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

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**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. Afrayi 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), cefazidime (30µg), cefazolin (30µg), imipenem (10µg), ceftriaxone (30µg), cefotaxime (30µg), pipercillin (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.

Table1. Nucleotide sequences of used primers

<table>
<thead>
<tr>
<th>Genus</th>
<th>Primer sequence</th>
<th>Amplicon Size (bp)</th>
<th>References</th>
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| chuA    | F: GACGAAACACAGGCTCAGGAT
         | R: TCGCCGCCAGTACCAAAGACA | 279         | [6]        |
| yjaA    | F: TGAAAGTGTCAGGAGACGCT
         | R: ATGGAGAATGCCTCCTCAAC   | 211         | [6]        |
| TSPE4.C2| F: GAGTAATGTCGGGGCATTCA
         | R: CGCGCCAACAAAGGATTAACG | 152         | [6]        |
PCR results showed that 64 cases (64%) of strains belonged to the phylogenetic group B2, 24 (24%) to the phylogenetic group D, and 12 (12%) to the phylogenetic group A. The phylogenetic group B1 was not observed among the strains. According to the results, the isolates from group D showed the higher resistance to the antibiotics, whereas the highest susceptibility was noticed among the isolates belonging to group A, and a moderate level of multidrug resistance was observed among the strains of group B2.

DISCUSSION

At present, the clinical management of UTI is one of the primary concerns worldwide due to the increased resistance of E. coli infections to commonly used antimicrobial agents. The resistance of E. coli to antibiotics showed to be related to their phylogenetic grouping [9]. The objective of this study was to describe the phylogenetic groups of E. coli based on the Clermont et al. (2000) method and to determine the relationship between these phylogenotypes and antibiotic resistance patterns.

The results of the study showed that 64%, 24%, 12% and 0% of strains were in the phylogenetic groups of B2, D, A, and B1, respectively. Distribution of strains in our study in the phylogenetic groups is consistent with the results and pattern of Clermont et al. (2000). They showed that most extra-intestinal pathogenic strains were in group B2, and then group D. It should be noted that the strains belonging to the phylogenetic group B1 was not found among the studied strains in the present study, being similar with the research of Grude et al. (2009) conducted in Russia (2007), and study of Sawma-Aouad et al. (2009) in Lebanon, in which none of the strains belonged to group B1 [10-11].

The findings are consistent with the results obtained in the study of Bashir et al. (2012) in Faisalabad, Pakistan on 59 UPEC isolates from patients, and one study by Abdi et al. (2014) on E. coli isolated from UTIs in Sistan region. These were also consistent with the study of Iranpour et al. (2015) who studied the phylogenetic typing of strains of E. coli isolated from UTIs, and with the study conducted by Zhao et al. (2009) in China on 202 strains of E. coli isolated from UTIs [12-15].

The present study results are consistent with those of the study of Moreno et al. (2006) and Johnson et al. (2005) in the United States in which groups A and D were dominant. Asadi et al. (2010) through phylotyping showed that the most common phylogenetic E. coli groups in southern Iran were D, A, and B1 with the frequencies of 70%, 23.3%, and 6.7%, respectively; they did not find group B2. The variation in prevalence of the phylogenetic groups in different studies might be attributed to the health status, diet, and genetic factors of the host, and environmental, social, and geographic conditions of sampling areas [16-19].

Evaluation of antibiotic resistance profile of UPEC strains showed that the strains from phylogenetic group D were significantly resistant to the majority of antibiotics compared to other phylogenetic groups. In addition, a moderate level of multidrug resistance was observed among the strains from group B2, while a low frequency of multidrug resistance was noticed among the isolates belonging to group A. Our findings were in agreement with the results of two separate studies in India and Sweden [20-21], but not with the studies conducted in southern Iran in cities of Shiraz and Bushehr [19, 14]. This issue might be due to bacterial characteristics in different geographic regions or use of antibiotics.

Our findings showed that group B2 was the most common phylogenetic group and the most resistant strain to generally used antibiotics among patients with UTI. Similar studies in other geographical regions are required to provide a better understanding of the prevalence and geographic distribution of E. coli phylogenetic groups. The routine monitoring of antibiotic resistance patterns will also help clinicians to prescribe the most effective antibiotic and to prevent further increase of antimicrobial drug resistance.

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CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest associated with this manuscript.

REFERENCES