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Characterization of (Uropathogenic) *E. coli* isolated from urinary tract infections: phylogenetic typing and distribution of virulence-associated traits

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Uropathogenic *Escherichia coli* (UPEC) is the pre-dominant bacterial pathogen causing Urinary Tract infections (UTIs). Pathogenicity of UPEC results from presence and expression of several virulence factors which facilitate bacterial adhesion and infection development. Virulence Factor Genes (VFGs) are located on bacterial chromosome, plasmids and even bacteriophages and could be disseminated horizontally and/or vertically between bacteria [1]. Virulence characteristics of *E. coli* isolates could be linked to the bacterial genetic background. Phylogenetic typing of UPEC isolates enables epidemiologists to determine surveillance guidelines, monitoring outbreak situations and tracking the spread of emerging pathogens [2]. Multiplex Polymerase Chain Reaction (PCR) is regarded as a convenient method commonly used to classify *E. coli* strains into four phylogenetic groups (A, B_1, B_2 and D) [3]. We investigated the phylogenetic distribution of UPEC isolates and the association of several virulence determinants including adhesions, iron uptake mediators, toxin and pathogenicity island, as well as antibiotic resistance profile to the genetic background of the isolates.

A total number of 100 UPEC were isolated from unrelated cases of community-acquired UTIs, Rasht city, Iran, from February to September, 2016. Patient's mean age was 38 (range 8–65): 74 were women (mean age 35), 26 were men (39 years). Bacterial isolates were identified using morphological and conventional biochemical assays, including Gram staining, lactose fermentation, MR-VP, Motility, H_2S and urease production and indole and oxidase. Bacterial DNA was extracted using CinnaGen™ DNA extraction kit according to the manufacturer's instruction. Bacterial phylogenetic groups were determined using a multiplex-PCR assay of the genes *chuA* and *yjaA* and the DNA fragment *TspE*$_4$.*C*$_2$, as described previously [3,4]. The multiplex-PCR primers for gene amplification were presented in Table 1. The amplified DNA fragments were mixed with 3 µl PowerLoad™ DNA stain and were visible after electrophoresis in a 1.5% agarose gel in TBE buffer and under UV illumination. The isolates were assigned to the phylogenetic groups of A, B_1, B_2 and D according to the following criteria [3,4]: A: (*chuA*-, *TspE*$_4$.*C*$_2$-); B_1: (*chuA*-, *TspE*$_4$.*C*$_2$+); B_2: (*chuA*+, *yjaA*+); D: (*chuA*+, *yjaA*-).

Prevalence of the genes corresponding to the following virulence factors were screened using PCR: pathogenicity island I (*PAI*), protectin (*traT*), mannose-specific type 1 fimbriae (*fimH*), P fimbriae (*papC*), haemolysin A (*hlyA*), S-fimbriae (*sfa-S*), F_1C fimbriae (*foc/G*) and iron acquisition components (*chuA* and *fyuA*). The PCR primers for each gene were presented in Table 1. In order to ensure the accuracy of the PCR products, the amplified genes were sequenced and the sequences were submitted to the GenBank (NCBI) and blasted with other published sequences from the GenBank database. Isolates were tested for antimicrobial susceptibilities by disc diffusion [5] with cephalothin (30 µg), imipenem (10 µg), gentamicin (10 µg), chloramphenicol (30 µg), ciprofloxacin (5 µg), tetracycline (30 µg), cefotaxime (30 µg), cefepime (30 µg), cefixime (5 µg) and piperacillin (100 µg). *E. coli* ATCC 25922 was the reference strains to control the quality of the applied antimicrobial agents [6]. In addition, isolates which displayed resistance to more than three chemotherapeutic groups were considered multi-drug resistant. An Antibiotic resistance score (ARS) for different phylogenetic groups was determined as median number of antibiotics resisted by the isolates from the different phylogenetic groups. Fisher's exact test was used to establish significance and *P* value of < 0.05 was considered significant.

Most UPEC isolates belonged to the group B_2 (52%), six isolates were assigned into the group B_1, and 14 and 28 isolates belonged to the phylogenetic groups A and D, respectively. Distribution of different VFGs

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Table 1. PCR primers for amplification of target genes.

Gene	Primers (5′ → 3′) (Size of product [bp])
chuA	F: GACGAACCAACGGTCAGGAT (279) R: TGCCGCCAGTACCAAAGACA
yjaA	F: TGAAGTGTCAGGAGACGCTG (211) R: ATGGAGAATGCGTTCCTCAAC
TspE$_4$.*C*$_2$	F: GAGTAATGTCGGGGCATTCA (152) R: CGCGCCAACAAAGTATTACG
fimH	F: TGCAGAACGGATAAGCCGTGG (508) R: GCAGTCACCTGCCCTCCGGTA
papC	F: GTGGCAGTATGATGAATGACCGTTA (200) R: ATATCCTTTCTGCAGGGATGCAATA
Sfa-S	F: GTGGATACGACGATTACTGTG (244) R: CCGCCAGCATTCCCTGTATTC
Foc/G	F: CAGCACAGGCAGTGGATACGA (363) R: GAATGTCGCCTGCCCATTGCT
PAI	F: GGACATCCTGTTACAGCGCGCA (930) R: TCGCCACCAATCACAGCCGAAC
traT	F: GGTGTGGTGCGATGAGCACAG (290) R: CACGGTTCAGCCATCCCTGAG
hlyA	F: AACAAGGATAAGCACTGTTCTGGCT (1177) R: ACCATATAAGCGGTCATTCCCGTCA
fyuA	F: TGATTAACCCCGCGACGGGAA (880) R: CGCAGTAGGCACGATGTTGTA

*All primer were used at a concentration of 1 µM except for the *chuA*, *yjaA* and *TspE*$_4$.*C*$_2$ which used 0.6 µM.

among UPEC isolates, regardless their phylogenetic groups, showed high prevalence (≥80%) of *papC*, *traT*, *fimH* and *chuA*. A moderate frequency was observed for *fyuA* (52%) and *sfa*-S (42%) and a low prevalence of *foc/G* (20%) and *hlyA* (12%). *PAI*, *fimA*, *papC* and *traT* were widely distributed among all groups, while *hlyA*, *sfa-S* and *chuA* were more prevalent among the isolates associated to the groups B$_2$ and D. Distribution of the VFGs among the isolates was presented in Table 2. Among 100 UPEC isolates, 52 displayed resistance to at least three cephalosporins (different generations). Only nine isolates were piperacillin-susceptible, while imipenem was found to be the most efficient antibiotic with 14% susceptibility of the isolates. In addition, the isolated strains indicated 76 and 56% resistance to ciprofloxacin and tetracycline, respectively. Moreover, 42 isolates were considered multi-drug resistant (MDR). Our results revealed that the isolates from group D showed higher resistance to different antibiotics (ARS = 9.0) whereas, highest susceptibility was noticed among the isolates belonged to the group B$_1$. MDR organisms were also more prevalent among the isolates from groups D and B$_2$ with frequency of 50 and 48%, respectively (Table 2).

A better understanding of the characteristics of microbial pathogens would be helpful to determine suitable preventive and therapeutic strategies. We present a study on phylogenetic grouping and prevalence of different virulence determinants genes among 100 UPEC isolates causing community-acquired UTIs. Generally, we found that prevalence of some virulence factors as well as antibiotic resistance patterns could be associated to the genetic background of the isolates. We confirm previously described data on predominance of the pathogenic groups B$_2$ and D UPEC [3,6,7]. The phylogenetic group B$_2$ was the most prevalent group, followed by group D. The phylogenetic groups A and B$_1$ showed the least prevalence, indicating their weak association with UTIs. In addition, we showed there is a link between bacterial phylogenetic group and presence of virulence determinants among UPEC. Highest prevalence of VFGs was noticed among the UPEC belonged to the phylogenetic group B$_2$. This finding was in agreement with previous studies [6–8]. The strains belonged to the phylogenetic groups D and A showed a moderate to high prevalence of VFGs, while the least prevalence of VFGs was recorded for group B$_1$. The weak association of the groups A and B$_1$ UPEC with UTIs could be attributed to the lower prevalence of the virulence determinants. Lower frequency of VFGs among

Table 2. Association of different VFGs and antibiotic resistance profile to bacterial pylogenetic groups.

	Phylogenetic groups (*n*,%)				
Gene	A (*n* = 14)	B$_1$ (*n* = 6)	B$_2$ (*n* = 52)	D (*n* = 28)	Total (*n* = 100)
fimH	14 (100)*	4 (66.6)	50 (96.1)*	18 (64.3)	86
papC	14 (100)	4 (66.6)	50 (96.1)	24 (85.7)	92
Sfa-S	4 (28.6)	0 (0)	32 (61.5)*	6 (21.4)	42
Foc/G	1 (7.1)	0 (0)	16 (30.7)*	1 (3.6)	18
PAI	14 (100)*	4 (66.6)	40 (76.9)	18 (64.3)	76
traT	14 (100)	6 (100)	46 (88.5)	26 (92.8)	92
hlyA	2 (14.3)	0 (0)	4 (7.7)	6 (21.4)*	12
fyuA	4 (28.6)	4 (66.6)	30 (57.7)	14 (50)	52
chuA	0 (0)	0 (0)	52 (100)*	28 (100)*	80
Antibiotic resistance					
Cefotaxime (30 µg)	7 (50)	3 (50)	29 (55.7)	19 (67.8)	58
Cefepime (30 µg)	5 (35.7)	3 (50)	21 (40.3)	13 (46.4)	42
Cefixime (5 µg)	10 (71.4)	5 (83.3)	45 (86.5)	25 (89.3)	80
Imipenem (10 µg)	3 (21.4)	0 (0)	7 (13.4)	4 (14.3)	14
Gentamicin (10 µg)	2 (14.3)	1 (16.6)	13 (25)	15 (53.5)	31
Chloramphenicol (30 µg)	2 (14.2)	0 (0)	7 (13.5)	6 (21.4)	15
Ciprofloxacin (5 µg)	10 (71.4)	4 (66.6)	38 (73)	24 (85.7)	76
Tetracycline (30 µg)	6 (42.8)	2 (33.3)	27 (52)	21 (75)	56
Piperacillin (100 µg)	11 (78.5)	6 (100)	49 (94.2)	25 (89.3)	91
Multi-drug resistance	3 (21)	0 (0)	25 (48)*	14 (50)*	42
Aggregate resistance score	4.5	3.0	5.0	9.0*	5.0

*Statistically significant ($p \leq 0.05$).

UPEC could reduce bacterial infectivity, survival inside host's body and infection development [3].

Investigating the prevalence of genes associated with adhesion and biofilm formation showed that *fimH* and *papC* were present in high percentages among all phylogenetic groups, indicating a crucial role during bacterial localization in the urinary tract [1,6]. The *fimH* showed positive relationship with the phylogenetic group B_2 ($p = 0.002$), in accordance with the results from Piatti et al. [1]. However, conversely to their report, *papC* showed uniform distribution among different phylogenetic groups. Moreover, low prevalence of *sfa-S* and *foc/G* was observed among all phylogenetic groups except for those of group B_2 which showed higher prevalence of 58% for *sfa-S* ($p = 0.003$) and 31% for *foc/G* ($p = 0.007$). Adhesion factors are major determinants in biofilm formation and infection development [9–11]. The high association of UTIs with the phylogenetic group B_2 could be attributed to the higher prevalence of the adhesion factors which mediates bacterial adhesion to the urinary tract and biofilm formation. In addition, none of the strains from the group B_1 harboured *sfa-S* and *foc/G*, indicating importance of F_1C fimbriae and S-fimbriae in bacterial infectivity and UTI development [12].

Prevalence of other VFGs including *PAI*, *traT*, *hlyA* and *fyuA* and *chuA* among UPEC was also investigated. No significant difference in the prevalence of *fyuA* and *traT* among different phylogenetic groups was observed, while *hlyA* showed positive association with group D. In addition, a higher frequency of *PAI* was observed among the strains from group A ($p = 0.017$), while *chuA* was only present among strains of groups B_2 and D. Our findings suggests a weak association between prevalence of the mentioned virulent factors and UTIs caused by UPEC strains.

Evaluation of antibiotic resistance profile of UPEC strains showed that strains from group D were significantly more resistant to the majority of antibiotics compared to other groups. This finding is contrary to the results from Lee et al. [6] who reported the highest antibiotic resistance among the isolates from group B_2. This difference could be due to the bacterial characteristics in different geographic regions, antibiotics usage or host genetic factors. In addition, moderate level of MDR was observed among the strains from groups B_2 and D, while low frequency of MDR was noticed among the isolates of groups A and B_1. Since the majority of UTIs are caused by the groups B_2 and D strains, high-antibiotic resistance complicates the treatment and results in therapeutic failure. Several intrinsic and acquisitive factors are involved in the antibiotic resistance of bacterial strains which need to be addressed in order to determine suitable preventive and therapeutic strategies against UPEC strains.

A strong association of UPEC strains from the phylogenetic groups B_2 and D with community-acquired UTIs was observed. High prevalence of VFGs could result in higher infectivity and pathogenicity of UPEC strains, which accounts for the higher prevalence of phylogenetic groups B_2 and D. This work represents an advance in biomedical science because it characterized virulence determinants of UPEC strains in association with UTIs.

Disclosure statement

No potential conflict of interest was reported by the authors.

References

[1] Piatti G, Mannini A, Balistreri M, et al. Virulence factors in urinary Escherichia coli Strains: phylogenetic background and quinolone and fluoroquinolone resistance. J clin microbial. 2008;46(2):480–487.
[2] Hu YY, Cai JC, Zhou HW, et al. Molecular typing of CTX-M-producing Escherichia coli isolates from environmental water, swine feces, specimens from healthy humans, and human patients. Appl Environ Microbial. 2013;79(19):5988–5996.
[3] Clermont O, Bonacorsi S, Bingen E. Rapid and simple determination of the Escherichia coli phylogenetic group. Appl Environ Microbial. 2000;66(10):4555–4558.
[4] Clermont O, Christenson JK, Denamur E, et al. The clermont Escherichia coli phylo-typing method revisited: improvement of specificity and detection of new phylo-groups. Environ Microbiol Rep. 2013;5(1):58–65.
[5] CLSI C. Performance standards for antimicrobial susceptibility testing; twenty-second informational supplement. CLSI/NCCLS Document M100-S22. Wayne, PA: Clinical and Laboratory Standards Institute; 2012.
[6] Lee JH, Subhadra B, Son YJ, et al. Phylogenetic group distributions, virulence factors and antimicrobial resistance properties of uropathogenic Escherichia coli strains isolated from patients with urinary tract infections in South Korea. Lett Appl Microbiol. 2016;62(1):84–90.
[7] López-Banda DA, Carrillo-Casas EM, Leyva-Leyva M, et al. Identification of virulence factors genes in Escherichia coli isolates from women with urinary tract infection in Mexico. Biomed Res Int. 2014;2014:1–10.
[8] Nowrouzian FL, Adlerberth I, Wold AE. Enhanced persistence in the colonic microbiota of Escherichia coli strains belonging to phylogenetic group B2: role of virulence factors and adherence to colonic cells. Microb Infect. 2006;8(3):834–840.
[9] Oelschlaeger TA, Dobrindt U, Hacker J. Virulence factors of uropathogens. Curr Opin Urol.12(1):33–38.
[10] Emő L, Kerenyi M, Nagy G. Virulence factors of uropathogenic Escherichia coli. Int J Antimicrob Agents. 2003;22:29–33.
[11] Ejrnæs K, Stegger M, Reisner A, et al. Characteristics of Escherichia coli causing persistence or relapse of urinary tract infections: phylogenetic groups, virulence factors and biofilm formation. Virulence. 2011;2(6):528–537.
[12] Khan AS, Kniep B, Oelschlaeger TA, et al. Receptor structure for F1C fimbriae of uropathogenic Escherichia coli. Infect Immune. 2000;68(6):3541–3547.