

The prevalence of plasmid-mediated quinolone resistance and ESBL-production in *Enterobacteriaceae* isolated from urinary tract infections

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Introduction: β -lactam and fluoroquinolone antibiotics are usually used for the treatment of urinary tract infections (UTIs). The aim of this study was to determine the prevalence of plasmid-mediated quinolone resistance (PMQR) and extended spectrum β -lactamases (ESBLs) in *Enterobacteriaceae* isolated from UTIs.

Materials and methods: Two hundred and nineteen samples of *Enterobacteriaceae* isolated from UTIs were collected in the Northwest of Iran. Antimicrobial susceptibility testing was determined by the disk diffusion method. ESBLs were detected by the double-disk test. ESBL and PMQR-encoding genes were screened using the polymerase chain reaction.

Results: The rate of resistance to moxifloxacin, nalidixic acid, gatifloxacin, ofloxacin, ciprofloxacin, and levofloxacin in ESBL-producing isolates was 89.3%, 88%, 84%, 80%, 78.7%, and 73.3%, respectively. PMQR-producing *Enterobacteriaceae* isolates were identified in 67 samples (89.1%). The most prevalent PMQR genes were *aac(6′)-Ib-cr* 120 (68.6%) followed by *oqxB* 72 (41.1%), *oqxA* 59 (33.7%), *qnrB* 36 (20.6%), *qnrS* 33 (18.9%), *qnrD* 19 (10.9%), *qepA* 13 (7.4%), *qnrA* 10 (5.7%), and *qnrC* 9 (5.1%). There was a strong association between PMQR genes and *bla*_{CTX-M-15} and *bla*_{TEM-116} and other ESBL genes.

Conclusion: High resistance rates were detected to quinolones among ESBL-producing isolates from UTIs. There is a high prevalence of PMQR genes in *Enterobacteriaceae* in Azerbaijan and Iran, and the most common PMQR is *aac(6′)-Ib-cr*. There is a significant association between PMQR and ESBL-producing isolates.

Keywords: *Enterobacteriaceae*, ESBLs, plasmid-mediated quinolone resistance, urinary tract infections

Introduction

Urinary tract infections (UTIs) are the most common infections around the world. It is estimated that 150 million UTIs occur each year worldwide, with about 70%–80% of uncomplicated UTIs caused by *Escherichia coli*.¹ Drugs commonly recommended for simple UTIs include cotrimoxazole, nitrofurantoin, cephalexin, and ceftriaxone. The fluoroquinolones, such as ciprofloxacin and levofloxacin are commonly recommended for complicated UTIs. However, β -lactam and fluoroquinolone antibiotics have been used for the treatment of UTIs.²

On the other hand, fluoroquinolones resistance and extended spectrum β -lactamases (ESBL)-producing *Enterobacteriaceae* have increased worldwide.³ The most important

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mechanism of quinolone resistance is chromosomal mutations in the quinolone resistance-determining region of genes encoding DNA gyrase (*gyrA* and *gyrB*) and topoisomerase IV (*parC* and *parE*) genes.⁴ In addition, plasmid-mediated quinolone resistance (PMQR) determinants have been reported. PMQR include Qnr (quinolone resistance) proteins (*qnrA*, *qnrB*, *qnrC*, *qnrD*, and *qnrS*), which protect the DNA gyrase and topoisomerase IV from quinolone inhibition, *aac* (6′)-*Ib-cr* (aminoglycoside acetyltransferase variant), which acetylates aminoglycoside, ciprofloxacin, and norfloxacin and reduces their activity. Additionally, *oqxAB* and *qepA* are plasmid-mediated efflux pumps.⁵ Although the PMQR determinants lead to low-level quinolone resistance, they facilitate the chromosome-encoded quinolone resistance.⁶ ESBL-producing *Enterobacteriaceae* has emerged as multidrug-resistant (MDR), especially resistant to trimethoprim/sulfamethoxazole, aminoglycosides, and fluoroquinolones.⁷

PMQR genes are often on the same plasmid as the ESBL genes.⁸ Resistance plasmids with genes encoding ESBLs can be transferred by the conjugation that helps dissemination of PMQR determinants in different *Enterobacteriaceae* species.⁹ Due to MDR establishment, co-existence of ESBLs and PMQR genes are a major concern. The infections caused by these MDR isolates are associated with high public health costs, therapeutic failures, restriction of the antibacterial agents choice, increased duration of hospitalization, rising morbidity, and mortality.⁷

On the other hand, *Enterobacteriaceae* is the most common cause of UTIs, and MDR in *Enterobacteriaceae* is a serious threat to community health as it limits the selection of antibiotics for the empirical treatment of UTIs caused by *Enterobacteriaceae*.

There are few studies regarding co-resistance of β -lactamas and quinolones in *Enterobacteriaceae* isolated from UTIs in Iran.¹⁰ Emergence of ESBLs and PMQR have lead to MDR *Enterobacteriaceae*, which is a serious hazard for community health. The aim of this study was to investigate the prevalence of PMQR and ESBLs determinants in *Enterobacteriaceae* isolated from UTIs in Azerbaijan and Iran.

Materials and methods

Bacterial isolates

This prospective study was conducted in the Department of Microbiology, Tabriz University of Medical Sciences, Iran, from December 2015 until August 2016. All patients were from the Azerbaijan and Iran. Urine samples were collected from inpatients and outpatients suspected of having a UTI, who had not received antibiotics within the previous

2 months. The method of samples collection was simple random sampling. Urine was collected in adult patients by clean-catch midstream and children aged <3 years were sampled using a sterile urine bag or suprapubic catheter.

All urine samples were inoculated on blood agar as well as MacConkey agar. A specimen was considered positive for UTIs if a single microorganism was cultured at a count of 10^5 cfu/mL and was included in this study. Two hundred and nineteen isolates of *Enterobacteriaceae* causing UTIs were isolated. *Enterobacteriaceae* was identified by the conventional biochemical tests and standard culture methods.

The local ethics committee, Tabriz University of Medical Sciences, approved this project, number 5/4/10393, and the participants provided written informed consent.

Antimicrobial susceptibility testing

The antibiotic susceptibility profile was determined on Muller–Hinton agar (Merck, Munchen, Germany) plates by the disk diffusion method (the modified Kirby–Bauer assay) as described by the Clinical and Laboratory Standards Institute (CLSI).¹¹ The used disks were amoxicillin–clavulanic acid (20/10 μ g), ampicillin (10 μ g), cefotaxime (30 μ g), ceftazidime (30 μ g), cefepime (30 μ g), cefuroxime (30 μ g), imipenem (10 μ g), aztreonam (30 μ g), gentamicin (10 μ g), amikacin (30 μ g), trimethoprim–sulfamethoxazole (30 μ g), nitrofurantoin (300 μ g), ciprofloxacin (5 μ g), nalidixic acid (30 μ g), levofloxacin (5 μ g), gatifloxacin (5 μ g), ofloxacin (5 μ g), and moxifloxacin (5 μ g). All the disks were obtained from MAST Company, Bootle, UK. The minimum inhibitory concentrations (MICs) of nalidixic acid, ciprofloxacin, and levofloxacin were determined using the agar dilution method and interpreted according to the guidelines of the CLSI.¹¹ *E. coli* American Type Culture Collection (ATCC) 25922 was used as a quality control strain.

ESBLs detection

The initial screening test to detect ESBL activity was carried out by the disk diffusion method according to the CLSI guidelines. Inhibition zone size of ≤ 22 mm with ceftazidime (30 μ g), ≤ 27 mm with cefotaxime (30 μ g), suggested ESBL production. The phenotypic confirmatory test for ESBL was done by double disk synergy using cefotaxime (30 μ g) and ceftazidime (30 μ g) alone and in combination with clavulanic acid (10 μ g). ESBL activities were identified by zone diameter increase of ≥ 5 mm around the disk with the antibiotic in combination with clavulanic acid.¹¹ *E. coli* ATCC 25922 and *Klebsiella pneumoniae* ATCC

700603 were used as the ESBL-positive and negative control strains, respectively.

Molecular detection of ESBLs

All isolates that were phenotypically resistant to β -lactams were screened for ESBL genes by the polymerase chain reaction (PCR) and sequencing of relevant encoding genes, including *bla*_{SHV}, *bla*_{CTX-M}, and *bla*_{TEM}. The multiplex PCR assays were used as described by Dallenne et al.¹² The QIA-quick PCR Purification Kit (Qiagen, Hilden, Germany) was used for amplified PCR products and sequencing of both strands was conducted using an ABI 3730XL DNA Analyzer. Each sequence was compared with known β -lactamase gene sequences using the multiple-sequence alignment of the Basic Local Alignment Search Tool program.

Detection of PMQR genes

All phenotypically fluoroquinolone-resistant isolates were screened by PCR for detection of *qnrA*, *qnrB*, *qnrC*, *qnrD*, *qnrS*, *aac(6')-Ib-cr*, *oqxAB*, and *qepA* genes.^{13,14}

Statistical analysis

The relationships between demographic characteristics of the 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 statistically significant. The data were analyzed using the Statistical Package for Windows v.19.0 (SPSS Inc., Chicago, IL, USA).

Results

The patients and bacteria

The mean age of patients was 50 ± 31 years (range, 1–93 years), and included 78 (35.6%) males and 141 (64.4%) females. Two hundred and nineteen samples of *Enterobacteriaceae* from urine specimens in different wards of the hospital (internal 135 (61.6%), surgery 47 (21.5%), intensive care unit (ICU) 22 (10%), and pediatrics 15 (6.8%)) were collected. *E. coli* was the most common isolate at 177 (80.8%), followed by *K. pneumoniae* 28 (12.8%), *Enterobacter cloacae* 7 (3.2%), *Proteus mirabilis* 2 (0.9%), *Morganella morganii* 2 (0.9%), *Proteus vulgaris* 1 (0.5%), *Citrobacter freundii* 1 (0.5%), and *Klebsiella oxytoca* 1 (0.5%).

Susceptibility testing

Total resistance rate of *Enterobacteriaceae* to antimicrobial agents was as follows: ampicillin 189 (86.3%), ceftazidime 174 (79.4%), nalidixic acid 150 (68.5%), moxifloxacin 143 (65.3%), trimethoprim–sulfamethoxazole 140 (63.9%), gatifloxacin 131 (59.8%), ofloxacin 130 (59.4%), ciprofloxacin 126 (57.5%), cefuroxime 117 (53.4%), levofloxacin 115 (52.5%), aztreonam 103 (47.1%), cefepime 79 (36.1%), gentamicin 77 (35.1%), nitrofurantoin 57 (26.1%), amoxicillin–clavulanic acid 49 (22.4%), cefotaxime 41 (18.7%), amikacin 18 (8.2%), and imipenem 7 (3.2%). Table 1 shows resistance patterns of various *Enterobacteriaceae* in patients with UTIs. High resistance to fluoroquinolones was found in the internal ward 42 (60%) followed by, surgery 10 (14%),

Table 1 Patterns of antibiotic resistance of *Enterobacteriaceae* species in urinary tract infections

Antibiotics	<i>Escherichia coli</i> (n=177)	<i>Klebsiella pneumoniae</i> (n=28)	<i>Enterobacter cloacae</i> (n=7)	<i>Proteus mirabilis</i> (n=2)	<i>Morganella morganii</i> (n=2)	<i>Klebsiella oxytoca</i> (n=1)	<i>Citrobacter freundii</i> (n=1)	<i>Proteus vulgaris</i> (n=1)
Amoxicillin–clavulanic acid	36.6%	75%	85.7%	0	0	0	100%	0
Ampicillin	85.8%	96.4%	85.7%	50%	0	100%	100%	100%
Cefotaxime	14.7%	25%	19.4%	0	0	0	2.8%	0
Ceftazidime	79.7%	75%	85.7%	50%	100%	100%	100%	100%
Cefepime	32.8%	64.3%	42.9%	0	0	0	0	0
Cefuroxime	52.5%	60.7%	57.1%	50%	1.7%	0	0	0
Imipenem	0	21.5%	0	50%	0	0	0	0
Aztreonam	46.9%	60.7%	42.9%	0	0	0	0	0
Gentamicin	33.9%	46.4%	42.9%	0	0	0	0	0
Amikacin	7.7%	17.9%	0	0	0	0	0	0
Trimethoprim–sulfamethoxazole	65%	67.9%	57.1%	100%	0	0	0	0
Nitrofurantoin	16.4%	64.3%	85.7%	100%	100%	0	0	0
Ciprofloxacin	62.1%	53.6%	14.3%	0	0	0	0	0
Nalidixic acid	72.3%	64.3%	57.1%	0	0	0	0	0
Levofloxacin	58.8%	39.3%	0	0	0	0	0	0
Gatifloxacin	63.8%	53.6%	14.3%	50%	0	0	100%	0
Ofloxacin	65%	42.9%	14.3%	50%	0	0	100%	0
Moxifloxacin	67.2%	60.7%	71.4%	50%	0	0	100%	0

ICU 9 (12.9%), and pediatrics 9 (12.9%). There was a significant relationship between resistance to fluoroquinolones and the different wards of the hospital ($P \leq 0.05$). The agar dilution results indicated that 44, 85, and 60 of the isolates were highly resistant; $MIC \geq 512 \mu\text{g/mL}$, $MIC \geq 64 \mu\text{g/mL}$, and $MIC \geq 128 \mu\text{g/mL}$ to nalidixic acid, ciprofloxacin, and levofloxacin, respectively. There were no significant relationships between the antimicrobial resistance, gender and age groups ($P > 0.05$).

Molecular analysis

The ESBLs were phenotypically detected in 75 (34.2%) of the isolates. ESBL-producing *Enterobacteriaceae* in internal, surgery, ICU, and pediatrics wards were 47 (62.7%), 16 (21.3%), 8 (10.7%), and 4 (5.3%), respectively. Table 2 shows the frequency of ESBL genes among isolates. *bla*_{CTX-M} group (38.4%) was the most frequent ESBL gene in tested

isolates followed by *bla*_{TEM} (20.6%) and *bla*_{SHV} (1.5%). We observed a high-level resistance to all tested quinolones in ESBL-producing isolates (67/75, 89.3%) compared with non-ESBL-producing isolates. The rate of moxifloxacin, nalidixic acid, gatifloxacin, ofloxacin, ciprofloxacin, and levofloxacin resistance in ESBL-producing isolates was 89.3%, 88%, 84%, 80%, 78%, and 73.3%, respectively. There was a significant relationship between the activity of ESBLs and fluoroquinolones resistance ($P \leq 0.05$). The prevalence of ESBLs was high in elderly and male patients ($P \leq 0.05$).

One hundred and fifty-six (89.1%) of the 175 fluoroquinolone-resistant isolates were positive for at least 1 PMQR gene. The most common PMQR gene was *aac* (6')-*Ib-cr* 120 (68.6%) followed by *oqx*B 72 (41.1%), *oqx*A 59 (33.7%), *qnr*B 36 (20.6%), *qnr*S 33 (18.9%), *qnr*D 19 (10.9%), *qep*A 13 (7.4%), *qnr*A 10 (5.7%), and *qnr*C 9 (5.1%) (Table 3).

Table 2 The prevalence of ESBL-producing genes among the members of *Enterobacteriaceae* isolated from urinary tract infections

Genes	<i>Escherichia coli</i> (n=177)	<i>Klebsiella pneumoniae</i> (n=28)	<i>Klebsiella oxytoca</i> (n=1)	<i>Enterobacter cloacae</i> (n=7)	<i>Proteus mirabilis</i> (n=2)	<i>Proteus vulgaris</i> (n=1)	<i>Morganella morganii</i> (n=2)	<i>Citrobacter freundii</i> (n=1)	Total (n=219)
<i>bla</i> _{TEM}	34 (75.6%)	6 (13.3%)	0	3 (6.7%)	1 (2.2%)	0	0	1 (2.2%)	45 (20.6%)
TEM-12	1	0	0	0	0	0	0	0	1
TEM-24	1	0	0	0	0	0	0	0	1
TEM-116	32	6	0	3	1	0	0	1	43
<i>bla</i> _{CTX-M}	66 (78.6%)	15 (17.9%)	0	3 (3.6%)	0	0	0	0	84 (38.4%)
CTX-M-3	5	5	0	0	0	0	0	0	10
CTX-M-9	1	0	0	0	0	0	0	0	1
CTX-M-14	3	0	0	1	0	0	0	0	3
CTX-M-15	45	8	0	2	0	0	0	0	55
CTX-M-22	1	1	0	0	0	0	0	0	2
CTX-M-27	5	0	0	0	0	0	0	0	5
CTX-M-28	1	0	0	0	0	0	0	0	1
CTX-M-55	2	1	0	0	0	0	0	0	3
CTX-M-79	3	0	0	0	0	0	0	0	3
<i>bla</i> _{SHV}	1 (33.3%)	2 (66.6%)	0	0	0	0	0	0	3 (1.5%)
SHV-2a	1	0	0	0	0	0	0	0	1
SHV-27	0	1	0	0	0	0	0	0	1
SHV-28	0	1	0	0	0	0	0	0	1

Table 3 Prevalence of plasmid-mediated quinolone resistance in *Enterobacteriaceae* species isolated from urinary tract infections

Genes	<i>Escherichia coli</i> (n=144)	<i>Klebsiella pneumoniae</i> (n=21)	<i>Enterobacter cloacae</i> (n=6)	<i>Proteus mirabilis</i> (n=2)	<i>Proteus vulgaris</i> (n=1)	<i>Citrobacter freundii</i> (n=1)	Total (n=175)
<i>qnr</i> A	8	1	0	1			10
<i>qnr</i> B	27	7	1			1	36
<i>qnr</i> C	9						9
<i>qnr</i> D	14	5					19
<i>qnr</i> S	26	3	2	1	1		33
<i>aac</i> (6')- <i>Ib-cr</i>	99	14	5	2			120
<i>oqx</i> A	36	19	2	2			59
<i>oqx</i> B	50	20	1	1			72
<i>qep</i> A	12		1				13

Abbreviation: Qnr, quinolone resistance gene.

The prevalence of PMQR genes was more in isolates with high-level quinolone MIC than low-level quinolone MIC. PMQR genes were detected from the internal ward in 97 cases (62.2%), surgery in 39 cases (25%), ICU in 14 cases (9%), and pediatrics ward in 6 cases (3.8%).

Among the 75 ESBL-producing isolates, 51 (68%), 32 (42.6%), 27 (36%), 17 (22.6%), 14 (18.6%), 8 (10.6%), 6 (8%), 4 (5.3%), and 4 strains (5.3%) carried the *aac(6)-Ib-cr*, *oqxB*, *oqxA*, *qnrB*, *qnrS*, *qnrD*, *qnrA*, *qnrC*, and *qepA* genes, respectively. There were no significant relationships between PMQR genes, gender, and age groups ($P>0.05$). In this study, at least 1 ESBL was found in 44% of PMQR-positive isolates. We found that PMQR genes could co-exist with *bla*_{CTX-M-15}, *bla*_{CTX-M-14}, and *bla*_{TEM-116} and other ESBL genes (Table 4).

Discussion

A high proportion of our isolates (68%) were resistant to fluoroquinolones. Our results showed that resistance to

Table 4 (Continued)

PMQR genes	Species	ESBL genes	Numbers of isolates
<i>aac(6)-Ib-cr</i>	<i>E. coli</i>	TEM-116	1
	<i>E. coli</i>	CTX-M-15	14
	<i>K. pneumoniae</i>	CTX-M-15	2
	<i>E. coli</i>	CTX-M-3	1
	<i>K. pneumoniae</i>	CTX-M-3	3
	<i>E. coli</i>	CTX-M-22	1
	<i>K. pneumoniae</i>	CTX-M-22	1
	<i>E. coli</i>	CTX-M-79	1
	<i>K. pneumoniae</i>	SHV-27+ TEM-116	1
	<i>K. pneumoniae</i>	SHV-28+ CTX-M-15	1
	<i>E. coli</i>	CTX-M-15+ TEM-116	13
	<i>K. pneumoniae</i>	CTX-M-15+ TEM-116	3
	<i>E. coli</i>	CTX-M-3+ TEM-116	1
	<i>K. pneumoniae</i>	CTX-M-3+ TEM-116	1
	<i>E. coli</i>	CTX-M-55+ TEM-116	1
	<i>E. coli</i>	CTX-M-55+ TEM-116	1
	<i>E. coli</i>	CTX-M-28+ TEM-116	1
	<i>E. coli</i>	CTX-M-14+ TEM-116	2
	<i>E. coli</i>	CTX-M-9+ TEM-116	1
	OqxA	<i>E. coli</i>	CTX-M-27+ TEM-12
<i>E. coli</i>		CTX-M-15+ TEM-24	1
<i>E. coli</i>		TEM-116	1
<i>E. coli</i>		CTX-M-15	7
<i>K. pneumoniae</i>		CTX-M-15	3
<i>K. pneumoniae</i>		CTX-M-3	4
<i>K. pneumoniae</i>		CTX-M-22	1
<i>K. pneumoniae</i>		SHV-27+ TEM-116	1
<i>K. pneumoniae</i>		SHV-28+ CTX-M-15	1
<i>E. coli</i>		CTX-M-15+ TEM-116	3
OqxB	<i>K. pneumoniae</i>	CTX-M-15+ TEM-116	3
	<i>K. pneumoniae</i>	CTX-M-3+ TEM-116	1
	<i>K. pneumoniae</i>	CTX-M-55+ TEM-116	1
	<i>K. pneumoniae</i>	CTX-M-14+ TEM-116	1
	<i>E. coli</i>	TEM-116	2
	<i>E. coli</i>	CTX-M-15	8
	<i>K. pneumoniae</i>	CTX-M-15	3
	<i>E. coli</i>	CTX-M-3	1
	<i>K. pneumoniae</i>	CTX-M-3	3
	<i>K. pneumoniae</i>	CTX-M-22	1
QepA	<i>E. coli</i>	CTX-M-14	1
	<i>K. pneumoniae</i>	SHV-27+ TEM-116	1
	<i>K. pneumoniae</i>	SHV-27+ CTX-M-15	1
	<i>E. coli</i>	CTX-M-15+ TEM-116	4
	<i>K. pneumoniae</i>	CTX-M-15+ TEM-116	3
	<i>E. coli</i>	CTX-M-15+ TEM-24	1
	<i>K. pneumoniae</i>	CTX-M-3+ TEM-116	1
	<i>E. coli</i>	CTX-M-27+ TEM-116	1
	<i>E. coli</i>	CTX-M-14+ TEM-116	1
	<i>E. coli</i>	CTX-M-15	1

Table 4 Co-existence of ESBLs and PMQR in *E. coli* (n=144) and *K. pneumoniae* (n=21) isolated from urinary tract infections

PMQR genes	Species	ESBL genes	Numbers of isolates
<i>qnrA</i>	<i>Escherichia coli</i>	CTX-M-15	1
	<i>E. coli</i>	CTX-M-27	1
	<i>Klebsiella pneumoniae</i>	TEM-116+SHV-27	1
<i>qnrB</i>	<i>E. coli</i>	CTX-M-15+ TEM-116	3
	<i>E. coli</i>	CTX-M-15	5
	<i>K. pneumoniae</i>	CTX-M-3	3
	<i>K. pneumoniae</i>	CTX-M-22	1
	<i>K. pneumoniae</i>	SHV-28+ CTX-M-15	1
	<i>E. coli</i>	CTX-M-15+ TEM-116	2
	<i>K. pneumoniae</i>	CTX-M-15+ TEM-116	1
<i>qnrC</i>	<i>E. coli</i>	CTX-M-15+ TEM-24	1
	<i>K. pneumoniae</i>	CTX-M-55+ TEM-116	1
	<i>E. coli</i>	CTX-M-14+ TEM-116	2
	<i>E. coli</i>	CTX-M-15	1
	<i>E. coli</i>	CTX-M-79	1
<i>qnrD</i>	<i>E. coli</i>	CTX-M-15+ TEM-116	2
	<i>E. coli</i>	TEM-116	2
	<i>K. pneumoniae</i>	TEM-116	1
	<i>E. coli</i>	CTX-M-15	3
	<i>K. pneumoniae</i>	CTX-M-15	1
	<i>E. coli</i>	CTX-M-3	1
<i>QnrS</i>	<i>E. coli</i>	CTX-M-3	1
	<i>K. pneumoniae</i>	CTX-M-15	2
	<i>K. pneumoniae</i>	CTX-M-15+SHV28	1
	<i>E. coli</i>	CTX-M-15+ TEM-116	6
	<i>K. pneumoniae</i>	CTX-M-3+ TEM-116	1
	<i>E. coli</i>	CTX-M-28+ TEM-116	1
	<i>E. coli</i>	CTX-M-27+ TEM-116	1
	<i>E. coli</i>	CTX-M-14+ TEM-116	1
	<i>E. coli</i>	CTX-M-15+ TEM-24	1

(Continued)

Abbreviations: ESBL, extended spectrum β-lactamases; PMQR, plasmid-mediated quinolone resistance.

tested fluoroquinolones in ESBL-producing isolates was significantly higher than in non-ESBL-producing isolates.

In the present study, 34.2% of isolates were ESBL-producing. The prevalence of ESBL-producing *E. coli* and *K. pneumoniae* was 33.8% and 53.5%, respectively. The prevalence of ESBL varies depending on species and geographical regions. In South Korea and Iran, 30% and 34.8% of isolates were reported positive for ESBL, respectively.^{15,16} While in North America, the prevalence of ESBL-producing *E. coli* and *K. pneumoniae* was low.¹⁷ However, a high prevalence of ESBL was reported in other countries.^{18,19} Differences between these results may be due to the length of ICU stay, inappropriate and excessive use of antibiotics, and length of hospitalization.²⁰

In the current study, the prevalence of ESBL genes was examined by the multiplex PCR and sequencing methods. We found that *bla*_{CTX-M} was the most prevalent ESBL gene followed by *bla*_{TEM} and *bla*_{SHV}. Similar to the present investigation, the frequency of *bla*_{CTX-M}, *bla*_{TEM}, and *bla*_{SHV} genes was reported as 40%, 20.3%, and 14%, respectively.^{21,22} ESBL producers are often resistant to other antibiotics, such as fluoroquinolones.²³ The presence of ESBL and some of the fluoroquinolone-resistant genes in the same mobile genetic elements may be the cause of co-resistance to β -lactams and fluoroquinolones. Our results showed that resistance to fluoroquinolones (89.3%) was significantly higher in ESBL-producing isolates than the non-ESBL-producing isolates, as previously described in other studies conducted in Pakistan, Nepal, and Asia/Pacific.^{18,22,24} Therefore, the incidence of multidrug resistance among ESBL-producing *Enterobacteriaceae* limits therapeutic options. However, some studies indicated that there was no significant association between resistance to the fluoroquinolone and ESBL-producing isolates.^{7,20}

Our study showed a high prevalence of PMQR (89.1%) among quinolone-resistant *Enterobacteriaceae*. The *aac(6)-Ib-cr* was the most prevalent PMQR gene in this study, in agreement with previous reports.^{25,26} In contrast, *qnrA* and *qnrC* were detected at low frequency. It has been shown that the presence of PMQR genes provides a favorable field for quinolone resistance. Our data indicated that *aac(6)-Ib-cr*, *oqxB*, *oqxA*, and *qnrB* genes were detected in a significant proportion of ESBL-producing *Enterobacteriaceae*. The presence of PMQR genes was significantly associated with ESBL genes, perhaps due to the common carriage on a plasmid in *Enterobacteriaceae*.²⁷ Interestingly, at least 1 ESBL was detected in PMQR-positive isolates (44%). Several

previous studies reported a high percentage of PMQR genes among ESBL genes.^{6,28}

Notably, we found that *bla*_{CTX-M-15} and *bla*_{TEM-116} were common among most of the PMQR-positive isolates. In this study, 34 and 26 of 51 *aac(6)-Ib-cr*-positive isolates produced *bla*_{CTX-M-15} and *bla*_{TEM-116}, respectively. In our study, several PMQR-positive isolates contained *bla*_{CTX-M-3} except for *qnrA* and *qnrC* isolates. In addition, at least 1 PMQR-positive isolate carried SHV-27, SHV-28, or CTX-M-14 genes, except for the *qnrA*, *qnrC*, and *qepA* isolates. The previous studies have reported a significant association among *aac(6)-Ib-cr* and *qnrB* with CTX-M-15 and CTX-M-14 in *Enterobacteriaceae* isolates.^{29,30} However, in a study from Korea, CTX-M-15 and CTX-M-3 were rare among *qnr*-positive isolates.²⁷ These genes are usually transported by the plasmid and can easily spread among the members of *Enterobacteriaceae*. The association between PMQRs and ESBLs could be clinically important since treatment options for these isolates are limited and may lead to failure of therapy and death of patients.

The limitations of our study were, no equal number of isolates from each bacterium and not performing molecular epidemiology and typing. Due to the high prevalence of *Enterobacteriaceae* in the UTIs, and co-resistance to fluoroquinolones and β -lactams in ESBL-producing isolates, we emphasize the correct and judicious use of fluoroquinolone. The determination of susceptibility testing may help to prevent the dissemination of MDR isolates.

Conclusion

The rate of resistance to β -lactams and fluoroquinolones in *Enterobacteriaceae* isolated from UTIs is high. Amikacin and imipenem are the most effective antibiotics for empirical therapy in our setting. The prevalence of PMQR genes is high in *Enterobacteriaceae* isolates and the most common PMQR is *aac(6)-Ib-cr*. The PMQR genes and their association with ESBL-producing plasmids contribute to the spread of multidrug resistance and may lead to serious problems for treatment. Therefore, detection of PMQR determinants and ESBL genes among non-susceptible fluoroquinolone *Enterobacteriaceae* is important for appropriate empirical treatment and infection control.

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Disclosure

The authors report no conflicts of interest in this work.

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