Comparative virulotyping and phylogenomics of *Escherichia coli* isolates from urine samples of men and women suffering urinary tract infections

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**ABSTRACT**

Objective(s): *Escherichia coli* strains are common pathogens that can cause urinary tract infections (UTI). This study aimed to assess *E. coli* phylogroups and virulence types in male and female UTI patients.

Materials and Methods: In the present study, 160 uropathogenic *E. coli* (UPEC) isolates (from both sexes) were assigned to phylogroups/types and some extraintestinal virulence factors were detected within them by multiplex-PCR.

Results: The isolates from women and men were predominantly distributed within phylogroup B2 and D, respectively. The presence of D, phylogroup was higher in men isolates than women, significantly ($P=0.045$). In male isolates *papEF* and *sfa/focDE* are more prevalent in B group than D, significantly ($P=0.048$; $P=0.035$). The prevalence of *hly* in B group is significantly higher than D ($P=0.034$) in female isolates.

Conclusion: This study highlighted different features of *E. coli* genotypes from phylogenetic and virulence point of view implicated in UTIs in both human genders.

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**Introduction**

*Escherichia coli* (*E. coli*) strains belonging to *enterobacteriaceae* family are normal habitat of gastrointestinal tract in the wide range of warm-blooded hosts. Many *E. coli* strains are harmless while some are pathogenic, meaning that they can cause illness, either diarrhea or infections in extraintestinal sites such as respiratory infections, the UTI's are the most common urinary tract infections (UTI) in human (1, 2). After diarrhea or infections in extraintestinal sites such as *urinary tract infections*. Iran J Basic Med Sci 2019; 22:211-214. doi: 10.22038/ijbms.2018.28360.6880

**Materials and Methods**

**Isolation of E. coli strains from patients with UTI**

One hundred and sixty *E. coli* strains were isolated from patients (25 - 45 years old/both sexes) suspected to suffering UTI based on urologist’s diagnosis referring to diagnostic laboratories according to the protocol described by Bonadio et al (13). Then stock cultures were prepared from the *E. coli* isolates and stored in Luria-Bertani broth (Sigma-Aldrich) with 15% (v/v) glycerol at -20°C until genotyping.

**DNA extraction, phylogenetic and virulence genotyping**

Genomic DNA was extracted from *E. coli* strains with the rapid one-step extraction (ROSE) method (14) based on alkaline lysis of bacteria and phylogenetic group and phenotype of isolates were identified using a modified multiplex-PCR-based assay optimized for detection of *chuA, yjaA, and tspE4.C2* gene markers as previously described by Staji (15). Finally, the detection of four extraintestinal putative virulence genes (*hly, iucD, sfa/focDE* and *pap*)

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was carried out using a modified Tetraplex-PCR program as described by Staji et al. previously (8). The primers sequences used in this study are present in Table 1.

**Statistical analysis**

Differences in frequencies of phylogenetic groups and virulence markers among men and women isolates was evaluated using chi-squared tests ($\chi^2$) on contingency tables and Fisher’s exact test with a significance level of $P=0.05$, using SPSS (version 21) software.

**Results**

A total of 160 *E. coli* isolates, 100 from females and 60 from male patients, were obtained from 240 urine samples (66.6%) of human cases suffering UTI, confirming that *E. coli* is dominant agent in development of urinary tract infections. All phylogenetic groups and phylotypes were found among isolates from both sex individuals in the general population, except for A0 phylotype which was absent in *E. coli* isolates from females (Table 2). Distribution of the phylogenetic group’s differed depending on the host sexuality. In females (n=100 strains), 46% of isolates belonged to group’s differed depending on the host sexuality. In females (Table 2). Distribution of the phylogenetic groups in male and female isolates. Comparison of prevalence of phylotypes between both sexes shows that D2 phylotype is more prevalent in male isolates than females ($P=0.045$), significantly.

The detection of virulence factors (*hly; iucD; pap; sfa/focDE*) using Multiplex-PCR revealed that 158 (98.75%) of all strains (n=160) were positive for at least 1 of the virulence genes tested; of these strains, 100 (100%) were female and 58 (96.6%) were male isolates. In the strains isolated from female patients, *iucD* and *sfa/focDE* were the most common virulence genes identified (47% and 43%, respectively), followed by *hly* (15%) and *pap* (13%). The only significant difference in the prevalence of virulence genes among female phylgroups showed that *hly* was significantly more prevalent in B1 compared to D phylogroup ($P=0.034$).

In the male strains, *iucD, pap* and *sfa/focDE* were the most common virulence genes detected, present in 36%, 16% and 13% of isolates, respectively, followed by *hly* (4%). In the *E. coli* isolates related to male patients *sfa/focDE* and *pap* ($P=0.035$ and $P=0.048$, respectively) were significantly more prevalent in B1 phylogroup compared to D. The prevalence of virulence genes in *E. coli* phylogroups and phylotypes of human UTI’s are present in Table 2.

Statistical analyses on the prevalence of virulence

**Table 1.** Polymerase chain reaction primers used to detect phylogenetic groups and extraintestinal virulence factors genes of *Escherichia coli* in samples collected from male and female patients suffering UTI

| Gene name | Primer sequence (5’ to 3’) | Product size (bp) | Ref |
|-----------|---------------------------|------------------|-----|
| chuA      | GAGGAAACGAGGCTTACAG      | 279              | 5   |
| yfaA      | TCGCGCCGATCAGCAAGAC       | 211              | 5   |
| tspE4.C2  | GATCAGTTGGGGGATCTCA       | 152              | 5   |
| hly       | CAAGCAGATAAGCACGTTCTCG    | 1177             | 16  |
| iucD      | ATCCGGATTGTTCTATAGCAACCTG| 602              | 16  |
| Pap       | GCAACGCAAGGCTGCGCTGATCA  | 336              | 16  |
| Sfa/focDE | CTCGCCAGAAGCTGGTGCATCTTA | 410              | 16  |

**Table 2.** Frequency of *Escherichia coli* phylogenetic Types (PT) and virulence genes (%) in relation to PT among urinary *E. coli* isolates from human female and male UTI cases

| Source     | PT       | No. Strains (%) | hly (%) | iucD (%) | Sfa/focDE (%) | pap (%) |
|------------|----------|----------------|---------|----------|---------------|---------|
| Female UTI | Ae       | 0              | 0       | 0        | 0             | 0       |
|            | A1       | 13 (13)        | 0       | 6 (62.5) | 1 (2.3)       | 1       |
|            | B1       | 0 (0)          | 1 (6.7) | 2 (4.2)  | 5 (11.6)      | 1       |
|            | B2       | 5 (5)          | 0       | 3 (6.4)  | 1 (2.3)       | 1       |
|            | B10      | 41 (41)        | 12 (80) | 19 (48.5)| 23 (53.5)     | 8       |
|            | D1       | 12 (12)        | 1 (6.7) | 5 (40.6) | 5 (40.6)      | 1       |
|            | D2       | 21 (21)        | 1 (6.7) | 12 (55.5)| 8 (38.1)      | 1       |
| Total      | 100 (100)| 15 (15)        | 47 (47) | 43 (43)  | 13 (13)       |         |
| Male UTI   | Ae       | 2 (3.3)        | 0       | 0        | 0             | 0       |
|            | A1       | 10 (16.7)      | 0       | 5 (50)   | 0             | 0       |
|            | B1       | 4 (6.7)        | 0       | 2 (50)   | 1 (20)        | 0       |
|            | B2       | 3 (5)          | 0       | 2 (50)   | 0             | 1 (20)  |
|            | B10      | 26 (43.3)      | 4 (16)  | 20 (50)  | 11 (42.3)     | 12 (50) |
|            | D1       | 10 (16.7)      | 0       | 5 (50)   | 0             | 1 (20)  |
|            | D2       | 5 (8.3)        | 0       | 2 (50)   | 1 (20)        | 1 (20)  |
| Total      | 60 (100) | 4 (100)        | 26 (43) | 13 (21.7)| 16 (27)       |         |
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Factors between E. coli isolates, stratifying by host gender, showed that pap is more prevalent in male isolates \( (P=0.0035) \) whereas sfa/focDE is more prevalent in female strains \( (P=0.006) \), significantly.

**Discussion**

E. coli is the predominant etiology of UTI and in this study a total of 160 E. coli isolates collected from the urine samples of female (62.5\%) and male (37.5\%) patients suffering from UTI caused by E. coli were examined to determine the phylogenetic group and phylotypes of each strains and the presence of the four extraintestinal pathogenic E. coli (ExPEC) related virulence genes. Finally, the distribution of phylogenetic characteristics and mentioned virulence genes were compared in E. coli strains of both sexes to investigate the differences in genotypes between the two sources.

All phylogenetic groups and subgroups were found among isolates from UTI’s and our results show that groups B\(_2\) (46.8\%) and D (30\%) are the most prevalent phylogenetic groups in E. coli strains implicated in human urinary tract infections (Table 2). These results are in parallel with some phylogenetic epidemiological studies about UPEC, as reported by Staji \textit{et al.} (8). It has been demonstrated that ExPEC strains usually belong to groups B\(_2\) and D. In our study about 23\% of isolates belonged to groups A and B\(_1\). These findings are in accordance with the results of Bingen \textit{et al.} and Pupo \textit{et al.} demonstrating that the route of infection can be intestinal pathogenic E. coli and commensal strains (17, 18). Although there are some evidences about the impact of host gender on the pathogenic aspects of E. coli in UTIs (12) but there is no data about comparative phylogenomics of this pathogenic agent between male and female patients. The comparison of phylogenetic groups and phylotypes distribution between isolates from male and female cases demonstrated that the only significant difference is in D\(_1\) phylotype while it was more prevalent in male E. coli isolates from male UTI cases. The difference between D\(_1\) and D\(_2\) phylotypes is harboring tspE4.C2 genetic marker by D\(_1\), which its role in the pathogenesis is unclear (19). Any way considering tspE4.C2 as a virulence factor and higher distribution of this element in male UPEC, it can be concluded that UPEC strains in male patients are more virulent than female ones.

Detection of virulence genes in the strains from UTI’s shows the presence of isolates harboring a combination of hly, iucD, pap and sfa/focDE genes, which could implicate the presence of Pathogenicity Islands (PAI’s) – typical chromosomal traits of the UPEC strains (8, 20). As expected, the majority of urinary strains (98.7\%) harbored extraintestinal virulence factor-encoding genes and only 2 isolates (1.2\%) in our study were negative for the mentioned virulence genes. Previous studies have assessed the prevalence of E. coli strains showing multiple virulence factors patterns implicated in extra-intestinal infections and they have concluded that this prevalence is strongly associated with strains belonging to phylogroup B\(_2\) (21, 22). Comparison of the virulence factors distribution in E. coli phylogroups from male and female’s demonstrated that in women isolates hly is significantly more prevalent in B\(_2\) group versus D, while in men isolates sfa/focDE and pap genes are more prevalent in B\(_2\) versus D, significantly. UPEC possess a diverse array of adherence factors and fimbriae such as type 1, P, F1C/S and AFA fimbriae represent the primary mediators for colonization of the urinary tract. An important step in the onset and expansion of UTI is adhesion of E. coli to uroepithelial cells by P fimbriae. S fimbriae has also been shown to attach efficiently to the epithelial and endothelial cells of the lower human urinary tract. Studies have shown that papEF and sfa/focDE are essential for cystitis and/or pyelonephritis (16, 23, 24). In the present study it is observed that papEF is more prevalent in male isolates, while in female isolates sfa/focDE is dominant, significantly. These results show that in men and women UTI caused by E. coli, the type of adhesion and fimbriae may quite be different and this can be because of anatomic differences and fimbrial receptors on the epithelial cells of urinary tract. Also the comparison of distribution of these two adhesins in B\(_2\) and D phylogroups of this both genders shows that there is no difference in the type of adhesins in female isolates, but in male isolates presence of papEF and sfa/focDE is dominant in B\(_2\) phylogroup versus D, significantly. These findings confer that UPEC having any type of related adhesins belonging to B\(_2\) and D groups may be able to onset UTI in women but E. coli strains with pap and sfa/focDE belonging to B\(_2\) are more prone to induce UTI in men.

Detection of iucD in E. coli isolates showed that 52\% of tested strains are positive for this virulence factor, and assessment of this VF in different phylogroups showed that 82\% of iucD are present in B\(_2\) and D groups. There was not any sex depended differences between distribution of iucD prevalence in E. coli isolates. About 48\% of our isolates are lacking iucD, considering that ExPEC strains have evolved multiple strategies for sequestering iron from the host, probably they have other strategies for iron uptake from the host organs (25).

**Conclusion**

The results show that phylogroups B\(_2\) and D, are the major E. coli strains causing human urinary tract infections and strains causing such infections in different genders can vary in virulence genotypes, especially in type of adhesins for the colonization and onset of the infection. More work seems to be necessary for full genotyping of E. coli strains isolated from both sexes and assessment of antimicrobial resistance profile of such isolates from these two sources.

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**Conflicts of Interest**

The authors declare that they have no conflict of interest.
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References

1. Kaper JB, Nataro JP, Mobley HL. Pathogenic Escherichia coli. Nat Rev Microbiol 2004;2:123-140.

2. Fratamico PM, DebRoy C, Liu Y, Needleman DS, Baranzoni GM, Feng P. Advances in molecular serotyping and subtyping of Escherichia coli. Front Microbiol 2016;7:1-10.

3. Hryniewicz K, Szczypta K, Sulikowska A, Jankowski K, Bedejewska K, Hryniewicz W. Antibiotic susceptibility of bacterial strains isolated from urinary tract infections in Poland. J Antimicrob Chemother 2001;47:773-780.

4. Farajinia S, Alkhani MY, Ghotasou R, Naghli B, Nahilband A. Causative agents and antimicrobial susceptibilities of urinary tract infections in the northwest of Iran. Int J Infect Dis 2009;13:140-144.

5. Carlos C, Pires MM, Stuppe NC, Hachich EM, Sato MI, Gomes TA, et al. Escherichia coli phylogenetic group determination and its application in the identification of the major animal source of fecal contamination. BMC Microbiol 2010;10:161-171.

6. Salehi TZ, Tonelli A, Mazza A, Staji H, Badagiacca P, Tamai IA, et al. Genetic characterization of Escherichia coli O157:H7 strains isolated from the one-humped camel (Camelus dromedarius) by using microarray DNA technology. Mol Biotechnol 2012;51:283-288.

7. Clermont O, Bonacorsi S, Bingen E. Rapid and simple determination of the Escherichia coli phylogenetic group. Appl Environ Microbiol 2000;66:4555-4558.

8. Staji H, Khoshgofar J, Vayeghan AJ, Bejestani MRS. Phylogenetic grouping and assessment of virulence genotypes, with antibiotic resistance patterns, of Escherichia coli strains isolated from wild bird feces and its application in the identification of the major animal source of fecal contamination. BMC Microbiol 2010;10:161-171.

9. John AS, Mboto CI, Agbo B. A review on the prevalence and predisposing factors responsible for urinary tract infection among adults. Euro J Exp Bio 2016;6:7-11.

10. Tandan M, Duane S, Cormican M, Murphy AW, Vellinga A. Reconsultation and antimicrobial treatment of urinary tract infection and its application in the identification of the major animal source of fecal contamination. BMC Microbiol 2010;10:161-171.

11. Carlos C, Pires MM, Stuppe NC, Hachich EM, Sato MI, Gomes TA, et al. Escherichia coli phylogenetic group determination and its application in the identification of the major animal source of fecal contamination. BMC Microbiol 2010;10:161-171.

12. Lee DS, Choe H-S, Kim HY, Yoo J-M, Bae WJ, Cho YH, et al. Role of age and sex in determining antibiotic resistance in febrile urinary tract infections. Int J Infect Dis 2016;51:89-96.

13. Bonadio M, Meini M, Spitaleri P, Gigli C. Current microbiological and clinical aspects of urinary tract infections. Eur Urol 2001;40:439-445.

14. Staji H, Shahaboddin E, Kaftshouzan K. Correlation of Escherichia coli strains isolated from wild bird feces and human urinary tract infections from phylogenetic point of view. Avicenna J Clin Microbiol Infect 2017;4:16-25.

15. Staji H. Detection of enterohemorrhagic Escherichia coli related genes in Escherichia coli strains belonging to B2 phylogroup isolated from urinary tract infections in combination with antimicrobial resistance phenotypes. J Med Microbiol 2017;65:2685-2692.

16. Adib N, Ghanbarpour R, Solatzaheh H, Alizade H. Antibiotic resistance profile and virulence genes of uropathogenic Escherichia coli isolates in relation to phylogeny. Trop Biomed 2013;31:17-25.

17. Bingen E, Picard B, Brahim N, Mathy S, Desjardins P, Elion J, et al. Phylogenetic analysis of Escherichia coli strains causing neonatal meningitis suggests horizontal gene transfer from a predominant pool of highly virulent B2 group strains. Int J Infect Dis 1998;17:642-650.

18. Pupo GM, Karaolis D, Lan R, Reeves PR. Evolutionary relationships among pathogenic and nonpathogenic Escherichia coli strains inferred from multilocus enzyme electrophoresis and mdh sequence studies. Infect Immun 1997;65:2685-2692.

19. Lee CCY. Genotyping Escherichia coli isolates from duck, goose, and gull fecal samples with phylogenetic markers using multiplex polymerase chain reaction for application in microbial source tracking. J Exp Microbiol Immun 2011;15:130-135.

20. Blum G, Falbo V, Caprioli A, Hacker J. Gene clusters encoding the cytotoxic necrotizing factor type 1, Prs-fimbriae and α-hemolysin form the pathogenicity island II of the uropathogenic Escherichia coli strain J96. FEMS Microbiol Lett 1995;126:189-195.

21. Johnson JR, Kuskowski MA, Owens K, Gajewska L, Winokur PL. Phylogenetic origin and virulence genotype in relation to resistance to fluoroquinolones and or extended-spectrum cephalosporins and cephamycins among Escherichia coli isolates from animals and humans. J Infect Dis 2003;188:759-768.

22. Zhang L, Foxman B, Marrs C. Both urinary and rectal Escherichia coli isolates are dominated by strains of phylogenetic group B2. J Clin Microbiol 2002;40:3951-3955.

23. Wurpel DJ, Totsika M, Allsopp LP, Webb RI, Moriel DG, Schembri MA. Comparative proteomics of uropathogenic Escherichia coli during growth in human urine identify UCA-like (UCL) fimbriae as an adherence factor involved in biofilm formation and binding to uroepithelial cells. J Proteom 2016;131:177-189.

24. Bień J, Sokolowa O, Bozko P. Role of uropathogenic Escherichia coli virulence factors in development of urinary tract infection and kidney damage. Int J Nephrol 2012;2012:681473.

25. Gao Q, Wang X, Xu H, Xu Y, Ling J, Zhang D, et al. Roles of iron acquisition systems in virulence of extraintestinal pathogenic Escherichia coli: salmochelin and aerobactin contribute more to virulence than heme in a chicken infection model. BMC Microbiol 2012;12:143-152.