Evaluating the prevalence of plasmid- mediated quinolone resistant and ESBL- production in Uropathogenic Escherichia coli and the commensal gut microbiota in pregnant women: A comparative analysis

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Abstract

Background: ß-lactam and fluoroquinolone antibiotics are frequently prescribed for urinary tract infections (UTIs). This study aimed to determine the prevalence of plasmid-mediated quinolone resistance (PMQR) and extended spectrum β-lactamases (ESBLs) in *E. coli* from UTIs in comparison with the *E. coli* isolates from gut microbiota (fecal flora).

Methods: A total of 54 *E. coli* urine isolates and 54 *E. coli* fecal flora isolates were collected from pregnant women (same host) from April to September 2018. Antimicrobial susceptibility testing was determined by disk diffusion method. ESBLs were detected *via* double-disk test (DDST). ESBL and PMQR-encoding genes were identified, using PCR.

Results: The highest resistance rate was found against nalidixic acid (42 isolates in urinary and 41 in fecal flora isolates) and the lowest resistance rate belonged to levofloxacin (23 isolates) and ofloxacin (25 isolates) in urinary and fecal flora isolates. The most prevalent PMQR genes were *qnrS* (29 isolates in urinary and 34 in fecal flora isolates) followed

by *qnrB, aac (6')- Ib-cr* and *qnrA* in urinary and fecal flora isolates. There was a significant association between *qnrS* gene and bla_{TEM} in urinary and fecal flora isolates.

Conclusions: Resistance to quinolones antibiotics was highest among fecal flora isolates, especially

qnrS among other determinants of the qnr gene. In addition, it seems that high load of PMQR genes in commensal flora has a potential to spread to pathogenic organisms.

Background

Urinary tract infections (UTIs) caused by Escherichia coli strains is referred to as uropathogenic E. coli (UPEC) as the most frequent types of infections amongst women. Around 20–30% of women with a first UTI will have recurring infections [1]. UTIs in pregnancy occurs in almost 8% of pregnant women, and without treatment, UTIs can have severe consequences for the mother as well as the unborn child, including pyelonephritis,, low birth weight, preterm delivery and sepsis [2]. Most recurrences are reinfections, caused by the introduction of a new microorganism from the fecal/perineal flora into the urinary tract. However, the contribiotinal the original strain as part of the intestinal flora might be a possible cause reinfection, even months after the index episode[3]. E. coli as one of the most normal constituent of the intestinal microbiota of humans (E. coli as one of the many species of bacteria in the intestinal microbiota of humans), and the causative E. coli strain can often be detected in the woman's fecal flora during a UTI episode [4]. Developing antimicrobial resistance among UPEC isolates is an important factor, which is related to the pathogenesis and major cause of morbidity and mortality of patients[5]. The most common choice in antibiotic therapies are quinolones and β-lactams. At the present time, fluoroquinolones resistance and extended spectrum β-lactamases (ESBL)-producing Enterobacteriaceae have increased worldwide[6]. Quinolones families are grouped in four generations; for example, nalidixic acid, ciprofloxacin and levofloxacin, which belongs to the first, second and third generation[7]. Quinolones hinder bacterial DNA synthesis is done by inhibiting DNA gyrase enzyme, which leads to the death of bacteria[7]. Mutations in the gyrase (gyrA and gyrB) and topoisomerase IV (parC and parE) genes are the major mechanisms of quinolone resistance[8]. Moreover, plasmid-mediated quinolone resistance (PMQR) have been reported to increase resistance by the DNA gyrase protection proteins (via qnrA, qnrB, qnrC, qnrD and qnrS genes), an aminoglycoside-modifying enzyme [via aac(6')-Ib-cr genes] and efflux pumps (via oqxAB and qepA genes)[9]. On the other hand, multi-drug resistant (MDR) and ESBLs producing isolates of E. coli have been identified worldwide, which has limited the treatment options for MDR isolates[10].

Since several studies showed that the woman's own faecal flora acts as an immediate source of infection, found in UTI [1], Therefore, the aim of this study was to investigate the relationship between E. coli strains that cause urinary tract infection in women, and to determine the dominant faecal flora of the same hosts in antibiotyping by evaluating the prevalence of quinolone-resistant uropathogenic E. coli.

Methods

Study population

From April to September 2018, women who provided urine samples to the laboratory of Hafez hospital affiliated with Shiraz University of Medical Sciences, Iran, for urinalysis and culture for suspected acute cystitis were invited to participate in this study. The study was approved by the Ethics Committee of Shiraz University of Medical Sciences (IR.SUMS.REC. 1398.959). After obtaining written informed consent from each participant, clinical data, and a self-collected rectal swab were collected.

Isolation, identification, and storage of E. coli strains

Bacterial isolates were obtained from urine (54) and fecal (54) samples from pregnant women diagnosed with acute urinary tract infection and confirmed by the clinical laboratory. Concurrent with urine collection and prior to antibiotic administration, a rectal swab with Cary-Blair transport medium (Himedia, India) was collected from each participant. The rectal swab was inoculated onto a MacConkey agar plate and incubated at 35–37ºC for overnight. The typical purple colonies were then streaked on Eosin Methylene Blue (EMB) agar plates and were incubated for 20 h at 37 °C.

Biochemical confirmation of the strains was performed and E. coli was defined as oxidase negative, indole positive, Simon's citrate negative, urease negative and hydrogen sulfide negative[11]. Isolates which exhibited a biochemical profile of E. coli were grown in LB broth (Merck- Germany), and kept as stock in a 20% glycerol solution at -70 °C until used.

Antimicrobial susceptibility testing

The antibiotic susceptibilities of E. coli isolates to 13 antibiotics were determined by the disk diffusion method (modified Kirby-Bauer method) on Mueller Hinton agar (Mast Co, UK) in accordance with the Clinical and Laboratory Standards Institute guidelines using commercial antimicrobial disks[12]. The antibiotics investigated in this study included, β-lactamases (amoxicillin–clavulanic acid), third-generation cephalosporins (ceftazidime and cefotaxime), trimethoprim (trimethoprim-sulfamethoxazole), aminoglycosides (gentamicin and amikacin), quinolones (ciprofloxacin, nalidixic acid, ofloxacin, norfloxacin and levofloxacin), carbapenems (imipenem), and nitrofurantoin (Mast Co, UK). For quality-control E. coli ATCC®25,922™ was used. ESBL-producing isolates were screened by DDST as described elsewhere[13].

Molecular detection of ESBLs and Qnr encoding genes

Genomic bacterial DNA was extracted from bacteria using a commercial DNA extraction kit (AccuPrep® Genomic DNA Extraction Kit, Bioneer, South Korea). All isolates were phenotypically resistant to β-lactams, and screened for carrying different beta-lactamase genes (bla_{TEM}, bla_{SHV}, bla_{CTX}) and also screened for to detect qnr resistance genes including qnrA, qnrB, qnrS and aac(6')- Ib-cr genes. PCR assays were used to reveal the prevalence of these genes, using specific primers [14, 15].

Data analysis

The relationship between demographic characteristics of patients and fluoroquinolones resistance, ESBL production, and PMQR determinants were evaluated by the Chi-square test or Fisher’s exact test. P values of ≤ 0.05 were considered to be statistically significant. The data were analyzed using the Statistical Package for Windows v.22.0 (SPSS Inc., Chicago, IL, USA).

Results

Subject characteristics

The recruited patients were 54 pregnant women. The mean age of the participants was 27.4 ± 3.1(ranging 19–37 years), and all patients were in their first trimester.

Pattern of antimicrobial resistance

Results of antimicrobial resistance have shown among E. coli isolates from urine and fecal flora in Table 1and 2. No significant differences in antimicrobial resistance were observed between the isolates from urine and fecal flora. Amongst all the classes of antibiotics evaluated in urinary and fecal flora isolates, except fluoroquinolons families, the highest and lowest antimicrobial resistance rates were against nalidixic acid (77.8% for UPECs and 76% for fecal flora isolates) and imipenem (11.1% for UPECs and 9.2% for fecal flora isolates). While in fluoroquinolons families the highest resistance rates were found against nalidixic acid(40 isolates in urinary and 36 in fecal flora isolates) and the lowest resistance rates were against levofloxacin (23 isolates) and ofloxacin (25 isolates) in urinary and fecal flora isolates, respectively. The incidence of resistance to other antibiotic in E. coli strains isolated from urinary and fecal flora isolates are shown in Table 1.

Table 1
Distribution of Antibiotic Resistance and ESBLs Producing in E.coli isolates

Antibiotic	Antibiotic of resistance N (%)		
	Total of antibiotic resistant	Sample	
Urine	Fecal Flora		
AUG	76(76.9)	40(74)	36(66.7)
IMP	11(10.1)	6(11.1)	5(9.2)
GEN	14(13)	7(13)	7(13)
AN	45(41.7)	23(42.6)	22(40.7)
CAZ	67(62)	32(59.3)	35(64.8)
CTX	62(57.4)	29(53.7)	33(61.1)
SXT	67(62)	34(63)	33(61)
NA	83(76)	42(77.8)	41(76)
CIP	61(56.5)	31(57.4)	30(55.6)
OFX	51(47.2)	24(44.4)	25(46.2)
LEV	50(46.2)	23(42.5)	27(50)
NOR	53(49)	27(50)	28(51.8)
NI	25(23.1)	14(26)	11(20.4)
ESBLs	50(70.5)	24(44.4)	26(48.1)

AUG : Amoxicillin–clavulanic acid, IMP :Imipenem, GEN=:Gentamicin, AN :Amikacin, CAZ :Ceftazidime, CTX: Cefotaxime, SXT: Trimethoprim-Sulfamethoxazole, NA : Nalidixic acid, CIP :Ciprofloxacin, OFX : Ofloxacin,, LEV :Levofloxacin, NOR: Norfloxacin, NI :Nitrofurantoin

Table 2

Distribution of Antibiotic Resistance, qnr genes and ESBLs among E. coli isolates from urine and fecal flora.

Sample number	Antibiotic													qnr genes				
	AUG	IMP	GEN	AN	CAZ	CTX	SXT	NA	CIP	OFX	LEV	NOR	NI	qnrA	qnrB	qnrS	aac(6')-Ib-cr	ESBLs
1 U	R	R	S	S	R	R	R	R	R	R	R	R	S	N	N	P	N	P
1 F	R	S	S	S	S	S	R	R	R	S	R	S	S	N	N	N	N	N
2 U	S	S	S	S	R	S	R	S	S	R	R	S	S	N	N	P	N	N
2 F	S	S	S	S	R	S	S	S	S	R	R	R	S	N	N	N	N	P
3 U	R	S	S	S	R	R	S	R	R	S	R	R	S	N	P	P	N	P
3 F	R	S	S	S	R	R	R	S	R	S	R	S	S	N	P	P	N	N
4 U	R	R	S	R	R	R	R	R	R	R	S	R	S	N	P	P	N	P
4 F	S	S	S	R	R	R	R	R	R	S	R	R	S	N	P	P	N	P
5 U	R	S	S	R	R	R	S	R	R	R	R	R	R	N	N	N	P	P
5 F	S	S	S	S	R	R	S	R	R	R	R	S	S	N	N	N	N	N
6 U	R	R	R	S	R	R	R	R	R	R	S	R	S	N	N	P	N	P
6 F	R	S	S	S	R	S	R	S	S	S	S	R	S	N	P	P	P	N
7 U	R	S	S	R	S	S	R	R	R	R	S	S	S	N	N	N	N	N
7 F	S	S	S	S	S	R	S	S	S	R	R	R	S	N	N	P	N	N
8 U	R	S	S	S	S	S	R	R	R	R	R	R	R	N	N	N	N	N
8 F	R	S	S	S	R	R	R	R	R	S	S	S	S	P	N	P	N	N
9 U	R	S	S	S	R	S	R	R	S	S	S	S	S	N	P	N	N	N
9 F	R	S	S	R	R	R	R	R	R	R	R	S	S	N	N	P	N	N
10 U	R	S	S	S	R	R	S	R	R	S	R	R	S	P	N	P	N	N
10 F	S	S	S	S	S	S	S	S	S	S	S	R	S	N	N	N	N	N
11 U	R	S	S	S	S	S	R	R	S	R	S	S	S	N	N	N	N	N
11 F	S	S	S	S	S	S	S	S	S	R	R	R	S	N	N	N	N	N
12 U	R	S	S	S	S	S	R	S	S	S	S	R	S	N	N	N	N	N
12 F	R	S	S	S	S	S	R	S	S	S	S	R	S	N	N	N	N	N
13 U	S	S	S	R	S	S	S	S	S	S	R	S	S	N	N	N	N	N
13 F	S	S	S	R	S	S	S	S	S	S	R	S	S	N	N	N	N	N
14 U	R	S	S	S	S	R	S	S	R	R	S	S	S	N	N	P	N	N
14 F	R	S	S	S	S	S	S	R	S	S	S	R	S	N	N	N	N	N
15 U	R	S	S	S	R	R	R	R	R	R	R	R	S	N	N	P	N	P
15 F	S	S	S	S	S	S	S	S	S	R	R	S	S	N	N	N	N	N

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	AUG	IMP	GEN	AN	CAZ	CTX	SXT	NA	CIP	OFX	LEV	NOR	NI	qnrA	qnrB	qnrS	aac(6')-Ib-cr	ESBLs
16 U	R	S	R	S	S	S	R	R	R	S	S	S	S	N	N	P	N	N
16 F	R	S	R	S	S	S	R	R	R	R	R	R	R	N	N	P	N	P
17 U	R	S	R	R	R	R	R	R	R	R	R	R	R	N	P	N	P	P
17 F	R	S	R	R	R	R	R	R	R	R	R	R	R	N	P	P	P	N
18 U	R	S	S	S	R	R	R	S	S	R	S	R	S	N	N	P	N	P
18 F	R	S	S	R	R	R	R	R	R	S	R	R	R	N	N	P	P	P
19 U	R	S	R	R	R	R	R	R	R	S	R	S	R	N	N	N	P	P
19 F	S	S	S	R	S	S	R	R	R	R	R	S	S	N	N	P	N	N
20 U	R	S	S	S	S	S	S	R	R	S	S	S	S	N	N	P	N	N
20 F	R	S	S	R	R	R	R	R	R	R	S	S	R	N	P	P	N	P
21 U	R	R	R	R	R	R	R	R	R	S	S	R	S	N	N	N	N	P
21 F	S	S	S	S	R	R	R	R	R	S	R	R	S	N	N	N	N	P
22 U	R	S	S	S	S	S	S	S	S	R	S	S	S	N	N	N	N	N
22 F	R	S	S	S	S	S	S	S	S	R	S	S	S	N	N	N	N	N
23 U	R	S	S	S	S	S	S	S	S	S	S	R	S	N	N	N	N	N
23 F	S	S	S	R	S	S	S	S	S	R	R	R	S	N	N	N	N	N
24 U	S	S	S	S	R	R	R	R	R	S	S	S	S	N	N	P	N	N
24 F	S	S	S	R	R	R	R	R	S	R	R	S	S	N	N	N	N	P
25 U	S	S	S	S	R	R	R	R	S	R	R	R	S	N	N	P	N	P
25 F	S	S	R	S	R	R	R	R	S	R	R	S	S	N	P	P	P	N
26 U	S	S	S	S	R	R	R	R	S	S	S	R	S	N	N	P	N	P
26 F	S	S	S	S	R	R	R	R	R	S	R	S	S	N	N	P	N	N
27 U	R	S	S	S	S	S	R	R	S	S	R	S	S	N	N	P	N	N
27 F	S	R	S	S	S	S	R	S	S	S	R	R	S	N	N	P	N	N
28 U	S	S	S	S	S	S	R	R	R	S	R	R	S	N	N	N	N	N
28 F	R	S	S	R	R	R	R	R	S	R	R	R	S	N	N	P	P	P
29 U	R	S	S	S	R	S	R	R	R	R	S	R	S	N	N	N	N	N
29 F	S	S	S	S	R	R	R	R	R	R	R	S	R	N	P	P	N	P
30 U	R	S	S	R	S	S	R	R	R	R	S	S	S	N	N	N	N	P
30F	R	R	S	S	S	S	R	S	S	S	S	S	S	N	N	N	N	N
31U	R	S	S	R	S	S	S	R	R	S	S	S	S	N	N	P	N	N
31 F	S	S	S	S	R	R	S	R	R	R	S	R	S	N	P	P	N	P
32 U	R	S	S	S	S	S	S	S	S	S	R	S	S	N	N	N	N	N
32 F	R	S	S	S	R	R	S	R	S	S	R	S	R	N	N	N	N	P
33 U	S	S	S	R	S	S	S	R	S	R	S	S	R	N	N	P	N	N
33 F	R	S	S	R	S	R	S	R	R	S	S	R	R	N	P	P	N	N

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	AUG	IMP	GEN	AN	CAZ	CTX	SXT	NA	CIP	OFX	LEV	NOR	NI	qnrA	qnrB	qnrS	aac(6')-Ib-cr	ESBLs
34 U	R	S	S	R	R	R	S	R	S	R	R	R	R	N	N	P	N	P
34 F	R	S	S	R	R	R	S	R	S	R	R	R	R	P	P	P	N	P
35 U	R	S	R	R	R	R	S	R	R	S	S	R	S	P	N	P	N	P
35 F	R	S	R	R	R	R	S	R	R	S	S	R	S	N	N	P	N	P
36 U	R	R	R	R	R	R	R	R	R	R	R	R	S	N	N	N	P	P
36 F	R	S	S	R	R	R	R	R	R	R	S	S	R	N	P	P	P	P
37 U	R	S	S	R	R	R	R	R	R	S	S	S	R	N	N	P	N	N
37 F	R	S	S	R	R	R	R	R	R	S	R	S	S	N	N	P	N	P
38 U	R	S	S	R	R	R	R	R	R	R	R	R	S	N	N	P	N	P
38 F	R	S	S	R	R	R	R	R	R	S	S	R	S	N	P	P	N	N
39 U	R	S	S	R	R	R	R	R	R	S	S	S	R	N	N	P	N	P
39 F	R	S	S	S	R	S	R	R	R	R	R	S	S	N	N	P	N	P
40 U	R	S	S	R	S	R	S	R	S	R	R	S	R	N	P	P	N	N
40 F	R	S	S	R	S	S	S	R	S	S	S	S	S	N	N	P	N	N
41 U	S	S	S	R	R	R	R	R	R	R	R	S	R	N	N	P	N	P
41 F	R	R	S	S	S	R	R	R	R	S	S	S	S	N	N	P	N	N
42 U	R	S	S	R	R	R	R	R	R	S	S	R	R	N	N	N	N	N
42 F	R	S	S	S	S	S	S	R	R	S	S	S	R	N	N	N	N	N
43 U	R	S	S	S	R	S	R	R	S	S	R	S	R	N	N	N	N	P
43 F	S	R	R	R	R	S	R	R	S	R	S	R	S	N	N	N	N	P
44 U	S	S	S	S	S	S	S	S	S	R	S	R	R	N	N	N	N	N
44 F	R	S	S	R	R	R	S	R	R	S	S	R	R	N	N	P	N	P
45 U	R	S	S	R	S	S	S	S	S	S	S	S	S	N	N	N	N	N
45 F	R	S	R	R	R	R	R	R	S	R	S	R	S	N	P	N	N	P
46 U	R	S	S	R	R	R	R	R	R	S	S	R	S	N	N	P	N	N
46 F	R	S	S	S	S	S	R	R	R	S	S	S	S	N	N	P	N	N
47 U	R	S	S	S	R	R	R	R	S	R	R	S	S	N	N	P	N	P
47 F	R	S	S	S	R	R	R	R	S	R	R	S	S	N	N	P	N	P
48 U	S	S	S	R	R	R	R	R	R	S	S	S	S	N	P	P	N	P
48 F	R	S	S	S	R	R	S	R	S	S	S	R	S	N	N	P	N	P
49 U	R	S	S	S	R	S	S	R	R	S	S	S	S	N	P	P	N	P
49 F	R	R	S	S	R	R	S	R	R	R	S	S	S	N	P	P	N	P
50 U	S	S	S	R	R	R	S	R	S	S	S	S	R	N	P	N	N	P
50 F	R	S	S	S	R	S	R	R	R	R	S	R	S	N	P	P	N	P
51 U	S	S	S	S	S	S	S	S	S	S	R	S	S	N	N	P	N	N
51 F	R	S	S	S	R	S	S	R	R	S	S	R	S	N	N	N	N	N

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52 F	S	R	S	S	R	R	R	R	R	S	S	S	S	N	N	P	N	N
53 U	S	S	S	S	S	S	S	R	S	S	R	R	S	N	N	N	N	N
53 F	R	S	S	S	R	R	R	R	S	R	S	R	S	N	N	N	N	P
54 U	R	S	S	S	S	S	R	S	S	S	S	S	S	N	N	N	N	N
54 F	R	S	R	R	R	R	R	R	R	S	S	R	S	N	N	P	N	N

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The ESBLs were phenotypically detected in 24 and 26 isolates of urine and fecal flora samples, respectively. bla_{TEM-1} (28 Urine, 29Fecal) was the most frequent ESBL gene in tested isolates followed by bla_{CTX-M} (21 Urine,22 Fecal) bla_{OXA} (Urine:3, Fecal:7) and bla_{SHV} (Urine:2,Fecal:2) in urinary and fecal flora isolates, respectively. Remarkably, 35.1% of ESBL-producing isolates were isolated from pregnant women who had recurrent UTI more than two cycles in past last 6 months.

We observed a high-level resistance to all tested quinolones in ESBL-producing isolates in comparison with non-ESBL-producing isolates (Table 3). High rate of quinolone antibiotic resistance was observed against nalidixic acid and ciprofloxacin in urinary and fecal flora isolates, respectively. Based on our statistical analysis, in fecal flora samples ESBL producing isolates showed significant relationships to resistance to nalidixic acid and ofloxacin in ($P < 0.05$); however, in urinary samples the relationship was only significant with nalidixic acid ($P = 0.003$). Also the results revealed that all ESBL-producing isolates exhibited resistance to amoxicillin–clavulanic acid and trimethoprim-sulfamethoxazole.

Table 3
Distribution of Quinolones antibiotic resistance among ESBLs producing isolates

	Urine				Fecal			
Antibiotic	Pattern	ESBL Positive N = 24	ESBL Negative N = 30	P-value	Pattern	ESBL Positive N = 26	ESBL Negative N = 28	P-value
Nalidixic acid	R: 42 S: 12	23 1	19 11	0.003	R: 41 S: 13	25 1	16 12	0.0008
Ciprofloxacin	R: 31 S: 23	17 7	14 16	0.07	R: 30 S: 24	15 11	15 13	0.7
Ofloxacin	R: 24 S: 30	14 10	10 20	0.06	R: 25 S: 29	16 10	9 19	0.03
Levofloxacin	R: 23 S: 31	13 11	10 20	0.12	R: 27 S: 27	14 12	13 15	0.5
Norfloxacin	R: 27 S: 27	15 9	12 18	0.1	R: 28 S: 26	15 13	13 13	0.7

Twenty six (89.6%) and thirty (93.75%) of the 29 and 32 fluoroquinolone-resistant isolates were positive for at least 1 PMQR gene. Molecular evaluation showed that the most prevalent PMQR gene family from fecal flora and urine samples were as follows: qnrS, qnrB, aac (6')- Ib-cr and qnrA(53.7%, 15%, 7.4%, 3.7% for UPECs and 63%, 27.7%, 13%, 5.5% for fecal flora isolates) respectively. Meanwhile, distribution of qnr genes among quinolone resistance isolates is shown in Table 4, 5.In addition, among PMQR genes, aac(6')-Ib-cr gene only was significantly related to the activity of ESBLs in urine samples ($P \leq 0.05$).

Table 4
Distribution of qnr genes in relation with quinolone resistance in urinary samples

Antibiotic	Pattern	Genes							
qnrS	P-value	qnrB	P-value	qnrA	P-value	aac(6')- Ib-cr	P-value		
Positive No (%)		Positive No (%)		Positive No (%)		Positive No (%)			
Nalidixic acid	R: 42 S: 12	26 (61.9) 3 (27.3)	0.45	8 (19) 0 (0)	0.07	2 (4.8) 0 (0)	0.47	4 (9.5) 0 (0)	0.5
Ciprofloxacin	R: 31 S: 23	20 (64.5) 9 (39.1)	0.45	5 (16.1) 3 (13)	0.46	2 (6.5) 0 (0)	0.6	4 (12.9) 0 (0)	0.2
Ofloxacin	R: 24 S: 30	13 (54.2) 16 (56)	0.54	5 (20.8) 3 (10)	0.38	2 (8.3) 0 (0)	0.5	3 (12.5) 1 (3.3)	0.53
Levofloxacin	R: 23 S: 31	14 (60.9) 15 (48.3)	0.42	6 (26.1) 2 (6.4)	0.63	2 (8.7) 0 (0)	0.7	3 (13) 1 (3.2)	0.21
Norfloxacin	R: 27 S: 27	15 (55.5) 14 (51.9)	0.57	8 (29.6) 0 (0)	0.2	2 (7.4) 0 (0)	0.6	4 (14.9) 0 (0)	0.53

Discussion

In this study each participant was allowed to serve as her own control; thus, avoiding the probability of between-population differences that might lead to comparisons of UTI isolates with fecal isolates from healthy hosts. This is an exceptionally powerful method to remove the effects of known or unrecognized potential confounders, such as behavioral, environmental, physiological, or genetic differences between infected and uninfected hosts. In this study, we assessed 54 pregnant women with recurrent UTI in their first trimester. We compared E. coli isolated urine and fecal flora samples in terms of quinolone-resistant and ESBL, and we also investigated whether recurrence could be predicted by the characteristics of the E. coli fecal flora.

The results of our study indicate that the prevalence of antibiotic resistance in UPEC in comparison with fecal flora samples is close to each other. In both groups the most effective antibiotic against E. coli isolates was imipenem. And the high incidence of resistance was observed for nalidixic acid resistance (77.8% for UPECs and 76% for fecal flora isolates). These data are in agreement with the results of Dellgren et al.[16] Sweden and Bahadori1 et al. [11, 17] in Iran. Hence, nalidixic acid has been widely used to treat acute lower UTI, which has become nearly ineffective to treat UTI in our country. Also, more than 50% of the isolates were insensitive to amoxicillin–clavulanic acid and co-trimoxazole in both groups. This finding is in line with an earlier study in Iran[18].

According to the findings, the ESBL-producing phenotype were detected in 44.4% (24/54) of the urinary isolates, which was slightly lower than a fecal flora E. coli isolates 48.1%(26/54). The incidence of ESBL phenotype might vary across geographical regions with low rates of about 1.5% reported in Denmark[19] and 5% in Canada [20] compared to much higher prevalence rates documented in other countries[21, 22]. In the current study, the presence and identification of ESBLs genes were determined by multiplex PCR. Our statistics showed that bla_{TEM-1} was the most prevalent ESBL gene followed by bla_{CTX-M} and bla_{SHV} in both groups. In comparison to a similar study conducted in Kerman, the most frequent ESBL gene was reported to be bla_{CTX-M}, bla_{TEM-1}, and bla_{SHV} genes, respectively[23]. Frequently, ESBL producers are resistant to other antibiotics, such as fluoroquinolones[6]. Based on the literature, fluoroquinolone resistance rate is ever increasing, even more than 50%, raising serious concerns in Iran and other parts of the world[24, 25]. In the present study, nearly half of the isolates were resistant to fluoroquinolones in UPEC and fecal flora samples (Table 2). In this study, higher resistance rate of strains against nalidixic acid and ciprofloxacin in UPEC and fecal flora samples are very close to the other findings of a recent research [26].

Table 5
Distribution of qnr genes in relation with quinolone resistance in fecal flora samples

Genes Antibiotic	Pattern	qnrS	P-value	qnrB	P-value	qnrA	P-value	aac(6')- Ib-cr	P-value
		Positive No.(%)		Positive No.(%)		Positive No.(%)		Positive No.(%)	
Nalidixic acid	R:41 S:13	30 (73.1) 4 (30.7)	0.45	13 (31.7) 2 (15.4)	0.07	3 (7.3) 0 (0)	0.47	6 (14.6) 1 (7.7)	0.5
Ciprofloxacin	R:30 S:24	25 (83.3) 9 (37.5)	0.45	11 (36.7) 4 (16.7)	0.46	2 (6.7) 1 (4.2)	0.6	7 (23.3) 0 (0)	0.2
Ofloxacin	R:25 S:29	15 (57.7) 19 (67.9)	0.54	9 (34.6) 6 (21.4)	0.38	3 (12) 0 (0)	0.5	6 (24) 1 (3.4)	0.53
Levofloxacin	R:27 S:27	16 (59.3) 18 (66.7)	0.42	10 (37) 5 (18.5)	0.63	2 (7.4) 1 (3.7)	0.7	5 (18.5) 2 (7.4)	0.21
Norfloxacin	R: 28 S:26	17 (65.4) 17 (60.7)	0.57	9 (32.1) 6 (23.1)	0.2	2 (7.1) 1 (3.9)	0.6	5 (17.9) 2 (7.7)	0.53

Our results showed that resistance to the tested fluoroquinolones in ESBL-producing isolates was significantly higher than in non-ESBL-producing isolates. Although some studies reported that there was no significant association between resistance to fluoroquinolone and ESBL-producing isolates[27, 28], our results revealed that there was a significant relationship between resistance to nalidixic acid and ofloxacin in ESBL-producing isolates in fecal flora and resistance to nalidixic acid in ESBL-producing isolates in UPEC samples. Co-resistance to beta-lactams and fluoroquinolones can be related to the presence of ESBL and some of the quinolone-resistant genes in the same mobile genetic elements[29].

The qnrS (53.7% in urinary and 63% in fecal flora isolates) was the most prevalent PMQR gene in this study, which is in agreement with previous reports [30, 31]. Meanwhile, significant association was only observed between the presence of qnrS genes and nalidixic acid and ciprofloxacin resistance in fecal flora samples. In contrast, qnrA, qnrB and aac(6')-Ib-cr were detected at low frequency. The rate of qnrB was more frequently was expressed in fecal flora (27.7%) in comparison with UPEC (15%) samples. It seems that the Qnr family can create a favorable condition for quinolone resistance. In addition, point mutations in the gyrA and parC genes might play a role as a principal mechanism of fluoroquinolones resistance [32]. Our data indicated that qnrS, qnrB and aac(6')-Ib-cr gene was detected in significant proportion of the ESBL-producing in UPEC and fecal flora samples. However, no significant association was observed between the presence of qnr genes and ESBL-producing isolates, but it was detected only between aac(6')-Ib-cr gene and ESBL-producing in UPEC samples (P = 0.034). Interestingly, at least 1 ESBL was detected in PMQR-positive isolates. Several previous studies reported a high prevalence of qnr genes among ESBL-producing isolates[33, 34].

Many studies have revealed that urine isolates collectively differed dramatically from fecal flora isolates, concerning antibiotic resistance content profiles, suggesting an increased resistance potential of the urine isolates[35]. In this study, comparing the results revealed a significant diversity in phenotypic drug sensitivity profiles as well as the distribution of ESBL-encoding genes and PMQR genes in urinary and fecal flora isolates. Nevertheless, phenotypic drug sensitivity profiles in 7 patients were similar to each other, and only four out of the 7 isolates had completely identical antibiotic resistance profiles and genotypes (Table 2).

In our search, a distinct difference between UPEC and commensal E. coli isolates was observed, with respect to their resistance pattern and distribution in qnr and ESBL genes. However, it is reasonable to say that UPEC and commensal E. coli isolates might have similar characteristics for adapting to an extraintestinal lifestyle, which in turn allows commensal E. coli to cause extraintestinal infections in humans as well as UPEC. As previously mentioned, commensal E. coli can serve as reservoirs of virulence and resistance genes for human pathogenesis.

Abbreviations

AUG : Amoxicillin–clavulanic acid, IMP :Imipenem, GEN::Gentamicin, AN :Amikacin, CAZ :Ceftazidime, CTX: Cefotaxime, SXT: Trimethoprim-Sulfamethoxazole, NA : Nalidixic acid, CIP :Ciprofloxacin, OFX : Ofloxacin,, LEV :Levofloxacin, NOR: Norfloxacin, NI: Nitrofurantoin, DDST: Double disk synergism test; ESBL: Extended-spectrumβ-lactamase; No: Number; PMQR: Plasmid-mediated quinolone resistance; qnr: Quinolone resistance gene; R: Resistant; S:Sensetive, UTIs: Urinary tract infections, UPEC: uropathogenic E. coli; E. coli: Escherichia coli; MDR :multi-drug resistant ,CLSI: Clinical and Laboratory Standards Institute guidelines, EMB: Eosin Methylene Blue ,

Declarations

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Author contributions

Authors' contributions ZH and SM conceived the project and designed the experiments. SKH and MH designed and collected samples. ZH and SM, analyzed the data.
ZH and SM supervised the collection of the samples. ZH and SM
wrote the manuscript. All authors reviewed and approved the manuscript

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All data associated with this manuscript is inclusive in this paper.

Ethics approval and consent to participate

This study was in accordance with the declaration of Helsinki and an ethical permission was sought from the institutional Ethics Committee of Shiraz University of Medical Sciences (Approval No. IR. SUMS. REC. 1398. 959). The informed consent was obtained from all the participants, and informed consent obtained was written.

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