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Original Article

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 cefixime (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.

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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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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), cefazoline (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 $MgCl_2$, 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

Genus	Primer sequence	Amplicon Size (bp)	References
chuA	F: GACGAACCAACGGTCAGGAT R: TGCCGCCCAGTACCAAAGACA	**279**	**[6]**
yjaA	F: TGAAGTGTCAGGAGACGCT R: ATGGAGAATGCGTTCCTCAAC	**211**	**[6]**
TSPE4.C2	F: GAGTAATGTCGGGGCATTCA R: CGCGCCAACAAAGTATTAACG	**152**	**[6]**

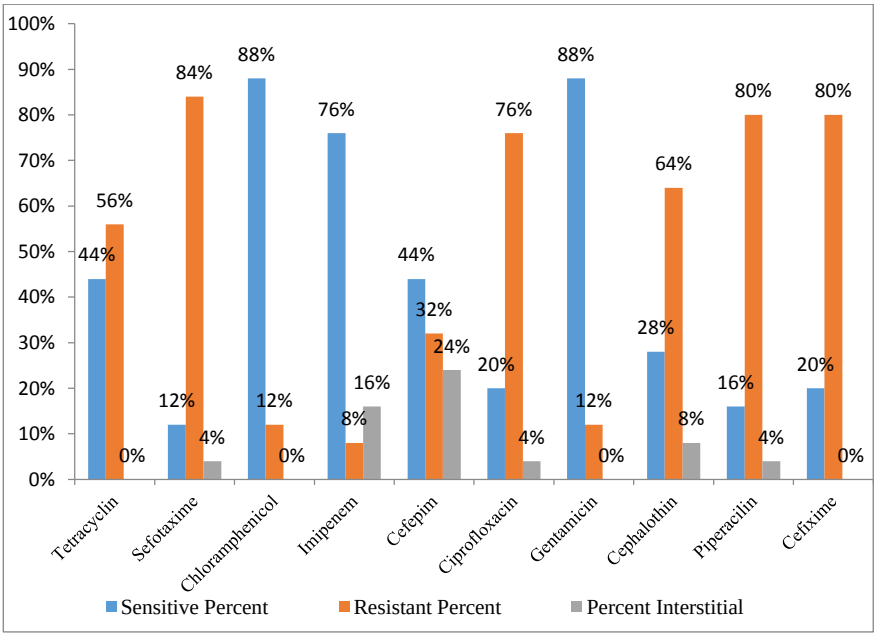


Fig. 1. The pattern of antibiotic resistance among tested isolates

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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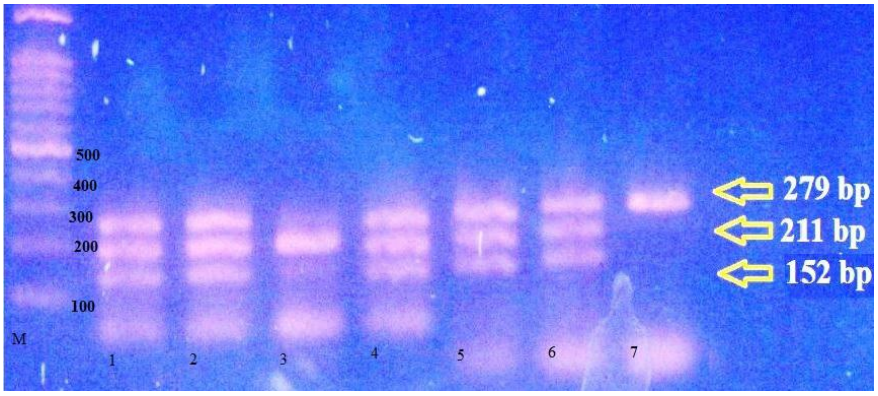


Fig. 2. The amplified fragment of isolates in 1% agarose gel

The size of *chuA* gene is 279 bp, *yjaA* gene 211 bp, and TSPE4.C2 152 bp. Lane M is 100 bp ladder; the lanes 1 to 7 are PCR products of 7 strains form 100 strains

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 phylogroups 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.

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