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The Phylogenetic Study of *Escherichia coli* Strains Isolated from Clinical Cases

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Urinary tract infection (UTI) is one of the common infectious diseases. *Escherichia coli* is one of the most important cause of UTI. The purpose of this research was to determine the frequency of some phylogenetic genes in *E. coli* strains isolated from patients with UTI. *E. coli* strains were recovered from urine culture of suspect patients with UTI and biochemical and bacterial tests have been utilized to identify the isolated bacteria. Detection of phylogenetic genes were done through Multiplex PCR. Approximately 94%, 92%, 82% and 72% of *E. coli* strains contained ChuA1, YjaA , TspE4C and arp genes respectively. Results indicate that PCR based phylogenetic molecular method is a simple and quick method for typing of our uropathogenic *E. coli*.

Keywords: Uropathogenic *E. coli*, Phylogenic, PCR.

UTI is one the common bacterial infectious diseases that involves people around the world (Basu *et al*, 2013). *E. coli* is the most important cause of UTI (Lee S *et al*, 2010). Clermont *et al* have introduced a method called Triplex PCR for determining genes of chuA, yjaA and TspE4 in 2000. Depending on presence or absence of these three genes *E. coli* can be categorized in one of phylogenic groups of A, B1, B2 or D (Clermont O *et al*, 2000). In 2013, Clermont *et al* have added a secondary gene called arpA to other three above mentioned genes and created a quarduplex PCR for classification of *E. coli* to one of A, B1, B2, C, D E, F and clad I phylo-groups (Clermont O *et al*, 2013). Phylogenetic studies in the world indicate that pathogenic *E. coli* outside intestine are mostly belonging to B2 and less to D group, while most of the commonsal pathogenic strains are relating to groups A and B1 (Moreno E *et al*, 2008). Sulfamethoxazol- trimethoprim (Cotrimoxazole), Fluoroquinolones, Beta-lactam, Nitrofurantoin and Phosphomycin are the most important antibiotics for treating UTI in society and hospitals (Ejrnaes K *et al*, 2011). Nowadays, many studies have indicated high resistance towards common antibiotics among pathogenic *E. coli* causing urinary tract infections (Santo E *et al*, 2007). In addition, during recent years, difference in resistance of antibacterial of phylogenetic groups of pathogenic *E. coli* is observed that it is a significant issue (K˜oljalg S etal, 2014). It was necessary to study the antibacterial sensitivity for assessing difficulties dimensions and it is necessary for choosing an appropriate antibacterial agent for infected people (Hassan SA *et al*, 2011). The purpose of this research was to determine the frequency of some phylogenetic genes in *E. coli* isolated from patients with UTI.

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MATERIALS AND METHODS

Bacterial strains

Research population includes all *E. coli* strains recovered in a major hospital in Tehran. According to standard method of clinical sample consisting of patients' urine suspected to UTI, urine was collected in sterile containers and after microscope observations and observing extreme WBC which indicates UTI, suspected samples were cultured on Chrome Agar and EMB agar. Then, all positive isolates were stored in skim milk.

DNA extraction and PCR

Boiling method was utilized in order to extract DNA. Utilized primers are shown in table 1. PCR was done in volume of 20 microliter including 7 microliter distilled water, 1 microliter extracted DNA, 10 microliter master mix ambliqon including Taq DNA polymerase and 1 microliter of each primer. Thermal cycles are respectively: 1 cycle of 59 centigrade for 5 minutes, 30 cycles including 94 centigrade for 30 seconds, 55 centigrade for 30 seconds, 72 centigrade for 40 seconds, and final cycle of 72 centigrade for 5 minutes. In order to manifest PCR products were electrophoresed and stained with ethidium bromide.

RESULTS

Eighty two and Eighteen percentage of the patients were female and male respectively with different ages from 15 to 75 years. Patients have been categorized based on their age into four groups, including: teenager (15-17 years old), Youth (18 to 39), middle age (40 to 59) and old (60 to 75) (Fig 1). In this research, the antibiotic sensitivity of pathogenic *E. coli* have been determined for following antibiotics including Ampicillin, Amikacin (30 mg), Amoxicillin (25mg), ceftizoxim (30 mg), ceftazidim (30 mg), cefalutin (30 mg), ciprofloxacin (5 mg), cefotaxim (30 mg), ceftriaxone (30 mg), gentamicin (10 mg), imipenem (10 mg), meropenem (10 mg), norfloxacin (10 mg), Nalidixic acid (30 mg), Nitrofurantoin (300 mg), Cotrimoxazole (23.75 mg sulfametoxsazole + 1.75 mg trimethoprim) and ofloxacin (5 mg). The highest sensitivity was to amoxicillin and the highest resistance was to Amikacin . Approximately 94%, 92%, 82% and 72% of *E. coli* strains contained ChuA1 , YjaA , TspE4C and arp genes respectively (Fig 2).

DISCUSSION

UTI is one of the most common infectious diseases in the world. *E. coli* is from the family of Enterobacteriaceae and the most important pathogen causing UTI. Inappropriate treatment of UTI in long term will cause renal failure (Cento *et al*, 2006). Scientists believe that the first step in starting urinary infection is the attachment of bacteria to euro epithelium. Better awareness from

Table 1. Primes used in the study

Target gene	Primer sequence
arpA	5-AACGCTATTCGCCAGCTTGC-3
	5-TCTCCCCATACCGTACGCTA-3
chuA	5-ATGGTACCGGACGAACCAAC-3
	5-TGCCGCCAGTACCAAAGACA-3
yjaA	5-CAAACGTGAAGTGTCAGGAG-3
	5-AATGCGTTCCTCAACCTGTG-3
TspE4C2	5-CACTATTCGTAAGGTCATCC-3
	5-AGTTTATCGCTGCGGGTCGC-3

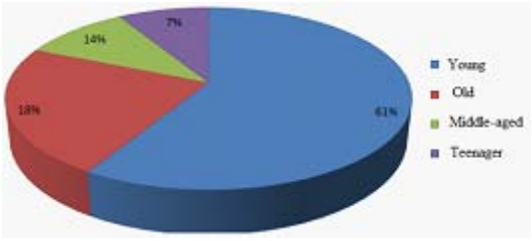

Fig. 1. Distribution of age among the patients

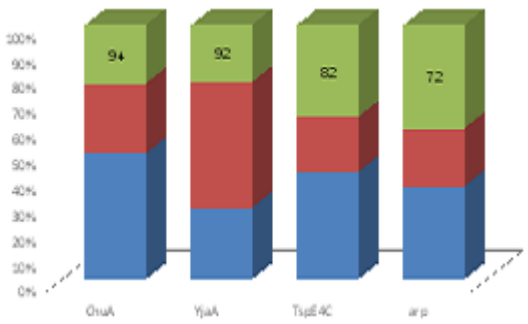

Fig. 2. Results of frequency of studied genes in *Escherichia coli*

the characteristics of virulence of pathogenic organisms gives this opportunity to physicians to estimate the progress flow of infection on patient and appropriate treatment (Behroozi *et al*, 2010). Nowadays, genetic molecular techniques like bacterial typing are important and useful tools in identifying and classification of bacteria. By molecular typing we can determine the hospital infections, identifying cases of infection from food and even distribution of plant pathogenic bacterial strains within environment and also these methods aware us more about epidemiologic principles and perfection and distribution most of bacterial diseases. In a study carried out by Rijavich *et al* in 2008 on 105 pathogenic *E. coli* in Slovenia, these results have been achieved: 51% of pathogenic strains have been related to group B2, 20% to D, 15% to A and 13% to B1.Also in studies by Kanamura *et al* in 2006 and Johnson *et al* in 2005 in U.S, also the highest frequency of isolated pathogens from patients with UTI were in B2 group of phylogenic. Hancock *et al* in 2009 in Denmark and Hughes *et al* in same year in Hong Kong indicated that most of urinary samples are belonging to B2 and D phylogenic groups. Recent study is referring to study of Ejrans in Denmark during 2011 on urinary E. coli ; it was similar that most of pathogens were in B2 group and then other groups. Piati *et al* in Italy 2008 reported 56% of *E. coli* strains were in group B2. But it did not match with study by Moreno and colleagues and the study by Johnson *et al* that their strains were mostly in group A and D. One of the reasons for non-compliance can be the difference of distribution of strains in different geographic areas. UTI in women is more than men. In general, the incidence of UTI in our study, the average age of our population is people over 25 years. Urinary tract infection is more related to the phylogenetic group D, respectively. Among the genes in our study, the highest prevalence was reported in the gene ChuA. In this study, the most abundant gene was 94 ChuA (% while the lowest gene was) 72 % arp

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