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Comparison of Virulence Factors Distribution in Uropathogenic *E. coli* Isolates From Phylogenetic Groups B2 and D

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Background: Urinary tract infections are a significant health problem, with *Escherichia coli* as a primary pathogen in approximately 80% of cases. The pathogenesis of *E. coli* in urinary tract infections is attributed to the production of virulence factors and phylogenetic background groups.

Objectives: The aim of this study was to determine differences in prevalence of virulence factors of *E. coli* isolates from phylogenetic groups B2 and D, collected from patients with urinary tract infections.

Materials and Methods: A total of 100 *E. coli* isolates were identified by conventional biochemical tests from patients with urinary tracts infections (UTIs) in teaching hospitals of Zabol, Iran. DNA was extracted using the boiling method. Analysis of phylogenetic groups, along with detection of virulence factor genes was performed by the multiplex-PCR method. Associations were assessed between type 1 fimberia-encoding gene, siderophore receptor encoding genes and hemolysin encoding gene among 55 B2 group *E. coli* isolates and 22 D group *E. coli* isolates. Statistical analysis was performed using the Fisher exact test.

Results: Phylogenetic analysis showed that 55 and 22 of 100 isolates belonged to the B2 and D phylogenetic groups, respectively. The *hlyA, iroN, iucD* and *fimH* genes were present in 29 (52.72%), 22 (40%), 46 (83.63%) and 55 (100%) isolates belonging to the phylogenetic group B2, whereas in 2 (9.09%), 2 (9.09%), 10 (45.45%) and 22 (100%) isolates belonging to the phylogenetic group D, respectively. The comparison showed that there was a significant difference between the presence of *hlyA* and *iroN* genes in isolates belonging to the phylogenetic group B2 and D ($P \leq 0.05$).

Conclusions: This study determined that strains belonging to group B2 are the most important and abundant *E. coli* strains causing urinary tract infections.

*Keywords:*Uropathogenic *E. coli* (UPEC); Urinary Tract Infections (UTIs); Virulence Factors; Phylogenetic Analysis

1. Background

Urinary tract infections (UTIs) are the most frequent bacterial infections among outpatients and inpatients. Acute uncomplicated UTIs are mainly due to extra intestinal pathogenic *Escherichia coli* (ExPEC) (1). However, management of patients with UTIs has become a particular problem in the recent decades due to the presence of various virulence factors among uropathogenic *E. coli* (UPEC) strains (2, 3). The severity of a UTI is amplified by the existence of a diverse range of virulence factors such as type 1 fimberia encoding genes, siderophore receptors encoding genes or iron intake system and hemolysin encoding gene conferring the pathogenicity of UPEC strains (4-6). The generally accepted hypothesis today is that UPEC strains evolved from commensal strains by acquiring new virulence factors from accessory DNA horizontal transfer at the chromosomal or plasmid level (7, 8). Many of these properties contribute to UPEC colonization and invasion (9). Uropathogenic *E. coli* strains, like other pathogenic forms of *E. coli* do not have very diverse origins (10, 11) and fall into four major phylogenetic groups. Phylogenetic analysis of *E. coli* strains separates them into four major phylogenetic groups (A, B1, B2 and D) (12). Most ExPEC are derived from group B2 and to a lesser extent from group D, while the other pathogenic *E. coli* are consistently distributed in all classes with the non-pathogenic strains belonging to group A and B1 (12). Results from molecular studies on the recognized ExPEC virulence factors are compatible with this classification, where B2 group strains are highly pathogenic with numerous virulence determinants responsible for extra intestinal infections and strains of phylogenetic group D seem to have fewer virulence determinants than B2 group strains, whereas strains of the group A and B1, are most often devoid of extra intestinal virulence factors (8). There is clear evidence that the association of virulence properties and phylogenetic background of *E. coli* is a complex phenomenon, resulting from their different interplays. Therefore, we thought that these may not be

mutually exclusive. To clarify whether the present virulence factors (VFs) are directly associated with phylogenetic background, we compared VFs distribution difference in two major ExPEC phylogenetic groups, including B2 and D in a collection of 100 *E. coli* strains.

2. Objectives

The aim of this study was to compare virulence factors distribution between two major ExPEC phylogenetic groups B2 and D in a collection of 100 *E. coli* strains using multiplex-PCR.

3. Materials and Methods

3.1. Bacterial Isolates

Urine samples were collected from patients with UTIs 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 MacConkey and Eosin-Methylene Blue agar. The isolates were identified based on standard methods (13, 14) and UTI was defined as cultures yielding greater than or equal to 105 cfu/mL.

3.2. Phylotyping

E. coli isolates were classified into phylogenetic biotypes (A, B1, B2, and D) according to the presence of the two virulence genes (*chuA*, encoding a hem transporter protein in *E. coli* O157: H7 and *yjaA*, initially identified in the genome of *E. coli* K-12) and one DNA fragment TspE4.C2 as described by Clermont et al. 2000 (12). The amplification of DNA was performed according to the methods provided by Clermont et al. 2000. Briefly, PCR was performed in a reaction mixture with total volume of 25 μL, containing 2 μL of bacterial DNA in a mix reaction containing 12.5 μL 2 × MasterMix Red Taq polymerase, and 30 pmol of each primer (Table 1).

Table 1. Primers Used for the Multiplex-PCR

(Primer Name) Primer Sequence (5-3)	Size of Amplicon, bp	Reference
hlyA	1361	This study
F-GTTAGCGGGTGTCACCAGAAAT		
R-GTGTGATTACCCTGCCGTCTTT		
iroN	1048	This study
F-CGGTTCCTGGCACGAATATCAT		
R-TTTTGGGATTTCCCCAACCTGG		
iucD	534	This study
F-ATGGCATCACTGCCGATTCTTT		
R-AGTGAGTTAAAGCAGCAGCCTC		
fimH	170	This study
F-ATTCCTCACAATCAGCGCACTT		
R-ATCAGCAGTACAGCAAACAGGG		
chuA	279	(12)
F-GACGAACCAACGGTCAGGAT		
R-TGCCGCCAGTACCAAAGACA		
yjaA	211	(12)
F-TGAAGTGTCAGGAGACGCTG		
R-ATGGAGAATGCGTTCCTCAAC		
TspE4C2	152	(12)
F-GAGTAATGTCGGGGCATTCA		
R-CGCGCCAACAAAGTATTACG		

Table 2. Distribution of Phylogenetic Groups Among 100 *Escherichia coli* Isolates From UTIs

Phylogenetic groups	No. of UPEC isolates (n = 77)	Distribution	*chuA*	*yjaA*	TSPE4.C2
Group B2	55	$B2_2 = 54$	+	+	+
		$B2_3 = 1$	+	+	-
Group D	22	$D_2 = 12$	+	-	+
		$D_1 = 10$	+	-	-

Table 3. Association of Virulence Factor Genes with Phylogenetic Groups Among *Escherichia Coli* Isolates From UTIs

Phy. gp	*hlyA*	*iroN*	*iucD*	*fimH*
B2	29	22	46	55
D	2	2	10	22
PValue	0.01	0.05	0.21	1

Table 4. Virulence Patterns Identified Among the Studied Isolates Belonged to Phylogenetic Group B2

Patterns	*hlyA*	*iroN*	*iucD*	*fimH*	No.
Ec1	+	+	+	+	10
Ec2	-	-	+	+	18
Ec3	+	-	+	+	13
Ec4	-	+	+	+	5
Ec5	+	+	-	+	6
Ec6	-	+	-	+	1
Ec7	-	-	-	+	2
Total	29	22	46	55	55

Table 5. Virulence Patterns Identified Among the Studied Isolates Belonged to Phylogenetic Group D

Patterns	*hlyA*	*iroN*	*iucD*	*fimH*	No.
Ec1	+	+	-	+	1
Ec2	-	-	+	+	9
Ec3	+	-	+	+	1
Ec4	-	-	-	+	10
Ec5	-	+	-	+	1
Total	2	2	10	22	22

3.3. Multiplex-PCR for Virulence Genes

A multiplex-PCR assay was designed for the simultaneous detection of four ExPEC-associated VFs, fimH, hlyA, iucD and iroN (encoding, respectively, type 1 fimbriae, hemolysin and the aerobactin iron transport system) in a total of 100 isolates.

Briefly, DNA was extracted from the logarithmic phase cultures by the boiling method. PCR was performed in a reaction mixture with total volume of 25 μL, containing 12.5 μL of 2 × MasterMix red Taq polymerase and 0.3 μL of each primer. The PCR procedure was as follows: initial denaturation step at 94°C for 5 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 minutes. The PCR products were evident after migration by gel electrophoresis on 2% agarose gel prepared in tris-acetate-EDTA (TAE) 1X, stained with ethidium bromide 0.5 μg/mL, visualized by UV and photographed with a Polaroid camera. The amplicon size of fimH-170bp, hlyA-1361 bp, iucD-534 bp and iroN-1084 bp genes were determined by comparison with a molecular size marker (Fermentas 100 bp) (Figure 1). Specific primers were designed to amplify sequences of the *fimH*, *hlyA*, *iucD*, and *iroN* using the MPprimer 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.

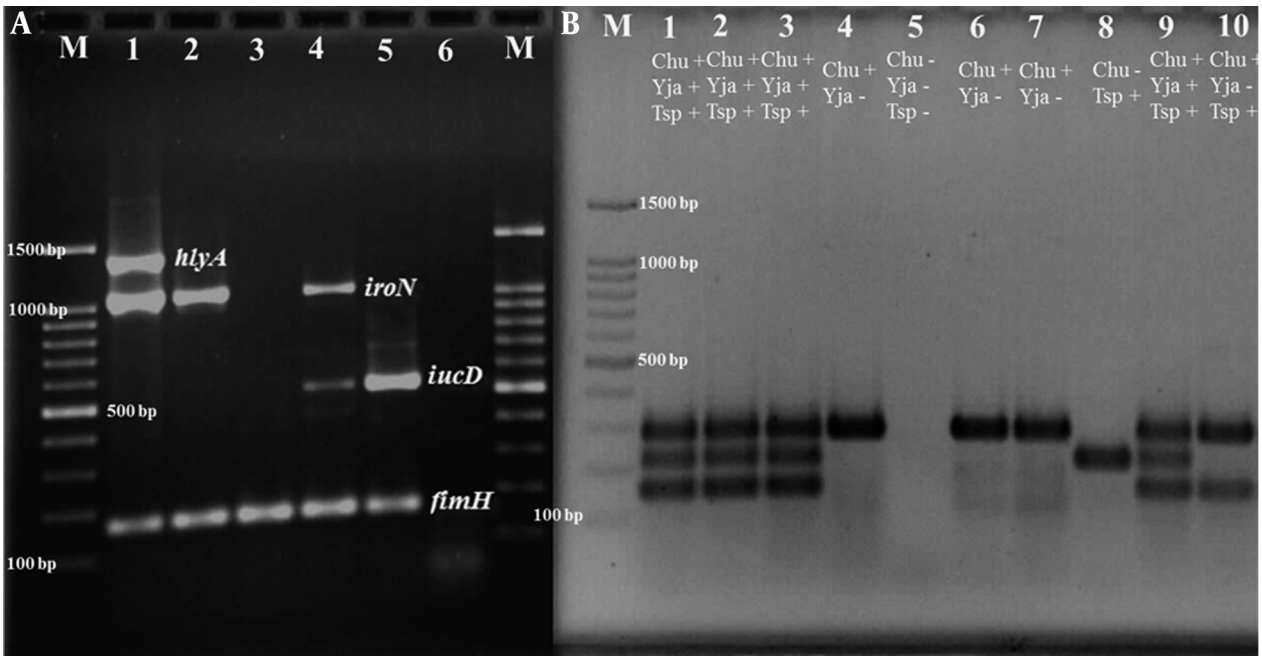

Figure 1. Positive Multiplex-PCR Results for the Detection of *E. coli* Virulence Factors (A) and Phylogenetic Groups (B) Among *E. coli* isolates

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 D (22%), comprised the majority of all isolated strains (Table 2).

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

The studied virulence genes were widely distributed among isolates belonging to the two phylogenetic groups B2 and D. The fimH genes were found in 100% of isolates from the B2 and D groups. Differences in the prevalence of the four studied ExPEC-associated virulence genes was observed between isolates belonging to phylogenetic groups B2 and D (Table 2). Among the 55 isolates from group B2, *iroN*, *iucD* and *hlyA* were found in 22, 46 and 29 isolates, respectively while among 22 isolates from group D, *iroN*, *iucD* and *hlyA* were present in two, ten and two isolates, respectively (Table 2).

4.3. Phylogenetic Distribution of Virulence Factors

According to the phylogenetic classification, *E. coli* isolates belonging to the extraintestinal phylogenetic group B2 possess 55 genes, as compared to those belonging to the phylogenetic group D with 22 virulence genes. The majority of isolates belonging to the phylogenetic group B2 harbor the complete set of four virulence factors (10 strains) (Table 4). For groups B2 and D, *fimH* was distributed in all isolates, while *hlyA* and *iroN* genes were predominating present in group B2 strains (29 out of 31 strains for *hlyA* and 22 out of 24 for *iroN*), followed by D (4 strains); in group B2 only four isolates harbored these genes (Table 3). The three virulence genes were observed in 24 isolates belonging to group B2, while in group D only two isolates harbored the three virulence genes (Tables 4 and 5). Considering the total prevalence of the four studied virulence determinants among the UPEC isolates, the *fimH* gene was present in all of the tested isolates (100%) belonging to groups B2 and D (Table 3), *iroN* in 31.16% (28.57% for group B2 and 2.59% for group D), *hlyA* in 40.25% (37.66% for group B2 and 2.59% for group D) and *iucD* in 72.72% (59.74% for group B2 and 12.98% for group D) of the studied strains. The analysis of the association between the presence of different combinations of virulence genes among the two studied phylogenetic groups B2 and D, allowed us to devide the tested strains into seven virulence patterns noted Ec1 to Ec7 (Table 4) for phylogenetic group B2 and five virulence patterns noted Ec1 to Ec5 (Table 5) for phylogenetic group D. The twelve strains (two isolates for B2 group and 10 isolates for D group) with only one virulence marker were included in patterns Ec7 and Ec5, while 27 isolates harboring *iucD* and *fimH* genes were included in pattern Ec2 (Table 4 and 5). The pattern Ec3 included strains simultaneously positive for $hlyA^+$, $iucD^+$ and $fimH^+$ (22 isolates). The two patterns Ec4 and Ec5 in group B2 included strains possessing a combination of three virulence genes; Ec4 with $fimH^+$, $iroN^+$ and $iucD^+$ (five isolates) and Ec5 with $fimH^+$, $hlyA^+$ and $iroN^+$ (six isolates). The two patterns Ec4 and Ec5 in group D included strains possessing a combination of different virulence genes; Ec4 with only *fimH* (10 isolates) and Ec5 with $fimH^+$ and $iroN^+$ (one isolate).

5. Discussion

In many UPEC strains, virulence is due to phylogenetic group background and to various virulence factors, such as hemolysins, adhesins and siderophore receptors (15). In this study, most of the uropathogenic *E. coli* isolates belonged to the phylogenetic group B2 and D. Previous research by Karimi Darehabi et al. (2013) (16) revealed a different result from that of the current research, identifying a higher prevalence of phylo-group A and D strains among *E. coli* isolates, which can be explained by the geographic effects of the *E. coli* population among hosts. Seventy-seven of the studied isolates were distributed between the phylogenetic groups (B2 and D), known for their higher virulence potential. The most virulent isolates belonged to the phylogenetic group B2, harboring the majority of tested virulence markers. We assumed that the virulence gene distribution pattern of phylogenetic group B2 would be similar to that of other ExPEC isolates belonging to the phylogenetic group D. Based on the distribution of the studied virulence genes, the studied isolates of phylogenetic group B2 exhibited seven virulence gene patterns, while group D isolates exhibited only five virulence gene patterns. Our results revealed the complexity of the association between phylogenetic groups and the properties of virulence markers in *E. coli* referred to as Ec (Tables 4 and 5). In the present study, the gene coding for type 1 fimbrial adhesion system (*fimH*) represented the most common factor for the virulence of *E. coli* isolates of both phylogenetic groups. The distribution of *E. coli* isolates possessing *fimH* gene, regardless of the phylogenetic group, was in good agreement with other published data (17-19). Our results illustrate a higher frequency of *fimH* compared with other virulence markers, which may suggest a crucial role of the virulence genes in *E. coli* causing UTIs. The distribution of the other studied virulence genes found among the studied isolates belonging to group B2 was also similar to other reported data (9, 20). The prevalence of *hlyA* gene encoding a toxin implicated in tissue damage and dysfunction of local immune responses was found more frequently in phylogenetic group B2. The distribution of this gene in our isolates is in agreement with other reports (21, 22). The prevalence of genes encoding the aerobactin and siderophore receptors (*iucD* and *iroN*), which confers the ability to take up iron, among isolates belonging to phy-

logenetic group B2 were fit with those published by other investigators (23, 24). Wereas we found a lower percent prevalence of iucD and iroN among isolates belonging to phylogenetic group D. In our study, phylogenetic distribution of virulence factors did allow the determination of a clear correlation between studied genes, phylogenetic background and the complexity of UTIs.

In conclusion, our study showed that: 1) a low number of *E. coli* virulence genes in isolates belonged to phylogenetic group D, 2) the characterization of *E. coli* isolates collected from patients with UTIs is of great interest to improve our knowledge regarding their virulence genetic markers and phylogenetic background, 3) further studies are needed to identify *E. coli* virulence factors responsible for UTIs and to determine the physiopathology of these infections to allow possible prevention measures and means.

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Authors' Contributions

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

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References

1. Piatti G, Mannini A, Balistreri M, Schito AM. Virulence factors in urinary Escherichia coli strains: phylogenetic background and quinolone and fluoroquinolone resistance. *J Clin Microbiol.* 2008;**46**(2):480–7.
2. 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.
3. Dhakal BK, Kulesus RR, Mulvey MA. Mechanisms and consequences of bladder cell invasion by uropathogenic Escherichia coli. *Eur J Clin Invest.* 2008;**38 Suppl 2**:2–11.
4. Li K, Zhou W, Hong Y, Sacks SH, Sheerin NS. Synergy between type 1 fimbriae expression and C3 opsonisation increases internalisation of E. coli by human tubular epithelial cells. *BMC Microbiol.* 2009;**9**:64.
5. Guyer DM, Gunther NWt, Mobley HL. Secreted proteins and other features specific to uropathogenic Escherichia coli. *J Infect Dis.* 2001;**183 Suppl 1**:S32–5.
6. Lindberg F, Lund B, Normark S. Gene products specifying adhesion of uropathogenic Escherichia coli are minor components of pili. *Proc Natl Acad Sci U S A.* 1986;**83**(6):1891–5.
7. Vilskersts R, Liepinsh E, Mateuszuk L, Grinberga S, Kalvinsh I, Chlopicki S, et al. Mildronate, a regulator of energy metabolism, reduces atherosclerosis in apoE/LDLR-/- mice. *Pharmacology.* 2009;**83**(5):287–93.
8. 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.
9. 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.
10. Pupo GM, Lan R, Reeves PR. Multiple independent origins of Shigella clones of Escherichia coli and convergent evolution of many of their characteristics. *Proc Natl Acad Sci U S A.* 2000;**97**(19):10567–72.
11. Torres AG, Blanco M, Valenzuela P, Slater TM, Patel SD, Dahbi G, et al. Genes related to long polar fimbriae of pathogenic Escherichia coli strains as reliable markers to identify virulent isolates. *J Clin Microbiol.* 2009;**47**(8):2442–51.
12. Clermont O, Bonacorsi S, Bingen E. Rapid and simple determination of the Escherichia coli phylogenetic group. *Appl Environ Microbiol.* 2000;**66**(10):4555–8.
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. Ben-Ami R, Rodriguez-Bano J, Arslan H, Pitout JD, Quentin C, Calbo ES, et al. A multinational survey of risk factors for infection with extended-spectrum beta-lactamase-producing enterobacteriaceae in nonhospitalized patients. *Clin Infect Dis.* 2009;**49**(5):682–90.
16. 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.
17. Licznar P, Eychenne I, Azema C, Decramer S, Bouissou F, Fayet O, et al. [Revised prevalence of afa+ Escherichia coli strains in acute pyelonephritis of children]. *Pathol Biol (Paris).* 2003;**51**(8-9):512–5.
18. Usein CR, Damian M, Tatu-Chitoiu D, Capusa C, Fagaras R, Tudorache D, et al. Prevalence of virulence genes in Escherichia coli strains isolated from Romanian adult urinary tract infection cases. *J Cell Mol Med.* 2001;**5**(3):303–10.
19. Sokurenko EV, Chesnokova V, Dykhuizen DE, Ofek I, Wu XR, Krogfelt KA, et al. Pathogenic adaptation of Escherichia coli by natural variation of the FimH adhesin. *Proc Natl Acad Sci U S A.* 1998;**95**(15):8922–6.
20. 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.
21. Koga VL, Tomazetto G, Cyoia PS, Neves MS, Vidotto MC, Nakazato G, et al. Molecular screening of virulence genes in extraintestinal pathogenic Escherichia coli isolated from human blood culture in Brazil. *Biomed Res Int.* 2014;**2014**:465054.
22. 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.
23. Takahashi A, Kanamaru S, Kurazono H, Kunishima Y, Tsukamoto T, Ogawa O, et al. Escherichia coli isolates associated with uncomplicated and complicated cystitis and asymptomatic bacteriuria possess similar phylogenies, virulence genes, and O-serogroup profiles. *J Clin Microbiol.* 2006;**44**(12):4589–92.
24. Lee S, Yu JK, Park K, Oh EJ, Kim SY, Park YJ. Phylogenetic groups and virulence factors in pathogenic and commensal strains of Escherichia coli and their association with blaCTX-M. *Ann Clin Lab Sci.* 2010;**40**(4):361–7.