)                       | 4 (6.5%)                      | 0.753*  |
| Diabetes mellitus       | 101 (36.6%)                     | 32 (51.6%)                    | 0.029   |
| Chronic lung disease    | 28 (10.1%)                      | 7 (11.3%)                     | 0.789   |
| Solid organ transplantation | 2 (0.7%)                    | 0 (0.0%)                      | 0.999*  |

BPH: benign prostate hyperplasia; UTI: urinary tract infection; *: Fisher's exact test
| Condition                                                                 | Phylogenetic group B2 (n = 276) | Phylogenetic group D (n = 62) | p value |
|--------------------------------------------------------------------------|---------------------------------|-------------------------------|---------|
| Pregnancy                                                                | 1 (0.4%)                        | 0 (0.0%)                      | 0.999*  |
| Neurogenic bladder                                                       | 24 (8.7%)                       | 5 (8.1%)                      | 0.873   |
| BPH or uterine prolapse                                                 | 26 (9.4%)                       | 2 (3.2%)                      | 0.110   |
| Urogenic anomaly                                                        | 4 (1.4%)                        | 0 (0.0%)                      | 0.999*  |
| Nephrectomy state (one kidney)                                          | 3 (1.1%)                        | 1 (1.6%)                      | 0.557*  |
| Neutropenia                                                             | 0                               | 0                             |         |
| Previous genitourinary surgery or procedure within 72 h                  | 0                               | 0                             |         |
| Recurrent UTI                                                           | 32 (11.6%)                      | 6 (9.7%)                      | 0.666   |
| Presence of urologic devices                                            | 1 (0.4%)                        | 0 (0.0%)                      | 0.999*  |
| Intermittent catheterization                                            | 2 (0.7%)                        | 0 (0.0%)                      | 0.999*  |
| Urinary catheter                                                        | 23 (8.3%)                       | 3 (4.8%)                      | 0.439*  |
| Prior antibiotics within 3 months                                       | 64 (23.2%)                      | 12 (19.4%)                    | 0.514   |
| Type of UTI                                                             |                                 |                               |         |
| Bacteremic UTI                                                          | 167 (60.5%)                     | 44 (71.0%)                    | 0.124   |
| Complicated UTI                                                         | 57 (20.7%)                      | 4 (6.5%)                      | 0.009   |
| Severe UTI                                                              | 94 (34.1%)                      | 21 (33.9%)                    | 0.978   |

BPH: benign prostate hyperplasia; UTI: urinary tract infection; *: Fisher’s extract test

**Comparison of virulence factors between phylogenetic groups B2 and D**

*FimH* and *fyuA* were the most common virulence factors in both groups. Adhesion molecules were identified in both groups, and their distribution was similar. *FimH* (99.6% vs. 90.3%, *P* < 0.001), *sfa/focED* (17.0% vs. 0.0%, *P* < 0.001), and *focG* (12.3% vs. 3.2%, *P* = 0.036) were more significant in phylogenetic group B2 than in D. Phylogenetic group B2 was the most closely related to virulence factors associated with adhesion, toxin, iron metabolism, and *PAI*; toxin-related virulence factors were more significantly identified in phylogenetic group B2 than in phylogenetic group D. Group B2 had higher levels of toxin-associated virulence: *hlyA* (phylogenetic group B2 = 33.3% vs. D = 6.5%, *P* < 0.001), *cnf1* (39.9% vs. 0.0%, *P* < 0.001), and *cvaC* (8.7% vs. 0.0%, *P* = 0.011); iron metabolism-associated virulence factors: *fyuA* (99.6% vs. 93.5%, *P* = 0.004); and *PAI* (88.8% vs. 19.4%, *P* < 0.001) than group D. In protection molecules,
no significant differences occurred between the two groups. The K1 serotype was prevalent in the phylogenetic group B2, whereas K5 was widespread in group D (Table 4).

Table 4. Virulence factors of uropathogenic *Escherichia coli* classified by phylogenetic group
|                      | Phylogenetic group B2 (n = 276) | Phylogenetic group D (n = 62) | p value |
|----------------------|---------------------------------|-------------------------------|---------|
| Adhesion molecule    |                                 |                               |         |
| papA                 | 186 (67.4%)                     | 41 (66.1%)                    | 0.848   |
| papEF                | 40 (14.5%)                      | 7 (11.3%)                     | 0.510   |
| papC                 | 195 (70.7%)                     | 43 (69.4%)                    | 0.840   |
| papG                 | 133 (48.2%)                     | 28 (45.2%)                    | 0.666   |
| papG allele I        | 1 (0.4%)                        | 0 (0.0%)                      | 0.999*  |
| papG allele II       | 196 (71.0%)                     | 46 (74.2%)                    | 0.616   |
| papG allele III      | 7 (2.5%)                        | 0 (0.0%)                      | 0.357*  |
| fimH                 | 275 (99.6%)                     | 56 (90.3%)                    | <0.001* |
| afa/draBC            | 38 (13.8%)                      | 12 (19.4%)                    | 0.263   |
| sfaS                 | 15 (5.4%)                       | 5 (8.1%)                      | 0.385*  |
| sfa/focED            | 47 (17.0%)                      | 0 (0.0%)                      | <0.001  |
| bmaE                 | 1 (0.4%)                        | 0 (0.0%)                      | 0.999*  |
| gafD                 | 0                               | 0                              |         |
| nfaE                 | 1 (0.4%)                        | 2 (3.2%)                      | 0.088*  |
| focG                 | 34 (12.3%)                      | 2 (3.2%)                      | 0.036   |
| Toxin                |                                 |                               |         |
| hlyA                 | 92 (33.3%)                      | 4 (6.5%)                      | <0.001  |
| cnf1                 | 110 (39.9%)                     | 0 (0.0%)                      | <0.001  |
| cvaC                 | 24 (8.7%)                       | 0 (0.0%)                      | 0.011*  |
| cdtB                 | 0                               | 0                              |         |
| Iron metabolism      |                                 |                               |         |
| fyuA                 | 275 (99.6%)                     | 58 (93.5%)                    | 0.004*  |
| iutA                 | 203 (73.6%)                     | 47 (75.8%)                    | 0.715   |
| Protection, Capsule  |                                 |                               |         |
| kpsMT II             | 159 (57.6%)                     | 39 (62.9%)                    | 0.444   |
| kpsMT III            | 3 (1.1%)                        | 2 (3.2%)                      | 0.228*  |
Comparison of antibiotic resistance, empirical antibiotics, and antibiotic adequacy

The rates of resistance to ciprofloxacin, cefotaxime, and trimethoprim/sulfamethoxazole were 50.5%, 45.1%, and 37.1% in group B2 and 22.6%, 29.0%, and 48.4% in group D, respectively ($P < 0.001$; $P < 0.001$; and $P = 0.100$, without significance difference). The proportions of ESBL-producing *E. coli* in Phoenix GN Combo Panels were 44.0% and 27.4% in groups B and D, respectively ($P = 0.016$): in the double-disk diffusion test, the proportions of ESBL-producing *E. coli* were 27.2% and 17.7% in groups B2 and D, respectively ($P = 0.123$) (Table 5). Among ESBL-producing *E. coli*, resistance rates to ciprofloxacin, piperacillin/tazobactam, and trimethoprim/sulfamethoxazole were 87.6%, 14.0%, and 56.2% in group B2 and 47.1%, 11.8%, and 58.8% in group D, respectively ($P < 0.001$; $P = 0.999$; and $P = 0.838$, without significance difference). For both groups, the most commonly used empirical antibiotic was ceftriaxone. Eighty-four (66.7%) and 48 cases (77.4%) in groups B2 and D, respectively, were evaluated to have used concordant initial antibiotics.
Table 5
Antibiotic resistance of uropathogenic *Escherichia coli* classified by phylogenetic group

| Resistance             | Phylogenetic group B2 (n = 276) | Phylogenetic group D (n = 62) | p value |
|------------------------|----------------------------------|-------------------------------|---------|
| Amikacin               | 2 (0.8%)                         | 0 (0.0%)                      | 0.999*  |
| Amoxicillin/clavulanate| 118 (42.9%)                      | 9 (14.6%)                     | < 0.001 |
| Ampicillin             | 210 (76.4%)                      | 47 (75.8%)                    | 0.926   |
| Aztreonam              | 120 (43.6%)                      | 17 (27.4%)                    | 0.019   |
| Cefazolin              | 134 (48.7%)                      | 18 (29.0%)                    | 0.005   |
| Cefepime               | 120 (43.6%)                      | 17 (27.4%)                    | 0.019   |
| Cefotaxime             | 124 (45.1%)                      | 18 (29.0%)                    | 0.022   |
| Cefoxitin              | 25 (9.1%)                        | 5 (8.1%)                      | 0.804   |
| Ceftazidime            | 121 (44.0%)                      | 16 (25.8%)                    | 0.008   |
| Ciprofloxacin          | 139 (50.5%)                      | 14 (22.6%)                    | < 0.001 |
| Ertapenem              | 0                                | 0                             |         |
| Gentamicin             | 94 (34.2%)                       | 17 (27.4%)                    | 0.315   |
| Imipenem               | 0                                | 0                             |         |
| Piperacillin/tazobactam| 25 (9.1%)                        | 3 (4.8%)                      | 0.276   |
| Tigecycline            | 0                                | 0                             |         |
| Trimethoprim/sulfamethoxazole | 102 (37.1%)       | 30 (48.4%)                    | 0.100   |
| ESBL                   | 121 (44.0%)                      | 17 (27.4%)                    | 0.016   |
| ESBL double disk       | 75 (27.2%)                       | 11 (17.7%)                    | 0.123   |

ESBL: extended-spectrum beta-lactamase; *: Fisher’s exact test

Comparison of treatment outcomes

In early outcomes, for group B2, 22.8% and 17.0% of cases had persistent fever and experienced acute kidney injury, respectively, during the hospital stay; for D, 22.6% and 17.7% of cases had persistent fever and acute kidney injury, respectively. Persistent fever and acute kidney injury differences were insignificant between the two groups. The duration of hospital stay, 30-day mortalities, and infection-related 30-day mortality were 14.90 days, 1.8%, and 0.7% in group B2 and 12.71 days, 1.6%, and 0.0% in group D, respectively (without significant difference; *P* = 0.999 and *P* = 0.999). Six and one patient died in
groups B2 and D, respectively. After they were diagnosed with UTI, the median period from diagnosis to death in group B2 was 9.5 days (interquartile range 7.0–25.75 days), and in group D, a patient died on day 3. Within 3 months, UTI events relapsed in 7.6% and 8.1% of B2 and D members, respectively, which were not significantly different (Table 6).

Table 6
Outcomes of uropathogenic *Escherichia coli* infection classified by phylogenetic group

| Outcome                                  | Phylogenetic group B2 (n = 276) | Phylogenetic group D (n = 62) | p value |
|------------------------------------------|---------------------------------|------------------------------|---------|
| Persistent fever                         | 63 (22.8%)                      | 14 (22.6%)                   | 0.967   |
| Acute kidney injury                      | 47 (17.0%)                      | 11 (17.7%)                   | 0.893   |
| 30-day mortality                         | 5 (1.8%)                        | 1 (1.6%)                     | 0.999*  |
| Infection-related 30-day mortality       | 2 (0.7%)                        | 0 (0.0%)                     | 0.999*  |
| Total duration of hospital stay, days    | 14.90 ± 10.70                   | 12.71 ± 7.74                 | 0.128   |
| Time to death, days                      | 9.50 (7.0-25.75)                | 3 (3–3)                      | 0.313   |
| Relapse within 3 months                  | 21 (7.6%)                       | 5 (8.1%)                     | 0.999*  |

*: Fisher’s exact test

**Discussion**

In this study, the phylogenetic groups B2 and D showed different characteristics. Phylogenetic group B2 had more virulence factors, especially higher presentation of adhesion-related (S fimbriae, F fimbriae), toxin-related (hemolysin A, cytotoxic necrotizing factor 1), and iron metabolism-related virulence factors (*fyuA*), than group D. A higher presentation of antimicrobial resistance and healthcare-associated infection was also noted in group B than in group D. Phylogenetic group D was associated with community-acquired UTI and exhibited a lower association with virulence and predisposing factors than group B. No significant differences in clinical manifestations and treatment outcomes between the phylogenetic groups B2 and D occurred.

Pathogenic strains of *E. coli* have been classified by the identification of O, K, and H antigens [13]. A phylogenetic study revealed that *E. coli* can be separated into four major groups: A, B1, B2, and D [9] and are classified into three main groups according to genetic and clinical criteria: commensal, intestinal pathogenic, and extraintestinal pathogenic strains [13]. Among the extraintestinal pathogenic *E. coli*, some strains such as uropathogenic *E. coli* could survive in the gut and colonize the periurethral area, resulting in UTIs. The uropathogenic *E. coli*, which are known as the virulent strains, belong to the phylogenetic group B2 or D, and the less virulent strains mainly belong to A or B1 and are commensal strains [10].

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The phylogenetic group of uropathogenic *E. coli* mainly comprised B2, but the distributions and proportions of phylogenetic groups and virulence factors have differed according to countries and study settings. In Italy, phylogenetic group B1 was the most prevalent in both community-acquired acute pyelonephritis and recurrent cystitis in females. The distribution and proportion of phylogenetic groups of acute pyelonephritis by uropathogenic *E. coli* were as follows: group B1, 68.7%; group A, 27.8%; and group D, 11.1%. Toxin-associated and siderofore-associated virulence factors were frequently observed in patients with recurrent cystitis [22]. In a study of community-acquired UTIs in Iran, phylogenetic group B2 was most frequently detected. The phylogenetic groups were as follows: group B2, 67.3%; group D, 21.4%; group A, 6.5%; and group B1, 4.8% [23]. In a study of community-acquired UTIs in Korea, phylogenetic group B2 was the most frequently detected, followed by groups D and A [11]. In an analysis of symptomatic UTIs in Mexico, phylogenetic group B2 was detected as the most frequent (51.0%), followed by groups A (13.4%) and B1 (10.3%) [24]. In Turkey, phylogenetic group B2 was the most prevalent in UTIs, including cystitis and pyelonephritis [12]. In a study of UTI in Mongolia, the proportion of phylogenetic groups was as follows: B2, 33.8%; D, 28.4%; A, 19.6%; and B1, 18.2% [25]. In this study, we included patients with acute pyelonephritis who needed hospitalization, including cases of community-acquired, healthcare-associated, and nosocomial infections, which may have influenced the distribution of the phylogenetic groups.

Uropathogenic *E. coli* have virulence factors, such as adhesion molecules, toxins, iron acquisition, immune evasion, and protectins [26]. In this study, virulence factors related to adhesion, iron metabolism, and protection were identified in both phylogenetic groups B2 and D. *FimH* in adhesion molecules and *fyuA* in iron metabolism-related virulence factors were the most and second most frequently detected virulence factors. The virulence factors that showed differences in distributions between the two groups were type I *fimbriae; focG, sfa/focED* in adhesion molecules; *hlyA, cnf1* in toxins; *fyuA* in iron metabolism; and *PAI*. Adhesion molecules, such as type I *fimbriae*, play an important role in the attachment of *E. coli* to the mucosal epithelium, initiation of biofilm formation, and persistence in the bladder [27]. In a comparative study of UTIs with and without bacteremia in Sweden, adhesion molecules such as *papG* (*P fimbriae*) were more frequently observed in bacteremic UTI than in non-bacteremic UTI [28]. In a study of UTI at outpatient clinics, risk factor analysis of virulence factors affecting phylogenetic groups revealed that strains with *papC* and *sfa* genes were associated with the phylogenetic group B2 [23]. In addition, biofilm formation in *E. coli* was observed in strains harboring adhesion-associated virulence genes [29]. Toxin-related virulence factors are important for mediating bacterial invasion and for the dissemination and persistence of bacteria in the bladder [30–32]. *HlyA* is needed for initial bacterial invasion, and *cnf1* is needed for bacteria dissemination and persistence [13, 31–33]. In a UTI-infected mouse model, *hlyA* accelerated bacteremia to fulminant sepsis [33]. *HlyA* expressing uropathogenic *E. coli* activated caspase-independent necroptosis, but not caspase-mediated apoptotic cell death, and the products released from damaged cells by necroptosis induced proinflammatory response in macrophages [31]. In *cnf1*- and *hlyA*-expressing uropathogenic *E. coli*, higher urinary levels of proinflammatory cytokines were detected than in pathogens not expressing such virulence factors [32]. Iron uptake systems and siderophores facilitate iron scavenging in the environment [13]. In a study of *E. coli* bacteremia in Spain,
strains expressing *fyuA* were associated with increased mortality during hospital stay [34]. *FyuA* causes invasion of the bloodstream from the urinary tract and is associated with highly pathogenic strains [19, 34]. Various vaccines are being developed according to the mechanisms of virulence factors, mainly targeting adhesion molecules and iron metabolism [18–19]. Vaccines related to toxins have not yet achieved significant results[16].

The phylogenetic group B2 has been associated with high antimicrobial resistance rates [23, 35]; this may have been influenced by a combination of several factors [36]. Several studies have reported that biofilm formation is associated with a high antibiotic resistance rate [22, 37]. Multiple virulence factors, such as *a-hemolysin*, lipopolysaccharides, proteases, adhesins, aerobactin, and fimbriae, significantly affect biofilm formation [13]. The phylogenetic group B2 was more associated with adhesion molecules and biofilm formation than other phylogenetic groups [37]. Drug resistance in uropathogenic *E. coli* strains is more likely caused by biofilm formation, and the biofilms have potential roles in recurrent infections and antibiotic resistance [22, 37–38]. Several studies have reported virulence factors associated with the antimicrobial resistance of uropathogenic *E. coli* [12, 22–23]. PAI is also associated with antimicrobial resistance [23]. In a study of symptomatic UTIs in outpatients in Iran, *hlyA*, *malX*, and *hlyA* were revealed as risk factors among virulence factors affecting antimicrobial resistance to ciprofloxacin and ceftriaxone [22]. Another study of UTIs including cystitis and pyelonephritis in Turkey showed that *afa/draC* and *iha* were the virulence factors associated with antimicrobial resistance [12].

There are several limitations to this study. First, this study was retrospective; therefore, we had to rely on the medical records, and it was difficult to evaluate urinary function and identify the subjective urinary symptoms in all patients. Second, we acknowledge that the patients included in this study were at a tertiary hospital, and their condition might have been more severe than that of patients in a primary medical center. Despite these limitations, we found differences in the virulence factors, antimicrobial susceptibility, and clinical presentations of uropathogenic *E. coli* according to the phylogenetic group.

In conclusion, in cases of pyelonephritis with uropathogenic *E. coli*, differences occurred in the virulence factors and antimicrobial resistance rates according to phylogenetic groups B2 and D. Further studies will be needed to elucidate the virulence factors of uropathogenic *E. coli* according to phylogenetic group and host interaction. Because differences in genetic and phenotypic characteristics occur based on strains, various therapeutic options targeting virulence factors may be considered along with antibiotics.

**Declarations**

**Ethics approval and consent to participate**

The study was reviewed and approved by the Institutional Review Board of Keimyung University Dongsan Medical Center (File No. 2020-02-003). The requirement for written informed consent was waived by the committee because of the retrospective nature of the study and the use of identifiable specimens.

**Consent for publication**
No applicable.

Availability of data and materials
The dataset of the current study are available from the corresponding author upon request.

Competing interests
The authors declare that there is no conflict of interest.

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Authors' contributions
Conceptualization & data curation: HMR
Laboratory experiment & methodology: HMR, KHA
Formal analysis: HMR, LJY
Writing - review & editing: HMR, LJY, KHA

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