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Determination of Phylogenetic Groups of *Escherichia Coli* Isolated from Human Urine in Urmia City

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Abstract

Objective: *Escherichia coli* is the member of *Enterobacteriaceae* family and is one of the most important and common species of Escherichia in medicine. Escherichia coli has high potential in creating different intestinal and extraintestinal diseases in human and animals. The goal of this research determination of phylogenetic groups of Escherichia Coli isolated from human urine in Urmia city.

Materials and Methods: In the present research, 950 urine samples were studied from hospitalized patients in Urmia city. The urine sample were inoculated on MacCankey agar and blood agar and incubated at 37°C. Positive urine cultures was identified by standard laboratory methods involving morphological characteristics and biochemical tests. To determine the phylogenetic group, multiplex-PCR (m-PCR) was used. Primers used in this study could successfully amplify genes, including *yjaA*, *chuA* and TSPE4.C2 with 211, 279 and 152 bp, respectively.

Results: Fifty samples were positive after culture and bacterial isolation. Fifteen of isolates belong to group A (30%), 6 belong to group B1 (12%), 20 belong to group B2 (40%) and 9 of them belong to group D (18%).

Conclusion: The high number of bacteria in group B2 as extra intestinal pathogenic agent, indicative of having a care about choosing a treatment for such infections.

Keywords: Urinary tract infection, *Escherichia coli*, Phylogenetic typing, m-PCR

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Introduction

As a member of *Enterobacteriaceae* family, *Escherichia coli* is one of the most important and most prevalent species of *Escherichia* genus in medicine and veterinary. The bacteria has a high potential to develop intestinal and extra-intestinal diseases in human and different animals, urinary tract infection, meningitis, sepsis, abdominal infection, osteomyelitis, cellulitis, avian colibacillosis, and wound infection are examples of diseases caused by this bacterium (1). Most urinary tract infection-causing gram-negative bacilli originate from the intestine, and ascend to the bladder after involvement of urethra; they may also involve the kidneys and prostate (2). Patients with *E. coli*-induced urinary tract infection may develop cystitis, pyelonephritis, or sepsis. Although cystitis is associated with frequency and dysuria, fever is rare and flank pain may not occur always. In contrast, pyelonephritis, i.e. infection of renal parenchyma and pelvis, is usually associated with fever, flank pain, dysuria, and frequency. Sometimes, patients experience chills, nausea, vomiting, diarrhea, leukocytosis with left shift, and bacteremia. Sepsis develops after entering of *E. coli* from the urinary tract into the blood (3).

Phylogenetic analysis has shown that *E. coli* strains can be categorized in 4 main phylogenetic groups. Acute strains, as the causing agents of extra-intestinal infection are included in group B2 and partly in group D, while symbiotic strains are included in group B1 and group A. There is another sub-group in this classification, called group E (4). Extra-intestinal infection-causing strains possess more abundant virulence genes and factors than symbiotic strains. According to studies conducted on laboratory mice, it seems that strains in group B2 are more virulent than all other groups; a relationship has been reported between this group and inflammatory bowel disease (5). Group A has been more reported from hospital-acquired infections as well as wound infections. It should be noted that in the phylogenetic Group A, *chuA* gene and DNA fragment TspE4.C2 are not present while *yjaA* gene is variably present; in the phylogenetic group B1, *chuA* gene is not present, *yjaA* gene is variably present, and DNA fragment TspE4.C2 is present; the phylogenetic group B2 is characterized with a positive *chuA* and *yjaA* genotype and variable DNA fragment TspE4.C2; and the phylogenetic group D is characterized with a positive *chuA* and negative *yjaA* genotype and variable DNA fragment TspE4.C2 (6). Considering the importance of the mentioned groups, this study aimed at determining the dominant phylogenetic groups of *E. coli* isolated from human urine in Urmia city.

Materials and Methods

In this study, 950 urine samples were collected from hospitals in Urmia. To isolate *E. coli*, the centrifuged sed-

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iment of urine samples was cultured on the McConKey agar medium. Red colonies on the media were cultured on Eosin methylene blue medium to purify the bacteria. After 24 hours incubation, the gram-negative bacilli were examined in terms of colonies and tests of oxidase, urease, indole, motility, methyl red, Voges–Proskauer (VP), triple sugar, and citrate, and were kept in nutrient broth for molecular works (7). To extract DNA in the next step, all *E. coli* positive samples were incubated at 37°C in nutrient agar medium (Scharlau Microbiology, Spain) for 24 hours. A full loop of each sample was mixed with 250 µL distilled water. All samples were vortexed to produce a uniform opacity. They were then boiled for 10 minutes, and centrifuged for 7 minutes at 6000 g. The supernatant was collected for PCR. Multiplex-PCR (m-PCR) was used in this study for phylogenetic analysis of the samples. Full specifications of primers for m-PCR are listed in Table 1 (8).
For negative control, distilled water was used instead of DNA in the reaction mixture. PCR was performed in a thermocycler (Eppendorf, Germany) with the following conditions:

- Initial denaturation at 95°C for 5 minutes
- Followed by 35 cycles, each consisting of denaturation at 94°C for 30 seconds, annealing at 59°C for 10 seconds, and extension at 72°C for 30 seconds
- Final extension at 72°C for 7 minutes

PCR products were electrophoresed on 1.8% agarose gel containing 10 mg/mL ethidium bromide at 80 V for 1 hour. The gels were observed in a transilluminator (Uvitec, Europe) and the images were recorded.

Results

Of 930 urine samples collected after culture in EMB medium, those with lustrous green colonies (Figure 1) were gram stained. Then urease-negative, indole-positive, motility-positive, MR-positive, VP-negative, and citrate-positive samples were recognized as *E. coli* contamination samples, which were totally 50 isolates.

Determination of phylogenetic groups

Using m-PCR, 50 detected isolates were examined to determine the phylogenetic groups. Primers listed in Table 1 were able to properly amplify the desired genes of *yjaA*, *chuA*, and TSPE4.C2 with product sizes of 211, 279, and 152 bp, respectively (Figure 2). No product was found in the negative control including distilled water instead of DNA.

Of 50 *E. coli* isolates, 15 belonged to group A (30%), 6 to group B1 (12%), 20 to group B2 (40%), and 9 to group D (18%).

Discussion and Conclusion

The majority of isolates of colibacillosis belonged to group B2 (symbiotic bacteria), while Ghanbarpour et al studied 96 isolates of human diarrhea and reported that 52.1% belonged to group A, 2.1% to group B1, 10.4% to group B2, and 35.4% to group D (9). In another study by Bukh et al, included 1533 unique isolates of *E. coli* from Danish patients during a 10 year period, results showed 65.9% of the 1533 *E. coli* isolates belonged to phylogroup B2, 16.6% to D, 13.1% to A and 4.4% to B1 (10). In a study for phylogenetic typing of urine samples, Ebrahimzadeh et al showed that 65% of isolates were in group B2, 19% in group D, 16% in group A, with no group B1 (11). In a study by Kazemnia et al (12) in 2014, most strains of *E. coli* belonged to groups A and B2, and no pattern of B1 was observed in human isolates; this is inconsistent with the findings of this research. In another study by Alizadeh et al on 155 isolates of *E. coli* in Bam city, 71.6% were in group A, 3.22% in group B1, 9.67% in group B2, and 15.48% in group D. It was also shown that about 29 isolates with ST-3 gene were distributed in 3 phylogenetic groups with a frequency of 48.28% group A, 41.38% group D, and 10.34% group B2 (13). But in the present study, of 930 collected urine samples, 50 isolates of *E. coli* were identified through culture, purification, and biochemical tests. Of 50 *E. coli* isolates, 15 belonged to group A (30%), 6 to group B1 (12%), 20 to group B2 (40%), and 9 to group D (18%). Intestinal pathogenic *E. coli* enter into intestine from fecal oral route.
Recent phylogenetic studies show that extra-intestinal pathogenic *E. coli* mostly belongs to group B2, and partly to group D; this is confirmed by the results of typing.
The relationship between phylogenetic groups and drug resistance was shown in studies, and multiple drug resistance is higher in group A of poultry samples. Presence of multiple drug resistance in poultry symbiotic isolates may arise from symbiosis with the hosts which are always faced with antibiotics used to control the disease in poultry.
Multiple drug resistance among pathogenic groups, which are highly prevalent and have a great number of virulence genes as well as drug resistance genes, necessitates careful selection of appropriate antibiotic treatment.
Finally, selection of treatment strategy based on continuous monitoring of health authorities is necessary to pre-

Table 1. Characteristics of Primers Used for Multiplex (PCR)

Primer Name	Genes	Primer Size	Primer Sequences	Segment Size
T1	TSPE4.C2	24	5'- gagtaatgtcggggcattca-3'	152
T2	TSPE4.C2	25	5'- cgcgccaacaaagtattacg-3'	
Y1	*yjaA*	20	5'- tgaagtgtcaggagacgctg-3'	211
Y2	*yjaA*	20	5'- atggagaatgcgttcctcaac-3'	
C1	*chuA*	20	5'- gacgaaccaacggtcaggat-3'	279
C2	*chuA*	20	5'- tgccgccagtaccaaagaca-3'	

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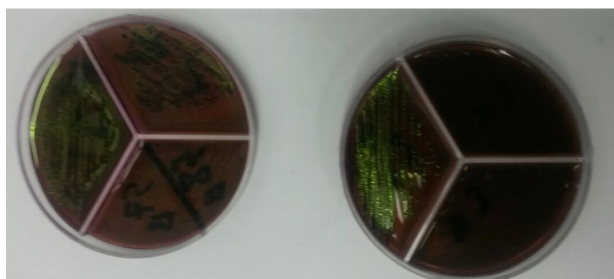

Figure 1. *E. coli* Isolated in EMB medium.

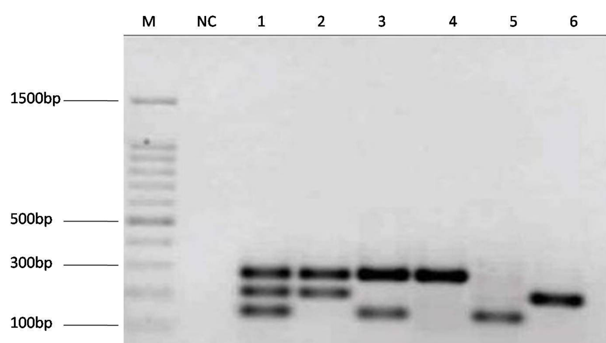

Figure 2. Results of multiplex PCR. Well M, marker; well NC, negative control; wells 1 and 2, group B2; wells 3 and 4, group D; well 5, group B1; well 6, group A.

vent the transmission of resistant bacteria from poultry to human.

Ethical issues

We have no ethical issues to declare.

Conflict of interests

The authors declare that they have no conflict of interest.

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