Genetic diversity and antibiotic susceptibility of uropathogenic Escherichia coli isolates from kidney transplant recipients

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Purpose: Uropathogenic Escherichia coli (UPEC) strains are a common cause of transplant rejection, morbidity, and mortality among kidney transplant recipients. The virulence of UPEC strains differs based on their pathogenicity islands (PAIs) and susceptibility to antibiotics. The present study evaluates the clonal relationship and antibiotic susceptibility of UPEC PAI-genotypes among Escherichia coli (E. coli) isolates from kidney transplant patients.

Patients and methods: A total of 115 Escherichia coli (E. coli) isolates were collected from kidney transplant recipients with acute urinary tract infections (UTIs). Isolates were typed based on the presence of PAI-markers, and random amplified polymorphic DNA (RAPD). The disk diffusion method was performed for the antibiotic susceptibility pattern of isolates.

Results: According to the PAI-specific virulence markers, 69 (60%), 21 (18.3%), and 25 (21.7%) isolates were identified as genotypes related to UPEC 536, UPEC J96, and UPEC CFT073 strains, respectively. PAI III536 genotypes were the most prevalent genotype in this study. The findings showed a high-sensitivity to imipenem (93.9%) and nitrofurantoin (91.3%) and a low-sensitivity to trimethoprim/sulfamethoxazole (36.5%). Clonal association and similar antibiotic susceptibility pattern were seen in the PAI-related genotypes.

Conclusion: Due to a similar pattern of antibiotic susceptibility of these clonal groups and increased resistance to some important antibiotics such as trimethoprim/sulfamethoxazole in the treatment of urinary tract infections, especially in kidney transplant patients, the spread of these clones should be considered as a serious concern.

Keywords: uropathogenic Escherichia coli, kidney transplantation, pathogenicity islands, random amplified polymorphic DNA

Introduction
Bacterial infections are the most serious post-transplantation complications in kidney transplant recipients, resulting in transplant rejection, morbidity, and mortality. An impaired immune system resulting from exposure to immunosuppressive therapies increases the susceptibility of these patients to infections. Uropathogenic E. coli (UPEC) is a very common etiologic agent of urinary tract infections (UTIs) among transplant recipients. Additionally, UPEC infections are the most common nosocomial and community-acquired UTIs worldwide. UPEC strains attach to bladder epithelial cells, initiate cystitis, and progress to pyelonephritis. Pyelonephritis may furthermore affect renal grafts, can lead to life-threatening urosepsis. The pathogenicity of UPEC
Bacterial DNA extraction

The isolates were cultured in Luria-Bertani broth overnight, and the DNA was extracted using a DNA extraction kit (Bioneer, South Korea), according to the kit protocol. To ensure extraction efficiency, 4 μL of extracted DNA samples were electrophoresed on 0.8% agarose gel (Merck, Germany) to detect related bands. Also, to obtain the concentration and purity of DNA, optical density (OD) of the samples 260/280 wavelengths was measured using a nanodrop system (Thermo, USA).

Detection of PAIs according to virulence markers

A polymerase chain reaction (PCR) assay was carried out for the detection of prsX, hek, sfaA, ybtA, cnf1, iucA, and sat genes, as well as for the PAI J96 marker, using specific primers. Each of these genes is specific for PAIs of UPEC and are used as a marker for their identification.

PCR amplification was performed using a 25-μL mixture, containing 12.5 μL of ready-to-use MasterMix (Fermentas, Germany), 9.5 μL of distilled water, 1 μL of 15-pmol forward and reverse primers, and 1 μL of DNA template (200 ng). PCR amplifications were performed in 30–35 cycles using a thermal Mastercycler system (Eppendorf, Germany). The primer sequences and size of the amplicons of PAI-markers and their encoded virulence factors have been described in Table 1 (PCR thermal conditions listed in Table S1). Electrophoresis was carried out in a 1.5% agarose gel (Merck, Germany), using a 50 bp DNA ladder (Fermentas, Germany). One positive PCR amplicon for each gene was submitted to a sequence service provider (Bioneer, South Korea). A Basic Local Alignment Search Tool (BLAST) was performed for each sequence using the National Centre for Biotechnology Information’s (NCBI) online services.

Antibiotic susceptibility

The antibiotic susceptibility test was carried out by the Kirby-Bauer disk diffusion method. The suspension of 0.5 MacFarland (1.5×10⁸ CFU/ml) was used for preparing each isolate and samples were cultured on Mueller-Hinton agar media (Merck, Germany), the antibiotic disks were placed on agar surface and plates were incubated in 37 °C overnight.

The antibiotic disks; Nitrofurantoin (300 μg), Ampicillin (10 μg), Cefazolin (30 μg), Cefotaxime (30 μg), Imipenem (10 μg), Gentamicin (10 μg), Ciprofloxacin (5 μg), and Trimethoprim/Sulfamethoxazole (1.25/23.75 μg) (Mast,
England) were used in this study according to protocols of the Clinical and Laboratory Standards Institute (CLSI 2017).

**RAPD-PCR analysis**

The RAPD-PCR method was applied to determine the clonal relationships of the UPEC isolates. In this method, short primers are used which randomly bind to complementary sequences of DNA, and amplification is performed between the primers. Accordingly, a pattern of amplified fragments with different sizes is formed for each isolate. These patterns are similar in genetically related isolates.

Amplifications were conducted in a 25-μL reaction mixture, containing 1 μL of DNA template (200 ng), 11 μL of ready-to-use Long PCR MasterMix (Fermentas, Germany), 11 μL of distilled water, and 2 μL of two 20-pmol Decamer primers (5'-CCGCCGCAACAA-3' and 5'-AAGCGCGCAAC-3'). The amplification conditions were as follows: one cycle for five minutes at 95 °C, 15 cycles for one minute at 95 °C, for one minute at 33 °C, and for 90 seconds at 72 °C, followed by 15 cycles for one minute at 95 °C, for one minute at 38 °C, and for 90 seconds at 72 °C, and a final extension for five minutes at 72 °C.

Electrophoresis of the products was performed in a 1% agarose gel (Merck, Germany) in the presence of a 1-kb DNA ladder (Fermentas, Germany). The RAPD patterns were analyzed using the Gel-Compare II software, version 6.6 (Applied Maths, Belgium), and the dendrogram was drawn. Isolates with more than 90% similarity, based on the Dice coefficient and UPGMA method, were considered as common type.

**Statistical analysis**

Chi-squared and Fisher’s exact tests were performed for analysis of the categorical data, and ANOVA was used for continuous data using the Statistical Package for the Social Sciences (SPSS) software, version 22.0 (IBM, USA). A P-value of less than 0.05 was considered significant.

**Results**

**Distribution of the UPEC isolates and related PAIs**

The UPEC isolates that had been obtained from urine culture of kidney transplant recipients were confirmed according to microbiological methods. In total, 83 (72.2%) isolates were collected from female patients while 32 (27.8%) isolates were obtained from male patients. Based on the laboratory records, 63 (54.8%) patients were above 50 years, while 52 (45.2%) patients were within the age range under 50 years (52±14). According to the laboratory records of blood culture, *E. coli* was detected in nine (7.8%) patients.

<table>
<thead>
<tr>
<th>Target</th>
<th>Primers (5’ to 3’)</th>
<th>Size (bp)</th>
<th>Gene product</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>prsX</em> (PAI J536)</td>
<td>F: TCACAGGGGAGTCTCCACCA R: GCGGTCGAGATGATTATGT</td>
<td>874 bp</td>
<td>F17 like adhesion</td>
</tr>
<tr>
<td><em>Hek</em> (PAI II536)</td>
<td>F: AGGCTGACAGTGACAGCGGAT R: TCAGACCAGCAGGTTTCTGTC</td>
<td>415 bp</td>
<td>Hek adhesion</td>
</tr>
<tr>
<td><em>sfaA</em> (PAI III536)</td>
<td>F: GTGTTGCGACAAATTGCGGCT R: CCGGTCGATGTTACCGGCT</td>
<td>454 bp</td>
<td>S fimbrisi</td>
</tr>
<tr>
<td><em>ybtA</em> (PAI IV536)</td>
<td>F: AACCCACTTAACGGCTAGG R: TTTCTGACATCCGAGCCGGCT</td>
<td>355 bp</td>
<td>Yersiniabactin</td>
</tr>
<tr>
<td>PAI J96</td>
<td>F: TGCTGGTCCAGTCCGGAAATT R: TGGCATCCCCATATTATG</td>
<td>400 bp</td>
<td>-</td>
</tr>
<tr>
<td><em>cnf1</em> (PAI II96)</td>
<td>F: TGGTTGCGACAAATTGCGGAT R: TACCTCCCCCGCGGTATGA</td>
<td>686 bp</td>
<td>Cytotoxic necrotizing factor I</td>
</tr>
<tr>
<td><em>iucA</em> (PAI I5CEF073)</td>
<td>F: ACGGACGAGTACGGGATGGA R: CCGTTGGAGAAAGCCTGGA</td>
<td>492 bp</td>
<td>Aerobactin</td>
</tr>
<tr>
<td><em>Sat</em> (PAI IICFT073)</td>
<td>F: GTCAAGGCGCATGAGTGTC R: TATCGATCTTGGTGACGGCAC</td>
<td>389 bp</td>
<td>Secreted autotransporter toxin</td>
</tr>
</tbody>
</table>
The UPEC PAI-genotypes were identified according to the virulence PAI markers; prsX, hek, sfaA, ybtA, PAI IJ96 marker, cnfl, iucA, and satA were detected in 20 (17.4%), 11 (9.6%), 30 (26.1%), 8 (6.9%), 3 (2.6%), 18 (15.6%), 13 (11.3%), and 12 (10.4%) isolates, respectively. According to the presence of aforementioned PAI-markers; 69 (60%), 21 (18.3%) and 25 (21.7%) isolates were identified as UPEC 536, J96 and CFT073 related genotypes. UPEC 536 related genotypes were more common than other genotypes in kidney transplant recipients with UTIs. Also, four (3.5%) III536 genotypes and five (4.3%) CFT073 related genotypes were isolated from patients who suffered E. coli-related sepsis. 

Due to statistics, no significant correlations were found between distribution UPEC genotypes of the sex and age range of the patients (Table 2).

Antibiotic susceptibility pattern of UPEC isolates

The antibiotic susceptibility test showed that the 103 (89.6%), 102 (88.7%), 91 (79.1%), 81 (70.4%), 80 (69.8%), 71 (61.7%), 69 (60%) and 36 (31.3%) of UPEC isolates were susceptible to Nitrofurantoin, Imipenem, Ampicillin, Cefazolin, Gentamicin, Cefotaxime, Ciprofloxacin and Trimethoprim/Sulfamethoxazole respectively. According to in vitro findings, Nitrofurantoin and Imipenem were more effective antibiotics for treatment of UPEC isolates, while Ciprofloxacin and Trimethoprim/Sulfamethoxazole had the least therapeutic effect. Two isolates (1.7%) were resistant to all antibiotics used in this study. UPEC J96 related genotypes showed higher resistance to most antibiotics rather than 536 and CFT073 genotypes. The total antibiotic susceptibilities of UPEC isolates according to PAI-genotypes are presented in Table 3.

Phylogenetic analysis

The dendrogram (Figure 1) represents five clusters (A-E). The UPEC isolates with common genotypes showed more similarity in the RAPD pattern. The UPEC 1536, II536, and IV536 genotypes were situated in cluster A, while cluster B includes III536 genotypes. UPEC ICFT073 and IICFT073 related genotypes were placed in clusters C and D, respectively. Subsequently, all UPEC J96 related genotypes stayed in cluster E. Considering the Dice Coefficient of 90%, 25 clonal groups were constructed. In total, A closer genetic relationships were found in the RAPD results among UPEC isolates that were categorized in the same genotypes. Moreover, these isolates showed more similarity in terms of antibiotic susceptibility. The antibiotic susceptibility pattern of all isolates integrated into the dendrogram is shown in Figure 1.

Discussion

The synchronic study of the distribution of PAIs, phylogenetic relationships, and antibiotic susceptibility of UPEC genotypes in UTIs among kidney transplant recipients is quite rare. Some studies about the distribution of PAIs in UPEC reported PAI IV536 as the most prevalent PAI in isolates from UTIs.16-19 In the present study, UPEC 536 related genotypes were the most common genotypes followed by CFT073 related genotypes, and UPEC III536 genotypes were the most frequent.

The standard treatment and antibiotic therapy for UPEC are complicated in kidney transplant patients. The intolerance of patients to some antibiotics, drug toxicity, and the acquisition of antibiotic resistance can make treatment of these patients quite challenging. Therefore, it is important to select an effective antibiotic agent to treat UTIs. Recently, increasing rates of antibiotic-resistant UPEC isolates, especially to Trimethoprim/Sulfamethoxazole were reported repeatedly.20-23 The high resistance to Trimethoprim/Sulfamethoxazole and Ciprofloxacin, as well as the effectiveness of Carbapenems and Nitrofurantoin, were seen in our study. Using an effective antibiotic regimen based on antibiotic susceptibility test is an important factor for a confined of the emergence of resistance to various antibiotics.24 Trimethoprim/Sulfamethoxazole and Fluoroquinolones commonly are used for the treatment of UTIs and gastrointestinal infections.

Table 2 Distribution of UPEC genotypes among kidney transplant patients, according to sex and age

<table>
<thead>
<tr>
<th>Strains</th>
<th>Patients</th>
<th>Sex</th>
<th>Age</th>
<th>P-value*</th>
<th>&lt;50 years (n=52)</th>
<th>&gt;50 years (n=63)</th>
<th>P-value**</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Male</td>
<td>Female</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>UPEC 536</td>
<td>69 (60%)</td>
<td>18 (15.6%)</td>
<td>51 (44.3%)</td>
<td>0.67</td>
<td>27 (23.5%)</td>
<td>32 (31.7%)</td>
<td>0.12</td>
</tr>
<tr>
<td>UPEC J96</td>
<td>21 (18.3%)</td>
<td>7 (6.1%)</td>
<td>14 (12.2%)</td>
<td>0.59</td>
<td>12 (10.4%)</td>
<td>9 (7.9%)</td>
<td>0.33</td>
</tr>
<tr>
<td>UPEC CFT073</td>
<td>25 (21.7%)</td>
<td>7 (6.1%)</td>
<td>18 (15.7%)</td>
<td>1.0</td>
<td>13 (11.3%)</td>
<td>12 (10.4%)</td>
<td>0.50</td>
</tr>
</tbody>
</table>

Notes: *Correlation of UPEC genotypes with gender of patients **Correlation of UPEC genotypes with age range of patients
Furthermore, Trimethoprim/Sulfamethoxazole is used as a prophylaxis for most kidney transplant patients. Antibiotic pressure also plays an important role in the development of resistant clones. Therefore, there may be causes of increased resistance to these antibiotics in bacterial isolates, especially in kidney transplant patients.

The genetic characteristics of bacteria can contribute to the resistance to various antibiotics. There is some evidence about the association between UPEC’s PAIs and virulence genes with antibiotic resistance. Estrada et al. showed an association of iutA/sat genes with quinolone resistance and iutA/cnf1/hlyA genes with amoxicillin/clavulanic acid resistance in UPEC. These virulence genes are encoded by UPEC different PAIs. Calhau et al. reported association of adhI/adhJ/adhK/adhL genes with amoxicillin/clavulanic acid resistance in UPEC. These genes with antibiotic resistance. Estrada et al. showed an association between UPEC’s PAIs and virulence genes with antibiotic resistance. Therefore, there may be causes of increased resistant clones in these antibiotics in bacterial isolates, especially in kidney transplant patients.

The RAPD-PCR analysis revealed that the isolates, which were categorized in the same PAI-genotypes, showed more similarity. Also, these isolates showed similar antibiotic susceptibility patterns. Based on some clonal groups, were similar in terms of PAI-genotype and antibiotic resistance pattern. The RAPD-PCR analysis revealed that the isolates, which were categorized in the same PAI-genotypes, showed more similarity. Also, these isolates showed similar antibiotic susceptibility patterns. Based on some clonal groups, were similar in terms of PAI-genotype and antibiotic resistance pattern. Based on some clonal groups, were similar in terms of PAI-genotype and antibiotic resistance pattern.

### Table 3 Pattern of antibiotic susceptibility among UPEC isolates according to genotype

<table>
<thead>
<tr>
<th>Strains</th>
<th>Antibiotics susceptibility</th>
<th>FMNc</th>
<th>AMPb</th>
<th>CFNd</th>
<th>CTXd</th>
<th>IMFc</th>
<th>GNf</th>
<th>CPg</th>
<th>SXTh</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>UPEC 536 (n=69)</strong></td>
<td>S*</td>
<td>64 (92.8%)</td>
<td>55 (79.7%)</td>
<td>46 (66.6%)</td>
<td>43 (62.3%)</td>
<td>60 (86.9%)</td>
<td>46 (66.6%)</td>
<td>40 (58%)</td>
<td>20 (29%)</td>
</tr>
<tr>
<td></td>
<td>I**</td>
<td>3 (4.3%)</td>
<td>1 (1.4%)</td>
<td>2 (2.9%)</td>
<td>3 (4.3%)</td>
<td>2 (2.9%)</td>
<td>7 (10.1%)</td>
<td>21 (30.4%)</td>
<td>4 (5.8%)</td>
</tr>
<tr>
<td></td>
<td>R***</td>
<td>2 (2.9%)</td>
<td>13 (18.8%)</td>
<td>21 (30.4%)</td>
<td>23 (33.3%)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>45 (65.2%)</td>
</tr>
<tr>
<td><strong>UPEC J96 (n=21)</strong></td>
<td>S*</td>
<td>16 (76.2%)</td>
<td>14 (66.7%)</td>
<td>14 (66.7%)</td>
<td>11 (52.4%)</td>
<td>18 (85.7%)</td>
<td>14 (66.7%)</td>
<td>12 (57.1%)</td>
<td>6 (28.6%)</td>
</tr>
<tr>
<td></td>
<td>I**</td>
<td>3 (14.3%)</td>
<td>2 (9.5%)</td>
<td>2 (9.5%)</td>
<td>3 (14.3%)</td>
<td>1 (4.8%)</td>
<td>1 (4.8%)</td>
<td>6 (28.6%)</td>
<td>2 (9.5%)</td>
</tr>
<tr>
<td></td>
<td>R***</td>
<td>2 (9.5%)</td>
<td>5 (23.8%)</td>
<td>6 (28.6%)</td>
<td>8 (38.1%)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>8 (38.1%)</td>
</tr>
<tr>
<td><strong>UPEC CFT073 (n=25)</strong></td>
<td>S*</td>
<td>23 (92.0%)</td>
<td>22 (88%)</td>
<td>21 (84%)</td>
<td>24 (96%)</td>
<td>-</td>
<td>-</td>
<td>17 (68%)</td>
<td>10 (40%)</td>
</tr>
<tr>
<td></td>
<td>I**</td>
<td>1 (4.0%)</td>
<td>3 (12%)</td>
<td>4 (16%)</td>
<td>1 (4.0%)</td>
<td>1 (4.0%)</td>
<td>1 (4.0%)</td>
<td>4 (16%)</td>
<td>2 (8%)</td>
</tr>
<tr>
<td></td>
<td>R***</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>6 (24%)</td>
<td>13 (52%)</td>
</tr>
</tbody>
</table>

Notes: *Sensitive. **Intermediate. ***Resistant. aNitrofurantoin. bAmpicillin. cCefazolin. dCefotaxim. eImipenem. fGentamicin. gCiprofloxacin. hTrimethoprim/sulfamethoxazole.
Figure 1 Genetic relationships and antibiotic susceptibility patterns of UPEC isolates. The susceptibility patterns of UPEC isolates to different antibiotics presented in front of the position of each isolate in the dendrogram. The isolates that are similar in regard to RAPD-PCR is more similar in terms of antibiotic susceptibility. Representative isolates for each cluster have been marked with Genbank accession numbers.
studies UTIs caused by *E. coli* usually originate from the normal flora of the colon; this is, in fact, the cause of genetic differences in isolates from different patients.\textsuperscript{32,33} However, sexual contact, the consumption of contaminated foods and especially urinary tract catheterization may be the transmission routes of UPEC between different peoples.\textsuperscript{34–36} Due to kidney transplant patients have a history of hospitalization and catheterization, may these isolates originate from non-flora sources.

**Conclusion**

PAI III\textsubscript{536} genotypes were dominant in UPEC genotypes which were isolated from kidney transplant patients; these findings suggest that these patients may be more vulnerable to infection with these genotypes. Furthermore, isolates with similar PAs exhibited more relation in RAPD patterns and antibiotic susceptibility, which confirm the clonal association of these isolates. The genetic relationship of some UPEC isolates was demonstrated in the present study. The isolates in the same clonal group carried common PAI makers and showed very similar antibiotic susceptibility patterns. Moreover, remarkable resistance was shown to some common antibiotics such as Trimethoprim/Sulfamethoxazole in these isolates. Due to the importance of antibiotic susceptibility in the treatment of urinary tract infections, especially in kidney transplant patients, the screening and control of these clones will be critical.

**Acknowledgments**

We wish to thank the Deputy of Research and Technology and Molecular Biology Research Center of Baqiyatallah University of Medical Sciences for supporting this study. Special thanks to the Microbiology Department of the Alborz University of Medical Sciences and the hospitals involved in this project.

**Disclosure**

The authors report no conflicts of interest in this work.

**References**


### Supplementary material

**Table S1** PCR conditions and accession number of submitted sequences of PAI markers to GenBank

<table>
<thead>
<tr>
<th>Virulence gene</th>
<th>Pathogenicity island</th>
<th>PCR thermal conditions</th>
<th>Accession number of marker genes</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>prsX</strong></td>
<td>PAI I536</td>
<td>(94 °C; 45 sec, 59 °C; 45 sec, 72 °C; 45 sec) x30</td>
<td>MH932676</td>
</tr>
<tr>
<td><strong>hek</strong></td>
<td>PAI I11536</td>
<td>(94 °C; 45 sec, 59 °C; 30 sec, 72 °C; 30 sec) x35</td>
<td>MH932677</td>
</tr>
<tr>
<td><strong>sfaA</strong></td>
<td>PAI III536</td>
<td>(94 °C; 45 sec, 58 °C; 30 sec, 72 °C; 30 sec) x35</td>
<td>MH932678</td>
</tr>
<tr>
<td><strong>ybtA</strong></td>
<td>PAI IV536</td>
<td>(94 °C; 45 sec, 56 °C; 30 sec, 72 °C; 30 sec) x35</td>
<td>MH932679</td>
</tr>
<tr>
<td><strong>PAI IJ96 marker</strong></td>
<td>PAI IJ96</td>
<td>(94 °C; 45 sec, 54 °C; 30 sec, 72 °C; 30 sec) x35</td>
<td>MH932680</td>
</tr>
<tr>
<td><strong>cnfI</strong></td>
<td>PAI IJ96</td>
<td>(94 °C; 45 sec, 60 °C; 30 sec, 72 °C; 30 sec) x35</td>
<td>MH932681</td>
</tr>
<tr>
<td><strong>iucA</strong></td>
<td>PAI I CFT073</td>
<td>(94 °C; 45 sec, 58 °C; 30 sec, 72 °C; 30 sec) x30</td>
<td>MH932682</td>
</tr>
<tr>
<td><strong>satA</strong></td>
<td>PAI II CFT073</td>
<td>(94 °C; 45 sec, 58 °C; 30 sec, 72 °C; 30 sec) x35</td>
<td>MH932682</td>
</tr>
</tbody>
</table>