Original Article

Antibiotic Resistance Pattern and Phylogenetic Groups of the Uropathogenic *Escherichia coli* Isolates Recovered from the Urinary Catheters of the Hospitalized Patients

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Introduction: Almost 80% of nosocomial urinary tract infections (UTIs) are due to catheterization. Catheter-associated UTI (CAUTI) is the primary source for colonization of antibiotic-resistant pathogens, and uropathogenic *Escherichia coli* (UPEC) is the most common causative bacteria. This study was conducted to determine the phylogenetic groups, and antibiotic resistance pattern as the two important features of pathogenicity of UPEC isolates collected from urinary catheters. **Methods:** The UPEC isolates were obtained from the urinary catheters of the patients without UTI, from two referral hospitals during 2015 to 2016. Phylogenetic grouping was performed using a multiplex PCR. Antibiotic susceptibility and extended spectrum beta-lactamase (ESBL) production were tested by the disc diffusion method. Multidrug resistance was determined based on a recent guideline. The presence of some resistance genes was examined by a PCR assay. **Results:** Thirty-eight percent of the isolates were UPEC, all of them belonged either to B2 (62.5%) or D (37.5%) phylogenetic groups. The UPEC isolates showed a very high resistance to ciprofloxacin (80%) and the third-generation cephalosporins (72.5%). Seventy percent of the isolates were ESBL-producing, and 90% of them were multiple drug resistant (MDR). Meanwhile, the frequency of the resistance genes: *ctxM, aacIV, sul1, shv,* and *qnrA* in the isolates were 95%, 82.5%, 77.5%, 72.5%, and 45%, respectively. **Conclusion:** High resistance to fluoroquinolones and third-generation cephalosporins, as well as high frequency of ESBL-producing and MDR UPEC isolates, are a great concern. This phenomenon is probably the consequence of the indiscriminate use and on the counter availability of antibiotics, which should be considered in empirical therapy of CAUTIs. *J Med Microbiol Infec Dis, 2016, 4 (3-4): 76-82.*

Keywords: Catheter-Related Infections, Uropathogenic *Escherichia Coli*, Bacterial Drug Resistance, Multiple Drug Resistance, beta-Lactam Resistance.

INTRODUCTION

The urinary tract is the most common site for hospital-acquired infections [1], generally due to catheterization or surgery [2]. About 10-15% of the patients receive urinary catheters during their hospitalization [3]. The catheter-associated urinary tract infections (CAUTIs) increase mortality in hospitalized patients up to 3 times [4]. In fact, these infections are the most common cause of nosocomial bacteremia [5]. The risk of CAUTI rises ≈5% in each day of catheterization, and virtually all patients are colonized by the day 30 [6].

In contrast to uncomplicated nosocomial urinary tract infection (UTI), CAUTI is a multi-pathogenic disease. However, like uncomplicated UTI, *Escherichia coli* is the main causative agent in CAUTIs [7]. This pathogen has been isolated from the urine of 20-50% of patients with urinary catheters in previous studies [8]. Up to now, some reasons have been introduced to explain the high frequency of uropathogenic *E. coli* (UPEC) in CAUTIs. Some studies have pointed out the presence of adhesins, flagellum-mediated motility, and toxins as responsible factors for colonization in rectal area [9]. Others have focused on the ability of biofilm formation on the surface of the urinary catheters, which not only prepares the main core for attachment of other pathogens but increases resistance to antimicrobials up to 1000 times more than planktonic status [10-12].

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Antibiotic resistance among gram-negative uropathogenic agents such as UPEC has become a great concern [13]. It not only makes bacterial eradication almost impossible but has led to antibiotic overuse [8]. Extended spectrum beta-lactamases (ESBLs), which are mainly produced by *Enterobacteriaceae* family members such as *E. coli,* has always been a major issue in choosing appropriate antibiotic against CAUTI during the recent years [14]. Besides, multidrug resistant (MDR) UPECs, defined as non-susceptible isolates to one or more agent(s) in three or more antimicrobial categories, are increasing worldwide [15]. Also, the resistance of UPECs to fluoroquinolones, third-generation cephalosporins, and carbapenems is growing globally [13] and several genes are responsible for conferring this resistance [16].

Previous studies have shown that while *E. coli* strains, in general, belong to four phylogenetic groups, A, B1, B2, and D [17], the uropathogenic strains mostly fit the B2 and D phylogroups [18]. It has been demonstrated that the strains of these two groups carry much virulence factors than the others [19]. Meanwhile, a definite association between antibiotic resistance and phylogenetic groups, especially B2 and D, has been identified in UPEC isolates [20, 21].

This study was conducted to investigate the phylogenetic groups and antibiotic resistance patterns of the UPEC isolates recovered from the urinary catheters of patients with no signs and symptoms of UTI during different days of their hospitalization. The main goals of the study were to evaluate these important features which are related to UPEC pathogenicity and to assess their relations not only with each other, but with the other factors like patients' gender and age, the hospitals and different wards, and the time that urinary catheters were removed.

MATERIAL AND METHODS

Sample collection. The *E. coli* isolates were collected from 164 urinary catheters of in-patients from two referral hospitals (Loghman Hakim and Imam Khomeini hospitals) in Tehran, Iran from January 2015 to January 2016. The patients had no signs and symptoms of UTI and were under treatment in the infectious diseases ward or the intensive care unit (ICU). The catheters were removed and cut into three pieces under a sterilized condition, and their outer parts were maintained at peptone water for further experiments. Later, these parts were transferred to the Molecular Biology Department of Pasteur Institute of Iran. There, they were sonicated (1.5 Volt for 2 min) to separate the bacteria from the outer surfaces of the catheters. All the separated bacteria were cultured on Maconkey and Eosin Methylene Blue agars at 37°C for 24 h, and then subcultured on TSI and IMViC mediums and incubated as before. The isolates were stored in LB broth with 80% Glycerol at -80°C.

Confirmation of UPEC isolates. The identity of UPEC strains was confirmed through the amplification of the highly specific *E. coli* universal stress protein A (*uspA*) gene as described elsewhere [22]. The DNA amplification reaction mixtures (25 µl) contained 12.5 µl *Taq* DNA

Polymerase Master Mix (Sinaclon, Iran), 1 µl of each forward and reverse primers, 1.5 µl DNA, and 9 µl double distilled water (DDW). The amplification program included a 5 min initial denaturation at 94°C, followed by 30 cycles of 2 min denaturation at 94°C, 1 min annealing at 70°C, and 1 min extension at 72°C. The amplifications were electrophoresed on 1.5% agarose in TBE 1X buffer, stained with ethidium bromide, and visualized under UV light.

Phylogenetic grouping. The phylogenetic grouping of the UPEC isolates was carried out, using a triplex PCR for *chuA* and *yjaA* genes, and the DNA fragment TspE4.C2 which allows determination of all 4 different groups [19]. The PCR mixture (25 µl) included 12.5 µl *Taq* DNA Polymerase Master Mix (Sinaclon, Iran), 1 µl of each forward and reverse primers, 3 µl template DNA, and 7.5 µl DDW. The primers sequences were the same as what was described before, and the PCR steps consisted of an initial 4 min denaturation at 94°C, following by 30 cycles of 5 s denaturation at 94°C and 10 s annealing at 59°C, and a 5 min final extension at 72°C [19].

Antibiotic susceptibility test. All the isolates were investigated for their resistance to 14 antibiotics which not only are commonly used in CAUTIs but represent most of the antimicrobial categories defining MDR, based on the Clinical and Laboratory Standards Institute and other guidelines [23, 15]. The antibiotic susceptibility was tested by disc diffusion method using the antibiotics, amikacin (AMI) 30 µg, ampicillin (AMP) 10 µg, aztreonam (AZT) 30 µg, cefazolin (CFZ) 30 µg, cefotaxime (CTX) 30 µg, ceftazidime (CAZ) 30 µg, ceftriaxone (CTR) 30 µg, ciprofloxacin (CIP) 5 µg, chloramphenicol (CLR) 30 µg, gentamicin (GEN) 10 µg, imipenem (IMP) 10 µg, piperacillin-tazobactam (PI+TZ) 100/10 µg, tetracycline (TET) 30 µg, and trimethoprim-sulfamethoxazole (SXT) 25 µg; the isolates were defined as susceptible, resistant, intermediate, and MDR [23, 15]. Meanwhile, the ESBL-producing isolates were recognized by the double disc synergy test (DDST) method using cefotaxime and ceftazidime plus cefotaxime + clavulanic acid 30/10 µg and ceftazidime + clavulanic acid 30/10 µg [23].

Detection of antibiotic resistance genes. The presence of 5 main antibiotic resistance genes (*ctxM*, *shv*, *qnrA*, *aacIV*, and *sul1*) was detected using PCR as described by others [24-28]. The primers and their sequences are shown in Table 1. The PCR mixtures and protocols were optimized for each gene.

RESULTS

One hundred and six isolates were collected during one year, among them, 40 isolates (38%) were uropathogenic *E. coli* (UPEC), all confirmed through amplification of *uspA* gene. The number of isolates collected from Loghman Hakim Hospital was twice as those of Imam Khomeini Hospital (27 *vs.* 13). Furthermore, 95% of the UPEC isolates were recovered from the infectious disease ward (38 out of 40). There were no significant differences in the distribution of the isolates regarding the patients' age and gender and the weeks that the urinary catheters were removed from the patients (Table 2).

Table 1. The antibiotic resistance genes and the primers used for their amplification

Targeted genes	Primers	Sequences
CTXM	ctxM.f	5'-GTGACAAAGAGAGTGCAACGG-3'
	ctxM.r	5'-ATGATTCTCGCCGCTGAAGCC-3'
SHV	shv.f	5'-TCGCCTGTGTATTATCTCCC-3'
	shv.r	5'-CGCAGATAAATCACCACAATG-3'
QNRA	qnrA.f	5'-GGATGCCAGTTTCGAGGA-3'
	qnrA.r	5'-TGCCAGGCACAGATCTTG-3'
AACIV	aacIV.f	5'-AGTTGACCCAGGGCTGTCGC-3'
	aacIV.r	5'-GTGTGCTGCTGGTCCACAGC-3'
SUL1	sul1.f	5'-TGAGATCAGACGTATTGCGC-3'
	sul1.r	5'-TTGAAGGTTCGACAGCACGT-3'

Table 2. Distribution of 40 collected UPEC isolates according to the patients' gender and age, the hospitals and their wards, and the time (in weeks) the urinary catheters were removed from the patients (when the samples were collected)

	Patients' gender		Patients' age		Hospitals				Catheter removal period (weeks)	
					Loghman Hakim (27)		Imam Khomeini (13)			
	Male	Female	< 65	≥ 65	Infectious diseases ward	ICU	Infectious diseases ward	ICU	1st week	2nd week
Number of the isolates	**17**	**23**	**18**	**22**	**26**	**1**	**12**	**1**	**16**	**24**

The phylogenetic analyses demonstrated that all the UPEC isolates, as expected, belonged either to B2 (25 isolates, 62.5%) or D phylogroups (15 isolates, 37.5%). None of the isolates belonged the phylogroups A or B.

The antimicrobial susceptibility pattern of the UPEC isolates showed a very high resistance (80%) to ciprofloxacin and resistance to 3 out of 4 cephalosporins including cefazolin, cefotaxime and ceftriaxone revealed to be 72.5%. The resistance to trimethoprim-sulfamethoxazole and tetracycline was very high (77.5%) as well. However, 90% of the isolates were sensitive to imipenem. Likewise, the sensitivity of the isolates to piperacillin-tazobactam and chloramphenicol was noticeable (72.5%, Table 3). Meanwhile, based on DDST results, 70% (28 out of 40) of the UPEC isolates were ESBL-producing.

Our antibiotic resistance assays revealed 90% of the isolates (36 out of 40) as MDR; only 3 isolates were sensitive to all the tested antibiotics, 2 from Imam Khomeini Hospital and 1 from Loghman Hakim Hospital. Two isolates (both from Loghman Hakim Hospital) were resistant to all the 14 antibiotics tested, which means they could be categorized as extensively drug-resistant (XDR) agents [15]. There was no significant relationship between the resistance to the antimicrobial agents (neither in the category nor the number of antibiotics) and the phylogenetic groups of the UPEC isolates (Table 4).

The detection of the antibiotic resistance genes revealed that 95% of the UPEC isolates had the *ctxM* gene, 82.5% *aacIV* gene, 77.5% *sul1* gene, and 72.5% *shv* gene (Fig. 1). The *qnrA* gene was present in 45% of the isolates. All the 5 genes were detected in 8 isolates, half of them were from Imam Khomeini Hospital, and the rest were from Loghman-e Hakim Hospital. Additionally, 7 out of 8 (87.5%) of these isolates, all from Imam Khomeini Hospital, belonged to the B2 phylogroup.

The comparison of the distribution of the antibiotic resistance genes in two phylogenetic groups demonstrated that the prevalence of all the detected genes was more in the B2 phylogroup than D (Fig. 2a). However, only regarding the *qnrA* gene, there was a very significant difference ($p \leq 0.01$) between B2 and D groups (60% *vs.* 20% respectively). Also, all the UPEC isolates of the phylogroup B2 (100%) had the *ctxM* gene, while the prevalence of this gene in the group D was 87%. Moreover, regarding the time the urinary catheters were removed from the patients, a very significant difference between the prevalence of the *qnrA* gene in the first and the second week (25% *vs.* 58% respectively) was observed (Fig. 2b).

Table 3. Susceptibility pattern of the isolates against different antibiotics

	Antibiotic	Sensitive	Intermediate	Resistant
1	Amikacin (AMI)	92.5%	-	7.5%
2	Ampicillin (AMP)	12.5%	-	87.5%
3	Aztreonam (AZT)	37.5%	12.5%	50%
4	Cefazolin (CFZ)	17.5%	10%	72.5%
5	Cefotaxime (CTX)	27.5%	-	72.5%
6	Ceftazidime (CAZ)	40%	12.5%	47.5%
7	Ceftriaxone (CTR)	25%	2.5%	72.5%
8	Ciprofloxacin (CIP)	20%	-	80%
9	Chloramphenicol (CLR)	72.5%	-	27.5%
10	Gentamicin (GEN)	55%	-	45%
11	Imipenem (IMP)	90%	2.5%	7.5%
12	Piperacillin-tazobactam(PI+TZ)	72.5%	5%	22.5%
13	Tetracycline (TET)	22.5%	-	77.5%
14	Trimethoprim-sulfamethoxazole (SXT)	22.5%	-	77.5%

Table 4. Antibiotic resistance pattern in each isolate based on the number and the category of the resistant antibiotics. Distribution of each group in the 2 different hospitals, as well as phylogenetic group of each isolate, is shown in separate columns

| Number of resistance and name of antibiotics	Total number of isolates	Loghman Hakim Hospital		Imam Khomeini Hospital	
		Number of isolates	Phylogenetic group	Number of isolates	Phylogenetic group
None (0)	3	1	D	2	B2, B2
2	1	-	-	1	B2
AMP/CIP	1			1	
4	4	4		-	-
AMP/CIP/TET/SXT	2	2	D, B2		
CFZ/CIP/TET/SXT	2	2	D, B2		
5	3	2		1	
AMP/CIP/CLR/TET/SXT	1	1	D	0	
AMP/CFZ/CIP/TET/SXT	2	1	D	1	B2
6	1	1		-	-
AMP/CFZ/CTX/CTR/TET/SXT	1	1	D		
7	3	2		1	
AMP/AZT/CFZ/CTX/CAZ/CTR/CIP	2	1	D	1	B2
AMP/CFZ/CTX/CTR/CIP/GEN/SXT	1	1	B2	0	
8	3	2		1	
AMP/AZT/CFZ/CTX/CAZ/CTR/TET/SXT	1	1	D	0	
AMP/AZT/CFZ/CTX/CTR/GEN/TET/SXT	1	1	B2	0	
AMP/AZT/CFZ/CTX/CAZ/CTR/CIP/PI+TZ	1	0		1	D
9	7	6		1	
AMP/AZT/CFZ/CTX/CTR/CIP/GEN/TET/SXT	1	1	B2	0	
AMP/AZT/CFZ/CTX/CAZ/CTR/CIP/IMP/PI+TZ	1	1	D	0	
AMP/AZT/CFZ/CTX/CAZ/CTR/CIP/TET/SXT	2	2	B2, B2	0	
AMP/AZT/CFZ/CTX/CAZ/CTR/CIP/PI+TZ/TET	1	1	D	0	
AMP/AZT/CFZ/CTX/CAZ/CTR/GEN/TET/SXT	1	1	B2	0	
AMP/AZT/CFZ/CTX/CAZ/CTR/CIP/GEN/SXT	1	0		1	B2
10	8	7		1	
AMP/AZT/CFZ/CTX/CAZ/CTR/CIP/GEN/TET/SXT	3	3	B2, B2, B2	0	
AMI/AMP/CFZ/CTX/CTR/CIP/CLR/PI+TZ/TET/SXT	1	1	D	0	
AMP/AZT/CFZ/CTX/CAZ/CTR/CLR/GEN/TET/SXT	2	2	D, B2	0	
AMP/AZT/CFZ/CTX/CAZ/CTR/CIP/GEN/PI+TZ/TET	1	1	B2	0	
AMP/AZT/CFZ/CTX/CAZ/CTR/CIP/CLR/TET/SXT	1	0		1	B2
11	3	-	-	3	
AMP/AZT/CFZ/CTX/CAZ/CTR/CIP/GEN/PI+TZ/TET/SXT	1			1	B2
AMP/CFZ/CTX/CAZ/CTR/CIP/CLR/GEN/PI+TZ/TET/SXT	1			1	B2
AMP/AZT/CFZCTX/CAZ/CTR/CIP/CLR/GEN/TET/SXT	1			1	D
12	1	-	-	1	
AMP/AZT/CFZ/CTX/CAZ/CTR/CIP/CLR/GEN/PI+TZ/TET/SXT	1			1	B2
13	1	-	-	1	
AMP/AZT/CFZ/CTX/CAZ/CTR/CIP/CLR/GEN/IMP/PI+TZ/TET/SXT	1			1	B2
All (14)	2	2		-	
AMI/AMP/AZT/CFZ/CTX/CAZ/CTR/CIP/CLR/GEN/IMP/PI+TZ/TET/SXT	2	2	B2, D		
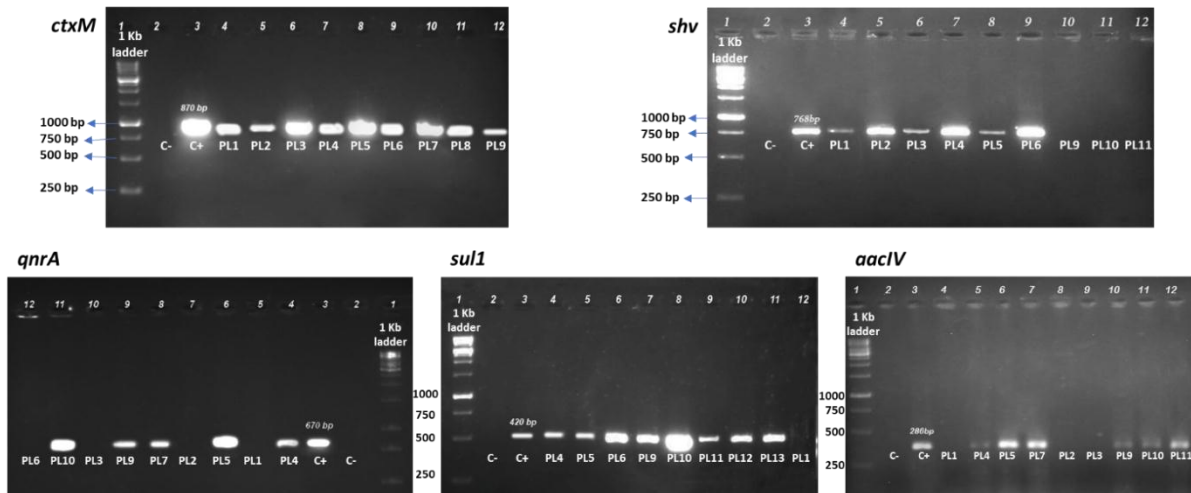
Fig. 1. Gel electrophoresis of some amplified antibiotic resistance genes. The names of genes are in the upper-left side of the gels. PL refers to each isolate which has been specified by a following number
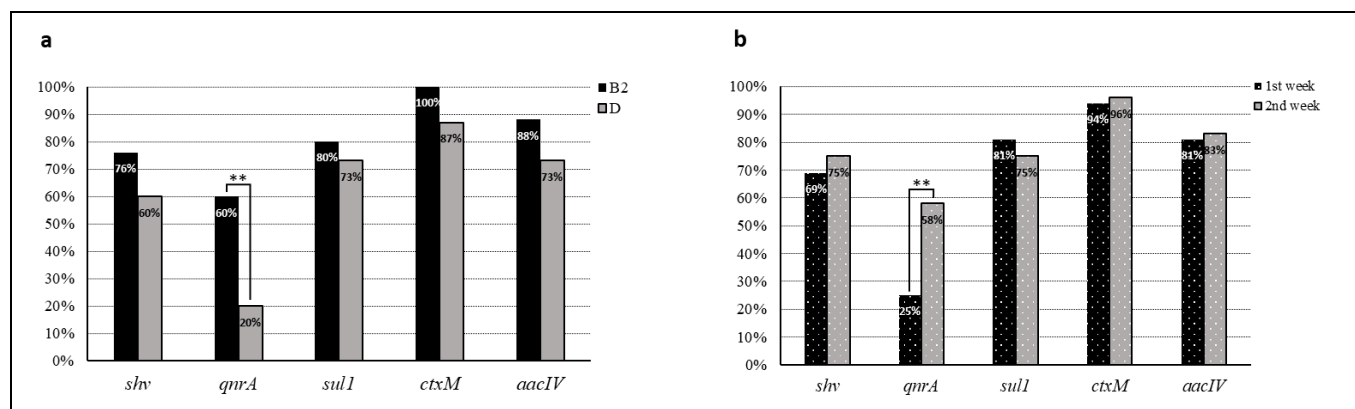
Fig. 2. Prevalence of the antibiotic resistance genes; a) among two phylogenetic groups (B2 and D); b) in two weeks that the urinary catheters were removed from the patients. *P*-values were calculated by Fisher's exact test (** represents *p*≤0.01)

DISCUSSION

About 80% of hospital-acquired UTIs are due to the urinary catheterization. In fact, CAUTI is the most common nosocomial infection worldwide [29]. Although asymptomatic in most cases, CAUTI is the primary source of antibiotic resistant pathogens, and many of infected patients suffer from its complications such as bacteremia [5]. *Escherichia coli* is the most common and the most important pathogen responsible for the hospital-acquired CAUTI [7].

As the initial step of CAUTI is the attachment of bacteria to the surface of urinary catheters, it is important to monitor this process from the first days of catheterization to reveal the specifications of the isolates. Hence, in this study, we collected the *E. coli* isolates from the urinary catheters in patients with no signs or symptoms of UTI at different days of hospitalization and evaluated their phylogenetic groups and antibiotic resistance patterns as the two principal features of their pathogenicity.

The UPEC strains, in general, fit mostly to the B2 and D phylogenetic groups [18]. The previous findings are compatible with the results of our study in which most of the isolates belonged to the phylogroup B2 (62.5%) followed by the phylogroup D (37.5%), while the phylogroups A or B were not detected. Almost all of the previous studies have shown that B2 phylogroup was dominant in UPEC [30-33]. In a study in Mofid Hospital of Tehran, Iran, the phylogenetic groups of the *E. coli* isolates obtained from the urine of the children with UTI and the stool of healthy adults were almost the same as (B2: 54%, D: 34%, A: 8%, B1: 4%) those of our study [32]. A recent survey in Zabol, Iran, showed the similar results too [33].

Development of resistance even to the new generation antibiotics such as fluoroquinolones, third-generation cephalosporins, and carbapenems among gram-negative uropathogenic agents like UPEC has become an emerging threat in UTI treatment during the recent years [13]. In our study, the UPEC isolates show an overall high resistance rate to most of the tested antibiotic which is considerable in comparison to previous studies. The isolates of our study revealed not only to be more resistant to the old conventional antibiotics such as trimethoprim-sulfamethoxazole compared with previous reports (77.5% *vs.* 50%) [34] but were more resistant to the new generation combinations. Particularly, about 80% of the isolates were resistant to ciprofloxacin (a fluoroquinolone), cefotaxime and ceftriaxone (third-generation cephalosporins, Table 3). These findings are in contrast to the previous studies, like that of Bi and colleagues in which about 90% of the *E. coli* isolates collected from the children with indwelling catheters were sensitive to the third-generation cephalosporins such as cefotaxime and ceftazidime [35]. However, this can be explained by the fact that the patients in our study were adults and more than half of them (22 out of 40) were in their old age (Table 2). Besides, 90% of the isolates were sensitive to imipenem; this rate is still less than those reported in other studies, from Iran (97.5%) [34], and southern France (100%) [36].

About 70% of our isolates were ESBL-producing, which is more than the rates (≈40%) obtained in the recent studies from Iran [37-39]. For instance, in a hospital, the ESBL rate was 40% in the infectious diseases ward, but 70.4% in the ICU [38]. It is noticeable that although 70% of our isolates were ESBL-producing, only 5% of them were from the ICU (Table 2).

In this study, 90% of the UPEC isolates were MDR, among them, 2 showed resistance to all of the tested antibiotics and can be considered XDR (Table 4). In 2011, in a similar study 84.2% of the *E. coli* isolates derived from different specimens in a hospital in Tabriz, Iran, showed to be MDR [40]. Later in 2015, another study in Tabriz by the same authors showed that 48.2% of the UPEC isolates obtained from the children with UTI were MDR [41]. Although this rate seems to be much lower than what we obtained in our study, the age group of the patients should be considered as an important interfering factor here. In general, regarding previous reports and the present study, the prevalence of MDR *E. coli* in Iran is worrisomely much higher compared to the European countries and USA [42].

Previous studies have determined the association between antibiotic resistance and phylogenetic groups in *E. coli* [21, 30]. In the present study, we did not find any relationship between these two in phenotypic level (Table 4). However, in genotypic level, all of the genes were more

prevalent in the B2 phylogroup; among them, the prevalence of the *qnrA* gene was significantly higher (Fig. 2a).

In conclusion, we selected a time for sample collection from the catheterized in patients when they had no UTI symptoms or signs. Even in this period, a majority of the collected isolates showed resistance to fluoroquinolones and third-generation cephalosporins which are the first-line antibiotics against CAUTI. Moreover, 90% of the isolates were MDR, and 70% were ESBL-producer. The reason for this enormous kind of resistance in referral hospitals of Tehran should further be investigated. As usual, the first suspects are indiscriminate use and availability of over-the-counter antibiotics. However, an additional comprehensive survey is needed to understand any other underlying mechanisms. The results of this study can be used to update the antibiotic resistance pattern of the UPEC isolates in Iran for any probable modifications in choosing the appropriate antibiotics for empirical treatment of CAUTIs.

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CONFLICT OF INTEREST

The authors declare no conflict of interest. The funding sponsors had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, and in the decision to publish the results.

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