Avicenna J Clin Microb Infec. 2014 October; 1(3): e22981. DOI: 10.17795/ajcmi-22981

Published online 2014 October 22. **Research Article**

Comparison of the Common Adhesin Coding Operons Distribution in Uropathogenic and Phylogenetic Group B2 and A *Escherichia coli* Isolates

Masoud Rahdar [1]; Ahmad Rashki [2,*]; Hamidreza Miri [1]

[1]Department of Biology, Faculty of Basic Science, University of Zabol, Zabol, IR Iran
[2]Department of Physiopathology, Faculty of Vet-Medicine, University of Zabol, Zabol, IR Iran

*Corresponding author: Ahmad Rashki, Department of Physiopathology, Faculty of Vet-Medicine, University of Zabol, Zabol, IR Iran. Tel: +98-9151970877, Fax: +98-5424822251, E-mail: ah_rashki@usal.es

Received: August 22, 2014; **Revised:** September 9, 2014; **Accepted:** September 11, 2014

Background: *Escherichia coli* is one of the most causative pathogen of urinary tract infection. Urinary tract infections (UTIs) are the second most common cause of morbidity and remain a serious health concern among the clinicians. The severity of UTI caused by uropathogenic *E. coli* (UPEC) is due to the expression of a wide spectrum of virulent factors such as adhesin coding operons. Little is known about the relationship between the *E. coli* genetic background and the acquisition of adhesin coding operons in UPEC isolates.
Objectives: The aim of this study was to determine the prevalence of adhesin coding operons in UPEC isolates belonged to phylogenetic group B2 and A collected from patients suffering from UTI.
Materials and Methods: A total 100 UPEC isolates were used for DNA extraction by the boiling lysis. The analysis of phylogenetic groups, along with detection of adhesin coding operons was performed by Multiplex-PCR method. Associations were assessed between *afa*, *fim*, *foc*, *pap* and *sfa* operons among to 55 B2 and 17 A groups *E. coli* isolates. Statistical analysis was performed using Fisher exact test.
Results: Phylogenetic analysis showed that 55 and 17 of 100 UPEC isolates belonged to the B2 and A phylogenetic groups, respectively. The *afa*, *fim*, *foc*, *pap* and *sfa* operons were present in five (9.09%), 55 (100%), 16 (29.09%), 46 (83.63%) and 46 (83.63%) of UPEC isolates belonged to phylogenetic group B2, and two (12.50%), 14 (87.50%), one (6.25%), two (12.5%) and 12 (75%) of isolates belonged to phylogenetic group A, respectively. Statistical analysis showed that *pap* gene was significantly more frequently detected in phylogenetic group B2 ($P < 0.05$).
Conclusions: The UPEC isolates belonging to group B2 harbored a greater number of adhesin coding operons than strains from phylogenetic groups A.

Keywords: Phylogenetic Analysis; Urinary Tract Infections (UTI); Uropathogenic *Escherichia coli*; Virulence Factors

1. Background

Escherichia coli is one of the most extensively studied Gram-negative bacteria in microbiology (1, 2). This species is also a common cause of extraintestinal infections, such as urinary-tract infection and septicemia (1, 3). Phylogenetic studies have shown that UPEC has four main phylogenetic groups, designated as A, B1, B2, and D (4). Group B2 strains carry more virulence-factor genes than other strains (5-7). Furthermore, strains that cause extraintestinal infections belong mostly to group B2 and, to a lesser extent, group D (4). Extraintestinal pathogenic *E. coli* (ExPEC) strains differ widely in their capacity to localize in the human urinary tract (8). It has been reported that adhesins and other virulence factors may contribute to the colonization of *E. coli* strains in the human urinary tract. The UPEC strains express adhesin-encoding operons (*pap*, *sfa* and *afa*) (9, 10), and have genes for P fimbriae and type one fimbriae (11, 12). Genes encoding virulence factors are also enriched in UPEC strains. Up to now, there have been very few published studies on association of the common fimbriae virulence factors and phylogenetic background of UPEC isolates in Iran.

2. Objectives

The objective of this study was to determine the distribution of adhesin-encoding operons between two phylogenetic groups of B2 and A in UPEC isolates collected from 100 patients suffering from UTI.

3. Materials and Methods

3.1. Bacterial Isolates

Urine samples were collected from patients with UTI from two major hospitals in Zabol, Iran. Specimens were taken from clean-catch sample, midstream urine and urinary catheters. One loop of specimens (10 μL) was seeded on Mac Conkey and Eosin-Methylene Blue agar (Biolife Laboratories, Milano, Italy). The isolates were identified

based on standard methods (2, 13, 14) and UTI was defined as cultures yielding greater than or equal to 10^5 cfu/mL.

3.2. Phylotyping

E. coli isolates were classified into phylogenetic biotypes (A, B1, B2 and D) according to present two virulence genes (*chuA*, encoding a heme transporter protein in *E. coli* O157: H7 and *yja*A, initially identified in the genome of *E. coli* K-12) and one DNA fragment TspE4.C2 as described by Clermont et al. (4). The amplification of DNA was performed according to the methods provided by Clermont et al. Briefly, PCR was performed in a reaction mixture with total volume of 25 μL, containing 2 μL of bacterial DNA was used in a mix reaction containing 12.5 μL 2 × MasterMix Red Taq polymerase (Ampliqon, Pishgam Iran), and 30 pmol of each primer (Pishgam Iran) (Table 1). The PCR products were observed in an agarose gel 2% (Figure 1 A).

3.3. Multiplex-PCR for Virulence Genes

The assessed virulent factors were represented by five adhesin-encoding operons, *afa*, *fim*, *foc*, *pap* and *sfa* were also sought by using Multiplex-PCR. Briefly, genomic DNA was prepared from overnight cultures grown in 5 mL Luria Bertani (LB) broth (Invitrogen, Paisley, Scotland), by the boiling method. PCR was performed in a reaction mixture with total volume of 25 μL, containing 12.5 μL 2 × Master Mix red Taq polymerase (Ampliqon, pishgam Iran) and 0.3 μL of each primer. The PCR procedure was as follows: initial denaturation step at 94°C for five minutes followed by 35 cycles consisting of denaturation (94°C for 30 seconds), annealing (59°C for 50 seconds) and extension (72°C for 70 seconds), followed by a final extension step at 72°C for five min. The PCR products were observed in an agarose gel 2% (Figure 1 B). The amplicon size of *afa*-750 bp, *fim*-400 bp, *foc*-388 bp, *pap*-328 bp and *sfa*-100 bp operons were appreciated by comparison with a molecular size marker (Fermentase 100 bp) (Figure 1 B). Specific primers were design to amplify sequences of the *fim*H, *hly*A, *iuc*D, and *iro*N using MP primer 2.0 software (Table 1).

3.4. Statistical Analysis

The Fisher exact test was used for statistical analysis. The threshold for statistical significance was a P value of ≤ 0.05.

4. Results

4.1. Phylogenetic Groups Among Isolates

E. coli isolates causing UTIs in patients attending teaching hospitals in Zabol belonged to all four phylogenetic groups; however, the phylogenetic groups, B2 (55%) and A (17%), comprised the majority of all isolated strains (Table 2).

4.2. Distribution of Virulence Genes Among the Studied Uropathogenic E. coli

The *fim* operon is widely distributed among isolates belonged to two phylogenetic groups B2 and A. The phylogenetic group B2 represents a 75%, higher than in group A. Differences in prevalence of five studied adhesin coding operons were observed between phylogenetic groups B2 and A isolates (Table 2). Among 55 B2 isolates, the prevalence of *afa*, *foc*, *pap* and *sfa* was five (9.09%), 16 (29.09%), 46 (83.63%) and 46 (83.63%) isolates, respectively; while among 17 type A isolates, the prevalence of *afa*, *foc*, *pap* and *sfa* was two (11.76%), one (5.88%), two (11.76%) and 12 (70.58%), respectively (Table 2).

4.3. Phylogenetic Distribution of VFs

According to the phylogenetic classification, *E. coli* isolates belonging to the extraintestinal phylogenetic B2 groups possess an accumulation of adhesin coding operons, as compared to those belonging to the phylogenetic group A. One of the isolates belonging to the phylogenetic B2 group harbor the complete set of five operons (Table 2). For B2 and A groups, the *fim* operon was distributed in 100% and 82.35% of isolates, respectively; while *foc*, *pap* and *sfa* operons are predominating in group B2 strains (16 out of 17 isolates for *foc*, 46 out of 48 isolates for *pap* and 46 out of 58 for *sfa*) (Table 2). Three adhesin coding operons were observed in 32 isolates belonging to B2 group; while in the A group, only one isolate existed with such operons (Tables 3 and 4). Concerning the total prevalence of five studied operons among the UPEC isolates, the *fim* operon was present in all of the tested isolates (100%) belonging to B2 and 82.35% of A group (Table 2); *afa* in 9.72% (6.94% for B2 group and 2.77% for A group), *foc* in 23.61% (22.22% for B2 group and 1.38% for A group), *pap* in 66.66% (63.88% for B2 group and 2.77% for A group) and *sfa* in 80.55% (63.88% for B2 group and 16. 66% for A group) of the studied isolates. The analysis of the association between the presence of different combinations of virulence genes among two studied phylogenetic group B2 and A, allowed us to include the tested isolates into 12 virulence patterns noted Ec1 to Ec12 (Table 3) for phylogenetic group B2 and eight virulent patterns noted Ec3-5 to Ec17 (Table 4) for phylogenetic group A. One isolate for phylogenetic group A with only one operon was included in Ec16, 33 isolates harboring *fim*, *pap* and *sfa* operons in Ec3 (Tables 3 and 4). The pattern Ec4 included the isolates simultaneously positive for fim^+ and sfa^+ (9 isolates; 1 isolate for B2 group and 8 isolates for A group). The pattern Ec2 in group B2 included the isolates simultaneously positive for fim^+, pap^+, foc^+ and sfa^+ (9 isolates). The two patterns Ec6 and Ec11 in B2 group were represented by the strains possessing a combination of three operon, Ec4 with fim^+, foc^+ and sfa^+ (3 isolates) and Ec11 with fim^+, pap^+ and afa^+ operon (1 isolate). The three patterns Ec12, Ec13 and Ec17 in A group were represented by the strains possessing a combination of different adhesin operons (Table 4).

Table 1. Primers Used in This Study

Primer Name (Sequences)	Size (bp)	Reference
fim	400	(15)
F-GTTGTTCTGTCGGCTCTGTC		
R-TAAATGTCGCACCATCCAG		
pap	328	(16)
F-GACGGCTGTACTGCAGGGTGTGGCG		
R-ATATCCTTTCTGCAGGGATGCAATA		
sfa	100	(15)
F-CCGTAAAGATGTCTGCGAG		
R-AGCAAGTCTGGCAACGAG		
foc	388	(17)
F-GGTGGAACCGCAGAAAATAC		
R-GAACTGTTGGGGAAAGAGTG		
afa	750	(16)
F-GCTGGGCAGCAAACTGATAACTCTC		
R-CATCAAGCTGTTTGTTCGTCCGCCG		
chuA	279	(4)
F-GACGAACCAACGGTCAGGAT		
R-TGCCGCCAGTACCAAAGACA		
yjaA	211	(4)
F-TGAAGTGTCAGGAGACGCTG		
R-ATGGAGAATGCGTTCCTCAAC		
TspE4C2	152	(4)
F-GAGTAATGTCGGGGCATTCA		
R-CGCGCCAACAAAGTATTACG		

Table 2. Distribution of Virulence Genes in Phylogenetic Groups B2 and A

Phylogenetic Groups	*fim*	*sfa*	*pap*	*foc*	*afa*
B2	55	46	46	15	5
A	14	12	2	1	2
Pvalue	0.68	0.83	0.004	0.17	0.67

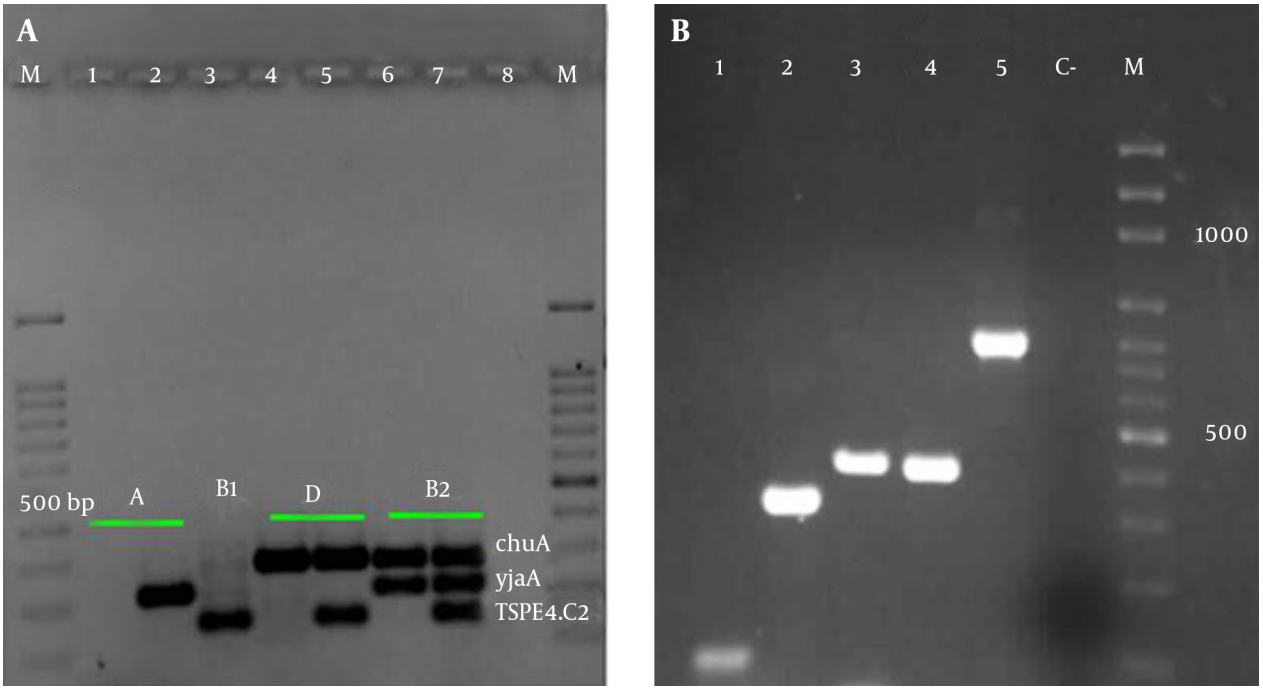
Table 3. Virulence Patterns Identified Among the Studied Isolates Belonged to Phylogenetic Group B2

Pattern	*fim*	*sfa*	*pap*	*foc*	*afa*	No. of Isolates
Ec1	+	+	+	+	+	1
Ec2	+	+	+	+	-	9
Ec3	+	+	+	-	-	32
Ec4	+	+	-	-	-	1
Ec5	+	-	-	-	-	2
Ec6	+	+	-	+	-	3
Ec7	+	+	+	-	+	1
Ec8	+	-	+	-	-	2
Ec9	+	+	-	-	+	1
Ec10	+	-	-	-	+	1
Ec11	+	-	+	-	+	1
Ec12	+	-	-	+	-	1
Total	55	46	46	15	5	55

Table 4. Virulence Patterns Identified Among the Studied Isolates Belonged to Phylogenetic Group A

Pattern	*fim*	*sfa*	*pap*	*foc*	*afa*	No. of Isolates
Ec3	+	+	+	-	-	1
Ec4	+	+	-	-	-	8
Ec5	+	-	-	-	-	3
Ec9	+	+	-	-	+	1
Ec12	+	-	-	+	-	1
Ec13	-	+	-	-	+	1
Ec16	-	-	-	-	-	1
Ec17	-	+	+	-	-	1
Total	14	12	2	1	2	17

Figure 1. Multiplex- PCR Profiles Specific for *E. coli* Phylogenetic Groups and Detection of Virulence Genes



Each combination of *chuA* and *yjaA* gene and DNA fragment TSPE4.C2. (Line 1-8) amplification allowed phylogenetic group determination of a strain (A). Rows 1-5 gene amplification result of *sfa*, *pap*, *fim*, *foc* and *afa* fimbriae genes run on 2% agarose gel (B).

5. Discussion

Certain genotypic traits associated with *E. coli* extraintestinal diseases are frequently found in human UTI. Epidemiologic investigations have shown a good correlation between the occurrence of certain human diseases and the presence of adhesin operon in *E. coli* (18-20). Operons encoding P/Prs fimbriae, S/FIC fimbriae and Dr/AFA adhesins contribute to the pathophysiology of UTI (10, 19), whereas gene encoding for S *fim*briae is correlated with the pathogenesis of neonatal meningitis (16). In the present study, we have identified the phylogenetic type of UPEC isolates collected from 100 patients who had UTIs. In phylogenetic analyses performed to date, it has been reported that UPEC strains mostly belong to group B2 (4, 21). As expected, in our study, similarly, the most common phylogenetic group in the UPEC isolates was B2. Seventy-two of studied isolates were distributed between the phylogenetic groups (B2 and A), known for their higher virulence potential. Previous research by Karimi Darehabi et al. (2013) (22) revealed a different result from that of the current research, identifying a higher prevalence of strains of phyla-group A and D among *E. coli* isolates, which can be usual because of geographic effects in the *E. coli* population among hosts. The most virulent isolates belonged to phylogenetic group B2, harboring the major-

ity of tested adhesin operons. We assumed that the virulence gene distribution pattern of phylogenetic group B2 would be similar to that of other UPEC isolates belonged to phylogenetic group A. Our results, based on the distribution of the studied adhesin operons, the studied isolates of phylogenetic group B2 exhibited 12 virulent gene patterns; while in the A group only eight virulent gene patterns existed. Our results revealed the complexity of the association between phylogenetic groups and the properties of adhesin operons in UPEC referred to as Ec (Tables 3 and 4). In the present study, adhesin-encoding operons *fim*, *foc*, *pap*, and *sfa* are prevalent in UPEC isolates belonged to phylogenetic group B2. Group B2 strains have an accumulation of virulence-factor genes, as has been demonstrated both elsewhere (23) and in the present study. The distribution of *E. coli* isolates possessing *fim*, *pap* and *sfa* operons, regardless of phylogenetic group, showed a good agreement with other published data (12, 24). Our results illustrated a higher frequency of *fim*, *pap* and *sfa* operons compared with other virulence markers, which may suggest a crucial role of the adhesin-encoding operons in UPEC causing UTI. The distribution of the other studied virulent genes found among the studied isolates belonged to group B2 was also similar to other reported data (25, 26). We found a smaller proportions among isolates belonged to phylogenetic group A. In our study, phylogenetic distribution of virulent factors did allow the determination of a clear correlation between studied operons, phylogenetic background and the complexity of the UTI.

In conclusion, our study showed that a low number of UPEC adhesin-encoding operons in isolates belonged to phylogenetic group A. The characterization of UPEC isolates collected from patient with UTI is of great interest to improve our knowledge regarding their adhesin-encoding operons and phylogenetic background. Further studies are needed to identify UPEC virulent factors responsible for UTI and to determine the pathophysiology of these infections to consider possible prevention measures and means.

Acknowledgements

This work was performed in partial fulfillment of the requirements for MSc student's thesis (Masoud Rahdar) and we wish to thank the staff of the laboratory of microbiology of faculty of vet-medicine.

Authors' Contributions

Study design, data collection and data interpretation: Masoud Rahdar; Study design, data collection, data interpretation, funds collection, literature review, manuscript preparation: Ahmad Rashki and Hamidreza Miri.

Funding/Support

This study was financially supported by the University of Zabol (MSc Grant), Zabol, Iran.

References

1. Toval F, Kohler CD, Vogel U, Wagenlehner F, Mellmann A, Fruth A, et al. Characterization of Escherichia coli isolates from hospital inpatients or outpatients with urinary tract infection. *J Clin Microbiol.* 2014;**52**(2):407–18.
2. Yah SC, Eghafona NO, Enabulele IO, Aluyi HSA. Ampicillin Usage and Ampicillin Resistant (Ampr) Plasmids Mediated Escherichia Coli Isolated from Diarrheagenic Patients Attending Some Teaching Hospital in Nigeria. *SEMJ.* 2004;**7**(4):1–12.
3. Vosti KL, Goldberg LM, Monto AS, Rantz LA. Host-Parasite Interaction in Patients with Infections Due to Escherichia Coli. I. the Serogrouping of E. Coli from Intestinal and Extraintestinal Sources. *J Clin Invest.* 1964;**43**:2377–85.
4. Clermont O, Bonacorsi S, Bingen E. Rapid and simple determination of the Escherichia coli phylogenetic group. *Appl Environ Microbiol.* 2000;**66**(10):4555–8.
5. 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. *Microbes Infect.* 2006;**8**(3):834–40.
6. Nowrouzian FL, Wold AE, Adlerberth I. Escherichia coli strains belonging to phylogenetic group B2 have superior capacity to persist in the intestinal microflora of infants. *J Infect Dis.* 2005;**191**(7):1078–83.
7. Abdi H, rashki A, rashki Z, shahkarami F, shahraki Z. The relationship between phylogenetic group and distribution of virulence genes hlyA, iroN, iucD, fimH in Escherichia coli isolated from female genital tract among women attending Gynecology clinics in Zabol-Iran by Multiplex-PCR) . *Iran J Med Microbiol.* 2014;**7**(4):9–15.
8. Rashki A. Cervico-vaginopathogenic Escherichia coli in Iran: Serogroup distributions, virulence factors and antimicrobial resistance properties. *Microbial Pathogenesis.* 2014;**x**(x):xx–xx (in press).
9. Blanco M, Blanco JE, Alonso MP, Mora A, Balsalobre C, Munoa F, et al. Detection of pap, sfa and afa adhesin-encoding operons in uropathogenic Escherichia coli strains: relationship with expression of adhesins and production of toxins. *Res Microbiol.* 1997;**148**(9):745–55.
10. Bogyiova E, Kmetova M, Biros E, Siegfried L. Detection of pap-, sfa- and afa-specific DNA sequences in Escherichia coli strains isolated from extraintestinal material. *Folia Microbiol (Praha).* 2002;**47**(6):723–6.
11. Momtaz H, Karimian A, Madani M, Safarpoor Dehkordi F, Ranjbar R, Sarshar M, et al. Uropathogenic Escherichia coli in Iran: serogroup distributions, virulence factors and antimicrobial resistance properties. *Ann Clin Microbiol Antimicrob.* 2013;**12**:8.
12. Bashir S, Haque A, Sarwar Y, Ali A, Anwar MI. Virulence profile of different phylogenetic groups of locally isolated community acquired uropathogenic E. coli from Faisalabad region of Pakistan. *Ann Clin Microbiol Antimicrob.* 2012;**11**:23.
13. Maluta RP, Stella AE, Riccardi K, Rigobelo EC, Marin JM, Carvalho MB, et al. Phenotypical characterization and adhesin identification in Escherichia coli strains isolated from dogs with urinary tract infections. *Braz J Microbiol.* 2012;**43**(1):375–81.
14. Kalantar E, Soheili F, Salimi H, Soltan Dallal MM. Frequency, antimicrobial susceptibility and plasmid profiles of Escherichia coli pathotypes obtained from children with acute diarrhea. *Jundishapur J Microbiol.* 2011;**4**(1):23–8.
15. Nazemi A, naderi M, jafarpour M. The Detection of Fimbrial Pathogenic Genes in E. coli Strains Isolated from Patients with Urinary Tract Infection. *Medical Laboratory Journal.* 2011;**2**(8):569–73.
16. Birosova E, Siegfried L, Kmet'ova M, Makara A, Ostro A, Gresova A, et al. Detection of virulence factors in alpha-haemolytic Escherichia coli strains isolated from various clinical materials. *Clin Microbiol Infect.* 2004;**10**(6):569–73.
17. Mitsumori K, Terai A, Yamamoto S, Yoshida O. Identification of S, F1C and three PapG fimbrial adhesins in uropathogenic Escherichia coli by polymerase chain reaction. *FEMS Immunol Med Microbiol.* 1998;**21**(4):261–8.
18. Archambaud M, Courcoux P, Ouin V, Chabanon G, Labigne-Roussel A. Phenotypic and genotypic assays for the detection and identification of adhesins from pyelonephritic Escherichia coli.

Ann Inst Pasteur Microbiol. 1988;**139**(5):557–73.
19. Rahman H, Deka M. Detection & characterization of necrotoxin producing Escherichia coli (NTEC) from patients with urinary tract infection (UTI). *Indian J Med Res*. 2014;**139**(4):632–7.
20. Harada K, Niina A, Nakai Y, Kataoka Y, Takahashi T. Prevalence of antimicrobial resistance in relation to virulence genes and phylogenetic origins among urogenital Escherichia coli isolates from dogs and cats in Japan. *Am J Vet Res*. 2012;**73**(3):409–17.
21. Bingen-Bidois M, Clermont O, Bonacorsi S, Terki M, Brahimi N, Loukil C, et al. Phylogenetic analysis and prevalence of urosepsis strains of Escherichia coli bearing pathogenicity island-like domains. *Infect Immun*. 2002;**70**(6):3216–26.
22. Karimi Darehabi H, Naseri MH, Menbari S, Mobaleghi J, Kalantar E. Antibiotic Resistance Pattern of Escherichia coli Groups A, B1, B2 and D Isolated from Frozen Foods and Children with Diarrhea in Sanandaj, Iran. *Int J Enterpathog*. 2013;**1**(1):1–4.
23. 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.
24. Szemiako K, Krawczyk B, Samet A, Sledzinska A, Nowicki B, Nowicki S, et al. A subset of two adherence systems, acute proinflammatory pap genes and invasion coding dra, fim, or sfa, increases the risk of Escherichia coli translocation to the bloodstream. *Eur J Clin Microbiol Infect Dis*. 2013;**32**(12):1579–82.
25. Cremet L, Corvec S, Bemer P, Bret L, Lebrun C, Lesimple B, et al. Orthopaedic-implant infections by Escherichia coli: molecular and phenotypic analysis of the causative strains. *J Infect*. 2012;**64**(2):169–75.
26. Totsika M, Beatson SA, Holden N, Gally DL. Regulatory interplay between pap operons in uropathogenic Escherichia coli. *Mol Microbiol*. 2008;**67**(5):996–1011.