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

Robab Azargun1,2
Mohammad Reza Sadeghi3
Mohammad Hossein Soroush Barahangi3
Hossein Samadi Kafii2,4
Fatemeh Yeganeh2
Mahin Ahangar Oskouee2
Reza Ghotaslou1,2

1Infectious and Tropical Diseases Research Center, Tabriz University of Medical Sciences, Tabriz, Iran; 2Microbiology Department, School of Medicine, Tabriz University of Medical Sciences, Tabriz, Iran; 3Azad University of Macu, Macu, Iran; 4Drug Applied Research Center, Tabriz University of Medical Sciences, Tabriz, Iran

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 (68.6%) followed by oqxA 59 (33.7%), 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 blaCTX-M-15 and blaTEM-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

Infection and Drug Resistance 2018:11 1007–1014

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, cephalaxin, and ceftiraxone. 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...
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. 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. Although the PMQR determinants lead to low-level quinolone resistance, they facilitate the chromosome-encoded quinolone resistance. ESBL-producing Enterobacteriaceae has emerged as multidrug-resistant (MDR), especially resistant to trimethoprim/sulfamethoxazole, aminoglycosides, and fluoroquinolones.

PMQR genes are often on the same plasmid as the ESBL genes. Resistance plasmids with genes encoding ESBLs can be transferred by the conjugation that helps dissemination of PMQR determinants in different Enterobacteriaceae species. 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.

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. 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 10⁵ 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 pattern 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). The used disks were amoxicillin–clavulanic acid (20/10 µg), ampicillin (10 µg), cefotaxime (30 µg), ceftazidime (30 µg), cefepime (30 µg), cefuroxime (30 µg), imipenem (10 µg), aztreonam (30 µg), gentamicin (10 µg), amikacin (30 µg), trimethoprim–sulfamethoxazole (30 µg), nitrofurantoin (300 µg), ciprofloxacin (5 µg), nalidixic acid (30 µg), levofloxacin (5 µg), gatifloxacin (5 µg), ofloxacin (5 µg), and moxifloxacin (5 µg). 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. 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 µg), ≤27 mm with cefotaxime (30 µg), suggested ESBL production. The phenotypic confirmatory test for ESBL was done by double disk synergy using cefotaxime (30 µg) and ceftazidime (30 µg) alone and in combination with clavulanic acid (10 µg). ESBL activities were identified by zone diameter increase of ≥5 mm around the disk with the antibiotic in combination with clavulanic acid. E. coli ATCC 25922 and Klebsiella pneumoniae ATCC
Association between PMQR and ESBLs-producing isolates

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 QIAquick 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%), 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                              | Escherichia coli (n=177) | Klebsiella pneumoniae (n=28) | Enterobacter cloacae (n=7) | Proteus mirabilis (n=2) | Morganella morganii (n=2) | Klebsiella oxytoca (n=1) | Citrobacter freundii (n=1) | Proteus vulgaris (n=1) |
|------------------------------------------|--------------------------|-------------------------------|----------------------------|-------------------------|--------------------------|-------------------------|--------------------------|------------------------|
| Amoxicillin–clavulanic acid             | 36.6%                    | 75%                          | 85.7%                      | 0                       | 0                        | 0                       | 100%                     | 0                      |
| Ampicillin                               | 85.8%                    | 96.4%                        | 85.7%                      | 50%                     | 0                        | 0                       | 0                        | 100%                   |
| Cefotaxime                               | 14.7%                    | 25%                          | 19.4%                      | 0                       | 0                        | 0                       | 0                        | 2.8%                   |
| 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                      |
| Levofoxacin                              | 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 ≥2512 µg/mL, MIC ≥64 µg/mL, and MIC ≥2128 µg/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 *qepA* 72 (41.1%), *qnrA* 59 (33.7%), *qnrB* 36 (20.6%), *qnrS* 33 (18.9%), *qnrD* 19 (10.9%), and *qnrC* 13 (7.4%), *qnrA* 10 (5.7%), and *qnrC* 9 (5.1%) (Table 3).

---

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

| Genes       | *Escherichia coli* (n=177) | *Klebsiella pneumoniae* (n=28) | *Klebsiella oxytoca* (n=1) | *Enterobacter cloacae* (n=7) | *Proteus mirabilis* (n=2) | *Proteus vulgaris* (n=1) | *Morganella morganii* (n=2) | *Citrobacter freundii* (n=1) | Total (n=219) |
|-------------|----------------------------|-------------------------------|-----------------------------|----------------------------|--------------------------|--------------------------|-----------------------------|-----------------------------|------------------|
| *blaTEM*    | 34 (75.6%)                 | 6 (13.3%)                     | 3 (6.7%)                    | 1 (2.2%)                   | 0                        | 0                        | 1 (2.2%)                    | 0                           | 45 (20.6%)       |
| TEM-12      | 1                          | 0                             | 0                           | 0                          | 0                        | 0                        | 1                           | 1                           | 1                |
| TEM-24      | 1                          | 0                             | 0                           | 0                          | 0                        | 0                        | 1                           | 1                           | 1                |
| TEM-116     | 32                         | 6                             | 3                           | 0                          | 0                        | 0                        | 0                           | 0                           | 43               |
| *bla CTX-M* | 66 (78.6%)                 | 15 (17.9%)                    | 3 (3.6%)                    | 0                          | 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                        | 1                           | 1                           | 1                |
| CTX-M-14    | 3                          | 0                             | 1                           | 0                          | 0                        | 0                        | 0                           | 0                           | 3                |
| CTX-M-15    | 45                         | 8                             | 2                           | 0                          | 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-55    | 2                          | 1                             | 0                           | 0                          | 0                        | 0                        | 0                           | 0                           | 3                |
| CTX-M-79    | 3                          | 0                             | 0                           | 0                          | 0                        | 0                        | 0                           | 0                           | 3                |
| *bla 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       | *Escherichia coli* (n=144) | *Klebsiella pneumoniae* (n=21) | *Enterobacter cloacae* (n=6) | *Proteus mirabilis* (n=2) | *Proteus vulgaris* (n=1) | *Citrobacter freundii* (n=1) | Total (n=175) |
|-------------|----------------------------|-------------------------------|----------------------------|--------------------------|--------------------------|-----------------------------|------------------|
| *qnrA*      | 8                          | 1                             | 0                           | 1                        | 1                        | 1                           | 10               |
| *qnrB*      | 27                         | 7                             | 1                           | 1                        | 1                        | 1                           | 36               |
| *qnrC*      | 9                          | 0                             | 0                           | 0                        | 0                        | 0                           | 9                |
| *qnrD*      | 14                         | 5                             | 5                           | 5                        | 5                        | 5                           | 19               |
| *qnrS*      | 26                         | 3                             | 1                           | 1                        | 1                        | 1                           | 33               |
| *aac(6’)-Ib-cr* | 99                   | 14                            | 14                           | 14                       | 14                       | 14                          | 146              |
| *qepA*      | 36                         | 19                            | 19                           | 19                       | 19                       | 19                          | 57               |
| *qepB*      | 50                         | 20                            | 20                           | 20                       | 20                       | 20                          | 70               |
| *qepA*      | 12                         | 1                             | 1                            | 1                        | 1                        | 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 \( aac(6')-Ib-cr \), \( qoxB \), \( qoxA \), \( qnrB \), \( qnrD \), \( qnrA \), 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

| PMQR genes | Species | ESBL genes | Numbers of isolates |
|------------|---------|------------|---------------------|
| qnrA       | *Escherichia coli* | CTX-M-15 | 1                   |
|            | *E. coli* | CTX-M-27  | 1                   |
|            | *Klebsiella pneumoniae* | TEM-116+SHV-27 | 1 |
|            | *E. coli* | CTX-M-15+ TEM-116 | 3 |
| qnrB       | *E. coli* | CTX-M-15 | 5                   |
|            | *K. pneumoniae* | CTX-M-3  | 3                   |
|            | *K. pneumoniae* | CTX-M-22 | 1                   |
|            | *K. pneumoniae* | SHV-28+ CTX-M-15 | 1 |
|            | *E. coli* | CTX-M-15+ TEM-116 | 2 |
|            | *K. pneumoniae* | CTX-M-15+ TEM-116 | 1 |
|            | *E. coli* | CTX-M-15+ TEM-24 | 1 |
|            | *K. pneumoniae* | CTX-M-55+ TEM-116 | 1 |
|            | *E. coli* | CTX-M-14+ TEM-116 | 2 |
| qnrC       | *E. coli* | CTX-M-15 | 1                   |
|            | *E. coli* | CTX-M-79  | 1                   |
|            | *E. coli* | CTX-M-15+ TEM-116 | 2 |
| qnrD       | *E. coli* | TEM-116  | 2                   |
|            | *K. pneumoniae* | TEM-116 | 1                   |
|            | *E. coli* | CTX-M-15 | 3                   |
|            | *K. pneumoniae* | CTX-M-15 | 1                   |
|            | *E. coli* | CTX-M-3  | 1                   |
| QnrS       | *K. pneumoniae* | CTX-M-15 | 2                   |
|            | *K. pneumoniae* | CTX-M-15+SHV-28 | 1 |
|            | *E. coli* | CTX-M-15+ TEM-116 | 6 |
|            | *K. pneumoniae* | CTX-M-3+ TEM-116 | 1 |
|            | *E. coli* | CTX-M-28+ TEM-116 | 1 |
|            | *E. coli* | CTX-M-27+ TEM-116 | 1 |
|            | *E. coli* | CTX-M-14+ TEM-116 | 1 |
|            | *E. coli* | CTX-M-15+ TEM-24 | 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 |
|------------|---------|------------|---------------------|
|            | *E. coli* | TEM-116  | 1                   |
|            | *K. pneumoniae* | CTX-M-15 | 14                  |
|            | *E. coli* | CTX-M-15 | 2                   |
|            | *E. coli* | CTX-M-3  | 1                   |
|            | *K. pneumoniae* | CTX-M-3 | 3                   |
|            | *E. coli* | CTX-M-22 | 1                   |
|            | *K. pneumoniae* | CTX-M-22 | 1                   |
|            | *E. coli* | CTX-M-79  | 1                   |
|            | *K. pneumoniae* | SHV-27+ TEM-116 | 1 |
|            | *E. coli* | CTX-M-15+ TEM-116 | 13 |
|            | *K. pneumoniae* | CTX-M-15+ TEM-116 | 3 |
|            | *E. coli* | CTX-M-3+ TEM-116 | 1 |
|            | *E. coli* | CTX-M-27+ TEM-12 | 1 |
|            | *E. coli* | CTX-M-15+ TEM-24 | 1 |
|            | *E. coli* | TEM-116  | 1                   |
|            | *K. pneumoniae* | CTX-M-15  | 7                   |
|            | *K. pneumoniae* | CTX-M-3  | 3                   |
|            | *K. pneumoniae* | CTX-M-22 | 1                   |
|            | *K. pneumoniae* | SHV-27+ TEM-116 | 1 |
|            | *K. pneumoniae* | SHV-28+ CTX-M-15 | 1 |
|            | *E. coli* | CTX-M-15+ TEM-116 | 13 |
|            | *K. pneumoniae* | CTX-M-3+ TEM-116 | 1 |
|            | *K. pneumoniae* | CTX-M-3 | 3                   |
|            | *K. pneumoniae* | CTX-M-22 | 1                   |
|            | *E. coli* | CTX-M-15 | 8                   |
|            | *K. pneumoniae* | CTX-M-15  | 3                   |
|            | *E. coli* | CTX-M-3  | 1                   |
|            | *K. pneumoniae* | CTX-M-3  | 3                   |
|            | *K. pneumoniae* | CTX-M-22 | 1                   |
|            | *E. coli* | CTX-M-14  | 1                   |
|            | *K. pneumoniae* | SHV-27+ TEM-116 | 1 |
|            | *K. pneumoniae* | SHV-27+ CTX-M-15 | 1 |
|            | *E. coli* | CTX-M-15+ TEM-116 | 4 |
|            | *K. pneumoniae* | CTX-M-15+ TEM-116 | 3 |
|            | *E. coli* | CTX-M-3+ TEM-24 | 1 |

**Abbreviations:** ESBL, extended spectrum \( \beta \)-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 regions. In South Korea and Iran, 30% and 34.8% of isolates were reported positive for ESBL, respectively.15,16 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 present study, the prevalence of ESBL genes was examined by the multiplex PCR and sequencing methods. We found that blaCTX-M was the most prevalent ESBL gene followed by blaTEM and blashv. Similar to the present investigation, the frequency of blaCTX-M, blaTEM, and blashv 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, oqxAB, 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 blaCTX-M-15 and blaTEM-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 blaCTX-M-15 and blaTEM-116, respectively. In our study, several PMQR-positive isolates contained blaCTX-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.

**Acknowledgments**

This project was financially supported by Infectious and Tropical Diseases Research Center, Tabriz University of Medical Sciences (grant no.95-02). This article was written based on...
a dataset of a PhD thesis (number: 95/5-7/1) registered at Tabriz University of Medical Sciences, Tabriz, Iran.

**Disclosure**

The authors report no conflicts of interest in this work.

**References**

1. Kresken M, Körber-Irrgang B, Biedenbach D, et al. Comparative in vitro activity of oral antimicrobial agents against *Enterobacteriaceae* from patients with community-acquired urinary tract infections in three European countries. *Clin. Microbiol. Infect.* 2016;22(1):63.e61–63.e65.

2. Bajaj P, Kanaujia PK, Singh NS, Sharma S, Kumar S, Virdi JS. Quinolone co-resistance in ESBL-or AmpC-producing *Escherichia coli* from an Indian urban aquatic environment and their public health implications. *Environ Sci Pollut Res Int.* 2016;23(2):1954–1959.

3. Oteo J, Campos J, Lázaro E, et al. Increased amoxicillin–clavulanic acid resistance in *Escherichia coli* blood isolates, Spain. *Emerg. Infect. Dis.* 2008;14(8):1259.

4. Ni Q, Tian Y, Zhang L, et al. Prevalence and quinolone resistance of *Escherichia coli* in 6 communities and 2 physical examination center populations in Shanghai, China. *Diagn. Microbiol. Infect. Dis.* 2016;86(4):428–433.

5. Strahilevitz J, Jacoby GA, Hooper DC, Robicsek A. Plasmid-mediated quinolone resistance: a multifaceted threat. *Clin. Microbiol. Rev.* 2009;22(4):664–689.

6. Yanat B, Machuca J, Diaz-De-Alba P, et al. Characterization of plasmid-mediated quinolone resistance determinants in high-level quinolone-resistant *Enterobacteriaceae* isolates from the community: first report of qnrD Gene in Algeria. *Microb. Drug Resist.* 2017;23(1):90–97.

7. Mansouri S, Abbasi S. Prevalence of multiple drug resistant clinical isolates of extended-spectrum beta-lactamase producing *Enterobacte-riaceae* in Southeast Iran. *Iran J Med Sci.* 2015;35(2):101–108.

8. García-Fulgueiras V, Bado I, Mota MI, et al. Extended-spectrum β-lactamases and plasmid-mediated quinolone resistance in enterobacterial clinical isolates in the paediatric hospital of Uruguay. *J Antimicrob Chemother.* 2011;66(8):1725–1729.

9. Rodríguez-Martínez JM, Cano ME, Velasco C, Martínez-Martínez L, Pascual A. Plasmid-mediated quinolone resistance: an update. *J Infect Chemother.* 2011;17(2):149–182.

10. Shams E, Firoozeh F, Moniri R, Zibaee M. Prevalence of plasmid-mediated quinolone resistance genes among extended-spectrum β-lactamase-producing *Klebsiella pneumoniae* human isolates in Iran. *J Pathog.* 2015;2015:7.

11. Wayne P. Clinical and Laboratory Standards Institute. Performance Standards for Antimicrobial Susceptibility Testing; Twenty-Fifth Informational Supplement. CLSI document M100-S25. 2015.

12. Dallenne C, Da Costa A, Decré D, Favier C, Arlet G. Development of a set of multiplex PCR assays for the detection of genes encoding important β-lactamases in *Enterobacteriaceae*. *J Antimicrob Chemother.* 2010;65(3):490–495.

13. Yang H, Duan G, Zhu J, Zhang W, Xi Y, Fan Q. Prevalence and characterisation of plasmid-mediated quinolone resistance and mutations in the gyrA and topoisomerase IV genes among Shigella isolates from Henan, China, between 2001 and 2008. *Int J Antimicrob Agents.* 2013;42(2):173–177.

14. Chen X, Zhang W, Pan W, et al. Prevalence of qnr, aac(6′)-Ib-cr, qepA, and oqxAB in *Escherichia coli* isolates from humans, animals, and the environment. *Antimicrob Agents Chemother.* 2012;56(6):3423–3427.

15. Kao C-Y, Udval U, Huang Y-T, et al. Molecular characterization of extended-spectrum β-lactamase-producing *Escherichia coli* and *Klebsiella* spp. isolates in Mongolia. *J Microbiol Immunol Infect.* 2015;49(5):692–700.

16. Ramazanzadeh R, Chitsaz M, Bahmani N. Prevalence and antimicrobial susceptibility of extended-spectrum beta-lactamase-producing bacteria in intensive care units of Sanandaj general hospitals (Kurdistan, Iran). *Chemotherapy.* 2009;55(4):287–292.

17. Hoban DJ, Lascals C, Nicole LL, et al. Antimicrobial susceptibility of *Enterobacteriaceae*, including molecular characterization of extended-spectrum beta-lactamase–producing species, in urinary tract isolates from hospitalized patients in North America and Europe: results from the SMART study 2009–2010. *Diagn. Microbiol. Infect. Dis.* 2012;74(1):62–67.

18. Lu P-L, Liu Y-C, Toh H-S, et al. Epidemiology and antimicrobial susceptibility profiles of Gram-negative bacteria causing urinary tract infections in the Asia-Pacific region: 2009–2010 results from the Study for Monitoring Antimicrobial Resistance Trends (SMART). *Int. J. Antimicrob. Agents.* 2012;40:S37–S43.

19. Rao SP, Rama PS, Gurushanthappa V, Manipura R, Srinivasan K. Extended-spectrum beta-lactamases producing *Escherichia coli* and *Klebsiella pneumoniae*: a multi-centric study across Karnataka. *J Lab Physicians.* 2014;6(1):7–13.

20. Sedighi I, Arabestani MR, Rahimbakhsh A, Karimtabar Z, Alikhani MY. Dissemination of extended-spectrum beta-lactamases and quinolone resistance genes among clinical isolates of uropathogenic *Escherichia coli* in children. *Indian J Microbiol.* 2015;5(7):e19184.

21. Giske CG, Monnet DL, Cars O, Carmeli Y. Clinical and economic impact of common multidrug-resistant gram-negative bacilli. *Antimicrob. Agents Chemother.* 2008;52(3):813–821.

22. Hussain M, Hasan F, Shah AA, et al. Prevalence of class A and AmpC β-lactamases in clinical *Escherichia coli* isolates from Pakistan Institute of Medical Science, Islamabad, Pakistan. *Jpn. J. Infect. Dis.* 2011;64(3):249252.

23. Yadav KK, Adhikari N, Khadka R, Pant AD, Shah B. Multidrug resistant *Enterobacteriaceae* and extended spectrum beta-lactamase producing *Escherichia coli*: a cross-sectional study in National Kidney Center, Nepal. *Antimicrob Resist Infect Control.* 2015;4:42.

24. Tumbarello M, Spanu T, Sanguinetti M, et al. Bloodstream infections caused by extended-spectrum-β-lactamase-producing *Klebsiella pneumoniae*: risk factors, molecular epidemiology, and clinical outcome. *Antimicrob. Agents Chemother.* 2006;50(2):498–504.

25. El-Badawy MF, Tawakol WM, El-Far SW, et al. Molecular identification of aminoglycoside-modifying enzymes and plasmid-mediated quinolone resistance genes among *Klebsiella pneumoniae* clinical isolates recovered from Egyptian Patients. *International Journal of Microbiology.* 2017;2017:12.

26. Karah N, Poirel L, Bengtsson S, et al. Plasmid-mediated quinolone resistance determinants qnr and aac (6′)-Ib-cr in *Escherichia coli* and *Klebsiella spp.* from Norway and Sweden. *Diagn Microbiol Infect. Dis.* 2010;66(4):425–431.

27. Xue G, Li J, Feng Y, et al. High prevalence of plasmid-mediated quinolone resistance determinants in *Escherichia coli* and *Klebsiella pneumoniae* isolates from pediatric patients in China. *Microb. Drug Resist.* 2017;23(1):107–114.

28. Jeong HS, Bae IK, Shin HJ, et al. Prevalence of plasmid-mediated quinolone resistance and its association with extended-spectrum beta-lactamase and AmpC beta-lactamase in *Enterobacteriaceae*. *Korean J Lab Med.* 2011;31(4):257–264.

29. Bado I, Gutiérrez C, García-Fulgueiras V, et al. CTX-M-15 in combination with aac (6′)-Ib-cr is the most prevalent mechanism of resistance both in *Escherichia coli* and *Klebsiella pneumoniae*, including *K. pneumoniae* ST258, in an ICU in Uruguay. *J Glob. Antimicrob. Resist.* 2016;6:5–9.

30. Vignoli R, García-Fulgueiras V, Cordeiro NF, et al. Extended-spectrum β-lactamases, transferable quinolone resistance, and virulotyping in extra-intestinal *E. coli* in Uruguay. *J Infect Dev Ctries.* 2016;10(01):43–52.
