Phylogenetic Classification of *Escherichia coli* Isolated from Urinary Tract Infections in the Central Regions of Guilan Province

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**Introduction:** Urinary tract infection (UTI) is one of the most common infectious diseases, and *Escherichia coli* is known as the most dominant causative agent of this infection in 90-80% of patients. There is not much information about the phylogenetic groups, types, and virulence factors of *E. coli*, causing UTIs from Iran. The objective of this study was to evaluate the antimicrobial susceptibility of *E. coli* strains isolated from patients with UTI and to determine the phylogenetic classification of the strains. **Methods:** In this cross-sectional study, 100 *E. coli* isolates were collected from patients with UTI from five laboratories in Rasht city. The isolates were confirmed by using morphological and biochemical common tests. The frequency of virulence genes and patterns of phylogenetic groups were performed using Multiplex PCR. Additionally, antimicrobial susceptibility of all isolates was evaluated by disk diffusion method. **Results:** Distribution of phylogenetic B2, D, A and B1 groups in the isolates were 64%, 24%, 12%, and 0%, respectively. The highest antibiotic resistance was reported to cefotaxime (84%), piperacillin, and ceftaxime (80%), and the lowest resistance was demonstrated to imipenem (8%), chloramphenicol and gentamicin (12%). **Conclusion:** Our findings showed that the B2 was the most prevalent phylogenetic group and the most resistant strain to generally used antibiotics among patients with UTI. *J Med Microbiol Infec Dis, 2017, 5 (1-2): 17-20.

**Keywords:** *Escherichia coli*, Multiplex PCR, Urinary Tract Infection.

**INTRODUCTION**

*Escherichia coli* is the normal flora of the gastrointestinal tract of humans and warm-blooded animals. Most *E. coli* strains are not pathogenic, but several strains have gained disease-causing genes that have enabled them to cause diseases in humans and animals. They can cause a variety of intestinal and extra-intestinal diseases such as meningitis, neonatal, gastroenteritis, septicemia, wound infections and urinary tract infections (UTIs) [1-3].

UTIs are the most common after the respiratory tract infections. Annually, approximately 150 million people suffer from UTIs around the world. Many bacteria are capable of causing UTIs, but among them, *E. coli* is known as the most common cause of UTI involving approximately 90% of these infections. Clinical signs of the infection are urinary frequency, dysuria, blood in the urine, and dirt in the urine [4].

Data from gene library of *E. coli* showed that it comprised different phylogenetic groups and that specific bacteria genes or DNA fragments could be specific markers for phylogenetic classification of *E. coli* [5-6].

Three selected markers, ChUa, YjaA, and TSPE4.C2 are used for phylogenetic classification of *E. coli*. The gene chUA is essential for transformation of *E. coli* O157: H7 EHEC, yjaA gene was the first identified in the full genome of *E. coli* K-12, but its function is still unknown, and TSPE4.C2 was obtained from the gene library of *E. coli* [5, 7, 8].

More than fifty years have passed since antibiotics were used in the quick and efficient treatment of diseases. During this period, many bacteria have developed resistance to antibiotics, and new generations of antibiotics were introduced to the market. For this purpose, one of the most critical issues in the treatment of infectious diseases is the resistance of pathogenic bacteria to the antibiotics. The basis for appropriate treatment of UTIs is choosing high performance and inexpensive antibiotics. The indiscriminate use of antibiotics has resulted in high levels of antibiotic resistance of *E. coli* in many parts of the world.

The objective of this study was to develop a phylogenetic classification of the *E. coli* isolates obtained from the individuals with UTIs and to evaluate the antibiotic resistance among them.

**MATERIAL AND METHODS**

This cross-sectional study included 100 *E. coli* isolates from UTIs collected from the outpatients and hospitalized patients whom samples were sent to five laboratories, Al-Zahra Hospital, Dr. Ashtiyani, Razi, Afrah, and Dr. Afroayi in Rasht city during February 2015 through January 2016. To obtain single colonies, the samples were cultured on the EMB and Blood agar medium cultures and incubated for 24 h at 37°C.

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http://jommid.pasteur.ac.ir
The culture media, TSI, SIM, and MR-VP were used to detect and confirm the identity of E. coli. The isolates were tested for their antimicrobial susceptibilities by the Kirby-Bauer disc diffusion technique according to the Clinical and Laboratory Standards Institute (CLSI) guideline using the antibiotics (CLSI 2015), ceftazidime (30µg), cefazolin (30µg), imipenem (10µg), ceftriaxone (30µg), cefotaxime (30µg), piperacillin (10µg), nalidixic acid (30µg), ciprofloxacin (5µg), cefoxitin (30µg). Two strains, E. coli ATCC 25922 and E. coli ATCC 35218 were used as the reference to control the quality of the applied antimicrobial agents (CLSI 2015).

Bacteria were cultured on the LB medium (Luriabrath), and for this purpose, a single clone of the bacteria was inoculated in LB medium according to instructions of the manufacturer (MIRMEDIA, Iran) and kept at 37°C for 12 to 16 h.

For molecular assessment of the isolated strains, the bacteria DNA was extracted using a DNA extraction kit (Cinnagen, Tehran, Iran). The quality of extracted DNA was evaluated by running 3µl of extracted DNA mixed with 1µl power load dye on 1% agarose gel. A multiplex PCR assay was used for typing and phylogenetic grouping of E. coli. The master PCR mix comprised 3µL of 10×PCR buffer, 3µL of 25mM MgCl2, 3µL of 10mM dNTP mix, 0.5µL of Taq DNA Polymerase, 9.5µL of MilliQ water and 1µL of each of the forward and reverse primers. Finally, 4µL of each DNA template was added to the tubes to make up the final reaction volume of 25µL. In this study, specific primers for chuA, yjaA genes and TSPE4.C2 segment were used (Table 1). After PCR, the products were sequenced (Macrogen Company, Korea).

RESULTS

The patients’ age ranged from 2 month old children to 79-years-old elderlies. All 100 samples were tested using biochemistry tests, and results showed that the isolated bacteria were Gram-negative, catalase positive and oxidase negative. In TSI medium, the isolates were Acid/Acid (A/A) (yellow/yellow) and H2S negative. In SIM medium, the motility and indole were positive and H2S negative. Also, MR was positive, and VP, Simon citrate and urea results were negative. As a result, the identity of the isolated bacteria was confirmed as E. coli.

The antibiogram test showed the highest rate of antibiotic resistance against cefotaxime (84%), piperacillin and cefixime (80%), and the lowest against imipenem (8%), chloramphenicol and gentamicin (12%) (Fig. 1).

The PCR bands in 83 isolates (83%) revealed gene chuA, in 66 (66%) yjaA gene, and in 76 (76%), the TSPE4.C2 fragment. According to the results, Figure 2 shows the PCR bands for the three genes.

Table 1. Nucleotide sequences of used primers

<table>
<thead>
<tr>
<th>Genus</th>
<th>Primer sequence</th>
<th>Amplicon Size (bp)</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>chuA</td>
<td>F: GACGAACCAACCGTGAGAT</td>
<td>279</td>
<td>[6]</td>
</tr>
<tr>
<td></td>
<td>R: TGCCGCCCAGTACAAAGACA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>yjaA</td>
<td>F: TGAAGTGTCAGGAGACGCT</td>
<td>211</td>
<td>[6]</td>
</tr>
<tr>
<td></td>
<td>R: ATGGAAATGCGTTCTCTCAAC</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>R: CGCGCCAACAAATTAACG</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Fig. 1. The pattern of antibiotic resistance among tested isolates

Group D showed the higher resistance to the antibiotics; group A showed the highest susceptibility; and group B2 showed the moderate level of multidrug resistance.
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CONFLICT OF INTEREST
The authors declare that there are no conflicts of interest associated with this manuscript.

REFERENCES