Phylogenetic Groups and Antibiotic Resistance Characteristics of Uropathogenic *Escherichia coli*

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**INFO**

**ABSTRACT**

**Background:** Different phylogenetic groups of uropathogenic *Escherichia coli* are associated with increased virulence and multidrug resistance, highlighting the importance of understanding the genetic characteristics of these isolates for effective treatment and prevention of UTIs. Genomic analysis was conducted on 100 *Escherichia coli* isolates obtained from multiple hospitals in Baghdad, Iraq, which showed extensive resistance to multiple drugs, including both multi-drug resistant (MDR) and extensively-drug resistant (XDR) isolates.

**Methodology:** Antibiotic susceptibility was evaluated using the disc diffusion technique. PCR was used to test for the presence of phylogenetic groupings and to characterise antibiotic resistance genes.

**Results:** Phylogroup F (36.66%) and Phylogroup C (23.33%) were the most common. Isolates of UPEC were found in every phylogroup, except for the six that were untypable. The study identified 54 out of 70 typed *Escherichia coli* isolates that belonged to phylogroups F (33/70) and C (21/70) as multidrug-resistant, with a high percentage (70.76%) of these isolates demonstrating extensive drug resistance (XDR). Based on the presence of particular genes or DNA, *E. coli* populations are classified into eight basic phylogenetic groups: A, B1, B2, C, D, E, F (belonging to *E. coli sensu stricto*), and clade I (belonging to *Escherichia clade*).

**Conclusion:** Based on our findings, certain types of *E. coli* belonging to the phylogenetic group F are more common, more dangerous, and more resistant to antibiotics than others when it comes to UTIs.

**Keywords:** *Escherichia coli*, Urinary Tract Infections, Extensively-Drug Resistant, Phylogenetic Group

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**Introduction**

Urinary tract infections are among the infectious diseases that impact individuals at a rate that is second only to common cold. Uropathogenic *Escherichia coli* is the strain of *Escherichia coli* bacteria that is responsible for these illnesses. Whittam and his colleagues described in the 1980s that *E. coli* has a genetic substructure that is unique to each species and in order to differentiate between the various *E. coli* species, multiple methods have been developed. Using electrophoresis, Selandar and his colleagues were the first to divide *E. coli* into six subgroups in 1987 (A to F). To classify *E. coli* into one of four categories (A, B1, B2, and D), Clermont and colleagues used triplex PCR in 2000 (cha, yjaA, and a DNA fragment...
The C2 phenotypic grouping was developed by using factors like virulence, resistance, serotype, and country of origin to categorise samples. The majority of E. coli outside of the intestines belonged to group B2, while just a small percentage belonged to group D. Communal bacteria in the colon were associated with A and B1 types. In 2013, Clermont and coworkers expanded triplex PCR with a fourth set of primers for detecting groups E and F and cryptic clades. This method is known as quadruplex. The use of an antibiotic susceptibility test to categorise isolates into groups based on their resistance to antibiotics constitutes an example of phenotypic analysis known as an antibiotype. The use of antibiotypes facilitates the study of species-specific resistance patterns and the assessment of disease transmission dynamics. The prevalence of extensive drug resistance patterns in uropathogenic E. coli isolates as well as multidrug resistance in these isolates are key problems that continue to develop with each passing year. Treatment failure and increased mortality rates are both direct results of antibiotic resistance. Hence, antimicrobial susceptibility monitoring is crucial for determining the full scope of the issue and choosing the most effective antimicrobial medications for treating infected persons. The goals of this study were to determine the genetic profiles of the most common UPEC and to analyse the varied patterns of resistance discovered in the isolates.

Materials and Methods

Collection of Specimens

One hundred Escherichia coli isolates were obtained from 150 midstream urine specimens (MUS) collected from patients diagnosed with urinary tract infections. The MUS specimens were stored in sterile screw-capped containers for analysis. Samples, in this study, were collected from outpatients (males and females) who were less than 18 years old and suffered from urinary tract infections. The patients were selected from Paediatric Teaching Hospital, Central Pediatric Teaching Hospital and Yarmouk Teaching Hospital, among those who visited the hospital during the period of October 2021 to January 2022. The urine specimens were plated immediately on Blood agar, MacConkey agar, and Eosin Methylene Blue plates by direct streaking methods, and were then examined for bacterial growth. Typical E. coli colonies were identified using the automated VITEK system (bioMérieux, France).

Antimicrobial Susceptibility

Clinical isolates of UPEC were tested for antibiotic susceptibility using the disc diffusion method in accordance with Clinical and Laboratory Standards Institute guidelines. Antibiotic susceptibility testing was done on Mueller-Hinton agar using the following antibiotic discs: tetracycline (30 µg), amikacin (30 µg), ampicillin (10 µg), co-trimoxazole (25 µg), ceftazidime (30 µg), imipenem (10 µg), piperacillin (10 µg), levofloxacin (5 µg), ciprofloxacin (5 µg), nalidixic acid (30 µg), nitrofurantoin (300 µg), cefepime, (10 µg), cefotaxime (10 µg), gentamicin (10 µg), ceftriaxone (10 µg), augmentin (100 µg), piperacillin/ tazobactam (100 µg), and colistin (10 µg) were carried out on.

The European Center for Disease Prevention and Control (ECDC) and the United States Centers for Disease Control and Prevention (CDC) convened an expert panel to classify bacterial isolates according to their resistance profiles. The study categorised the bacteria into four groups based on their resistance patterns. Group 1 included bacteria that were either not resistant to any antibiotics or had resistance to only one antibiotic in ≤ 2 classes. Group 2 comprised MDR microorganisms that were resistant to ≥ 1 antibiotic in ≥ 3 drug classes and belonged to a distinct class of XDR. Group 3 consisted of pandrug-resistant bacteria (PDR) that were resistant to all medications and classes of antibiotics. Finally, group 4 contained MDR bacteria that were resistant to at least three classes of antibiotics.

Phylogenetic Grouping by Quadruplex PCR

The presence or absence of four genes, arpA, chuA, yjaA, and TspE4.C2, is evaluated using a technique called quadruplex PCR, which is a method for evaluating isolates. To do multiplex PCR, a 25 µl reaction mixture including 12.5 µl of the master mix (Promega, USA), 25 pmol of each primer (forward and reverse), and 3 µl of template DNA was utilised. Then arpA, chuA, yjaA, and TspE4.C2 genes were amplified using primers described by Clermont et al., as shown in Table 1.

Table 1. Primers used in the Quadruplex PCR Technique

<table>
<thead>
<tr>
<th>Primers Id</th>
<th>Gene</th>
<th>Primer Sequence (5’-3’)</th>
<th>Product Size (bp)</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>chuA</td>
<td>F: ATGGTACCGGACGAACCAAC</td>
<td>288</td>
<td>Clermont et al., 4</td>
</tr>
<tr>
<td></td>
<td>chuA</td>
<td>R: TGCCGCAAGTACAAAGACA</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>yjaA</td>
<td>F: CAAACGTGAAGTGTCAGGAG</td>
<td>211</td>
<td></td>
</tr>
<tr>
<td></td>
<td>yjaA</td>
<td>R: AATGCGTTCCTCAACCCTGT</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>TspE4C2</td>
<td>F: CACTATTCTGAAGTGTCATCC</td>
<td>152</td>
<td></td>
</tr>
<tr>
<td></td>
<td>TspE4C2</td>
<td>R: AGTTTATCGCTGCGGGTCGC</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
The assay conditions for the PCR programme were as follows: four minutes at 94 °C; 30 cycles of five seconds at 94 °C; 20 seconds at 57 °C for group E or 59 °C for quadruplex and group C; one minute at 72 °C; followed by 5 minutes at 72 °C. Following that, an amplification product was divided, tagged with ethidium bromide, and imaged under UV illumination on a 1% agarose matrix. The sequence and size of the primers described in Table 1 were used to allotype E. coli into distinct phylogenetic groups.

Statistical Analysis

The analysis of the gathered data was performed in Stata 14.0. Both groups were tested for the presence of integrons and the antimicrobial resistance profile, and any link between the two was examined using the Chi-square test. The level of significance was set at p < 0.05.

Ethical Review and Approval

Mustansiriyah University’s Biological Sciences Research Ethics Committee approved the study. Isolates were prepared and diagnosed at Paediatric Teaching Hospital, Central Pediatric Teaching Hospital and Yarmouk Teaching Hospital after gaining permission to collect samples from the Ministry of Health and Environment. All participants agreed to have their personal information collected and to have their samples used in scientific studies.

Results

Socio-demographic Information of Patients

In our study, there were documented 100 patients with E. coli isolates from 150 people suspected of UTI, with 66 (66%) isolates of females and 34 (34%) isolates of males, ranging in age from 1 month to 18 years. The majority of UTI cases were females, with the age group of 12-18 years. Table 2 summarises the social information and symptoms of participants.

Antimicrobial Susceptibility Testing

The study’s utilisation of antibiotics and the corresponding resistance are shown in Table 3.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Description</th>
<th>Number (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age groups (years)</td>
<td>&lt; 1-6</td>
<td>24 (24)</td>
</tr>
<tr>
<td></td>
<td>6-12</td>
<td>31 (31)</td>
</tr>
<tr>
<td></td>
<td>12-18</td>
<td>45 (45)</td>
</tr>
<tr>
<td>Gender</td>
<td>Female</td>
<td>66 (66)</td>
</tr>
<tr>
<td></td>
<td>Male</td>
<td>34 (34)</td>
</tr>
<tr>
<td>Attendance</td>
<td>Outpatient</td>
<td>24 (24)</td>
</tr>
<tr>
<td></td>
<td>Ward</td>
<td>76 (76)</td>
</tr>
<tr>
<td>UTI recurrence</td>
<td>New infection</td>
<td>52 (52)</td>
</tr>
<tr>
<td></td>
<td>Recurrent infection</td>
<td>48 (48)</td>
</tr>
<tr>
<td>Clinical symptoms</td>
<td>Symptomatic</td>
<td>61 (61)</td>
</tr>
<tr>
<td></td>
<td>Asymptomatic</td>
<td>39 (39)</td>
</tr>
<tr>
<td>Hospitals</td>
<td>Medical City</td>
<td>32 (32)</td>
</tr>
<tr>
<td></td>
<td>Alyarmok</td>
<td>24 (24)</td>
</tr>
<tr>
<td></td>
<td>Iskan Teaching for Kids</td>
<td>44 (44)</td>
</tr>
</tbody>
</table>
Quinolones antibiotics, such as nalidixic acid, were resistant to 86.6% of the isolates, fluoroquinolone antibiotics, such as ciprofloxacin to 66.6% of the isolates, and levofloxacin to 50% of the isolates in the current investigation. Sixty-three per cent, 73%, 86.6%, 43%, and 63% of bacteria were resistant to cephalosporins I, II, III, and IV, which include cefoxitin, cefotaxime, ceftazidime, cefepime, and ceftriaxone. Carbapenems, such as meropenem and imipenem, were resistant to cephalosporins I, II, III, and IV, which include cefoxitin, cefotaxime, ceftazidime, cefepime, and ceftriaxone.
were encountered with 20% and 30% resistance among the isolates, respectively. Augmentin and piperacillin-tazobactam, two-lactam combinations, were reported to have resistance rates of 18.8 and 20%, respectively. On the other hand, 45.6% resistance to the penicillin class of antibiotics, which includes piperacillin, was documented. The resistance rate was 46.6% for both tetracycline and chloramphenicol. Twenty-three per cent of the population showed resistance to the lipopeptide antibiotic colistin.

**Phylogenetic Grouping of Uropathogenic E. coli**

Phylogroup F was discovered to be ubiquitous among isolates in our current analysis. UPEC isolates depicted a strong association with phylogroup F (33, 36.6%) followed by C (21, 13.3%), B2 (12, 13.3%), E (7, 7.8%), UP (6, 6.6%), B1 and D (4, 4.4% each), and A (3, 3.3%).

Our findings revealed that antibiotic resistance patterns were most widespread in phylogenetic group F (36.6%), followed by phylogenetic groups C, B2, and E (23.3%, 13.3%, and 7.8%, respectively). The phylogenetic groupings with less widespread resistance patterns were UP (6.6%), B1 and D (4.4% each), and A (3.3%), as shown in Table 4.

Three or more UTIs in the past year, with at least two of those occurring during the past six months, were considered to be a case of recurrent UTI. Nuutinen and Uhar had estimated that between 20% and 30% of adult women who have had UTI initially will have a recurrence within the next 3 to 4 months, while between 13% and 18% of children who have had UTI before the age of 1 year will have a recurrence within the next 3 years. In contrast to this, the likelihood of a recurrence is significantly lower in adult women. The same or a different strain of UTI-causing bacteria in the stomach can (re)inoculate the bladder, which is why recurrent UTIs can be introduced from several locations. Around half of all UTIs (urinary tract infections) go undetected. These results were in agreement with those found by Silverman et al., who discovered that bacteria living in the bladder epithelium can re-emerge on a regular basis and cause UTI recurrence. In pre-menopausal women, the increased proportion of recurrence of UTIs may be related to sexual activities three or more times a week, use of spermicides, and new or multiple sexual partners. Systemic hormone therapy is not an effective way of prophylaxis in menopausal women, and asymptomatic bacteriuria during this period often does not call for treatment. This finding is consistent with the study that was documented by Milart et al. They found that women who had gone through menopause did not require treatment for asymptomatic bacteriuria. According to studies, an increase in risk occurs in women after menopause mostly as a result of the aftereffects of low oestrogen levels, which are commonly associated with vaginal atrophy. Majority of the research on this topic

### Table 5. Distributions of Antibiotic Resistance Patterns among Phylogeny of E. coli Isolates

<table>
<thead>
<tr>
<th>Genotype Groups</th>
<th>Multi-drug resistance (N = 23)</th>
<th>Extensively-drug resistance (N = 65)</th>
<th>Pan-drug resistance (N = 2)</th>
<th>Total (%)</th>
<th>χ²</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n (%)</td>
<td>n (%)</td>
<td>n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Phylogenetic A</td>
<td>1 (4.34)</td>
<td>2 (3.07)</td>
<td>0 (0.0)</td>
<td>3 (3.33)</td>
<td>0.33</td>
<td>0.5 NS</td>
</tr>
<tr>
<td>Phylogenetic B₁</td>
<td>2 (8.69)</td>
<td>2 (3.07)</td>
<td>0 (0.0)</td>
<td>4 (4.44)</td>
<td>1.0</td>
<td>0.6 NS</td>
</tr>
<tr>
<td>Phylogenetic B₂</td>
<td>5 (21.73)</td>
<td>7 (10.76)</td>
<td>0 (0.0)</td>
<td>12 (13.33)</td>
<td>0.33</td>
<td>0.5 NS</td>
</tr>
<tr>
<td>Phylogenetic C</td>
<td>4 (17.39)</td>
<td>16 (24.61)</td>
<td>1 (50)</td>
<td>21 (23.33)</td>
<td>18.0</td>
<td>0.001**</td>
</tr>
<tr>
<td>Phylogenetic D</td>
<td>1 (4.34)</td>
<td>3 (4.61)</td>
<td>0 (0.0)</td>
<td>4 (4.44)</td>
<td>1.0</td>
<td>0.3</td>
</tr>
<tr>
<td>Phylogenetic E</td>
<td>5 (21.73)</td>
<td>2 (3.07)</td>
<td>0 (0.0)</td>
<td>7 (7.77)</td>
<td>1.2</td>
<td>0.2 NS</td>
</tr>
<tr>
<td>Phylogenetic F</td>
<td>2 (8.69)</td>
<td>30 (46.15)</td>
<td>1 (50)</td>
<td>33 (36.66)</td>
<td>49.2</td>
<td>0.002**</td>
</tr>
<tr>
<td>Phylogenetic UP</td>
<td>3 (13.0)</td>
<td>3 (4.61)</td>
<td>0 (0.0)</td>
<td>6 (6.6)</td>
<td>0.0</td>
<td>1 NS</td>
</tr>
</tbody>
</table>

χ² (df) = 6.5 (df = 7) | 87 (df = 7) | NA | 70 (df = 7) | χ²: Chi-square, df: Degree of freedom, p: Probability, NA: Not Available, NS: Non-significant, ** p < 0.01.

### Discussion

This study indicated that females were more likely to have urinary tract infections (UTIs) than males (66% versus 34%). This is similar to the findings of another study by Mohsin et al. which demonstrated that females are more likely to get UTIs than males. Certain individuals are prone to experiencing recurrent infections of the urinary tract.
has been conducted on both sides of Baghdad, Karkh and Rusafa. Despite the fact that these two areas have significant demographic, social, and economic differences, research has shown that UTIs are significantly more prevalent, complicated, and poorly documented. These findings are comparable to those of another study conducted in Iraq, albeit with different percentages. The results of this investigation indicated an antibiotic resistance that was comparable with the findings of Ali et al., regarding nalidixic acid, ceftazidime, and amoxicillin/clavulanic acid (80%, 85%, and 52%, respectively). In contrast, our research revealed that resistance to levofloxacin and imipenem was 73%, while resistance to cefotaxime and cefepime was 78%, and levofloxacin resistance was determined to be 55.5%. The findings of this research contrast with those of a previous study by Maleki et al. They discovered that resistance to ceftazidime had reached 26.1% in Iran, while resistance to cefotaxime had reached 30%. They also explained that efflux pumps were the cause of the increase in resistance to these antibiotics. The results of our research are consistent with those of Shah and colleagues. The increase in resistance to beta-lactam antibiotics is due to the production of beta-lactamase, which includes cephalosporinase and penicillinase. These enzymes work on breaking the beta-lactam ring, inhibiting antibiotics that belong to the groups penicillin and cephalosporins. They found that resistance to meropenem was 26%, but resistance to ceftriaxone was 49%. Regarding the susceptibility of the isolates to antimicrobial drugs, all isolates showed resistance to at least one of the antibiotics that were tested and 23 isolates showed multidrug resistance (Table 5). It was discovered that 13.8-21.3% of the *E. coli* isolates that were obtained from the urine samples of hospitalised patients in England were resistant to the third-generation cephalosporins known as cefotaxime and ceftazidime. According to Ciontea et al., the percentage of UPEC strains in Romania that are susceptible to cephalosporins of the third generation was 87%.

In the course of our recent investigation, we found that phylogroup F was present in every single isolate. These findings are consistent with the findings of previous studies conducted in Iraq by Mohsen et al. According to these studies, the most common and virulent isolates that infect food-producing animals and transmit largely to humans are attributed to animal-to-human transmission via the food chain. Additionally, these studies revealed that phylogroup F was found to be prevalent throughout the country. According to the findings of the phylogenetic analyses that are outlined in Table 4, the majority of the *E. coli* isolates that were discovered in UPEC belonged to one of eight major phylogenetic families. The results from the study suggested that the isolates obtained from patients with urinary tract infections belonged to Uropathogenic *Escherichia coli* (UPEC). The results from the study suggested that the isolates obtained from patients with urinary tract infections belonged to Uropathogenic *Escherichia coli* (UPEC). This conclusion was drawn based on the investigation of the phylogenetic groups. According to the findings of the phylogenetic context, *E. coli* isolates exhibited significant levels of genetic recombination within the species. Monitoring and analysing the *E. coli* genotypic characteristics that are found in urine can provide beneficial data on the epidemiology of diseases in a number of geographical areas. These data can be used to formulate suitable public health policies. According to the research conducted by Waters et al., variations in phylogenetic grouping among *Escherichia coli* isolates may be influenced by several factors. These factors include physical health and nutritional status of the host, genomic determinants, ecological and topographical factors, changes in collection sites and techniques, virulence factors, antibiotic resistance, growth rate, carbohydrate fermentation, and genome size. All these factors may contribute to differences in the frequency of phylogenetic groupings observed in various studies. Clermont et al. discovered that 1% of isolates could not be assigned to one of the eight major phylogroups. Nonetheless, in the current study, 4% of isolates from UTI patients were untypeable, which is significantly lower than the results of Iranpour et al., which showed that 27% of the isolates were unclassified. Isolates that are untypeable are a product of phylogroup recombination or highly uncommon phylogroups. Certain isolates may not have been classified because of their rarity or because of the high frequency of gene loss that results in the low presence of these phylogroups, as discussed by Touchon et al. F and C phylogenetic groups made up over half of our sample pool (54/90), which is in line with the fact that F and C are the most common urovirulent phylogenetic groups in humans in Iraq. This similarity may have arisen via instances of transmission between humans and their pets. The broad frequency of the isolates belonging to phylogroup F was also seen in a study that identified *E. coli* isolates from poultry colibacillosis and human illnesses (sepsis, meningitis, and UTI) in China. To our knowledge, this is the first investigation to link UPEC pathotypes and zoonotic potential to *E. coli* isolates from human sources within phylogroup F. As cited by Mohsin et al., it is believed that animal-to-human transmission through the food chain is responsible for the most common and virulent isolates that infect food-producing animals and transmit to humans in large numbers. This theory is supported by the discovery that phylogroup F was found to be widespread across isolates. On the other hand, isolates belonging to the phylogroup F possess a significant number of virulence factors, which make it easier for *E. coli* to colonise a host and cause UTIs. There is a possibility that *E. coli* bacteria
belonging to phylogroups F and C can be transmitted from animals to humans, resulting in UTIs. Because of the presence of an anthropozoonotic relationship, the findings of our research suggest that F and C UPEC isolates are more frequently isolated from “animals that dwell with people”. The vast majority of research that has been undertaken on the phylogenetic grouping of UPEC has reported a similar distribution, including studies that have been carried out in China,30 South Korea,31 Denmark,32 Pakistan,33 Ethiopia,34 Mexico,35 and France,36 in which the majority of UPEC isolates belonged to phylogroup B2, followed by group D. In contrast to previous studies, the findings from our study showed that the majority of the Uropathogenic Escherichia coli (UPEC) isolates belonged to less common phylogenetic groups, namely A, B1, D, and E. These findings suggested that we should compare the dangers of phylogenetic groups F and C to those of phylogroup B2 and take into account all potential risks. Additionally, phylogenetic groups (F and C) often associated with commensal bacteria, also predominated in UPEC isolates, suggesting that the gastrointestinal tract is the primary origin of strains colonising urinary tracts. The presence of both virulence or resistance genes, according to the results of our study, is not limited to phylogroups by themselves, as has also been indicated by many researchers,37 rather the evolution within a species depends on the environment and the ability of bacteria to evolve within the same group by developing their skills by acquiring and maintaining genes to enable them to survive and develop themselves.

Conclusion

The results of our research revealed that certain types of E. coli belonging to the phylogenetic group F were the most frequent, aggressive, and insensitive to antibiotics routinely recommended for individuals with urinary tract infections. For better knowledge about the prevalence and geographic distribution of E. coli phylogenetic groupings, analogous research in other regions is required. The widespread use of antibiotics, often without antibiotic susceptibility testing, is one of the causes of the emergence of multidrug-resistant pathogens, which severely impedes therapeutic activities.

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Conflict of Interest: None

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