Gastroenterology and Hepatology From Bed to Bench.

ORIGINAL ARTICLE

Study on the presence of resistant diarrheagenic pathotypes in *Escherichia coli* isolated from patients with urinary tract infection

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ABSTRACT

Aim: This article aimed to analyze the diarrheagenic potential of *E. coli* isolated from urinary tract infection (UTI) and to recognize the presence of antibiotic resistance genes.

Background: The marked genome plasticity of *Escherichia coli* has allowed the emergence of resistant pathogenic strains displaying an unusual arrangement of genes.

Methods: In this cross-sectional study, 110 *E. coli* were isolated from patients with the symptoms of UTI in Sanandaj, west of Iran between July and September - 2015. The isolates were examined by the disk diffusion method for antibiotic susceptibility test and by polymerase chain reaction for the presence of genes characteristic of diarrheagenic *E. coli* (DEC), Uropathogenic *E. coli* (*UPEC*) virulence genes, extended-spectrum β-lactamase *bla*$_{\text{CTX-M}}$ and plasmid-mediated quinolone resistance determinants, *qnrA*, *qnrB*, and *qnrS*.

Results: The most and the least effective antibiotics were nitrofurantoin and cefotaxime (96.4% and 27.3% sensitivity, respectively). Of the 110 UTI isolates, 57.3% carried diarrheagenic genes. The bundle-forming pilus *bfpA* was the most prevalent diarrheagenic gene (39.1%). The most commonly detected DEC pathotype was enterotoxigenic *E. coli* (-ETEC, 12.7%). All the pathotypes carried the *bla*$_{\text{CTX-M}}$ and *qnr*. The -UPEC *hly* hemolysin and *pap* adhesin genes were mainly detected among ETEC isolates

Conclusion: Our results indicated the presence of resistant diarrheagenic pathotypes in UTI-associated *E. coli*. Such isolates may have the capacity of causing both extraintestinal and intestinal infections. Based on our knowledge, this is the first report of the presence of *qnr* in ETEC from urine.

Keywords: Diarrheagenic *Escherichia coli*, Resistance, Urinary tract infection, Virulence factors.
(Please cite as **Derakhshan S, Farhadifar F, Roshani D, Ahmadi A, Haghi F. Study on the presence of resistant diarrheagenic pathotypes in *Escherichia coli* isolated from patients with urinary tract infection. Gastroenterol Hepatol Bed Bench 2019;12(4):348-357).**

Introduction

Escherichia coli is a commensal inhabitant of the human gastrointestinal tract. However, some strains of *E. coli* can acquire specific virulence factors, taking on a more pathogenic nature. The pathogenic *E. coli* strains are classified as either diarrheagenic *E. coli* (DEC) or extraintestinal *E. coli* (1). DEC is a leading bacterial cause of diarrhea, mostly in children younger than five years. Currently, six major groups of DEC have been recognized: enterotoxigenic *E. coli* (ETEC), enterohemorrhagic *E. coli* (EHEC), enteropathogenic *E. coli* (EPEC), enteroinvasive *E. coli* (EIEC),

Received: 24 July 2019 *Accepted*: 18 September 2019
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enteroaggregative *E. coli* (EAEC) and diffusely adherent *E. coli* (DAEC) (2, 3).

ETEC strains produce a variety of adhesins and secret heat-labile (LT) and/or heat-stable (ST) enterotoxins. EHEC strains secret Shiga toxin (Stx), with receptors found on intestinal cells and in the kidney, which can lead to the hemolytic uremic syndrome. EPEC strains express a type IV pilus, named the bundle-forming pilus, which is involved in adhesion (3). Moreover, EPEC and some EHEC strain express the outer membrane adhesin intimin that promotes actin accumulation in eukaryotic cells. EIEC strains are involved in invasive intestinal infections and dysentery. EAEC strains, which cause persistent diarrhea (> 14 days), produce various types of adhesins, and a heat-stable enterotoxin. The most recently identified pathotype is DAEC; however, there is limited information on pathogenic characteristics of this pathotype (4).

E. coli strains have also been associated with extraintestinal infections, including urinary tract infections (UTIs). The primary causative agent of UTIs is uropathogenic *E. coli* (UPEC). UTI is one of the most common infections in humans and is more common among females. The UPEC strains carry different virulence genes such as adhesins and toxins that contribute to the development of the infectious process (1). Pyelonephritis-associated pilus (Pap), α-hemolysin (HlyA), -afimbrial adhesin (Afa) and cytotoxic necrotizing factor 1 (CNF1) are among the most important virulence factors of *E. coli* causing UTI (5, 6). Pap and Afa adhesins are involved in the tissue damage and development of chronic UTIs. Exotoxins such as HlyA and CNF1 are also implicated in the pathogenesis of UTIs and play a role in the invasion and dissemination of bacteria in the urinary tract (1).

In general, UTIs are caused by strains that are present in the intestinal tract and bear specific virulence genes. If these strains colonize the perineum, they may ascend the urethra and colonize the urinary tract, causing disease. Some UPEC strains have been found to carry characteristic markers of DEC pathotypes, becoming a potential cause of diarrhea. Alternatively, some DEC strains might represent potential uropathogens (4). Antibiotic treatment of bacterial infections plays a vital role in reducing morbidity and mortality; however, misuse and overuse of antibiotics have resulted in increased resistance. Because many patients with gastroenteritis are treated empirically, knowledge regarding the antimicrobial resistance in diarrheagenic pathogens is vital in selecting the most appropriate treatment (7). The spread of resistance among *E. coli* has resulted in limited effects of agents, such as trimethoprim-sulfamethoxazole, and increased reliance on newer broad-spectrum agents, such as fluoroquinolones and extended-spectrum cephalosporins. Unfortunately, the emergence of antibiotic resistance now threatens the use of these newer agents (8, 9). This mainly includes resistance caused by extended-spectrum β-lactamases (ESBLs). ESBLs comprise diverse groups, among which the CTX-M group is now the most prevalent ESBL type in most regions of the world (10). The genes encoding ESBLs are usually located on mobile plasmids that can harbor other resistance genes, such as plasmid-mediated quinolone-resistance (PMQR) genes. The PMQR determinants, termed *qnr*, encode small proteins that protect DNA topoisomerases from quinolones. Three major groups of *qnr* determinants are *qnrA*, *qnrB* and *qnrS*. Exposure to multi-drug resistant pathogenic *E. coli* may result in hard-to-treat infections (11).

In this study, we aimed to 1) determine the presence of four diarrheagenic pathotypes (EAEC, ETEC, EPEC, and EHEC) among 110 *E. coli* isolated from patients with the symptoms of UTI, 2) detect the presence of ESBL $bla_{\text{CTX-M}}$ and *qnr* genes in DEC pathotypes and 3) investigate the distribution of the most important UPEC virulence genes (*hly*, *pap*, *afa*, and *cnf*) among DEC pathotypes.

Methods

Bacterial isolates

In this cross-sectional study, we collected 110 nonduplicates *E. coli* from urine samples of patients with the symptoms of UTI in two teaching affiliated hospitals of Kurdistan University of Medical Sciences in Sanandaj, the center of Kurdistan Province in the west of Iran between July and September 2015. UTI was defined according to the 2015 European Association of Urology guidelines (12). *E. coli* isolates were identified according to the standard tests such as Gram staining, lactose fermentation, motility, indole test, citrate utilization, methyl red, Voges-Proskauer,

Table 1. Primer sequences, annealing temperatures and predicted length of PCR products

Category	Target gene	Primer sequence (5′ to 3′)[a]	Fragment size (bp)	Annealing temperature (°C)	Ref.[b]
DEC type					
ETEC	*eltB*	TCTCTATGTGCATACGGAGC CCATACTGATTGCCGCAAT	322	54	(17)
	estA	GCTAAACAAGTARGGTCTTCAAAA CCCGGTACARGCAGGATTACAACA	147	60	(17)
EPEC	*eaeA*	CTGAACGGCGATTACGCGAA CCAGACGATACGATCCAG	917	55	(18)
	bfpA	TTCTTGGTGCTTGCGTGTCTTTT TTTTGTTTGTTGTATCTTTGTAA	367	50	(17)
EHEC	*stx1*	ATAAATCGCCATTCGTTGACTAC AGAACGCCCACTGAGATCATC	180	62	(18)
	stx2	GGCACTGTCTGAAACTGCTCC TCGCCAGTTATCTGACATTCTG	255	62	(18)
EAEC	pCVD	CTGGCGAAAGACTGTATCAT CAATGTATAGAAATCCGCTGTT	630	56	(17)
	astA	CCATCAACACAGTATATCCGA GGTCGCGAGTGACGGCTTTGT	111	59	(4)
Antibiotic resistance					
CTX-M	bla_{CTX-M}	ATGTGCAGYACCAGTAARGT TGGGTRAARTARGTSACCAGA	593	56	(19)
qnr	*qnrA*	ATTTCTCACGCCAGGATTTG GATCGGCAAAGGTTAGGTCA	516	56	(20)
	qnrB	GATCGTGAAAGCCAGAAAGG ACGATGCCTGGTAGTTGTCC	469	58	(20)
	qnrS	ACGACATTCGTCAACTGCAA TAAATTGGCACCCTGTAGGC	417	56	(20)
Urovirulence factors					
P-fimbriae	*pap*	GACGGCTGTACTGCAGGGTGTGGCG ATATCCTTTCTGCAGGGATGCAATA	328	65	(21)
Afimbrial adhesin	*afa*	GCTGGGCAGCAAACTGATAACTCTC CATCAAGCTGTTTGTTCGTCCGCCG	750	65	(21)
Hemolysin	*hly*	AACAAGGATAAGCACTGTTCTGGCT ACCATATAAGCGGTCATTCCCGTCA	1177	65	(22)
Cytotoxic necrotizing factor 1	*cnf1*	AAGATGGAGTTTCCTATGCAGGAG CATTCAGAGTCCTGCCCTCATTATT	498	65	(22)

[a] R: G/A, S: G/C, Y: C/T; [b] Ref.: References

and lysine decarboxylation (13). All *E. coli* isolates were stored at −70°C in Trypticase soy broth (Q-lab, USA), containing 15% v/v glycerol.

Susceptibility testing

Susceptibility of isolates was determined to 13 antibiotics by the disk diffusion method on Mueller - Hinton agar plates (Q-lab, USA) according to the 2017 Clinical and Laboratory Standards Institute (CLSI) guidelines (14). The following antibiotic disks (Rosco, Denmark) were used: imipenem (IPM) (10 µg), cefotaxime (CTX) (30 µg), ceftazidime (CAZ) (30 µg), amoxicillin/clavulanic acid (AMC) (20/10 µg), aztreonam (AZT) (30 µg), ciprofloxacin (CP) (5 µg), tetracycline (TE) (30 µg), gentamicin (GM) (10 µg), cefepime (FEP) (30 µg), cefoxitin (CFO) (30 µg), nalidixic acid (NA) (30 µg), SXT, and nitrofurantoin (FM) (300 µg).

Briefly, agar plates were inoculated with a standardized inoculum (0.5 McFarland, 1.5 $\times 10^8$ colony-forming unit (Cfu)/ml) of the isolates. Then paper disks containing antimicrobial compounds were placed on the agar surface. The plates were incubated at 35°C for 16-18 h, and then the diameter of inhibition growth zones was measured (14). *E. coli* ATCC 25922 was used as quality control.

Preparation of DNA templates

Total DNA was obtained by the freeze-thaw method (15). Briefly, bacteria were pelleted from an overnight culture, suspended in sterile distilled water, and boiled at 100 °C for 10 min. The suspensions were then immediately placed on ice for 5 min. Samples taken

through a total of three freezing-thawing cycles were centrifuged. The supernatants were collected and after quality check on a 1% agarose gel and quantitative assessment by measuring absorbance at the wavelength of 260 nm and by calculating A260/A280 ratio to determine the purity of DNA (16), were stored at -20 °C as DNA template stocks.

Detection of genes characteristic of diarrheagenic pathotypes of *E. coli*

Polymerase chain reaction (PCR) assays were performed with specific primers (SinaClon, Iran) to detect the following eight genes associated with four DEC pathotypes (EPEC, EHEC, ETEC, and EAEC) (Table 1) (4, 17): *eaeA* (structural gene for intimin of EPEC and EHEC), *bfpA* (structural gene for bundle-forming pilus of EPEC), *stx1* and/or *stx2* (Shiga toxins 1 and 2 of EHEC), *eltB* and/or *estA* (heat-labile (LT) and heat-stable (ST) enterotoxin genes of ETEC), pCVD (the nucleotide sequence of the EcoRI–PstI DNA fragment of pCVD432 of EAEC), and *astA* (heat-stable enterotoxin of EAEC).

The minimum criteria for determination of DEC were defined as follows: the presence of *bfpA* and *eaeA* for typical EPEC (the presence of only *eaeA* for atypical EPEC), the presence of *stx1* and/or *stx2* for EHEC (the additional presence of *eaeA* for a typical EHEC), the presence of *eltB* and/or *estA* for ETEC, and the presence of pCVD for EAEC (17).

PCR was performed using a thermal cycler (Eppendorf, Germany) with the following conditions: initial denaturation of 5 min at 94 °C followed by 35 cycles of denaturation of 1 min at 94 °C, annealing of 1 min at different temperatures (Table 1), extension of 1 min at 72 °C, and a final extension of 7 min at 72 °C.

The following strains were used as controls: EPEC ATCC 43887 (*eaeA*, *bfpA*), ETEC ATCC 35401 (*eltB*, *estA*), EHEC ATCC 43890 (*stx1*, *eaeA*), EHEC ATCC 43889 (*stx2*, *eaeA*), EAEC strain 97R (pCVD), and *E. coli* K-12 (negative control). For the *astA*, clinical *E. coli* isolate containing the target gene was used as positive control.

Detection of *bla*$_{\text{CTX-M}}$ and *qnr* resistance genes

PCR tested the total DNA template for the presence of a *bla*$_{\text{CTX-M}}$ type of ESBLs, *qnrA*, *qnrB*, and *qnrS*. The primers (SinaClon, Iran) and the predicted sizes of amplicons are presented in Table 1.

PCR amplifications were carried out on a thermal cycler (Eppendorf, Germany) under the following conditions: initial denaturation at 94 °C for 5 min and 35 cycles of denaturation at 94 °C for 1 min, annealing for 1 min at different temperatures (Table 1), extension at 72 °C for 1 min, and final extension at 72 °C for 5 min.

Detection of UPEC virulence genes

Specific primers (SinaClon, Iran) were used to amplify the sequences of *pap*, *afa*, *hly*, and *cnf-1* genes. Details of primer sequences and predicted sizes of the amplified products are given in Table 1.

The amplification was performed in a thermal cycler (Eppendorf, Germany) under the following

Table 2. Diarrheagenic profiles of 110 *Escherichia coli* isolated from patients with urinary tract infection

Profile	*stx2*	*stx1*	*bfpA*	*eaeA*	*eltB*	*estA*	pCVD	*astA*	Total strains (%)
DP1	+	-	-	-	+	-	-	-	2 (1.8)
DP2	+	-	+	-	-	-	-	-	3 (2.7)
DP3	+	-	-	-	-	-	-	-	2 (1.8)
DP4	-	-	+	-	-	-	-	-	32 (29.1)
DP5	-	-	+	-	-	-	+	-	1 (0.9)
DP6	-	-	+	-	-	-	-	+	2 (1.8)
DP7	-	-	+	-	+	-	-	-	3 (2.7)
DP8	-	-	+	-	+	-	+	-	1 (0.9)
DP9	-	-	+	+	-	-	-	-	1 (0.9)
DP10	-	-	-	+	-	-	+	-	1 (0.9)
DP11	-	-	-	+	+	-	-	-	2 (1.8)
DP12	-	-	-	+	-	-	-	-	2 (1.8)
DP13	-	-	-	-	+	-	-	-	4 (3.6)
DP14	-	-	-	-	+	-	+	+	1 (0.9)
DP15	-	-	-	-	+	-	+	-	1 (0.9)
DP16	-	-	-	-	-	-	+	-	1 (0.9)
DP17	-	-	-	-	-	-	-	+	4 (3.6)

DP: Diarrheagenic profile, +: Positive, -: Negative

conditions: Initial denaturation at 94°C for 5 min; then 35 cycles of denaturation at 94°C for 1 min, annealing at 65°C for 1 min, and elongation at 72°C for 1 min followed by a final elongation at 72°C for 7 min. Conditions were the same for all the virulence genes.

Separation of PCR products

All PCR assays were performed in a final volume of 25 µL, containing 3 µL DNA template, 1.5 mM MgCl2, 0.2 mM of each dNTP, 1 U Taq DNA polymerase, 1X PCR buffer, and 0.4 µM of each primer (SinaClon, Iran). PCR products were separated by gel electrophoresis on a 1.5% agarose in 0.5X Tris-Borate EDTA (TBE) buffer. A molecular size marker (100-bp Plus DNA ladder, SinaClon, Iran) was included on each gel. The amplified DNA was then stained with safe stain (SinaClon, Iran) and visualized under UV transillumination.

Data analysis

The data were analyzed using SSPS software version 16.0. Pearson Chi-square test and Fisher's exact test were used to determining statistical significance. A P value of < 0.05 was considered significant.

Results

A total of 110 *E. coli* isolates were collected from patients with the symptoms of UTI in Sanandaj, west of Iran. The male to female ratio of the patients was approximately 1:4 (21 males and 89 females), and the average age of the patients was approximately 38 years; the oldest patient was 93 years old (one patient), and three one-year-old was the youngest patients. Seventy-five of the 110 isolates were from outpatients and thus, 35 isolates were from hospitalized patients admitted to different wards including women (n = 13), pediatrics (n = 10), emergency (n = 6), men (n = 4), internal (n= 1), and infectious diseases (n = 1) wards.

Antibiotic susceptibility

Antibiotic susceptibility of the isolates was carried out using different classes of antibiotics. Of the 110 isolates, 96.4% were susceptible to nitrofurantoin (n= 106), 91.8% to cefoxitin (n= 101), 85.4% to imipenem (n = 94), 68.2% to amoxicillin-clavulanic acid (n= 75), 63.6% to aztreonam (n= 70), 62.7% to ceftazidime (n= 69), 57.3% to ciprofloxacin (n = 63), 52.7% to gentamicin (n= 58), 47.3% to nalidixic acid (n= 52), 44.5% to cefepime (n= 49), and 42.7% to tetracycline (n= 47). The least effective antibiotics were trimethoprim/sulfamethoxazole and cefotaxime, with the susceptibility rates of 30.9% (n= 34) and 27.3% (n= 30), respectively.

Detection of DEC pathotypes in urine samples

Among the 110 *E. coli* isolated from UTI, the most commonly detected DEC pathotype was ETEC (n= 14 isolates, 12.7%), followed by EHEC (n= 7, 6.4%; no typical EHEC), EAEC (n= 6, 5.45%), and EPEC (n= 6, 5.45%; 1 typical and 5 atypical isolates).

The heat-labile toxin gene (*eltB*), carried by ETEC, was detected in 14 of the 110 isolates (12.7%). The heat-stable toxin gene (*estA*) was not found. The *stx2* and *stx1* genes carried by EHEC were found in 7 (6.4%) and 0 isolates, respectively. The marker pCVD, associated with EAEC, was found in 6 (5.45%) isolates. The *astA* gene carried primarily by EAEC (but also by other pathotypes) (23) was detected in 7 (6.4%) isolates. From the seven *astA* -positive isolates, only one was EAEC. Among the 110 isolates, 43 isolates (39.1%) carried the EPEC bundle-forming pilus gene *bfpA*, and 6 (5.45%) carried *eaeA*, which codes for intimin.

Eight patients (7.3%) were colonized with more than one DEC pathotype. The following combinations were detected: ETEC + EAEC (n= 3 isolates), ETEC + EHEC (n= 2), ETEC + atypical EPEC (n= 2), and EAEC + atypical EPEC (n= 1).

Sixty- three of the 110 isolates (57.3%) carried at least one of the diarrheagenic genes; thus, 47 isolates (42.7%) did not harbor any of the target genes. No isolate was found to carry more than three genes. A total of 45 isolates (40.9%) carried one gene; 16 isolates (14.5%) two genes; and a combination of three genes was observed in only two isolates (1.8%). Table 2 represents the diarrheagenic profile (DP) of isolates. Seventeen DP were found. The most prevalent profile was DP4, which was found in 32 isolates (29.1%) and characterized by the presence of *bfpA*. The most prevalent combination patterns were *bfpA* - *eltB* and *bfpA* - *stx2* (n= 3 isolates, 2.7%; each).

Distribution of ESBL *bla*$_{\text{CTX-M}}$ and *qnr* genes

Among the 110 isolates, 38 isolates (34.5%) carried *bla*$_{\text{CTX-M}}$, 16 isolates (14.5%) *qnrS* and 3 (2.7%) *qnrB*. The *qnrA* was not found. The prevalence of resistance genes was higher in the 63 isolates carrying diarrheagenic genes than the 47 isolates without these

genes (*bla*$_{CTX-M}$: 38.1% vs. 29.8%; *qnrB*: 4.8% vs. 0%; and *qnrS*: 15.9% vs. 12.8%, respectively), although significant differences were not found.

The *bla*$_{CTX-M}$ was found in EHEC (4 of 7), ETEC (6 of 14), and EAEC and EPEC (2 of 6, each) isolates. The *qnrB* was detected in EHEC and ETEC (n=1, each), and it was not found in EPEC and EAEC. The *qnrS* was found in all the pathotypes (3 ETEC, 1 EAEC, 2 EHEC, and 2 EPEC). One ETEC isolate and 1 EHEC harbored the three resistance genes, simultaneously. Two EPEC, 2 ETEC, 1 EAEC, and 1 EHEC harbored the *qnrS* and *bla*$_{CTX-M,}$ simultaneously.

Of the 13 tested antibiotics, nitrofurantoin was the most effective antibiotic against all the DEC pathotypes (100% sensitivity). Among the individual groups, EHEC isolates were mostly sensitive to amoxicillin/clavulanic acid and imipenem. In EPEC, ETEC, and EAEC isolate, sensitivity was found mostly for cefoxitin and imipenem (Table 3). For all the DEC pathotypes, less sensitivity was observed for trimethoprim-sulfamethoxazole, tetracycline, and cefotaxime. Multidrug resistance (non-susceptibility to at least one agent in three or more antimicrobial categories (24)) was seen in ETEC (12 of 14), EHEC (5 of 7), and EPEC and EAEC (5 of 6, each) isolates.

Distribution of UPEC virulence genes among the DEC isolates

Of the 110 isolates, the *pap* was found in 38 (34.5%) isolates, the *cnf* in 31 (28.2%), the *hly* in 22 (20%), and the *afa* in 11 (10%) isolates. Forty- five isolates (40.9%) were negative for all the UPEC virulence genes. Significant associations were detected

Table 3. Antibiotic susceptibility patterns of diarrheagenic pathotypes of 110 *Escherichia coli* isolated from patients with urinary tract infection

DEC pathotype	Susceptibility pattern[a]
EPEC (n=6)	IPM,FM,AMC,CFO (2 isolates)
	CP,CTX,IPM,CAZ,NA,FM,AZT,CFO
	CP,SXT,GM,IPM,CAZ,FM,AZT,CFO
	CP,GM,CTX,CAZ,NA,FM,FEP,AMC,AZT,CFO
	CP,SXT,GM,TE,IPM,CAZ,NA,FM,FEP,AMC,AZT,CFO
EHEC (n=7)	GM,IPM,FM,AMC
	IPM,FM,AMC,CFO
	CP,TE,FM,AMC,CFO
	CP,GM,IPM,FM,AMC,CFO
	IPM,CAZ,FM,FEP, AMC,AZT
	CP,SXT,TE,IPM,CAZ,NA,FM,FEP,AMC,AZT,CFO
	CP,SXT,GM,TE,IPM,CAZ,NA,FM,AMC,AZT,CFO
EAEC (n=6)	GM,FM,CFO
	CP,IPM,NA,FM,CFO
	CP,SXT,GM,IPM,CAZ,FM,AZT,CFO
	CP,TE,IPM,CAZ,FM,FEP,AMC,AZT,CFO
	CP,GM,TE,IPM,CAZ,FM,FEP,AMC,AZT,CFO
	CP,SXT,CTX,TE,IPM,CAZ,NA,FM,AMC,AZT,CFO
ETEC (n=14)	FM,AZT,CFO
	GM,IPM,FM
	IPM,FM,AMC,CFO
	CP,IPM,NA,FM,CFO
	CP,TE,FM,AMC,CFO
	CP,GM,IPM,FM,AMC,CFO (n=2 isolates)
	CP,CTX,IPM,CAZ,NA,FM,AZT,CFO
	CP,TE,IPM,CAZ,FM,FEP,AMC,AZT,CFO
	CP,GM,CTX,CAZ,NA,FM,FEP,AMC,AZT,CFO
	CP,CTX,IPM,CAZ,NA,FM,FEP,AMC,AZT,CFO
	CP,SXT,GM,IPM,CAZ,FM,FEP,AMC,AZT,CFO
	CP,SXT,CTX,TE,IPM,CAZ,NA,FM,AMC,AZT,CFO
	CP,SXT,GM,CTX,TE,IPM,CAZ,NA,FM,FEP,AMC,AZT,CFO

[a]CP, Ciprofloxacin ; SXT, Trimethoprim-sulfamethoxazole; GM, Gentamicin; CTX, Cefotaxime; TE, Tetracycline; IPM, Imipenem; CAZ, Ceftazidime; NA, Nalidixic acid; FM, Nitrofurantoin; FEP, Cefepime; AMC, Amoxicillin/clavulanic acid; AZT, Aztreonam; CFO, Cefoxitin

between the presence of *hly* and *pap* (P= 0.001), *hly* and *cnf* (P= 0.044), *pap* and *cnf* (P= 0.018), and *pap* and *afa* (P= 0.015).

The prevalence of the studied UPEC genes was higher in the 63 isolates carrying diarrheagenic genes than the 47 isolates without these genes. Of the 63 isolates, 28 (44.4%) carried *pap*, 20 (31.7%) carried *cnf*, 15 (23.8%) *hly*, and 7 (11.1%) *afa*. Significant positive associations were seen between the presence of *pap* and *bfp* (P = 0.034) and *pap* and *astA*(P = 0.007). Eighteen of the 63 isolates (28.6%) did not carry any of the studied urovirulence genes.

Among the toxins, the *hly* was mainly observed in ETEC (4 of 14) isolates. It was also found in EHEC (1 of 7), and EPEC and EAEC (1 of 6, each) isolates. The *cnf* was found in 3 EPEC, 3 ETEC, and 2 EHEC. For the adhesin genes, the *pap* was mainly detected in ETEC (4 of 14), and EHEC (2 of 7) isolates. It was also found in EPEC, and EAEC (1 of 6, each) isolates. The *afa* was found in 2 EHEC, 1 ETEC, 1 EPEC, and 1 EAEC

Discussion

The marked genome plasticity of *E. coli* has allowed the emergence of strains displaying an unusual arrangement of virulence genes. The ability of such strains to cause infection is not only dependent on their virulence traits, but also risk factors, such as age, immunosuppression, high levels of antimicrobials, and long-term indwelling catheterization (23).

In this study, we evaluated the presence of four diarrheagenic pathotypes (EAEC, ETEC, EPEC, and EHEC) in *E. coli* isolated from UTI. Relatively limited data are available regarding the existence of DEC pathotypes in UTI. Most other investigators have found EAEC as the most frequent DEC isolated from UTIs (4, 25, 26). We discovered that ETEC was the most frequent DEC pathotype in our isolates from UTI in Iran and a study in Germany (23) reported three EHEC isolates, one atypical EPEC, and one EAEC among 265 *E. coli* isolated from UTIs. In addition, Khaleque *et al.* found three ETEC isolates, one EHEC, and one EAEC among 56 UPEC strains (27).

To our knowledge, no previous study in Iran has investigated the simultaneous presence of four diarrheagenic pathotypes (EAEC, EPEC, ETEC, and EHEC) in UTI-associated *E. coli* and almost all of them have focused on the presence of some pathotypes especially EHEC in UTI. Navidinia *et al.*, in Iran, investigated the prevalence of EHEC from UTI in children. They found that only 5 (1.3%) of 378 *E. coli* were EHEC according to their PCR results (28). In the study of Salmani *et al.*, out of 100 *E. coli* isolated from UTI in Iran, 22% of the isolates were ETEC, while EPEC was not found (29). In another study from Iran, only 3% of the studied sample were EHEC, and 2% were EPEC (30).

Some of our UTI-associated DEC did not carry any of the four studied urovirulence genes. Although other urovirulence genes may be present in our strains which are not looked at in the present study, several studies have reported the uropathogenic role of diarrheagenic genes. The results of Toval *et al.* indicated that Stxs produced by EHEC could contribute to the uropathogenicity of EHEC (31). Furthermore, the *eae* which codes for adhesin intimin was reported to accumulate actin in kidney cells (4). Although a uropathogenic role for the bundle-forming pilus has not been demonstrated, the widespread presence of the *bfpA* (39.1%) in our UTI isolates may warrant efforts to understand its potential role in UTI. The urovirulence mechanism of ETEC has not been identified. Since adherence to epithelial surfaces is a crucial first step in the development of UTI (25); thus, adhesins of ETEC (32) may contribute to the uropathogenicity. Besides, the *hly* and *pap* were mainly detected among the ETEC. Seven (6.4%) of our isolates carried the *astA*. Some studies have also reported the presence of the *astA* in UTI isolates (23, 33) and its role in UPEC pathogenesis needs to be clarified. Significant associations were found between the presence of *pap – bfp* and *pap - astA* in our study. Taking into account the fact that the gut is the primary source of *E.* coli causing UTI (31), the presence of different adhesins, with affinity to distinct receptors, confers advantages to pathogens (34). Furthermore, the production of α-hemolysin among EAEC has been associated with the development of persistent diarrhea (2).

Patients with UTI need prompt antibacterial treatment, and empirical antibiotic therapy is usually started in patients with suspected UTI (1). However, the presence of DEC pathotypes in UTI isolates may pose a serious clinical challenge. Most notably, antibiotic

therapy for EHEC infections is generally not recommended because antibiotics induce increased levels of Stxs production (35, 36). On the other hand, Stxs contribute to the uropathogenicity of EHEC (31). Also, the presence of resistance genes in UTI-associated DEC isolates means that these strains might play a role in therapeutic failures of both UTI and diarrhea. Among our UTI-associated DEC isolates, less sensitivity was observed for trimethoprim-sulfamethoxazole, cefotaxime, and tetracycline, and this may be due to the widespread use of these antibiotics in the treatment of UTIs (37). Antimicrobial therapy is indicated for diarrhea in a variety of circumstances (10), and in developing countries, where the overuse and misuse of antibiotics are common, the widespread use of antibiotics such as tetracycline and trimethoprim-sulfamethoxazole to treat diarrhea, has resulted in a high level of resistance among DEC (7, 10, 38). Due to the emergence of resistance, effective antibiotics such as quinolones can be used for the treatment of diarrhea (39). However, the first occurrence of *qnrB* and *qnrS* in EHEC and atypical EPEC isolates from diarrhea was reported from Spain in 2016 (39). Moreover, Riveros *et al.* in Peru, reported the presence of *qnr* in EAEC isolated from diarrhea (40). Our study showed the first presence of *qnrB* and *qnrS* in ETEC from UTI. The *qnr* genes are commonly associated with the other genes, such as ESBL $bla_{\text{CTX-M}}$ (11). Although ESBL-positive EHEC still seems to be a rare phenomenon (41), we found the presence of CTX-M in EHEC and also the other DEC pathotypes from UTI, and this may indicate a clinical challenge.

In conclusion, our results indicated ETEC as the most prevalent DEC pathotype in *E. coli* isolated from UTIs in the west of Iran and the presence of resistant diarrheagenic pathotypes. Such isolates may have the capacity of causing both extraintestinal and intestinal infections. Although our study reports the occurrence of resistance genes in the DEC pathotypes isolated from UTI, whether these resistant UTI- associated DEC pathotypes play a role in diarrhea need to be elucidated. The possibility that some resistant UPEC may have acquired DEC markers, or that some resistant DEC may have acquired UPEC properties, might play a significant role in therapeutic failures of human infections. The appropriate use of antimicrobials is essential given the threats posed by antimicrobial resistance because many patients with diarrhea and UTI are treated empirically and antibiotics may not be effective.

Acknowledgment

The authors would like to thank all people who helped in the collection of *E. coli* isolates. This work was supported by a grant [IR.MUK.REC.1395.57] from Social Determinants of Health Research Center, Research Institute for Health Development, Kurdistan University of Medical Sciences, Sanandaj, Iran.

Conflict of interests

The authors declare that they have no conflict of interest.

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