Comparative Immunology, Microbiology and Infectious Diseases 65 (2019) 148–153

Contents lists available at ScienceDirect

Comparative Immunology, Microbiology and Infectious Diseases

journal homepage: www.elsevier.com/locate/cimid

Serogroup distribution, diversity of exotoxin gene profiles, and phylogenetic grouping of CTX-M-1- producing uropathogenic *Escherichia coli*

Seyyed Khalil Shokouhi Mostafavi[a], Shahin Najar-Peerayeh[a,*], Ashraf Mohabbati Mobarez[a], Mehdi Kardoust Parizi[b]

[a] Department of Bacteriology, Faculty of Medical Sciences, Tarbiat Modares University, Tehran, Iran
[b] Department of Urology, Shariati Hospital, Tehran University of Medical Sciences, Tehran, Iran

ARTICLE INFO

Keywords:
Uropathogenic *E. coli*
Urinary tract infection
Serogroups
Virulence genes
CTX-M-1

ABSTRACT

The emergence of CTX-M-1 producing Uropathogenic *Escherichia coli* (UPEC) has become a serious challenge. In addition to antimicrobial resistance, a number of virulence factors have been shown. Therefore, this study was designed to determine the prevalence of O- serogroups, phylogenetic groups, exotoxin genes, and antimicrobial resistance properties of CTX-M-1- producing UPEC. A total of 248 UPEC isolates were collected. The antibiotic resistance was performed, and PCR was used to detect the $bla_{\text{CTX-M1}}$, exotoxins, serogroups and phylogroups of UPEC. Of 248 isolates, 95 (38.3%) harbored $bla_{CTX\text{-}M\text{-}1}$. Of them, serogroups O1 and O25 were predominant, accounting for 20% and 13.7%, respectively. The *hlyA* was the dominant exotoxin gene (32.6%), followed by *sat* (28.4%), *vat* (22.1%), *cnf* (13.7%), *picU* (8.4%), and *cdt* (2.1%). The *hlyA* gene was significantly associated with pyelonephritis ($P = 0.003$). Moreover, almost half of the isolates (45.4%) belonged to phylogenetic group B2. Most of exotoxin genes were present in significantly higher proportions in group B2 isolates except *cdt* gene ($P < 0.05$). All of the isolates were susceptible to imipenem, nitrofurantoin, and fosfomycin. The CTX-M-1-producing UPEC strains causing nosocomial infections are more likely to harbor certain exotoxin genes, raising the possibility that this increase in virulence genes may result in an increased risk of complicated UTI.

1. Introduction

Urinary tract infections (UTIs) encountered in clinical practice with an estimated annual incidence of at least 150 million worldwide. Uropathogenic *Escherichia coli* (UPEC) strains have associated with a large portion of both hospital and community-acquired UTIs [1]. Unfortunately, the ability of UPEC to acquire multiple drug resistance, particularly extended-spectrum β-lactamases (ESBLs), can hamper the therapeutic management of infections [1]. Over the last decade, CTX-M family has rapidly emerged and became the predominant ESBL type globally. Based on CTX-M amino acid sequences, these enzymes have classified into five major groups, including CTX-M-1, CTX-M-2, CTX-M-8, CTX-M-9, and CTX-M-25/26 groups. Of these groups, CTX-M-1 is the most prevalent one, which widely disseminated throughout the world [2]. In Iran, the frequency of CTX-M1-producing UPEC has dramatically increased over the past years [3,4].

Traditionally, *E. coli* clones including both pathogenic and commensal ones identified by serological typing of their H (flagellar), O (lipopolysaccharide), and, in some cases, K (capsular) surface antigens. To date, more than 180 different serogroups have described for *E. coli*. However, some O antigen types frequently represented in uropathogenic *Escherichia coli* (UPEC) clones, including O1, O2, O4, O6, O7, O8, O16, O18, O25, and O75 [5]. Furthermore, previous studies showed that some of this O- serogroups related to certain ESBL types and virulence factors in UPEC clones [6,7].

Nowadays, it generally believed that UPEC has evolved from non-pathogenic *E. coli* ancestors by an acquisition of new virulence traits through horizontal gene transfer. UPEC possesses a diverse repertoire of virulence factors, which facilitate their growth and persistence within the adverse settings of the host urinary tract. In this respect, some potential virulence factors have been shown to play an important role in the pathogenesis of UPEC, including adhesins, hemolysins, siderophores, and toxins [8]. Production of toxins causes an inflammatory response, a possible pathway for UTIs symptoms. For instance, α-haemolysin (HlyA), an RTX pore-forming exotoxin, has been associated with most severe and symptomatic UTIs especially pyelonephritis. Similar to HlyA, cytotoxic necrotizing factor 1 (CNF1) is involved in kidney invasion and produced by one-third of all pyelonephritis-associated strains [9]. SPATEs (Serine Protease Autotransporters of Enterobacteriaceae) are another category of secreted proteins implicated

* Corresponding author.
E-mail address: najarp_s@modares.ac.ir (S. Najar-Peerayeh).

https://doi.org/10.1016/j.cimid.2019.05.003
Received 28 January 2019; Received in revised form 1 May 2019; Accepted 6 May 2019

Table 1
PCR and Annealing Temperatures Used for the Various Genes.

Target gene	Sequence (5' to 3')	Amplicon Size, bp	Optimal annealing temperature (°C)	Reference
hlyA	F: GCATCATCAAGCGTACGTTCC R: AATGAGCCAAGCTGGTTAAGCT	534	60	Paton, and Paton, 1998
cnf	F: AAGATGGAGTTTCCTATGCAGGAG R: CATTCAGAGTCCTGCCCTCATTAT	498	52	Yamamoto et al., 1995
sat	F: TATCACGCAATGCCAATGTT R: GACCCGGCGTTACAGTTTTA	393	58	Idress et al., 2010
vat	F: AACGGTTGGTGGCAACAATCC R: AGCCCTGTAGAATGGCGAGTA	420	58	Restieri et al., 2007
picU	F: ACTGGATCTTAAGGCTCAGGAT R: GACTTAATGTCACTGTTCAGCG	572	58	Restieri et al., 2007
cdt	F: AAATCACCAAGAATCATCCAGTTA R: AAATCTCCTGCAATCATCCAGTTTA	430	60	Chen and Griffiths, 1998
chuA	F: GACGAACCAACGGTCAGGAT R: TGCCGCCAGTACCAAAGACA	279	55	Clermont et al., 2000
yjaA	F: TGAAGTGTCAGGAGACGCTG R: ATGGAGAATGCGTTCCTCAAC	211	55	Clermont et al., 2000
TspE4.C2	F: GAGTAATGTCGGGGCATTCA R: CGCGCCAACAAAGTATTACG	152	55	Clermont et al., 2000
rfbO1	F: ATACCGACGACGCCGATCTG R: CCAGAAATACACTTGGAGAC	189	59	Clermont et al., 2007
rfbO2	F: ATACCGACGACGCCGATCTG R: GTGACTATTTCGTTACAAGC	274	59	Clermont et al., 2007
rfbO18	F: ATACCGACGACGCCGATCTG R: GAAGATGGCTATAATGGTTG	360	59	Clermont et al., 2007
rfbO16	F: ATACCGACGACGCCGATCTG R: GGATCATTTATGCTGGTACG	450	59	Clermont et al., 2007
rfbO6	F: ATACCGACGACGCCGATCTG R: AAATGAGCGCCCACCATTAC	584	59	Clermont et al., 2007
rfbO7	F: ATACCGACGACGCCGATCTG R: CGAAGATCATCCACGATCCG	722	59	Clermont et al., 2007
rfbO4	F: ATACCGACGACGCCGATCTG R: AGGGGCCATTTGACCCACTC	193	67	Clermont et al., 2007
rfbO12	F: ATACCGACGACGCCGATCTG R: GTGTCAAATGCCTGTCACCG	239	59	Clermont et al., 2007
rfbO25	F: ATACCGACGACGCCGATCTG R: GAGATCCAAAAACAGTTTGTG	313	59	Clermont et al., 2007
rfbO75	F: ATACCGACGACGCCGATCTG R: GTAATAATGCTTGCGAAACC	419	58	Clermont et al., 2007
rfbO15	F: ATACCGACGACGCCGATCTG R: TGATAATGACCAACTCGACG	536	59	Clermont et al., 2007
rfbO157	F: ATACCGACGACGCCGATCTG R: TACGACAGAGAGTGTCTGAG	672	59	Clermont et al., 2007

in the virulence of UPEC. Three SPATE members in UPEC strains which have received the greatest attention are Sat (serine protease autotransporter toxin), PicU (a homologe of mucinase in *Shigella flexneri*), and Vat (vacuolating autotransporter toxin recently described for avian pathogenic *E. coli*) [10].

To our knowledge, few studies reported the frequencies of O- serogroups, and exotoxins in CTX-M-1- producing UPEC strains in Iran. Therefore, this study was proposed to determine the prevalence of O serogroups, phylogenetic groups, exotoxin genes (i.e., *hlyA*, *cnf1*, *sat*, *vat*, *picU*, and *cdt*), and antimicrobial resistance among CTX-M-1- producing UPEC strains isolated from UTI patients in Tehran, Iran.

2. Materials and methods

2.1. Patients and bacterial strains

From March 2016 to January 2017, a total of 350 bacterial isolates were collected from urine specimens of patients with hospital and community- acquired UTI at a university hospital in Tehran, Iran.

Out of 350 bacterial isolates, 248 (70.8%) were *E. coli*. The *E. coli* isolates were obtained from patients of both genders (60% female vs. 40% male) aged between 12 and 76 years, with a mean age of 48.5 ± 23.5 years. All of the mentioned *E. coli* isolates were identified using standard biochemical and routine laboratory tests including Gram staining, oxidase test, indole production, H2S production, carbohydrate utilization on TSI agar, MRVP reaction, urease production, etc. UTI was defined as the presence of a positive urine culture ($\geq 10^5$ colony forming units/ml) and pyuria ($\geq 10^4$ leukocytes/ml of urine). Cystitis (inflammation of the bladder) describes the syndrome involving dysuria, frequency, urgency, and occasionally suprapubic tenderness. Pyelonephritis(inflammation of renal parenchyma) caused by bacterial invasion of a kidney. The symptoms of this clinical syndrome are chills and fever, flank pain and constitutional [11]

2.2. Detection of $bla_{CTX\text{-}M\text{-}1}$ by polymerase chain reaction

Genomic DNA was prepared using a boiling lysis method. For this purpose, several colonies of the pure isolate were suspended in 500 µL of distilled water and heated at 100 °C for 15 min. Then, it was centrifuged at 6000 *g* for 8 min. The supernatant was used as the template DNA. ESBL- producing UPEC isolates were screened for $bla_{CTX\text{-}M\text{-}1}$ gene by PCR, as previously described [11]. The PCR products were analyzed on 1% agarose gels stained with gel red (Pishgam Biotech Company, Iran).

2.3. Antimicrobial susceptibility testing

The antibiotic resistance patterns of the CTX-M1- producing UPEC isolates were determined by Kirby-Bauer disc- diffusion method on Mueller-Hinton agar plates, and the results were interpreted according

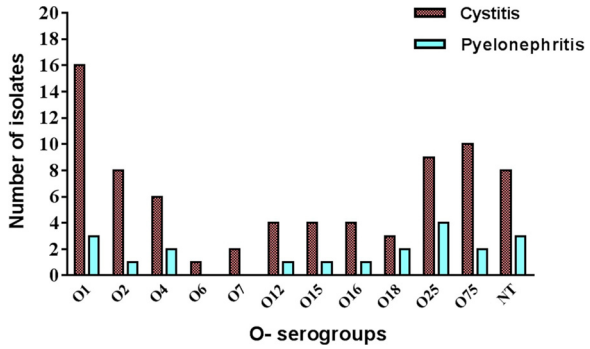

Fig. 1. Distribution of different O- serogroups among CTX-M-1-producing UPEC isolates causing cystitis and pyelonephritis.

to Clinical and Laboratory Standard Institute (CLSI) guidelines [12]. The antimicrobial agents (MAST, Co., United Kingdom) tested in this study included ampicillin (10 µg), amoxicillin-clavulanic acid (20/10 µg), cefazolin (30 µg), cefotaxime (30 µg), cefotaxime (30 µg), ciprofloxacin (5 µg), co-trimoxazole (25 µg), fosfomycin (50 µg), gentamicin (10 µg), imipenem (10 µg), and nitrofurantoin (100 µg).

2.4. Serogrouping

All of the CTX-M1- producing UPEC isolates were subjected to monoplex PCR assays for detection of O1, O2, O4, O6, O7, O12, O15, O16, O18, O25, O75, and O157 serogroups using a universal forward gndbis.f primer and O-specific reverse primers [13].

2.5. Detection of exotoxin genes

PCR assays were performed for all of the CTX-M1- producing UPEC isolates to reveal the frequencies of exotoxin genes including *hlyA*, *cnf1*, *sat*, *vat*, *picU*, and *cdt*. Primer sequences predicted sizes of the PCR products, and specific annealing temperatures are shown in Table 1.

2.6. Phylogenetic grouping

For determination of major UPEC phylogenetic group (A, B1, B2, and D), the *chuA* and *yjaA* genes and TspE4.C2 fragments of DNA were examined by triplex PCR assay [14]. The UPEC isolates were assigned to the clonal groups A ($chuA^-$, TspE4.C2$^-$), B1 ($chuA^-$, TspE4.C2+), B2 ($chuA^+$, $yjaA^+$) or D ($chuA^+$, $yjaA^-$).

2.7. Multi-locus sequence typing

In order to perform this technique, seven genes were firstly amplified by PCR method and then sent to the sequencing, and the sequencing results were analyzed. The primers used and the conditions for performing PCR were located on the MLST site of *E. coli* (http://enterobase.warwick.ac.uk/species/ecoli/allele_st_search). The phylogenetic relationship between isolates was investigated by UPGMA online algorithm.

In this method, the isolates of serotype O1, being the predominant serotypes, were identified by housekeeping genes, including *adk, fumC, gyrB, icd, mdh, purA* and *recA* and their sequence types (ST) and related clones were identified [15].

2.8. Statistical analysis

Chi-square and ANOVA tests were performed to examine the significance of the association between different variables such as the presence of the exotoxin genes, phylogenetic groups, O-serogroups, etc. A *p*-value less than 0.05 was considered statistically significant.

3. Results

3.1. Prevalence of $bla_{CTX\text{-}M\text{-}1}$ among UPEC isolates

In this study, a total of 248 UPEC isolates were recovered, comprising 130 isolates from inpatients and 70 isolates from outpatients. Among them 213 patients suffered from cystitis, while 35 afflicted by pyelonephritis. PCR assays revealed that out of 248 UPEC isolates, 95 isolates carried $bla_{CTX\text{-}M\text{-}1}$ gene. Of 95 CTX-M1- producing UPEC isolates, 74 isolates (77.9%) were obtained from hospitalized patients. The majority of CTX-M1- producing UPEC isolates (n = 75) belonged to patients with cystitis, whereas 20 isolates have been recovered from patients with pyelonephritis.

3.2. Antibiotic susceptibilities of CTX-M1-producing UPEC

All of the CTX-M1-producing UPEC isolates (100%) were resistant to cefotaxime, amoxicillin, and cefazolin. Resistance to ceftazidime was observed in 93 isolates (97.9%), followed by co-trimoxazole (n = 81, 85.3%), tetracycline (n = 76, 80%), ciprofloxacin (n = 69, 72.6%), and gentamicin (n = 23, 24.2%), and amoxicillin-clavulanic acid (n = 5, 5.3%). Fortunately, no resistance against imipenem, nitrofurantoin, and fosfomycin was observed among the isolates.

3.3. Serogroups of the CTX-M1-producing UPEC

Among 95 CTX-M-1-producing UPEC isolates tested, serogroups O1, O25, and O75 were the most prevalent, accounting for 20%, 13.7%, and 12.6%, respectively. However, 11.6% of the isolates were not typeable with the PCR method based on O-antigen-specific genes. Also, serogroup O157 was not found in tested strains. The distribution of O-serogroups among CTX-M-1-producing UPEC isolates causing cystitis and pyelonephritis was depicted in Fig. 1.

3.4. Prevalence of exotoxin genes among CTX-M1-producing UPEC

Regarding exotoxin genes, *hlyA* was the most prevalent gene (n = 31, 32.6%), followed by *sat* (n = 27, 28.4%), *vat* (n = 21, 22.1%), *cnf* (n = 13, 13.7%), *picU* (n = 8, 8.4%), and *cdt* (n = 2, 2.1%). A statistically significant association was observed only between the presence of *hlyA* gene and pyelonephritis ($P = 0.003$). Of 95 isolates, 63 (66.3%) harbored at least one exotoxin gene, while the rest (n = 32) did not carry any exotoxin genes. Also, 18 exotoxin profiles (EPs) were defined by a different combination of exotoxin genes. EP1, EP2, EP6, and EP7 were only observed in UPEC isolates recovered from patients with pyelonephritis (Table 2). The distribution of virulence genes among O- serogroups was also shown in Table 3.

3.5. Phylogenetic typing of the CTX-M1-producing UPEC

The CTX-M-1-producing UPEC isolates belonged to phylogenetic group B2 (n = 45, 45.4%), with the other isolates belonging to phylogenetic group B1 (n = 16, 16.8%), D (n = 24, 25.3%), and A (n = 10, 10.5%). Distribution of phylogenetic groups in CTX-M-1-producing UPEC isolates based on exotoxin genes was also shown in Fig. 2. However, chi-square analysis indicated that phylogenetic group distribution did not differ significantly between exotoxin positive (n = 63) and exotoxin negative (n = 32) isolates ($P = 0.122$), as shown in Table 4.

3.6. Genotyping using the MLST technique

Following the genotyping of the serotypes O1 of CTX-M1 beta-lactamase-producing UPEC and the results of the analysis after sequencing of the Housekeeping genes and sending samples for sequencing, according to the Warwick site and automatically and automatically

Table 2
Exotoxin profiles of CTX-M-1- producing UPEC isolates harboring at least one exotoxin gene (n = 63).

Exotoxin gene (s)	Exotoxin profile	Pyelonephritis No.	Cystitis No.	Total No. (%)
cnf-vat-sat-picU	EP1	1	0	1 (1.6)
cnf-vat-sat	EP2	1	0	1 (1.6)
cnf-sat-hlyA	EP3	1	1	2 (3.1)
vat-sat-hlyA	EP4	1	1	2 (3.1)
cdt-vat	EP5	0	1	1 (1.6)
cnf-vat	EP6	1	0	1 (1.6)
cnf-sat	EP7	1	0	1 (1.6)
cnf-hlyA	EP8	0	3	3 (4.7)
vat-sat	EP9	3	1	4 (6.2)
vat-hlyA	EP10	0	3	3 (4.7)
vat-picU	EP11	0	3	3 (4.7)
sat-hlyA	EP12	0	9	9 (14.1)
cdt	EP13	0	1	1 (1.6)
cnf	EP14	0	4	4 (6.2)
vat	EP15	0	5	5 (7.8)
sat	EP16	0	7	7 (10.9)
hlyA	EP17	10	1	11 (18.7)
picU	EP18	1	3	4 (6.2)

provided genotyping results. The ST648 was predominant and the ST10 was lowest rate.

4. Discussion

UPEC strains participate as the dominant agents of both community and hospital- acquired UTIs. Over the past decades, researchers witnessed the spread of antimicrobial resistance worldwide, which is hampering the therapeutic management of UTIs [16,17]. Initially, resistance was limited to a low number of antibiotics, such as trimethoprim or ampicillin. However, overuse of broad-spectrum antibiotics, such as third-generation cephalosporins, greatly hastened the appearance, in particular, of ESBLs. During the last decade, CTX-M family has gradually replaced the classical TEM and SHV- type ESBLs in many regions throughout the world. Amongst the CTX-M family, the CTX-M-1 is still by far the most important group worldwide [18,19]. Our results revealed that the frequency of $bla_{CTX\text{-}M\text{-}1}$ in UPEC isolates was 38.3%, which is higher than those of other studies from Columbia, Cambodia, and Pakistan [20–22]. In Iran, recent studies showed that the frequencies of $bla_{CTX\text{-}M1}$ among UPEC isolates ranged between 20.5% and 74% [23,24]. On the other hand, the $bla_{CTX\text{-}M}$ genes usually associated with resistance to other antibiotics especially aminoglycosides, quinolones, and sulfonamides. Not surprisingly, co-resistance to these antibiotic agents has frequently observed in our strains, which is almost similar to the findings of some previous studies [20,21,23–25]. In this context, carbapenems such as imipenem and fosfomycin still represent a good choice when therapy is needed for serious infections [26].

Although serogrouping is a valuable typing method for pathogenic *E. coli* isolates in epidemiological investigations, it is labor-intensive, time-consuming, and expensive for the analysis of large numbers of specimens. Also, cross-reactions between different O- serogroups usually occur giving equivocal results. Recently, several PCR assays based on O-antigen-specific genes have been developed for the identification of *E. coli* serogroups [27]. In this study, the highest distribution of serogroups was exhibited for O1 (20%), O25 (13.7%), and O75 (12.6%) among CTX-M1- producing UPEC isolates. Abe et al. showed that O6 (19.5%), O2 (7.1%), and O15 (5.8%) were the major O- serogroups among UPEC isolates in Brazil [28], whereas another study from India reported O6 (33.3%), O1 (15.1%), and O15 (15.1%) as the most common O-serogroups [7]. In a very recent study from our country, Tajbakhsh et al. demonstrated that O25, (27.7%), O15 (20.8%), and O6 (12.3%) were the most prevalent O- serogroups among *E. coli* isolated from UTI patients [29]. Similarly, Momtaz et al. showed that O25 (26%), O15 (21.1%), and O16 (10.6%) had the highest distributions among UPEC isolates, which is somewhat different from those of our study [30]. In general, O-serogroups can vary depending on the type of infection, region, or even different settings (hospital or community). Among O-serogroups in pyelonephritis, the distribution of O25 ($p = 0.037$) and O1 ($p = 0.041$) was significantly higher than other serogroups, followed by O18, O75, O4, O15, O16, O12, O2, O6, and O7.

Exotoxins have demonstrated to mediate invasion, dissemination, and persistence of *E. coli* in host cells. HlyA is required for the initial invasion of *E. coli* through the epithelial barrier, while CNF facilitates the spread and persistence of *E. coli* in the urinary tract [31]. In this study, the existence of *hlyA* ($p = 0.017$) was significantly associated with pyelonephritis, while *sat* ($p = 0.034$) was significantly associated with cystitis. Our study outlined a lower frequency of *hlyA* (32.6%) and, *cnf* (13.7%) genes among UPEC isolates in comparison with other previous studies in Iran [6]. Cytolethal distending toxin (CDT) was firstly identified from *E. coli* among children with diarrhea [32]. There is, however, scant information on the prevalence of this gene among Extra-intestinal pathogenic *E. coli* (ExPEC). In the current study, our results showed that the occurrence of *cdt* among CTX-M-1-producing UPEC isolates was rare (2.1%), which corroborates the findings of previous studies [28,33]. As for SPATEs, the *sat*, *vat*, and *picU* genes were present in 28.4%, 22.1%, and 8.4% of the UPEC isolates, respectively. To our knowledge, only a few studies have carried out to investigate the prevalence of SPATEs among *E. coli* isolated from patients with UTI. For instance, in a survey conducted by Abe et al., 13.8% of UPEC isolates harbored *picU* gene, which is lower than that of our study [28]. However, Momtaz et al. did not detect *vat* gene among UPEC strains in Iran [30]. By contrast, another report from our country indicated that the frequencies of *sat* and *vat* genes were 75% and 36% in UPEC isolates, respectively [34]. In our study, of six exotoxin genes studied in CTX-M-1-producing UPEC isolates, only the presence of *hlyA* gene was significantly associated with pyelonephritis ($P = 0.003$), which is in accordance with the findings of some previous studies from our country [35,36]. However, further studies with larger sample sizes are needed to clarify the role of different virulence genes in ESBL-producing *E. coli* isolates.

The ExPEC strains primary belong to phylogenetic groups B2 and, to a lesser extent, group D, whereas commensal *E. coli* isolates mainly belong to groups A and B1 [37]. The higher percentage of the

Table 3
Distribution of exotoxin genes among different O- serogroups of CTX-M-1-producing UPEC isolates.

		O-serogroups, No. (%)												
		O1	O2	O4	O6	O7	O12	O15	O16	O18	O25	O75	NT	Total
Exotoxin genes	*cdt*	0 (0)	0 (0)	0 (0)	0 (0)	1 (50)	0 (0)	0 (0)	1 (50)	0 (0)	0 (0)	0 (0)	0 (0)	2 (100)
	cnf	3 (23.1)	0 (0)	0 (0)	1 (7.7)	0 (0)	0 (0)	0 (0)	1 (7.7)	0 (0)	3 (23.1)	5 (38.5)	0 (0)	13 (100)
	vat	5 (23.8)	7 (33.3)	0 (0)	1 (4.8)	2 (9.5)	0 (0)	2 (9.5)	0 (0)	1 (4.8)	0 (0)	3 (14.3)	0 (0)	21 (100)
	sat	6 (22.2)	2 (7.4)	0 (0)	1 (3.7)	1 (3.7)	2 (7.4)	2 (7.4)	0 (0)	2 (7.4)	6 (22.2)	3 (11.1)	2 (7.4)	27 (100)
	hlyA	7 (22.6)	2 (6.5)	5 (16.1)	0 (0)	1 (3.2)	2 (6.5)	1 (3.2)	1 (3.2)	3 (9.7)	6 (19.4)	1 (3.2)	2 (6.5)	31 (100)
	picU	1 (12.5)	2 (25)	0 (0)	1 (12.5)	0 (0)	0 (0)	1 (12.5)	0 (0)	1 (12.5)	0 (0)	0 (0)	2 (25)	8 (100)

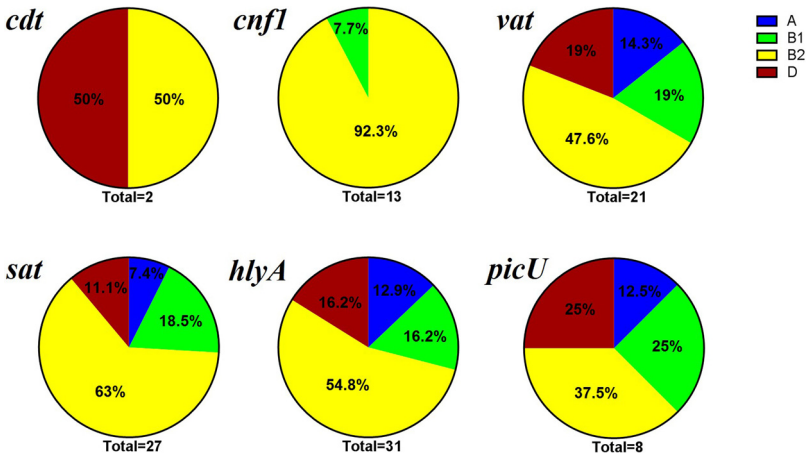

Fig. 2. Distribution of phylogenetic groups in CTX-M-1-producing UPEC isolates according to exotoxin genes.

Table 4
Distribution of phylogenetic groups among exotoxin positive and exotoxin negative UPEC isolates harboring $bla_{CTX\text{-}M\text{-}1}$.

Phylogenetic group	Exotoxin positive isolates, No. (n = 32)	Exotoxin negative isolates, No. (n = 63)	Total, No (%) (n = 95)
A	6	4	10 (10.5)
B1	10	6	16 (16.8)
B2	35	10	45 (47.4)
D	12	12	24 (25.3)

phylogenetic group B2 in our UPEC strains is in agreement with that observed in previous studies by other researchers [21,31,38]. Also, in the current study, most of exotoxin genes were present in significantly higher proportions in phylogenetic group B2 isolates except *cdt* gene ($P < 0.05$). Our results showed that the presence of exotoxins was considerably higher in the B2 phylogroup, followed by D, B1 and A phylogroups (table4). However, no obvious link found between phylogenetic groups and most of the O- serogroups in our isolates. However, the majority of O25 strains belonged to phylogenetic group B2. Moreover, O1 serogroup was mostly detected in B2 phylogroup (p = 0.033), followed by D, B1 and A phylogroups. *E. coli* sequence type 131 (ST131) serotype O25:H4 (B2 phylogroup), which is known as the highly virulent and antimicrobial resistant group worldwide, belongs to ExPEC. It is notable that ST648 was the predominant ST among strains with O1 serogroup and there was a significant relation between this ST and nitrofurantoin resistance. According to our recent previous results, this clone existed among the UPEC isolates as observed by sequencing results [39].

The identification of *E. coli* STs and lineages using MLST (multilocus sequence typing) and predominant phylogroup will helpful for epidemiological studies.

5. Conclusion

In summary, CTX-M1-producing *E. coli* causing UTI is a growing problem and is spreading in Tehran according to this study. This report the first to provide information on the prevalence of O -serogroups, exotoxin genes, and antibiotic susceptibility patterns in UPEC isolates harboring $bla_{CTX\text{-}M\text{-}1}$. We also found that UPEC causing inpatient infections are more likely to carry exotoxin genes, raising the possibility that this increase in virulence properties may result in an increased risk of complicated UTI. Further studies will be necessary to survey the presence of other UPEC virulence factors responsible for UTI and to determine the physiopathology of this infection to consider possible prevention measures.

Acknowledgments

The authors acknowledge the Shariati hospital laboratory for providing the clinical isolates.

References

[1] A.L. Flores-Mireles, J.N. Walker, M. Caparon, S.J. Hultgren, Urinary tract infections: epidemiology, mechanisms of infection and treatment options, Nat. Rev. Microbiol. 13 (5) (2015) 269.
[2] M. Baroud, G.F. Araj, G.M. Matar, Spread of CTX-M-15 extended spectrum β-lactamases encoding genes among Enterobacteriaceae in the Middle Eastern Region, Int. Arab. J. Antimicrob. Agents 1 (1) (2011).
[3] M.N. Moghaddam, M.R. Zolfaghari, N. Tavakoli-Hoseini, CTX-m-15 type β-lactamases from clinical isolates of Escherichia coli by polymerase chain reaction and DNA sequencing, Zahedan J. Res. Med. Sci. 19 (3) (2017).
[4] S.N. Peerayeh, E. Rostami, M. Eslami, M.A. Rezaee, High frequency of extended-spectrum β-Lactamase-Producing Klebsiella pneumoniae and Escherichia coli isolates from male patients' urine, Arch. Clin. Infect. Dis. 11 (2) (2016).
[5] D. Li, B. Liu, M. Chen, D. Guo, X. Guo, F. Liu, L. Feng, L. Wang, A multiplex PCR method to detect 14 Escherichia coli serogroups associated with urinary tract infections, J. Microbiol. Methods 82 (1) (2010) 71–77.
[6] B. Dormanesh, F.S. Dehkordi, S. Hosseini, H. Momtaz, R. Mirnejad, M.J. Hoseini, E. Yahaghi, V. Tarhriz, E.K. Darian, Virulence factors and o-serogroups profiles of uropathogenic Escherichia coli isolated from Iranian pediatric patients, Iran. Red Crescent Med. J. 16 (2) (2014).
[7] S. Sharma, N. Kaur, S. Malhotra, P. Madan, W. Ahmad, C. Hans, Serotyping and antimicrobial susceptibility pattern of Escherichia coli isolates from urinary tract infections in pediatric population in a tertiary care hospital, J. Pathog. 2016 (2016).
[8] H. Mobley, M. Donnenberg, E. Hagan, Uropathogenic Escherichia coli, EcoSal Plus 3 (2) (2009).
[9] J. Bien, O. Sokolova, P. Bozko, Role of uropathogenic Escherichia coli virulence factors in development of urinary tract infection and kidney damage, Int. J. Nephrol. 2012 (2012).
[10] R.A. Welch, Uropathogenic Escherichia coli-associated exotoxins, Microbiol. Spectr. 4 (3) (2016).
[11] Y. Doi, J.M. Adams-Haduch, A. Endimiani, H.E. Sidjabat, S.M. Gaddad, D.L. Paterson, High prevalence of CTX-M-15-producing Klebsiella pneumoniae among inpatients and outpatients with urinary tract infection in Southern India, J. Antimicrob. Chemother. (2008).
[12] P. Wayne, Performance Standards for Antimicrobial Suceptibility Testing Clinical and Laboratory Standards Institute, (2016).
[13] O. Clermont, J.R. Johnson, M. Menard, E. Denamur, 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. 57 (2) (2007) 129–136.
[14] O. Clermont, S. Bonacorsi, E. Bingen, Rapid and simple determination of the Escherichia coli phylogenetic group, Appl. Environ. Microbiol. 66 (10) (2000) 4555–4558.
[15] S.Y. Tartof, O.D. Solberg, A.R. Manges, L.W. Riley, Analysis of a uropathogenic Escherichia coli clonal group by multilocus sequence typing, J. Clin. Microbiol. 43 (12) (2005) 5860–5864.
[16] F. Hitzenbichler, M. Simon, T. Holzmann, M. Iberer, M. Zimmermann, B. Salzberger, F. Hanses, Antibiotic resistance in E. coli isolates from patients with urinary tract infections presenting to the emergency department, Infection (2018) 1–7.
[17] G. Raman, B. McMullan, P. Taylor, K.-A. Mallitt, S.E. Kennedy, Multiresistant E. coli urine infections in children: a case–control study, Arch. Dis. Child. 103 (4) (2018) 336–340.

[18] J.D. Pitout, K.B. Laupland, Extended-spectrum β-lactamase-producing Enterobacteriaceae: an emerging public-health concern, Lancet Infect. Dis. 8 (3) (2008) 159–166.
[19] S.N. Peerayeh, M. Navidinia, F. Fallah, B. Bakhshi, J. Jamali, Pathogenicity determinants and epidemiology of uropathogenic E. coli (UPEC) strains isolated from children with urinary tract infection (UTI) to define distinct pathotypes, Biomed. Res. 29 (10) (2018).
[20] P. Martinez, D. Garzón, S. Mattar, CTX-M-producing Escherichia coli and Klebsiella pneumoniae isolated from community-acquired urinary tract infections in Valledupar, Colombia, Braz. J. Infect. Dis. 16 (5) (2012) 420–425.
[21] E. Ruppé, S. Hem, S. Lath, V. Gautier, F. Ariey, J.-L. Sarthou, D. Monchy, G. Arlet, CTX-M β-lactamases in Escherichia coli from community-acquired urinary tract infections, Cambodia, Emerging Infect. Dis. 15 (5) (2009) 741.
[22] S. Abrar, A. Vajeeha, N. Ul-Ain, S. Riaz, Distribution of CTX-M group I and group III β-lactamases produced by Escherichia coli and klebsiella pneumoniae in Lahore, Pakistan, Microb. Pathog. 103 (2017) 8–12.
[23] I. Sedighi, M.R. Arabestani, A. Rahimbakhsh, Z. Karimitabar, M.Y. Alikhani, Dissemination of extended-spectrum β-Lactamases and quinolone resistance genes among clinical isolates of uropathogenic Escherichia coli in children, Jundishapur J. Microbiol. 8 (7) (2015).
[24] P. Mohajeri, Z. Rostami, A. Farahani, B. Norozi, Distribution of ESBL producing Uropathogenic Escherichia coli and carriage of selected β-lactamase genes in hospital and community isolates in west of Iran, Ann. Trop. Med. Public Health 7 (5) (2014) 219.
[25] S.N. Peerayeh, M. Eslami, M. Memariani, S.D. Siadat, High prevalence of blaCTX-M-1 group extended-spectrum β-lactamase genes in Escherichia coli isolates from Tehran, Jundishapur J. Microbiol. 6 (7) (2013).
[26] H.M. Zowawi, P.N. Harris, M.J. Roberts, P.A. Tambyah, M.A. Schembri, M.D. Pezzani, D.A. Williamson, D.L. Paterson, The emerging threat of multidrug-resistant Gram-negative bacteria in urology, nature reviews, Urology 12 (10) (2015) 570.
[27] A. Iguchi, S. Iyoda, K. Seto, T. Morita-Ishihara, F. Scheutz, M. Ohnishi, Escherichia coli O-genotyping PCR: a comprehensive and practical platform for molecular O serogrouping, J. Clin. Microbiol. 53 (8) (2015) 2427–2432.
[28] C.M. Abe, F.A. Salvador, I.N. Falsetti, M.A. Vieira, J. Blanco, J.E. Blanco, M. Blanco, A.M. Machado, W.P. Elias, R.T. Hernandes, Uropathogenic Escherichia coli (UPEC) strains may carry virulence properties of diarrhoeagenic E. coli, FEMS Immunol. Med. Microbiol. 52 (3) (2008) 397–406.
[29] E. Tajbakhsh, P. Ahmadi, E. Abedpour-Dehkordi, N. Arbab-Soleimani, F. Khamesipour, Biofilm formation, antimicrobial susceptibility, serogroups and virulence genes of uropathogenic E. coli isolated from clinical samples in Iran, Antimicrob. Resist. Infect. Control 5 (1) (2016) 11.
[30] H. Momtaz, A. Karimian, M. Madani, F.S. Dehkordi, R. Ranjbar, M. Sarshar, N. Souod, Uropathogenic Escherichia coli in Iran: serogroup distributions, virulence factors and antimicrobial resistance properties, Ann. Clin. Microbiol. Antimicrob. 12 (1) (2013) 8.
[31] J. Lee, B. Subhadra, Y.J. Son, D. Kim, H. Park, J. Kim, S. Koo, M. Oh, H.J. Kim, C. Choi, Phylogenetic group distributions, virulence factors and antimicrobial resistance properties of uropathogenic Escherichia coli strains isolated from patients with urinary tract infections in South Korea, Lett. Appl. Microbiol. 62 (1) (2016) 84–90.
[32] I. Tóth, F. Hérault, L. Beutin, E. Oswald, Production of cytolethal distending toxins by pathogenic Escherichia coli strains isolated from human and animal sources: establishment of the existence of a new cdt variant (type IV), J. Clin. Microbiol. 41 (9) (2003) 4285–4291.
[33] M. Mirzarazi, S.E. Rezatofighi, M. Pourmahdi, M.R. Mohajeri, Occurrence of genes encoding enterotoxins in uropathogenic Escherichia coli isolates, Braz. J. Microbiol. 46 (1) (2015) 155–159.
[34] J. Saraylu, J. Fallah-Mehrabadi, A. Imani-Fooladi, A. Sabbaghi, H. Molla-Aghamirzaei, M. Hasankhani, Prevalence and evaluation of toxin genes among uropathogenic Escherichia coli clinical isolates by duplex PCR, J. Med. Bacteriol. 1 (1-2) (2015) 17–22.
[35] M. Tabasi, M.R.A. Karam, M. Habibi, E. Mostafavi, S. Bouzari, Genotypic characterization of virulence factors in Escherichia coli isolated from patients with acute cystitis, pyelonephritis and asymptomatic bacteriuria, J. Clin. Diagn. Res.: JCDR 10 (12) (2016) DC01.
[36] F. Firoozeh, M. Saffari, F. Neamati, M. Zibaei, Detection of virulence genes in Escherichia coli isolated from patients with cystitis and pyelonephritis, Int. J. Infect. Dis. 29 (2014) 219–222.
[37] J.R. Johnson, P. Delavari, M. Kuskowski, A.L. Stell, Phylogenetic distribution of extraintestinal virulence-associated traits in Escherichia coli, J. Infect. Dis. 183 (1) (2001) 78–88.
[38] M. Navidinia, S.N. Peerayeh, F. Fallah, B. Bakhshi, R.S. Sajadinia, Phylogenetic grouping and pathotypic comparison of urine and fecal Escherichia coli isolates from children with urinary tract infection, Braz. J. Microbiol. 45 (2) (2014) 509–514.
[39] S.K.S. Mostafavi, S. Najar-Peerayeh, A.M. Mobarez, M.K. Parizi, Characterization of uropathogenic E. coli O25b-B2-ST131, O15: K52: H1, and CGA: neutrophils apoptosis, serum bactericidal assay, biofilm formation, and virulence typing, J. Cell. Physiol. (2019).