VIRULENCE FACTORS AND MOLECULAR EPIDEMIOLOGY OF UROPATHOGENIC *ESCHERICHIA COLI* ISOLATED FROM PAIRED URINE AND RECTAL SWAB SAMPLES OF PATIENTS WITH URINARY TRACT INFECTIONS IN THAILAND

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Abstract. The role of uropathogenic *Escherichia coli* (UPEC) pathotypes and genotypes, including their specific virulence factors, in the pathogenesis of infection remains unclear. We aimed to find the role of UPEC in the pathogeneses of the patients with urinary tract infections. Ninety urine and corresponding rectal swab *E. coli* samples from patients with community-acquired (CAI), hospital-acquired (HAI) and asymptomatic (AUTI) urinary infections ($n = 30$ per group) admitted to a hospital in Thailand were subjected to characterization of virulence phenotypes and genotypes. Serogroup O25 was most prevalent (18%) among 6 serogroups (including O1, O6, O8, O18, and O15) and phylogenetic group B2 (39%) among 4 groups (including A, B1 and D) of *E. coli* isolates, with those from urine significantly higher than in rectal swab from all three types of UTIs. Three virulence-associated gene profiles (*fim*H$^+$, *fimH*$^+$*aer*$^+$ and *fimH*$^+$*aer*$^+$*usp*$^+$) were the most common in *E. coli* strains isolated from both urine and rectal swab samples of all three UTIs. Six out of eight randomly amplified polymorphic DNA patterns of paired urine and rectal swab *E. coli* strains with identical serogroup, phylogenetic group and virulence-associated gene profile isolated from AUTI, CAI and HAI groups (two in each group) showed the same pattern. These findings should contribute to a better understanding of the transmission of commensal *E. coli* through the urethral route.

Keywords: uropathogenic *Escherichia coli*, phylogenetic group, RAPD profiling, serogroup, virulence-associated gene

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INTRODUCTION

Urinary tract infections (UTIs) constitute one of the major problems in public health globally (Foxman, 2002, Ejrnaes *et al*, 2011). Uropathogenic *Escherichia coli* (UPEC), a subgroup of extra-intestinal pathogenic *E. coli* that causes cystitis or highly invasive pyelonephritis, is the most common cause of hospital (HA)- and community-acquired (CA) UTIs (Nicolle, 2002). Approximately 80-90% of this UTI-causing pathogen are involved in infection from the bacteria colonizing the perineum (Zalmanovici *et al*, 2010). More than 80% of CAUTI and around 30-50% of HAUTI are caused by UPEC (Ejrnaes *et al*, 2011). However, UTIs can be asymptomatic (AUTI) or symptomatic infections, ranging from mild symptom to bacteremia, sepsis, or even death (Foxman, 2002).

It has been shown that more than 90% of bacteria that are responsible for UTIs cases are often identified in the fecal flora of the same host (Katouli, 2010). However, similarities between *E. coli* isolated from fecal flora and urine of the same hosts have rarely been documented.

UPEC O serogroups (O-specific antigen) are related to particular virulence factor of each strain (Yamamoto, 2007), with O1, O6, O8, O15, O18, and O25 serogroups preferentially associated with virulence strains (Yamamoto, 2007; Momtaz *et al*, 2013). O25 is one of the most commonly occurring O serogroups among UPEC (Momtaz *et al*, 2013, Sarkar *et al*, 2014, Issazadeh *et al*, 2015). In addition to O serogroups, UPEC possesses a broad range of virulence-associated genes encoding fimbrial adhesins, *eg*, type 1 (*fim*H), P (*pap*), S/F1C (*sfa* adjacent to *foc*), and a non-fimbrial adhesin (*afa*); toxins, *eg*, hemolysin (*hly*), cytotoxic necrotizing factor (*cnf*); and uropathogenic specific protein (*usp*) (Momtaz *et al*, 2013). Type 1 and P fimbriae or pilus are the most common fimbriae found on UPEC surface (Lo *et al*, 2014). Adhesin located at the tip of type 1 fimbriae can be detected in both urine and fecal samples (Najar *et al*, 2007). While 71% of UPECs causing pyelonephritis carry *pap* (Qin *et al*, 2013), other adhesion genes including *sfa*, *foc* and *afa*, as well as *aer* (encoding aerobactin, a bacterial siderophore) are also found (Nowicki *et al*, 1986; Yun *et al*, 2014; Hojati *et al*, 2015; Zaki and Elewa, 2015).

Phylogenetic analysis has shown that UPECs have four major phylogenetic groups (A, B1, B2, and D) based on PCR analysis. Briefly, genomic DNA of bacterial strains was amplified by triplex PCR using primers targeted to three markers, *chuA*, *yjaA* and *tspE4*.C2. The phylogenetic grouping was made on the basis of the presence of specific PCR amplified fragments (Clermont *et al*, 2000). Group A and B1 are commensal strains and carry few virulence-associated genes while pathogenic groups B2 and D usually possess these genes that enhance colonic persistence and adhesion. It is widely accepted that the gastrointestinal tract of healthy humans act as a reservoir for UPEC strains that belong to phylogenetic group B2 and group D (*ie*, the lesser virulence groups). UPECs have remarkable abilities to endure and survive in the gut of humans and can spread to cause extra-intestinal infections (Clermont *et al*, 2000, Zhezang *et al*, 2004, Dhakal and Mulvey, 2009, Katouli, 2010).

In this study, O serogroups, virulence-associated gene profile and phylogenetic group of 90 paired *E. coli* strains isolated from urine and rectal swab samples of adult patients with HAI, CAI and AUTI were characterized to understand the relationship between *E. coli* strains present in urine and rectal swab from the same patient with UTI.

MATERIALS AND METHODS

Patients

Ninety patients (30 each with AUTI, CAI and HAI; 49-95 years old of both sexes) based on the criteria of Centers for Disease Control and Prevention (2013) were recruited at a general hospital in a central province, Thailand during March – July, 2014. Patients with UTIs and discharged within the previous month were included. Exclusion criteria were patients with a clinical history of severe complications, such as vesicoureteral reflux, neurogenic bladder, diabetes mellitus or malignant neoplasm. Ninety paired urine and rectal swab samples were collected from each patient.

The study protocol was reviewed and approved by the Human Research Ethics Committee, Faculty of Public Health, Mahidol University (no. MUPH 2014-073).

Sample collection method

Approximately 2-3 ml of a clean-catch or midstream urine samples were collected in a sterile container under sterile condition. Rectal swab of the same patient was subsequently collected by inserting a cotton swab into rectum approximately 2- 4 cm and rotated gently. The rectal swab sample was kept in 5 ml of Cary-Blair transport medium (B-Medical Supply, Bangkok, Thailand). The urine and rectal swab samples were immediately transported within one hour to a hospital-based microbiology laboratory for further bacterial isolation and identification.

Isolation and identification of *E. coli* isolates

The urine and rectal swabs were cultured directly onto MacConkey agar (MC; Difco, Detroit, MI), and then incubated at 37ºC for 18-24 hours. Lactose fermenting brick red or pink colonies were subjected to triple sugar iron (TSI) agar, lysine-indole-motility medium (LIM) and indole-Methyl Red (MR) -Voges Proskauer (VP) - citrate (IMViC) tests to identify *E. coli* isolates (Koneman *et al*, 2006). Biochemical characteristics of *E. coli* were positive glucose and lactose fermentations, gas production (CO_2), motility test, lysine decarboxylation, indole production, MR test, but negative for H_2S production, VP test and citrate utilization. All *E. coli* isolates were cultured overnight in Luria-Bertani (LB) broth (LB; Difco, Detroit, MI) and inoculums were stored in LB broth containing 20% glycerol at -80ºC for further analysis.

O serogroups typing

Six O antigens commonly associated with *E. coli* causing UTIs *viz*, O1, O6, O8, O15, O18, and O25 (Gibreel *et al*, 2012; Momtaz *et al*, 2013) were determined using six UPEC O antisera (Denka Seiken, Tokyo, Japan). Serogrouping was performed by a slide agglutination method (Momtaz *et al*, 2013).

Determination of *E. coli* phylogenetic group

Each strain of overnight culture on trypticase soy agar (TSA; Difco) was inoculated into 3 ml of LB broth and grown at 37°C with shaking for 15-18 hours. DNA extraction was performed using Qiagen QIAamp® DNA mini kit (Qiagen, Hilden, Germany). Four main *E. coli* phylogenetic groups, namely, A, B1, B2, and D, were identified using triplex PCR technique as previously described (Clermont *et al*, 2000) using three primer sets targeting *chu*A, *yja*A, and *tspE4*.C2. Each reaction was carried out in a 20-µl mixture containing 2 µl of 10X Go®*Taq* Flexi PCR buffer

(Promega, Madison, WI), 0.2 mM dNTPs (Promega), 0.2 μM of each forward and reverse primer, 0.125 U Go®*Taq* Flexi DNA polymerase (Promega), and 200 ng of genomic DNA. Thermocycling was carried out in MyCycler™ Thermal Cycler (BIO-RAD, Hercules, CA) under the following conditions: 94°C for 10 minutes; 30 cycles of 94°C for 30 seconds, 55°C for 30 seconds and 72°C for 30 seconds; and a final step at 72°C for 5 minutes. PCR amplicons (279, 211 and 152 bp of *chu*A, *yja*A, and *tspE4*.C2, respectively) were analyzed by 2% agarose gel-electrophoresis followed by ethidium bromine staining.

The phylogenetic groups were interpreted by the presence and absence of specific amplicons as follows: group A (*chu*A$^-$, *yja*A$^-$, *tspE*.C2$^-$), group B1 (*chu*A$^-$, *yja*A$^+$, *tspE*.C2$^-$), group B2 (*chu*A$^+$, *yja*A$^+$, *tspE*.C2$^{+/-}$), and group D (*chu*A$^+$, *yja*A$^-$, *tspE*.C2$^-$). *E. coli* strain UE001 was used as a positive control for B2 phylogenetic group (Dhakal and Mulvey, 2009).

Virulence-associated genes profiling

Eight virulence-associated genes, namely, *aer*, *afa*, *cnf*, *fimH*, *hly*, *pap*, *sfa/foc*, and *usp* were identified using established PCR assay as previously descried (Martin-Farmer and Janssen, 1999; Codruta-Romanita *et al*, 2001; Bauer *et al*, 2002; Licznar *et al*, 2003; Soto *et al*, 2011). Each reaction was carried out in a 25-μl mixture containing 2 μl of 10X Go®*Taq* Flexi PCR buffer (Promega), 0.2 mM dNTPs (Promega), 0.2 μM of each forward and reverse primer, 0.125 U Go®*Taq* Flexi DNA polymerase (Promega), and 20 ng of genomic DNA. Thermocycling was performed as described above using the following conditions: 94°C for 10 minutes; 35 cycles of 94°C for 2 minutes, gradient temperature increase from 45ºC to 65°C for 30 seconds, and 72°C for 2 minutes; and a final step at 72°C for 5 minutes. Amplicons (440, 465, 328, 410, 672, 693, 556, and 269 bp of *usp*, *fim*H, *pap*, *sfa/foc*, *afa*, *hly*, *cnf*, and *aer)* were analyzed as described above. *E. coli* strain UE001 was used as a positive control for *aer, fim*H*, hly*, and *usp*; *E. coli* strain UE255 as a positive control for *cnf* and *sfa/foc*; and *E. coli* strain UE046 and UE034 as positive control for *afa* and *pap,* respectively.

Phylogenetic analysis by randomly amplified polymorphic DNA (RAPD) PCR

E. coli isolates from urine and rectal swab paired samples that showed similar phenotypic and genotypic patterns were selected and subjected to RAPD PCR (Ready-To-Go RAPD analysis kit, GE Healthcare, Bristol, UK). In brief, genomic profile of each selected strain was generated by using two separate arbitrary decamer primers, No. 3 and No. 4. PCR mixture (25 μl) contained 25 pmol of a primer, 1 bead of the RAPD kit (which included buffer and DNA polymerase), 50 -100 ng of purified genomic DNA and 19 μl of MilliQ water. The mixture were mixed well and subjected to PCR Thermal Cycler (iCycler™, BIORAD) under the following conditions: pre-denaturation at 95°C for 5 minutes; 45 cycles of 95°C for 1 minute, 36°C for 1 minute and 72°C for 2 minutes; and a final step at 72°C for 5 minutes. Amplicons were analyzed by gel-electrophoresis as follows: 5 μl of each PCR product were mixed with 2 μl of 1X gel loading dye (TriDye™ 100 bp; Biolabs, Massachusetts, NE). The mixture was loaded into each individual well of 2% agarose gel (Isc Bio Express, Kaysville, Spain), subjected to electrophoresis and visualized by ethidium bromide staining.

The banding patterns of individual strains were scored based on the presence and absence of the bands. Scoring was made in the form of binary code with the score "A" indicating absence of band and "T" presence of band. The score data were analyzed by Genius bioinformatics software (Genius 8.1.5; Biomatters, Wellington, New Zealand). Similarity of patterns was performed by UPGMA (unweighted pair group method with arithmetic mean) clustering method. A phylogenetic tree was constructed using MEGA 5 version 6.0 software (Kearse *et al*, 2012).

Statistical analysis

Prevalence of virulence factors and types of infections were compared using chi-square and the Fisher's exact tests. A p-value <0.05 is considered significantly different.

RESULTS

O serogroups

Among the 180 *E. coli* strains isolated from urine and rectal swab samples O25 serogroup was the most predominant serogroup (27%) of *E. coli* isolated from urine samples among all types of UTIs, while O8 serogroup was the most prevalent (11%) serogroup in rectal swab samples (Table 1). In patients with HAI and AUTI, the prevalence of O25 in *E. coli* isolated from urine samples is significantly higher than in rectal swab samples (p = 0.02 and 0.05, respectively), while the prevalence of O25 serogroup in urine and rectal swabs of patients with CAI were similar (Table 1). O25 serogroup was highest (15%) in CAI patients while O1 (12%) and O18 (10%) was the most common serogroup in HAI and AUTI patients, respectively. All six O serogroups tested in this study were detected in *E. coli* from urine samples of all types of UTIs, but O6 and O15 serogroup was not observed in *E. coli* from rectal swab samples of patients with AUTI and CAI, respectively.

Phylogenetic groups

All 4 phylogenetic groups (A, B1, B2, and D) of *E. coli* were detected in urine and rectal swab samples of patients with all three types of UTIs (Table 1). The majority of *E. coli* strains belonged to B2 (39%) and D (32%). There are no significant differences in prevalence among the four phylogenetic groups of *E. coli* from urine or rectal swabs of HAI, CAI, and AUTI patients.

Distribution of virulence-associated genes

Among 8 virulence-associated genes (*aer*, *afa*, *cnf*, *fim*H, *hly*, *pap*, *sfa/foc*, and *usp*) examined in *E. coli* from urine and rectal swabs, the most frequent were *fim*H (85%), *aer* (72%) and *usp* (63%) (Table 1). *E. coli* isolates from urine samples were positive for *aer*, *fim*H and *usp*, with higher frequencies than those from rectal swab samples. The prevalence of *afa* (47%) in urine samples of patients with HAI is significantly higher than that of patients with AUTI (30%) (p = 0.03). Similarly, the prevalence of *usp* in *E. coli* isolates from urine samples of patients with HAI (93%) is significantly higher than that of CAI (53%) and AUTI (63%) (p = 0.002 and 0.001, respectively). The prevalence of *afa*, *hly*, *sfa/foc*, and *usp* in *E. coli* isolates from all 3 types of UTIs are significantly different (p < 0.05). The prevalence of the 8 virulence-associated genes of *E. coli* isolates from urine and rectal swab samples were similar except for *afa* and *usp* that is significantly higher in patients with HAI (47% and 93%, respectively) than the other two groups (43% and 77%, respectively) (p <0.05) suggesting that they were commonly found in HAI. Conversely, the prevalence of the remaining virulence-associated genes (*aer*, *cnf*, *fim*H, and *pap*) in *E. coli* strains isolated from patients with AUTI, CAI and HAI are not significantly different among the three groups, indicating that these

Table 1
Phenotypic and genotypic characteristics detected in *Escherichia coli* strains isolated from urine and rectal swab samples of 90 patients with urinary tract infections in Thailand.

Characteristic	Number (%) of *E. coli* strains detected								
	Urine				Rectal swab				Total (180)
	HAI (30)	CAI (30)	AUTI (30)	Total	HAI (30)	CAI (30)	AUTI (30)	Total	
O serogroup									
O1	3 (10)	2 (7)	1 (3)	6 (7)	4 (13)	1 (3)	1 (3)	6 (7)	12 (7)
O6	1 (3)	3 (10)	2 (7)	6 (7)	4 (13)	2 (7)	0 (0)	6 (7)	12 (7)
O8	2 (7)	2 (7)	3 (10)	7 (8)	3 (10)	5 (17)	2 (7)	10 (11)	17 (9)
O15	1 (3)	1 (3)	2 (7)	4 (4)	1 (3)	0 (0)	2 (7)	3 (3)	7 (4)
O18	2 (7)	1 (3)	2 (7)	5 (6)	2 (7)	1 (3)	4 (13)	7 (8)	12 (7)
O25	12 (40)*	4 (13)	8 (27)*	24 (27)	2 (7) *	5 (17)	2 (7)*	9 (10)	33 (18)
Total	21 (70)	13 (43)	18 (60)	52 (58)	16 (53)	14 (47)	11 (37)	41 (46)	93 (52)
Undetermined	9 (30)	17 (57)	12 (40)	38 (42)	14 (47)	16 (53)	19 (63)	49 (54)	87 (48)
Phylogenetic group									
Group A	3 (10)	4 (13)	5 (17)	12 (13)	4 (13)	4 (13)	6 (20)	14 (16)	26 (14)
Group B1*	4 (13)	2 (7)	1 (3)	7 (8)	2 (7)	8 (27)	9 (30)	19 (21)	26 (14)
Group B2*	14 (16)	11 (37)*	19 (21)*	44 (49)	13 (43)*	9 (30)*	5 (17)*	27 (30)	71 (39)
Group D	9 (30)	13 (43)	5 (17)	27 (30)	11 (37)	9 (30)	10 (33)	30 (33)	57 (32)
Virulence-associated gene									
*fim*H	26 (87)	27 (90)	28 (93)	81 (90)	25 (83)	21 (70)	26 (87)	72 (80)	153 (85)
usp	28 (93)*	16 (53)*	19 (63)*	63 (70)	23 (77)*	16 (53)*	11 (37)*	50 (56)*	113 (63)
aer	25 (83)	26 (87)	21 (70)	72 (80)	23 (77)	17 (57)	17 (57)	57 (63)	129 (72)
afa	14 (47)*	1 (3)	9 (30)	24 (27)	13 (43)*	1 (3)	9 (30)	23 (26)	47 (26)
pap	7 (23)	9 (30)	6 (20)	22 (24)	7 (23))	5 (17)	5 (17)	17 (19)	39 (22)
Sfa/foc	3 (10)	3 (10)	12 (40)	18 (20)	3 (10)	0 (0)	13 (14)	16 (18)	34 (19)
hly	2 (7)	8 (27)	7 (23)	17 (19)	1 (3)	8 (27)	4 (13)	13 (14)	30 (17)
cnf	2 (7)	1 (3)	4 (13)	7 (8)	0 (0)	1 (3)	2 (7)	3 (4)	10 (6)

*$p < 05$, comparing between urine and rectal swab samples.

Table 2
Phenotypic and genotypic characteristics detected among *Escherichia coli* strains in each individual paired urine and rectal swab samples of 90 patients with urinary tract infection in Thailand.

Characteristic	No. (%) of *E. coli* strains detected in each individual paired sample			
	Total (*n* = 90)	HAI (*n* = 30)	CAI (*n* = 30)	AUTI (*n* = 30)
O serogroup				
O1	4 (4)	3 (10)	1 (3)	0 (0)
O6	1 (1)	1 (3)	0 (0)	0 (0)
O8	1 (1)	0 (0)	1 (3)	0 (0)
O15	1 (1)	1 (3)	0 (0)	0 (0)
O18	3 (3)	1 (3)	AI1 (3)	1 (3)
O25	4 (4)	2 (7)	0 (0)	2 (7)
Total	14 (16)	8 (27)	3 (10)	3 (10)
Phylogenetic group				
Group A	2 (2)	0 (0)	0 (0)	2 (7)
Group B1	5 (6)	2 (7)	2 (7)	1 (3)
Group B2	21 (23)	10 (33)	7 (23)	4 (13)
Group D	12 (13)	7 (23)	3 (10)	2 (7)
Total	40 (44)	19 (63)	12 (40)	9 (30)
Virulence-associated gene				
*fim*H	73 (80)	24 (80)	26 (87)	23 (77)
afa	13 (14)	9 (30)	0 (0)	4 (13)
pap	8 (9)	3 (10)	3 (10)	2 (7)
Sfa/foc	11 (12)	1 (3)	0 (0)	10 (33)
usp	42 (47)	22 (73)	10 (33)	10 (33)
hly	6 (7)	0 (0)	5 (17)	1 (3)
cnf	1 (1)	0 (0)	1 (3)	0 (0)
aer	51 (57)	20 (67)	16 (53)	15 (50)

AUTI, asymptomatic urinary tract infection; CAI, community-acquired urinary tract infection; HAI, hospital-acquired urinary tract infection.

virulence-associated genes were common in all 3 types of UTIs. There were 54 different patterns of virulence-associated gene profiles the 180 *E. coli* isolated from urine and rectal swab samples of 90 patients with AUTI, CAI and HAI, ranging from single to 6 virulence-associated genes, among which double *fim*H^{+}*aer*$^{+}$ has the highest prevalence (13%), followed by *fim*H^{+}*aer*$^{+}$*usp*$^{+}$(12%), and single virulence gene (11%) (data not shown).

Association of phenotype and genotype of *E. coli* strains from paired urine and rectal swab samples

Fourteen (16%) urine and rectal paired swabs contained *E. coli* strains had the same O serogroups. with the highest prevalence being serogroups O1 and O25 (4 patients each), followed by O18 (3 patients). Among these 14 patients, 8 (57%) had HAI, followed by CAI and AUTI (1 patient each) (Table 2). Forty (44%)

Table 3
Virulence associated gene profiles of *Escherichia coli* strains detected in each individual paired urine and rectal swab samples of 90 patients with urinary tract infections in Thailand.

Virulence-associated gene profile	Number (%) of paired *E. coli* strains detected in patients			Total (n = 90)
	HAI (n = 30)	CAI (n = 30)	AUTI (n = 30)	
Single virulence gene	1 (3)	2 (7)	0	3 (3)
*fim*H$^+$	1 (100)	2 (100)	0	3 (100)
Double virulence genes	0	1 (3)	1 (3)	2 (2.2)
*fim*H$^+$*aer*$^+$	0	1 100)	1 (100)	2 (100)
Triple virulence genes	1 (3)	3 (10)	1 (3)	5 (5.6)
*fim*H$^+$*usp*$^+$*aer*$^+$	1 (100)	2 (67)	1 (100)	4 (80)
usp$^+$*aer*$^+$*hly*$^+$	0	1 (33)	0	1 (20)
Quadruple virulence genes	1 (3)	1 (3)	0	2 (2.2)
*fim*H$^+$*usp*$^+$*aer*$^+$*afa*$^+$	1 (100)	0	0	1 (50)
*fim*H$^+$*usp*$^+$*aer*$^+$*cnf*$^+$	0	1 (100)	0	1 (50)
Quintuple virulence genes	2 (7)	0	1 (3)	3 (3)
*fim*H$^+$*usp*$^+$*aer*$^+$*afa*$^+$*pap*$^+$	1 (50)	0	0	1 (33)
*fim*H$^+$*usp*$^+$*aer*$^+$*afa*$^+$*sfa/foc*$^+$	1 (50)	0	1 (100)	2 (67)
Hexatruple	0	0	1 (3)	1 (1)
*fim*H$^+$*usp*$^+$*aer*$^+$*afa*$^+$*pap*$^+$*hly*$^+$	0	0	1 (100)	1 (100)
Total	5 (17)	7 (23)	4 (13)	16 (18)

AUTI, asymptomatic urinary tract infection; CAI, community-acquired urinary tract infection; HAI, hospital-acquired urinary tract infection.

patients had matched urine and rectal swab *E. coli* strains belonging to the same phylogenetic group, the highest prevalence being group B2 [21 (23%) patients], followed by group D [12 (13%) patients] (Table 2). HAI group [19 (63%)] contained the highest number of patients, followed by CAI [12 (40%)] and AUTI [9 (30%)]. Thus among matched urine and rectal swab samples *E. coli* belonging to phylogenic groups B2 and D were the most common in UTIs. As regards virulence-associated gene profiles of *E. coli* strains, 16 (18%) patients harbored bacteria from urine and rectal swabs that matched, which could be classified into six different patterns, namely, single gene (3 samples), and combination of two (2 samples), three (5 samples), four (2 samples), five (3 samples), and six (1 sample) genes (Table 3). In AUTI, CAI and HAI groups, virulence-associated gene profiles of matched virulence-associated gene profiles of *E. coli* strains contained 4 (different) patterns, with *fim*H$^+$*usp*$^+$*aer*$^+$ present in matched *E. coli* strains from all 3 types of infections.

Phylogenetic tree of matched *E. coli* strains

Eight matched *E. coli* strains having identical O serogroup, phylogenetic group, and virulence-associated gene

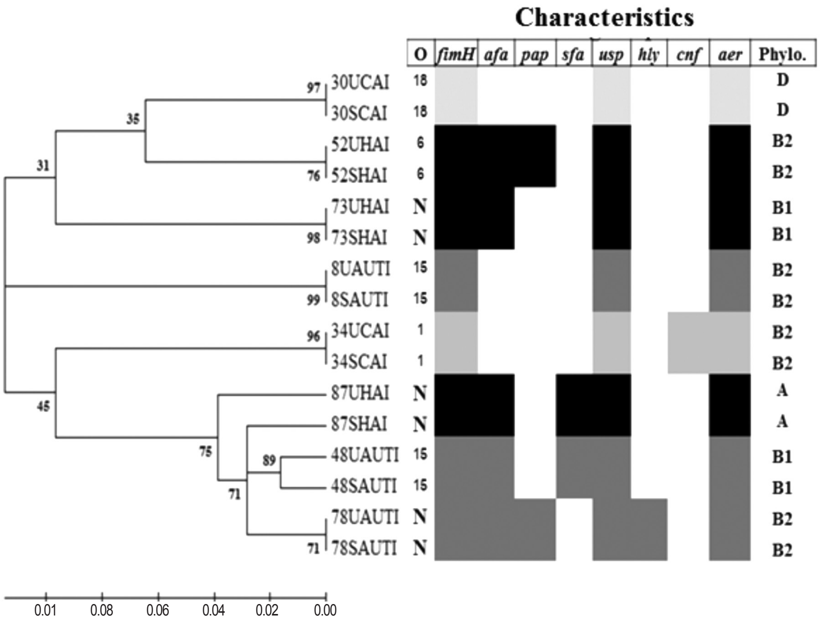

Fig 1–Dendogram of sixteen *Escherichia coli* strains based on randomly amplified polymorphic DNA profiles. The *E. coli* strains were those from paired urine and rectal swab samples with the same O serogroup, phylogenetic group and virulence-associated gene profile. RAPD profiles were generated by PCR using primer No. 4 (Ready-To-Go RAPD analysis kit, GE Healthcare, Bristol, UK). Dendogram was constructed using MEGA 5 version 6.0 software (Kearse *et al*, 2012). Solid block indicates the presence of virulence gene profiles. The code number in front of strain name indicates the source of isolation (SBL: 0.7217). Numeral at branch site refers to percent similarity of RAPD profile. O, O serogroup; Phylo., phylogenetic group.

profile (3, 2 and 2 strains from AUTI, CAI and HAI group, respectively) were subjected to molecular typing by RARD analysis and subsequent phylogenetic tree construction. The RAPD dendogram demonstrate close relationship between RAPD pattern and virulence gene profile in 6/8 paired *E. coli* strains (Fig 1). However, these *E. coli* strains were dispersed throughout the phylogenetic tree rather than belonging to two clusters in the tree (sum of branch lengths = 0.7217).

DISCUSSION

Virulence factors help in bacterial colonization in urinary epithelium and cause severe acuity in UPEC strains that are responsible for severe UTIs. O serogroup is one of the most important virulence factors of UPEC strains associated with severe UTIs (Momtaz *et al*, 2013). In this study, among the six O serogroups examined, O18 and O25 serogroups were common in the three UTIs. O25 serogroup is predominant in CAI in many countries, *eg*, Iran, Mexico and Syria (Jadhav *et al*, 2011; Morales-Espinosa *et al*, 2016; Sharma *et al*, 2016) and also causes severe symptoms in UTI patients in Iran an Mexico City (Molina-López *et al*, 2011; Paniagua-Contreras *et al*, 2015). O25 serogroup strains carried higher numbers of virulence-associated genes than those of the other 5 O serogroups in agreement with a previous study (Yamamoto, 2007). O6, O15 and O18 serogroups have been reported to account for the major O *E. coli* serogroups in UTI from different parts of the world (Blanco *et al*, 1997). O18 might be a dominant serogroup in this country. O6, O8, O15, and O25 are dominant serogroups in intestinal *E. coli* reported in a number of studies (Nataro and Kaper, 1998; Scheutz *et al*, 2004). Our findings also showed the presence of O1, O6, O18, and other O serogroups including other virulence factors in paired urine and rectal swab strains. These UTI patients might have extra-intestinal tract *E. coli* or UPEC derived from their stool (Katouli, 2010).

Among the four phylogenetic groups (A, B1, B2, D), group B2 was the predominant in *E. coli* from urine of 3 types of UTIs, in agreement with previous reports (Cao *et al*, 2011; Luo *et al*, 2012). Although resident strains belonging to phylogenetic group B2 and carrying a number of virulence-associated genes were reported (Nowrouzian *et al*, 2006), the majority belong to phylogenetic groups A and B1 (Moreno *et al*, 2008). At present, only phylogenetic group B2 might be a potential genetic marker for identifying UPEC of UTI patients.

The virulence-associated genes are important factors mediating bacterial invasion, dissemination and persistence (Johnson *et al*, 2001). There is a lower percent positivity of virulence genes in commensal *E. coli* than those in UPEC, including adhesin, toxin and aerobactin genes (Johnson *et al*, 2001; Abe *et al*, 2008; Molina-Lopez *et al*, 2011; Momtaz *et al*, 2013; Dormanesh *et al*, 2014). On the other hand, the eight virulence-associated genes (*aer*, *afa*, *cnf*, *fim*H, *hly*, *pap*, *sfa*/*foc*, and *usp*) studied in *E. coli* strains are almost equally distributed among UPEC and commensal strains (Farshad *et al*, 2009; Karimian *et al*, 2012; Momtaz *et al*, 2013). In our study all tested *E. coli* strains harbored virulence-associated genes ranging from a single gene and up to six genes, generating 54 different virulence-associated genes profiles. *fim*H encoding type 1 fimbriae causes invasion into the bladder (Wright *et al*, 2007). The distribution of virulence properties can also vary depending on host characteristics and the type of infection. Possession of adherence factors, toxins and aerobactin are important factors responsible for pathogenesis of UPEC (Mulvey, 2002; Marrs *et al*, 2005; Ejrnaes *et al*, 2011). Asymptomatic bacteriuria strains are well suited for growth in human urinary tract, without causing any clinical symptoms (Roos *et al*, 2006). However, extra-intestinal *E. coli* in AUTI or UTI patients have been established as constituting a reservoir in fecal flora (Schlager *et al*, 2002; Katouli, 2010).The present observation using RAPD PCR showed 100% identity in six paired urinary and rectal *E. coli* isolates indicate the strains were closely related. It could be possible that these six *E. coli* strains might be a potential source of UTI in AUTI, CAI and HAI under appropriate condition (Alteri and Mobley, 2012). The six RAPD patterns from paired urine and rectal AUTI, CAI and HAI samples should be compiled for epidemiological studies in hospital in question. The findings that *E. coli* strains causing UTIs have also been consistently isolated from fecal flora of the same patients strongly supports that these bacteria were derived from the gut (Karimian *et al*, 2012).

In conclusion, concurrent urine and rectal swabs among adult UTI patients showing close relatedness of virulence-associated gene profile and same virulence factors strongly support the notion of transmission of gut-flora *E. coli* to cause UTIs. This information regarding UPEC should be useful in further studies of the pathogenesis and transmission of these virulence strains from commensal *E. coli*. However, the attributes of intestinal *E. coli* pertinent to UTI remains to be further elucidated.

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CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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