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O-serotyping of *Escherichia coli* Strains isolated from Patients With Urinary Tract Infection in Southeast of Iran

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Background: Uropathogenic *Escherichia coli* (UPEC) O-serogroups with their phylogenetic background are the most prevalent causes of urinary tract infections (UTIs).
Objectives: The association of O types with phylogenetic background was assessed among *E. coli* isolates collected from patients with UTI.
Patients and Methods: In this study, 186 patients with UTI, referred to two hospitals affiliated to Zabol University of Medical Sciences in southeast of Iran, were enrolled during January to July 2013. Phylogenetic groups and serotyping were performed using multiplex-PCR method.
Results: A total of 100 *E. coli* strains were isolated from the urine samples. The most common types of O antigens were O2 (16.43%), O6 (16.43%) and O18 (13.69%). The phylogenetic analysis showed that 63 O-antigen-positive isolates were mainly segregated from the phylogenetic group B2 (56%) and the substantial prevalence (30%) belonged to the phylogenetic group D.
Conclusions: This was the first report of *E. coli* serotyping in patient with UTI from southeast of Iran as well as investigation of their relation with phylogenetic pattern by multiplex-PCR. Further studies from other parts of Iran and on other serotypes are recommended.

*Keywords:*Multiplex-Polymerase Chain Reaction; O-Antigen; *Escherichia coli*

1. Background

Urinary tract infection (UTI) is one of the most common infections and *E*scherichia *coli* is so far the most common causative agent of this disease (1-5). O antigen is exposed on the very outer surface of bacterial cell (6-8) and is an essential component of the lipopolysaccharides on the surface of Gram-negative bacteria; its variation provides a major basis for serotyping schemes (9, 10). For the first time, *E. coli* was classified based on various types of O antigen by Kauffmann (11, 12). O antigen is one of the most variable structures on the cell surface, leading to major antigenic variability due to the variation in types of sugar arrangement within the O unit and the linkages within and between the O units. Consequently, O antigen has become a major basis for serotyping schemes for many Gram-negative bacteria (13). *E. coli* clones, including both intestinal and extra-intestinal types, are normally identified by the combination of their O and H (and sometimes K) antigens (14). To date, more than 180 forms of O antigen have been recognized for *E. coli* (9). Each serotype of *E. coli* has an important role in clinical presentation of UTI and the prevalence of different serotypes varies in different regions. In Iran, despite the high prevalence of UTI, very few studies have been conducted to determine the prevalence of various O types of *E. coli* by multiplex-polymerase chain reaction (PCR).

2. Objectives

This study was performed to determine the association of O types with phylogenetic background among *E. coli* isolates collected from patient with UTI in southeast of Iran.

3. Patients and Methods

E. coli strains were isolated from urine samples of patients attending a teaching hospital in Zabol, Iran, during January to July 2013 and were identified using standard methods. The diagnosis of UTI was established by the hospital medical staff based on clinical symptoms and positive urine culture.

3.1. DNA Extraction

E. coli isolates were grown overnight (16 hours) in 5 mL Luria Bertani (LB) broth at 37°C. Two mL of bacterial isolates were then pelleted, resuspended in 200 μL of sterile double-distilled water and boiled at 95°C for 10 minutes. After centrifugation, the supernatants were stored as DNA template at -20°C until used for PCR.

3.2. Phylogenetic Classification

In this study, we used the PCR-based phylogenetic typing method because it is simple, rapid and easily applied for a large number of *E. coli* strains, as described by Clermont et al. (15). This phylogenetic grouping method, which uses a combination of three DNA markers (*chuA*, *yjaA*, and tspE4.C2 DNA fragment), can accurately classify *E. coli* isolates into one of the four major phylogenetic lineages. As described by Clermont et al. we used the primer pairs *ChuA.1* (5´-GACGAACCAACGGTCAGGAT-3´) and *ChuA.2* (5´-TGCCGCCAGTACCAAAGACA-3´), as well as *YjaA.1* (5´-TGAAGTGTCAGGAGACGCT-3´) and *YjaA*.2 (5´-ATGGAGAATGCGTTCCTCAAC-3´), and finally TspE4C2.1 (5´-GAGTAATGTCGGGGCATTCA-3´) and TspE4C2.2 (5´-CGCGCCAACAAAGTATTACG-3´), which generated 279-, 211-, and 152-bp fragments, respectively. We performed a triplex PCR using the above six primers in a single reaction in a gradient Eppendorf's Master cycler® pro (Eppendorf, Hamburg, Germany) under the following conditions: denaturation for four minutes at 94°C, 30 cycles of five seconds at 94°C and 10 seconds at 59°C, and a final extension step of five minutes at 72°C. The PCR products were analyzed on a 2% agarose gel. The grouping decision was made based on the presence or absence of specified amplifications as follows: *chuA*$^-$, TspE4.C2$^-$, group A; *chuA*$^-$, *yjaA*$^-$, TspE4.C2+, group B1; *chuA*$^+$, *yjaA*$^+$, group B2; *chuA*$^+$, *yjaA*$^-$, group D.

3.3. O-Typing by Multiplex-Polymerase Chain Reaction

O-typing of the *E. coli* isolates was determined by a multiplex-PCR-based method, recently developed by Clermont et al. (16). This simple and rapid O-typing method was performed in two separate PCR reactions comprised of six reverse primers representative of six O antigens and one universal forward primer (Table 1) as described by Clermont et al. Each 25-μL PCR mixture contained 12.5 μL Taq DNA polymerase master mix red (amplicon), 0.2 μM /μL of each primer (1 μL) (Pishgam, Iran), 2 μL (approximately 100 ng/μL) of genomic DNA, and 9.5 μL dd H_2O. Amplification conditions were four minutes at 95°C, 30 cycles of 40 seconds at 95°C, 30 seconds at 57°C, and 30 seconds at 72°C, with a final elongation of six minutes at 72°C, performed using a gradient Eppendorf's Mastercycler® pro (Eppendorf, Hamburg, Germany). The PCR products were electrophoresed in 1.5% agarose gels in 1 x Tris/borate/EDTA (TBE) (0.1 M Tris, 0.09 M boric acid and 1 mM EDTA) and the gels were then stained with ethidium bromide and photographed using UV light (Figure 1).

4. Results

A total of 100 isolates of *E. coli* were collected from the urine samples of patient with UTI. Among the total of 100 *E. coli* isolates analyzed, 55 (55%) belonged to phylogenetic group B2 (Table 2). Of the remaining isolates, 22 (22%), 17 (17%), and 6 (6%) isolates belonged to D, A, and B1 groups, respectively. Group B2 strains were the most common and group B1 strains were the least common among the UTI specimens.

Table 1. Primers Used in This Study

Primer	Primer Sequence (5'-3')	Size of PCR Product, bp
gndbis.F	ATA CCG ACGACGCCGATCTG	189
rfbO1.R	CCAGAAATACACTTGGAGAC	-
rfbO2a.R	GTGACTATTTCGTTACAAGC	274
rfbO18.R	GAAGATGGCTATAATGGTTG	360
rfbO16.R	GGATCATTTATGCTGGTACG	450
rfbO6a.R	AAATGAGCGCCCACCATTAC	584
rfbO7.R	CGAAGATCATCCACGATCCG	722
rfbO4.R	AGGGGCCATTTGACCCACTC	193
rfbO12.R	GTGTCAAATGCCTGTCACCG	239
rfbO25a.R	GAGATCCAAAAACAGTTTGTG	313
rfbO75.R	GTAATAATGCTTGCGAAACC	419
rfbO15.R	TGATAATGACCAACTCGACG	536
rfbO157.R	TACGACAGAGAGTGTCTGAG	672

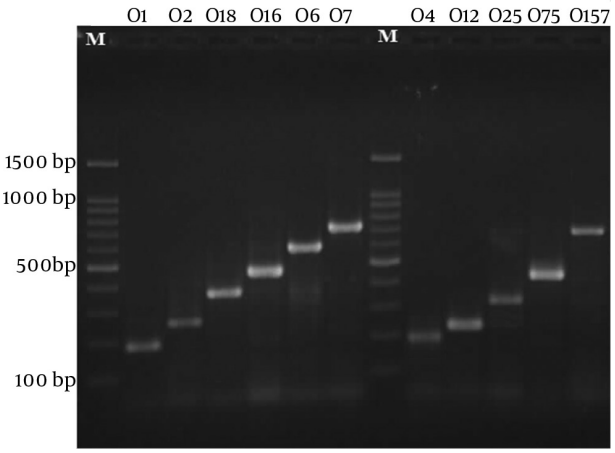

Figure 1. O-Type Profiles Obtained by Multiplex-Polymerase Chain Reaction

Table 2. Phylogenetic Groups Distribution of *E. coli* O-Types

O-Types	NO.	A	B1	B2	D
O4	6	0	0	3	3
O12	2	0	2	0	0
O25	3	1	0	2	0
O75	7	1	0	4	2
O15	0	0	0	0	0
O157	8	3	2	3	0
O18	10	0	0	7	3
O6	12	0	0	9	3
O1	6	0	0	1	5
O16	5	1	0	4	0
O2	12	0	0	8	4
O7	2	1	0	0	1
Total	73	7	4	41	22

4.1. O-Typing

Of 100 samples, 73 were positive for one of the tested O antigen types (73%). The most common types of O antigen were O2 (16.43 %), O6 (16.43 %) and O18 (13.69%), followed by O157 (10.95%), O75 (9.58%), O4 and O1 (each 8.21%), O16 (6.84%), O25 (4.10%), O7 and 012 (each 2.73%). There was no positive isolate for O15. Among the O antigen-positive isolates, 63 (86.3%) belonged to the phylogenetic groups B2 and D. Of the remaining, 4 (5.47%) and 7 (9.58%) isolates belonged to groups B1 and A (Table 2).

5. Discussion

We studied 12 types of O antigen in UTI-isolated *E. coli* strains. Totally, 73 samples were positive for one type of O antigen (73%), the most common of which were O2 and O6 (12% each). *E. coli* strains were classified based on various types of O antigen for the first time by Kauffmann (12) Until now, 180 types of O antigen have been detected (9). In Iran, various studies have been conducted for O-serotyping of the uropathogenic *E. coli* (UPEC). One of the earliest studies was conducted by Emamghorashi and coworkers in 2011 (6, 17). Their study on O serotype determination of 96 *E. coli* strains in children with pyelonephritis or cystitis led them to consider the relationship between some O serotypes and virulent genes in *E. coli*, causing UTIs. In that study, O1 was commonly seen in patients with pyelonephritis or cystitis (17). Johnson studied the relationship between bacterial characteristics and the clinical source of *E. coli*. One of the predictors of UTIs was the O75 antigen (18). In the present study, 11 O-antigen groups (O1, O2, O4, O6, O7, O12, O16, O18, O25, O75 and O157) were accounted for 6 (8.21%), 12 (16.43%), 6 (8.21%), 12 (16.43%), 2(2.73%), 2 (2.73%), 5 (6.84%), 5(6.84%), 10 (13. 69), 3 (4.10), 7 (9.58%) and 8 (10.95%) of the UTIs strains, respectively. O15 was not detected in UPEC isolates. In accordance with our study, Blanco and coworkers reported that most of the UPEC belonged to 10 (O1, O2, O4, O6, O7, O14, O18, O22, O75 and O83) of the 12 serogroups (19). Although serotype O6 was the most common type found in many studies, in our study O6, O2 and O18 were the most common types, while O6 was a common serotype in *E. coli* strains isolated from children with UTI in Slovakia (20). In a study of community-acquired UTI in Santiago, the characterized UPEC belonged to 27 different O serogroups; 68% of which were from one of the ten serogroups (O1, O2, O4, O6, O9, O18, O27, O73, O75 and O77) and 36% from one of the three serogroups (O2, O4 and O6) (19). The UTI-associated O antigens were also distributed widely between the phylogenetic groups A (9.58%), B1 (5.47%), B2 (56.16%) and D (30.13%). In the present study, 86.30% of O-antigen-positive isolates also belonged to the phylogenetic groups B2 and D, suggesting that certain O-antigen-positive strains were phylogenetic ally related. This is the first report of *E. coli* serotyping in patients with UTI from south of Iran using multiplex-PCR also assessing their relation with phylogenetic groups. O2 (19.04%), O6 (19.04%) and O18 (15.87%) were the three common types that correlated with the phylogenetic groups B2 and D.

One of the major findings of the present study was the demonstration of a striking phylogenetic distribution of various O types. Specifically, when group B2 was compared to other phylogenetic groups combined, significant differences in prevalence, favoring group B2, were seen for O6 (9 of 12), O2 (8 of 12) and O18 (7 of 10). This multiplex-PCR assay was an efficient and convenient strategy for serotyping the UPEC predominant strains, avoiding the disadvantages of traditional serologic assays. Therefore, development of this multiplex-PCR assay can be beneficial for clinical diagnostics, epidemiology studies and disease control.

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Authors' Contributions

Study design, data collection and data interpretation: Hussein Ali Abdi and Ahmad Rashki; funds collection, literature review and manuscript preparation: Ahmad Rashki.

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References

1. Yun KW, Kim HY, Park HK, Kim W, Lim IS. Virulence factors of uropathogenic Escherichia coli of urinary tract infections and asymptomatic bacteriuria in children. *J Microbiol Immunol Infect.* 2013.
2. Rudick CN, Berry RE, Johnson JR, Johnston B, Klumpp DJ, Schaeffer AJ, et al. Uropathogenic Escherichia coli induces chronic pelvic pain. *Infect Immun.* 2011;**79**(2):628–35.
3. Jacobsen SM, Stickler DJ, Mobley HL, Shirtliff ME. Complicated catheter-associated urinary tract infections due to Escherichia coli and Proteus mirabilis. *Clin Microbiol Rev.* 2008;**21**(1):26–59.
4. Beyene G, Tsegaye W. Bacterial uropathogens in urinary tract infection and antibiotic susceptibility pattern in jimma university specialized hospital, southwest ethiopia. *Ethiop J Health Sci.* 2011;**21**(2):141–6.
5. Demilie T, Beyene G, Melaku S, Tsegaye W. Urinary bacterial profile and antibiotic susceptibility pattern among pregnant women in north west ethiopia. *Ethiop J Health Sci.* 2012;**22**(2):121–8.
6. Emamghorashi F, Farshad S, Kalani M, Rajabi S, Hoseini M. The prevalence of O serogroups of Escherichia coli strains causing acute urinary tract infection in children in Iran. *Saudi J Kidney Dis Transpl.* 2011;**22**(3):597–601.
7. Domenico P, Tomas JM, Merino S, Rubires X, Cunha BA. Surface antigen exposure by bismuth dimercaprol suppression of Klebsiella pneumoniae capsular polysaccharide. *Infect Immun.* 1999;**67**(2):664–9.
8. Kalynych S, Yao D, Magee J, Cygler M. Structural characterization of closely related O-antigen lipopolysaccharide (LPS) chain length regulators. *J Biol Chem.* 2012;**287**(19):15696–705.
9. Wang Q, Perepelov AV, Beutin L, Senchenkova SN, Xu Y, Shashkov

AS, et al. Structural and genetic characterization of the Escherichia coli O180 O antigen and identification of a UDP-GlcNAc 6-dehydrogenase. *Glycobiology.* 2012;**22**(10):1321–31.
10. Brady PS, Feng YX, Brady LJ. Transcriptional regulation of carnitine palmitoyltransferase synthesis in riboflavin deficiency in rats. *J Nutr.* 1988;**118**(9):1128–36.
11. Gruneberg RN. Antibiotic sensitivities of urinary pathogens, 1971-82. *J Antimicrob Chemother.* 1984;**14**(1):17–23.
12. Kauffmann F. Ueber neue themolabile Korper antigene der colibakterien. *Acta Pathol Microbiol Scand.* 1943;**20**(21):21–44.
13. Reeves PP, Wang L. Genomic organization of LPS-specific loci. *Curr Top Microbiol Immunol.* 2002;**264**(1):109–35.
14. Nataro JP, Kaper JB. Diarrheagenic Escherichia coli. *Clin Microbiol Rev.* 1998;**11**(1):142–201.
15. Clermont O, Bonacorsi S, Bingen E. Rapid and simple determination of the Escherichia coli phylogenetic group. *Appl Environ Microbiol.* 2000;**66**(10):4555–8.
16. Clermont O, Johnson JR, Menard M, Denamur E. Determination of Escherichia coli O types by allele-specific polymerase chain reaction: application to the O types involved in human septicemia. *Diagn Microbiol Infect Dis.* 2007;**57**(2):129–36.
17. Emamghoraishi F, Farshad S, Kalani M. Relationship between O serotype and virulent genes in Escherichia coli causing urinary tract infections. *Iran J Kidney Dis.* 2011;**5**(4):234–7.
18. Johnson JR, Owens K, Gajewski A, Kuskowski MA. Bacterial characteristics in relation to clinical source of Escherichia coli isolates from women with acute cystitis or pyelonephritis and uninfected women. *J Clin Microbiol.* 2005;**43**(12):6064–72.
19. Blanco M, Blanco JE, Alonso MP, Blanco J. Virulence factors and O groups of Escherichia coli isolates from patients with acute pyelonephritis, cystitis and asymptomatic bacteriuria. *Eur J Epidemiol.* 1996;**12**(2):191–8.
20. Siegfried L, Kmetova M, Puzova H, Molokacova M, Filka J. Virulence-associated factors in Escherichia coli strains isolated from children with urinary tract infections. *J Med Microbiol.* 1994;**41**(2):127–32.