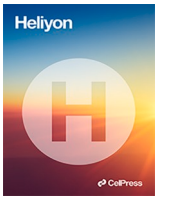
Heliyon 10 (2024) e37534

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon



Research article

Prevalence of plasmid-mediated quinolone resistance genes in extended-spectrum beta-lactamase producing *Klebsiella pneumoniae* isolates in northern Iran

Maedeh Hoseinzadeh [a], Mansour Sedighi [b,c], Yousef Yahyapour [d], Mostafa Javanian [d], Maryam Beiranvand [e], Mohsen Mohammadi [f], Sepide Zarei [a], Abazar Pournajaf [d,*], Amirmorteza Ebrahimzadeh Namvar [d,**]

[a] *Student Research Committee, Babol University of Medical Sciences, Babol, Iran*
[b] *Department of Microbiology, Faculty of Medicine, Kurdistan University of Medical Sciences, Sanandaj, Iran*
[c] *Zoonoses Research Center, Research Institute for Health Development, Kurdistan University of Medical Sciences, Sanandaj, Iran*
[d] *Infectious Diseases and Tropical Medicine Research Center, Health Research Institute, Babol University of Medical Sciences, Babol, Iran*
[e] *Division of Pulmonary, Critical Care and Sleep, College of Medicine-Jacksonville, University of Florida, Jacksonville, FL, USA*
[f] *Non-Communicable Pediatric Disease Research Center, Health Research Institute, Babol University of Medical Sciences, Babol, Iran*

ARTICLE INFO

Keywords:
Klebsiella pneumoniae
Extended-spectrum β-lactamase
Plasmid-mediated quinolone resistance
Fluoroquinolones
Multidrug-resistant

ABSTRACT

Plasmid-mediated quinolone resistance (PMQR) in extended-spectrum β-lactamase (ESBL)-producing *Klebsiella pneumoniae* (*K. pneumoniae*) contributes to treatment failures, extended hospital stays, and increased mortality percentages. We aimed to determine the prevalence of PMQR genes in ESBL-producing *K. pneumoniae* isolates from clinical samples in Babol, North of Iran region. This is the first study in this region to investigate this specific association. A total of 95 *K. pneumoniae* isolates were obtained from hospitalized patients with various clinical infections during March 2022 to February 2023. Disk diffusion and Combination disk method were performed to identification of antimicrobial resistance profiles and ESBL-producing strains. The presence of ESBL and PMQR genes among *K. pneumoniae* isolates was assessed using polymerase chain reaction (PCR) method. Of the isolates, 68 (71.57 %) were considered as ESBL-producers. The bla_{TEM}, bla_{SHV} and bla_{CTX-M} genes were detected in 74.73 %, 57.89 %, and 41.05 % of *K. pneumoniae* isolates, respectively. Among the PMQR encoding genes, the highest and lowest frequency was associated to *qepA* (67.3 %) and *qnrA* (4.2 %), respectively. The frequency of *qnrA*, *qnrB*, *qnrS*, *acc* (*6'*)*-Ib-cr*, *qepA*, *oqxA*, and *oqxB* genes in 26 MDR-Kp isolates was 11.53 % (n; 3), 69.23 % (n; 18), 65.38 % (n; 17), 73.07 % (n; 19), 80.76 % (n; 21), 84.61 % (n; 22), and 76.92 % (n; 20), respectively. Our result revealed of the 68 ESBL gene-positive isolates, 60 (88.23 %) were positive for the PMQR gene. The co-occurrence of these genes within resistant isolates suggests potential linkage on mobile genetic elements such as plasmids. These findings highlight the significant burden of PMQR determinants in ESBL-producing *K. pneumoniae* and underscore the urgent need for effective control measures. Implementing robust antimicrobial stewardship

* Corresponding author.
** Corresponding author.
E-mail addresses: m.hoseinzadeh2013@gmail.com (M. Hoseinzadeh), mansour.sedighi60@yahoo.com (M. Sedighi), uyahyapoor@yahoo.com (Y. Yahyapour), mjavanian@gmail.com (M. Javanian), maryam.rozgol@gmail.com (M. Beiranvand), dr.mohamadi61@yahoo.com (M. Mohammadi), sepidezarei6971@gmail.com (S. Zarei), amirmorteza.namvar@gmail.com (A. Pournajaf), abazar_pournajaf@yahoo.com (A. Ebrahimzadeh Namvar).

https://doi.org/10.1016/j.heliyon.2024.e37534
Received 13 February 2024; Received in revised form 20 August 2024; Accepted 4 September 2024
Available online 11 September 2024

programs and strengthening drug-resistance surveillance and control protocols are crucial to prevent the spread of resistant isolates.

1. Introduction

Klebsiella pneumoniae is a gram-negative bacilli that cause a wide range of community and healthcare-acquired infections [1]. A wide range of antimicrobials belongs to different families such as quinolones and β-lactams are used in treatment of these infections, but the main concern is the emergence of multidrug-resistant *K. pneumoniae* (MDR-Kp) [2].

Extensive antibiotic use in hospitalized patients has facilitated the emergence of MDR-Kp, posing a significant challenge for treatment and infection control [3]. MDR-Kp is defined by resistance to at least three antibiotic classes [3]. Studies in Iran report increasing MDR-Kp prevalence, particularly resistant to aminoglycosides, fluoroquinolones (FQs), cephalosporins, and carbapenems [4]. MDR-Kp infections are associated with severe outcomes, including high mortality (around 50 %), treatment failure, quick and more spread of resistance within Gram-negative bacteria and emergence of untreatable infections, prolonged hospitalization, and increased healthcare costs [5]. Furthermore, recent years have seen a rise in MDR-Kp strains harboring transferable resistance genes across Iran, with particularly high prevalence reported in the north [6]. The pooled prevalence of clinical MDR-Kp was estimated at 32.8 % worldwide [2].

FQs are a class of broad-spectrum bactericidal agents that have a bicyclic core linked to a 4-quinolone and are emerging as a common treatment option [7]. FQs resistance is facilitated by various mechanisms such as, chromosomal point mutation at the *gyrA/B* and *parC/E* genes in the quinolone resistance-determining regions (QRDR), and alteration in the outer membrane and efflux pumps [8,9]. Currently, plasmid-mediated quinolone resistance (PMQR) has been shown to play an important role in FQs resistance, and its prevalence is globally growing [10].

PMQR genes can confer low-level resistance to FQs. The first report of a PMQR gene originated from a *K. pneumoniae* isolate in the United States in 1998 [11]. Studies in Iran have documented a high prevalence of PMQR genes among clinical isolates of quinolone-resistant *K. pneumoniae* (QR-Kp) [11–14]. Notably, a study in northern Iran reported that a staggering 90 % of QR-Kp isolates harbored PMQR genes [15].

Furthermore, a separate review article in Iran identified a concerning prevalence of 34.8 % for QR-Kp strains [16]. These findings, along with additional studies conducted throughout Iran, highlight the significant challenge posed by the high level of resistance to quinolones in *K. pneumoniae* [14,15,17,18].

As a warning, it is imaginable to transfer these resistance plasmids to the other strains through horizontal gene transfer (HGT) [19]. To date, several pathways have been defined for PMQR, including (i) qnr protein-encoding genes (qnrA/B/C/D/S), (ii) aac(6')-Ib-cr (an aminoglycoside acetyltransferase), and (iii) FQs-exporting efflux pumps (qepA and OqxAB) [9,20].

Qnr (quinolone resistant) proteins, such as Qnr A, Qnr B, and QnrS, bind to bacterial enzymes, DNA gyrase and topoisomerase IV, preventing FQs from inhibiting their activity. This mechanism is widespread among various bacterial genera globally [14].

AAC(6′)-Ib-cr enzyme modifies fluoroquinolone structure, reducing its effectiveness. It adds an acetyl group to the piperazinyl ring of the FQs molecule, rendering it less effective against bacteria. Notably, *aac(6′)-Ib-cr* can also modify aminoglycosides, highlighting its potential for broad-spectrum resistance [11,14]. The *aac(6′)-Ib-cr* gene encodes an aminoglycoside acetyltransferase that modifies not only aminoglycosides but also ciprofloxacin and norfloxacin. The *aac(6′)-Ib-cr* (cr for ciprofloxacin resistance) is a variant of *aac(6′)-Ib* (responsible for resistance to kanamycin, tobramycin and amikacin) with two amino acid substitutions compared to the wild-type allowing it to acetylate and subsequently reduce the activity of norfloxacin and ciprofloxacin [21,22]. The *aac(6′)-Ib-cr* responsible for low-level ciprofloxacin resistance.

Studies conducted in different regions of Iran report varying prevalence of PMQR genes. The *aac(6′)-Ib-cr* gene appears to be more prevalent than others. A study in northern Iran has estimated a prevalence of 44 % for *aac(6′)-Ib-cr* gene [11–13,15].

OqxAB (olaquindox-carbadox AB), a multidrug efflux pump, is related to reduced fluoroquinolone susceptibility and resistance to multiple agents. QepA (quinolone efflux pump A), an efflux pump belonging to the major facilitator subfamily, is related to a decrease in susceptibility to hydrophilic fluoroquinolones [23]. QepA and OqxAB proteins act as proton-dependent transporters, actively pumping FQs out of the bacterial cell. QepA specifically targets FQs like norfloxacin, ciprofloxacin, and enrofloxacin, while the multidrug efflux pump OqxAB confers resistance to a wider range of antibiotics. The presence of these efflux pumps is concerning as they can promote the development of multidrug resistance and facilitate the spread of resistance genes horizontally between bacteria [11].

Extended-spectrum beta-lactamases (ESBLs) are enzymes produced by bacteria that can break down certain beta-lactam antibiotics, rendering them ineffective. These enzymes were first identified in different countries between 1983 and 1990 [24].

Since then, ESBL-producing *K. pneumoniae* (ESBL-Kp) has become a significant global threat, particularly in healthcare settings. The rapid spread of ESBLs has resulted in over 300 identified variants worldwide [25].

Studies reveal a concerning trend. While the global prevalence of ESBL-Kp isolated from clinical samples is estimated at 32.7 %, Iran reports a substantially higher pooled prevalence of 43.5 % for ESBL-Kp [26,27]. The reports show that the prevalence of ESBL-Kp is increasing over time in Iran [27,28].

ESBLs such as CTX-M (Cefotaximase), TEM (Temoneira) and SHV (Sulfhydryl variable) families are diverse enzymes of Ambler's class A or group 2b and 2be of Bush- Jacoby- Medeiros classification, which are able to hydrolyze penicillin, oxyimino-cephalosporins such as 3ed-generation cephalosporins, and aztreonam [29,30].

The genes for ESBLs are often carried on plasmids, mobile DNA elements easily shared between bacteria. These plasmids can harbor resistance genes to various antibiotic classes, creating multidrug-resistant pathogens. Examples include PMQR and aminoglycoside resistance genes. A significant shift has occurred in the prevalence of different ESBL types. In Africa and Europe, $bla_{\text{CTX-M}}$ ESBL genes have become dominant, dramatically increasing compared to bla_{TEM} and bla_{SHV} variants [31].

CTX-M-type beta-lactamases have become the most prevalent ESBL worldwide in *K. pneumoniae* isolates, with a corresponding decrease in TEM and SHV prevalence [32].

Interestingly, studies in Iran suggest a different trend. While the prevalence of the bla_{SHV} gene remains relatively high and stable, there has been a recent surge in bla_{TEM} and $bla_{\text{CTX-M}}$ genes compared to previous years [27].

Fast and accurate identification of ESBL-producing strains is crucial for prompt patient treatment and halting the further spread of resistant strains. The phenotypic identification of ESBL involves the Combination Disc Test (CDT), Double Disc Synergy Test (DDST), and E-test Strip. Among these methods, the E-test demonstrates higher sensitivity and accuracy compared to CDT and DDST, offering a straightforward workflow. However, it is notable that the E-test method is costlier than other techniques [33]. To confirm the presence of resistant genes, molecular tests like PCR, a widely used method, are recommended. Additionally, DNA sequencing or High-Resolution Melt (HRM) methods can be employed to detect mutations within these genes.

Infections caused ESBL-Kp are a global challenge among physicians because these strains are also resistant to other classes of antibiotics such as aminoglycosides, FQs, and trimethoprim/sulfamethoxazole [34].

Considering to the importance of the complex problem of antibiotic resistance and the lack of a comprehensive study in the northern Iran, the purpose of this cross-sectional study was to determine the prevalence of PMQR genes in ESBL-Kp isolates from clinical samples in Babol, Northern Iran, for the first time.

2. Materials and methods

This descriptive cross-sectional study was approved by the Ethics Committee of the Babol university of medical sciences with the Ethical code number IR.MUBABOL.HRI.REC.1398.039. Samples were collected from three teaching general hospitals including Rohani, Beheshti and Yahyanejad. In total, 95 non-duplicative clinical *K. pneumoniae* isolates obtained from hospitalized patients during March 2022 to February 2023.

2.1. Sample size and bacterial isolation

We calculate sample size using Stata software version 17.0 (STATA Corp, College Station, Tex). The sample size was calculated using single population proportion with the following assumptions: 5 % level of significance ($\alpha = 0.05$), 80 % power of study, 92 % proportion of PMQR. Therefore, in total 95 non-duplicative *K. pneumoniae* were isolated from various clinical samples at three large educational hospitals in Babol, north of Iran. Suspected bacterial colonies were identified by standard methods such as, microscopic examination, motility test, biochemical test including H2S production, indole production, sugar fermentation, citrate and urease tests, lysine decarboxylase test, Methyle red test, Voges-Proskauer test using SIM, Triple-sugar iron agar (TSI), citrate and urea agars, LDC agar and MR-VP medium. All pure *K. pneumoniae* colonies were conserved in the Brain-Heart Infusion (BHI) broth (Becton Dickinson, Franklin Lakes, NJ) including 15 % (v/v) glycerol (Merck Co., Germany) at −70 °C for further use. *K. pneumoniae* ATCC 13883 was used as quality control.

2.2. Antimicrobial susceptibility testing (AST)

AST was performed using the agar disk diffusion method on Mueller-Hinton agar (MHA) (Merck, Darmstadt, Germany) and according to the guidelines of the Clinical and Laboratory Standards Institute (CLSI- 2023). The following antimicrobial discs were used; nalidixic acid (NA, 30 μg, S: ≥19, I: 14–18, R: ≤13), ciprofloxacin (CIP, 5 μg, S: ≥21, I: 18–20, R: ≤17), ofloxacin (OFX, 5 μg, S: ≥16, I: 13–15, R: ≤12), norfloxacin (NOR, 10 μg, S: ≥17, I: 13–16, R: ≤12), levofloxacin (LEV, 5 μg, S: ≥17, I: 14–16, R: ≤13), ceftazidime (CAZ, 30 μg, S: ≥21, I: 16–20, R: ≤15), cefotaxime (CTX, 30 μg, S: ≥26, I: 23–25, R: ≤22), cefepime (FEP, 30 μg, S: ≥25, I: 19–24, R: ≤18), imipenem (IPM, 10 μg, S: ≥23, I: 20–22, R: ≤19), aztreonam (ATM, 30 μg, S: ≥21, I: 18–20, R: ≤17), amoxicillin-clavulanate (AMC, 20/10 μg, S: ≥18, I: 14–17, R: ≤13), gentamicin (GM, 10 μg, S: ≥15, I: 13–14, R: ≤12), amikacin (AN, 30 μg, S: ≥17, I: 15–16, R: ≤14), tetracycline (TET, 30 μg, S: ≥15, I: 12–14, R: ≤11), and trimethoprim-sulfamethoxazole (SXT, 1.25/23.75 μg, S: ≥16, I: 11–15, R: ≤10) (Padtan Teb Co, Iran). Isolates resistant to at least three or more different antimicrobial classes are considered MDR [35].

2.3. ESBL phenotypic confirmation

The Combination disk method was used as a reference approach. On Mueller-Hinton agar, ceftazidime (30 μg), ceftazidime clavulanate (30/10 μg), cefotaxime (30 μg), and cefotaxime clavulanate (30/10 μg) were employed in compliance with CLSI guidelines 2023. For 16–18 h, the plates were incubated at 37 °C. ESBL producers were identified as a 5-mm increase in zone diameter for indicator cephalosporins with clavulanate instead of indicator cephalosporins alone [36].

Combination disk method was performed using CTX (30 μg) and CAZ (30 μg) with and without clavulanic acid (CLA; 10 μg). The diameter of the resulting zone of inhibition was compared [37]. The test was considered positive when the difference of zone diameters between alone and combined disks was ≥ 5 mm.

2.4. Genomic DNA extraction

Genomic DNA was extracted based on Chen et al. (1993) study with brief modification [38]. The quantity of extracted genome was checked by 1.0 % agarose gel electrophoresis, while the purity and concentration were assessed using Thermo Scientific NanoDrop 2000 Spectrophotometer ($OD_{260/280}$ = 1.8–2.0 nm). Template DNA kept at −20 °C for more used.

2.5. Molecular detection of resistance determinants

As listed in Table 1, a set of primers are used for PCR. The reactions were carried out in a Techne TC-512 thermocycler (Eppendorf, Hamburg, Germany) in a final volume of 25 μl according to Table 2. PCR products were subjected to the ultraviolet trans-illuminator (Bio-Rad, Hercules, USA) after running at 100 V for 60 min on a 1.0 % agarose/TBE 0.5X (45 mM-Tris-borate, 1 mM-EDTA, pH = 8.0) gel stained with DNA safe stain (SinaClon, Tehran, Iran).

2.6. Statistical analysis

The statistical analysis was done by SPSS software, version 22.0 (IBM, Armonk, NY, USA) and Chi-square test was used to measure the level of significance. A *P-value* less than 0.05 were considered statistically significant.

3. Results

In total, 95 non-duplicative *K. pneumoniae* isolates were obtained from the various clinical samples including, wound (n; 39, 41.05 %), urine (n; 33, 34.73 %), blood (n; 10, 10.52 %), sputum (n; 9, 9.47 %), bronchoalveolar lavage (n; 3, 3.15 %) and cerebrospinal fluid (n; 1, 1.05 %). The mean age of the patients was 32 ± 1.2 years, including, 3 months-4 years (4.2 %, n; 4/95), 5–14 years (11.6 %, n; 11/95), 15–25 years (8.4 %, n; 8/95), 26–36 years (13.7 %, n; 13/95), 37–47 years (15.7 %, n; 15/95), 48–58 years (11.6 %, n; 11/95), 59–69 year (20.0 %, n; 19/95), and >70 years (14.7 %, n; 14/95). Fifty-four (56.8 %) of patients were male. Twenty-one (22.1 %) of cases were smokers. Three patients were diagnosed with cancer and one patient was suspected of having cystic fibrosis (CF). Six patients were on dialysis. The most commonly comorbidities were diabetes mellitus (DM) (15.7 %, n; 15/95), hypertension (HP) (14.7 %, n; 14/95), hyperlipidemia (HL) (9.5 %, n; 9/95), cardiovascular disease (CVD) (8.4 %, n; 8/95) and thyroid disorders (7.4 %, n; 7/95). The samples were obtained from different wards of the hospitals, including inpatients emergency (n; 22, 23.2 %), infectious (n; 19, 20.0 %), ICU (n; 15, 15.7 %), surgery (n; 13, 13.6 %), internal medicine (n; 12, 12.6 %), gynecology (n; 6, 6.3 %), dialysis (n; 6, 6.3 %) and neurology (n; 2, 2.1 %).

As shown in Table 3, the highest and lowest resistance percentages were related to AMC (n; 76, 80 %) and IPM (n; 3, 3.2 %). The pattern of resistance to FQs was as follows; NA (n; 23, 24.21 %), CIP (n; 21, 22.1 %), NOR (n; 27, 28.42 %), OFX (n; 18, 18.94 %) and LEV (n; 20, 21.05 %). Out of 95 *K. pneumoniae* isolates, 38 (40.0 %) isolates showed resistance to FQs. Among all strains, 26 (27.36 %) *K. pneumoniae* isolates were multi-drug resistance (MDR). The results of the combination disk method showed that 71.57 % (n; 68/95) isolates had an inhibition zone diameter ≥5 mm and therefore were considered as ESBL-producers.

Resistance to FQs was higher in ESBL-producing *K. pneumoniae* (Table 3). Only 3.2 % of the total isolates were resistant to IPM,

Table 1
Sequence of primer pairs used in this study.

Target Genes		Primer sequences (5'→3')	Product size (bp)	Ref
FQs-resistance genes	*qnrA*	F; 5' - AGAGGATTTCTCACGCCAGG -3' R; 5' - GTCAAGATCTGTGCCTGGCA -3'	571	[72]
	qnrS	F; 5' - ACGACATTCGTCAACTGCAA -3' R; 5' - GCCTACAGGGTGCCAATTTA -3'	417	[73]
	acc (6′)-Ib-cr	F; 5' - TTGCGATGCTCTATGAGTGGCTA -3' R; 5' - AAACACGCCAGGCATTCGAG -3'	482	[22]
	qepA	F; 5' -GCAGGTCCAGCAGCGGGTAG-3' R; 5' -CTTCCTGCCCGAGTATCGTG-3'	218	[74]
	qnrB	F; 5' - ATGACGCCATTACTGTATAA -3' R; 5' - GATCGCAATGTGTGAAGTTT-3'	408	[75]
	oqxA	F; 5' - CTCGGCGCGATGATGCT -3' R; 5' - CCACTCTTCACGGGAGACGA -3'	392	[37]
	oqxB	F; 5' - TTCTCCCCCGGCGGGAAGTAC -3' R; 5' - CTCGGCCATTTTGGCGCGTA -3'	512	
ESBLs	bla_{SHV}	F:5′- ATGCGTTATATTCGCCTGTG -3' R:5′- GTTAGCGTTGCCAGTGCTCG -3'	862	[76]
	bla_{TEM}	F:5′- AGTATTCAACATTTCCGTGTC -3′ R:5′- GCTTAATCAGTGAGGCACCTATC -3′	850	
	bla_{CTX-M}	F:5′- TTTGCGATGTGCAGTACCAGTAA-3′ R:5′- CGATATCGTTGGTGGTGCCATA -3′	544	

qnr; quinolone resistant, *acc (6′)-Ib-cr*; aminoglycoside 6′-N-acetyltransferase type Ib-cr, *qep*; quinolone efflux pump, *oqx*; olaquindox-carbadox, *bla*; β-lactamase.

Table 2
PCR programs and cycles adjusted in the present study.

Reaction set	Amplified genes	Reaction compounds	Multiplex-PCR program	Cycles of amplification
1	*qnrA, qnrS, qepA, oqxA*	1.5 μL of template, 12.5 μL of PCR Master Mix, 1.0 μL of each primer, and 9.0 μL of ddH2O.	Initial denaturation at 94 °C for 7 min, denaturation at 95 °C for 40 s, annealing at 57 °C for 60 s, extension at 72 °C for 60 s and a final extension at 72 °C for 5 min.	32
2	*acc (6′)-Ib-cr, oqxB*	1.0 μL of template DNA, 13.5 μL of CinnaGen PCR Master Mix, 1.1 μL of each primer, and 8.3 μL of ddH2O	initial denaturation at 95 °C for 7 min, denaturation at 94 °C for 55 s, annealing at 57 °C for 50 s, extension at 72 °C for 1 min and a final extension at 72 °C for 7 min.	
3	*qnrB*	1.2 μL of template DNA, 12.6 μL of CinnaGen PCR Master Mix, 0.9 μL of each primer, and 9.7 μL of ddH2O	initial denaturation at 94 °C for 5 min, denaturation at 95 °C for 55 s, annealing at 54 °C for 50 s, extension at 72 °C for 60 s and a final extension at 72 °C for 5 min.	35
4	bla_{SHV}, bla_{TEM}, bla_{CTX-M}	1.8 μL of template DNA, 13.6 μL of CinnaGen PCR Master Mix, 1.5 μL of each primer, and 6.6 μL of ddH2O.	initial denaturation at 95 °C for 5 min, denaturation at 95 °C for 60 s, annealing at 58 °C for 45 s, extension at 72 °C for 60 s and a final extension at 72 °C for 5 min.	33

which were considered as carbapenem-resistant *K. pneumoniae* (CRKp). All CRKp isolates were ESBL-producers. As can be deduced from the PCR results, the prevalence of bla_{TEM}, bla_{SHV} and bla_{CTX-M} genes were 74.73 % (n; 71/95), 57.89 % (n; 55/95) and 41.05 % (n; 39/95), respectively. So, the prevalence of β-lactamase genes in the ESBL-producing isolates was as follows, bla_{TEM} (77.94 %, n; 53/68), bla_{SHV} (60.29 %, n; 41/68) and bla_{CTX-M} (48.52 %, n; 33/68). The frequency of genes encoding PMQR was higher in ESBL-Kp isolates (Table 4). In total, the distribution of PMQR genes was as follows; *qnrA*; 4.21 % (n; 4/95), *qnrB*; 53.68 % (n; 51/95), *qnrS*; 54.73 % (n; 52/95), *acc (6′)-Ib-cr*; 58.94 % (n; 56/95), *qepA*; 67.36 % (n; 64/95), *oqxA*; 49.47 % (n; 47/95) and *oqxB*; 60.00 % (n; 57/95), respectively. The frequency of PMQR-encoding genes among ESBL-producing-Kp isolates is listed in Table 4. The *qnrA* gene was found only in ESBL-positive isolates. The prevalence of PMQR-encoding genes in the quinolone-resistant *K. pneumoniae* isolates are shown in Table 5. Out of 26 MDR-Kp isolates, the frequency of *qnrA*, *qnrB*, *qnrS*, *acc (6′)-Ib-cr*, *qepA*, *oqxA* and *oqxB* genes were 11.53 % (n; 3/26), 69.23 % (n; 18/26), 65.38 % (n; 17/26), 73.07 % (n; 19/26), 80.76 % (n; 21/26), 84.61 % (n; 22/26) and 76.92 % (n; 20/26), respectively. No significant difference was observed in the distribution of PMQR genes in MDR isolates compared to non-MDR (*P* value >0.05).

4. Discussion

K. pneumoniae, a common nosocomial opportunistic pathogen, presents a growing challenge due to its increasing antibiotic resistance. Horizontal gene transfer (HGT) of resistance plasmids, particularly those conferring PMQR, significantly contributes to this problem [39–41]. These plasmids often carry additional resistance genes, further escalating the threat. The global rise in PMQR prevalence underlines its crucial role in *K. pneumoniae* resistance development [42]. Our study detected a substantial 40.0 % FQ resistance rate in *K. pneumoniae* isolates. Reported percentages vary regionally, ranging from 41.3 % in Egypt [43] to 60.4 % in Iran [44], and even 89 % in India [45]. Notably, our study revealed the following resistance pattern: NA (24.21 %), CIP (22.1 %), NOR (28.42 %), OFX (18.94 %), and LEV (21.05 %). In Iran, the high prevalence of FQ-resistant *K. pneumoniae* infections poses a significant medical challenge. While excessive antibiotic use likely contributes to this resistance through genetic factors, further research in this area is crucial.

ESBLs production is a major resistance mechanism that impedes the antibacterial treatment of *K. pneumoniae* infections and is a significant threat to the currently available antibiotic therapy and human health [40,46]. Numerous studies report a strong co-occurrence of ESBL and plasmid-mediated quinolone resistance PMQR genes, facilitating the emergence of MDR isolates and further amplifying resistance [11,47–51]. Our study observed a high prevalence of ESBL-producing *K. pneumoniae* (71.57 %, n = 68/95), in agreement with reports from Iran and other countries [13,52,53].

In a study performed by Feizabadi et al. in 2010 [54], 69.7 % of *K. pneumoniae* isolates were ESBL producers. They found that bla_{SHV} (67.4 %) was the most prevalent gene detected and followed by bla_{TEM} (54 %), $bla_{CTX-M-I}$ (38.2 %), and $bla_{CTXM-III}$ (27 %), while in our study the most prevalent ESBL gene among *K. pneumoniae* isolates was bla_{TEM} (74.7 %). In fact, our results indicated that bla_{TEM} is the main gene responsible for *K. pneumoniae* with ESBL phenotype (77.94 %). Goudarzi et al. (2015) revealed that a high presence of bla_{CTX-M} (74.9 %), followed by bla_{TEM} (70 %) and bla_{SHV} (59.9 %), respectively [11]. In study carried out by Sedighi et al. (2017), the prevalence of the bla_{TEM}, bla_{CTX-M} and bla_{SHV} was 38 %, 24 %, 19 %, respectively [40]. Sadeghi et al. confirmed that the bla_{TEM} is the most dominant gene among ESBL-producing *K pneumoniae*. These findings suggest a concerningly high prevalence of ESBL genes in Iran, possibly influenced by regional differences, inadequate antibiotic stewardship, and dissemination of antimicrobial resistance determinants. Further research is warranted to pinpoint the precise factors contributing to these trends and inform targeted interventions.

This study confirms the global trend of widespread distribution of plasmid-mediated quinolone resistance (PMQR) genes in *K. pneumoniae*. Notably, ESBL-positive isolates displayed higher prevalence of PMQR genes than ESBL-negative isolates, which aligns with previous reports from Iran and other countries [13,55–57]. This further supports the notion that PMQR determinants and ESBL genes are likely co-localize on plasmids, facilitating their co-transmission via horizontal gene transfer such as plasmid conjugation [51]. Among our PMQR genes *qnrB*, *oqxA* and *oqxB* were the most prevalent genes that frequently higher in ESBL producer isolates than non-ESBL isolates. Interestingly, we observed no significant difference in PMQR gene distribution within MDR isolates compared

Table 3
Antibiotic resistance pattern in *K. pneumoniae* isolates.

Strains	No [(%)] of resistance to antimicrobials in *K. pneumoniae*														
	CIP	CAZ	CTX	IPM	ATM	FEP	GM	AN	AMC	TET	LEV	NOR	NA	SXT	OFX
ESBLs-positive (n; 68)	12 (17.5)	21 (30.8)	24 (35.3)	3 (4.4)	19 (27.9)	20 (29.4)	48 (70.6)	50 (73.5)	60 (88.2)	42 (61.7)	15 (22.0)	18 (26.5)	18 (72.0)	61 (89.7)	13 (19.1)
ESBLs-negative (n; 27)	9 (33.3)	14 (51.8)	6 (22.2)	0 (0.0)	8 (29.6)	4 (14.8)	4 (14.8)	8 (29.6)	16 (59.2)	20 (74.1)	5 (18.5)	9 (33.3)	5 (18.5)	11 (40.7)	5 (18.5)
P-value	0.198	0.209	0.364	0.281	0.903	0.243	0.048[a]	0.037[a]	0.271	0.609	0.757	0.623	0.52	0.046[a]	0.956

CIP; ciprofloxacin, CAZ; ceftazidime, CTX; cefotaxime, IPM; imipenem, ATM; aztreonam, FEP; cefepime, GM; gentamicin, AN; amikacin, AMC; amoxicillin-clavulanate, TET; tetracycline, LEV; levofloxacin, NOR; norfloxacin, NA; nalidixic acid, OFX; Ofloxacin and SXT; trimethoprim-sulfamethoxazole.

[a] Significant at 0.05 level.

Table 4
The distribution of resistance genes in *K. pneumoniae* isolates.

Selected genes	ESBLs-positive (n; 68)	ESBLs-negative (n; 27)	*P-value*
qnrA	4 (5.88)	0 (0.0)	0.214
qnrB	41 (60.29)	10 (37.03)	0.245
qnrS	38 (55.88)	14 (51.85)	0.847
acc (6′)-Ib-cr	42 (61.76)	14 (51.85)	0.649
qepA	47 (69.11)	17 (62.96)	0.798
oqxA	38 (55.88)	9 (33.33)	0.234
oqxB	49 (72.05)	8 (29.62)	0.042[a]
bla_{TEM}	53 (77.94)	18 (66.66)	0.661
bla_{SHV}	41 (60.29)	14 (51.85)	0.695
bla_{CTX-M}	33 (48.52)	6 (22.22)	0.113

[a] Significant at 0.05 level.

Table 5
The prevalence of PMQR genes in the quinolone-resistant and MDR *K. pneumoniae* isolates.

Bacterial Strains	No [(%)] of PMQR -encoding genes						
	qnrA	qnrB	qnrS	acc (6′)-Ib-cr	qepA	oqxA	oqxB
Quinolone resistant Kp (n; 38)	4 (10.52)	29 (76.31)	28 (73.68)	30 (78.94)	34 (89.47)	28 (73.68)	35 (92.1)
MDR-Kp (n; 26)	3 (11.53)	18 (69.23)	17 (65.38)	19 (73.07)	21 (80.76)	22 (84.61)	20 (76.92)
P-value	0.91	0.805	0.766	0.843	0.787	0.718	0.636

to non-MDR ones isolates (*P value* < 0.37). The *qnrB* (69.23 %), *qnrS* (65.38 %), *acc (6′)-Ib-cr* (73.07 %), *qepA* (80.76 %), *oqxA* (84.61 %) and *oqxB* (76.92 %) being most frequent. These findings are partially consistent with Malek Jamshidi et al.'s findings in 2019, which indicated the prevalence of PMQR genes among MDR-Kp isolates as follows: *qnrB* (25.0 %), *qnrS* (18.75 %), *aac(6′)-Ib-cr* (50.0 %) and *oqxAB* (93.75 %). Overall, these findings imply that the spread of PMQR determinants is largely attributed to plasmid transmission through horizontal exchange.

Our results revealed a high prevalence (58.9 %) of *acc (6′)-Ib-cr* gene among all isolated *K. pneumoniae* isolates. In line with our results, studies carried out in various parts of Iran by Vaziri et al. (2020), Eftekhar et al. (2015), Malek Jamshidi et al. (2019) and Shams et al. (2015) demonstrated that he frequency of *aac(6′)-Ib-cr* was 55.6 %, 53.2 %, 50 % and 70.1 %, respectively [13,52,58,59]. The prevalence of *qnrB* gene (53.6 %) in our study is agreement with Shams et al. (2015) at 46 % and Dehghan Banadkouki et al. (2017) at 45.7 %; however, it differed from other studies in Iran [13,47,52,58–61]. Moreover, Studies carried out by Tahou et al. (2017) in Abidjan and Selah et al. (2019) in Togo explained higher frequency of *qnrB* gene (71.73 % and 60 %, respectively) in *K. pneumoniae* isolates [48,62]. This highlights the potential geographical variations in PMQR gene epidemiology. In our survey, the prevalence of the *oqxA* (49.5 %) and *oqxB* (60 %) genes was notably high. This aligns with the study conducted by Amereh et al. (2023) in Iran, which reported 95 % frequency for *oqxA* and 98 % for *oqxB* genes [60]. Similarly, Malek Jamshidi et al. (2019) reported high prevalence of *oqxAB* (93.75 %) gene among *K. pneumoniae* isolates in Iran [59]. The high-level expression of the *oqxAB* pump has been linked to reduced susceptibility to FQ in ESBL-producing *K. pneumoniae* isolates, contributing to the emergence of PMQR strains and ultimately MDR bacteria [63,64]. Contrastingly, the presence of the *qnrA* gene (4.2 %) and the high frequency of the *qnrS* gene (54.7 %) observed in our *K. pneumoniae* isolates differed from other research conducted in Iran and other countries [13,58–60,65,66]. Studies by Amereh et al. (2023), Eftekhar et al. (2015), Malek Jamshidi et al. (2019), and Shams et al. (2015) revealed no *K. pneumoniae* isolates harboring the *qnrA* gene, while *qnrS* was detected in 9 %, 2.5 %, 18.75 %, and 5.7 % of their investigated isolates, respectively. Vaziri et al. (2020) reported a prevalence of 7.93 % for the *qnrS* gene. Conversely, Abossedgh et al. (2020) in Iran found a frequency of 33.3 % for *qnrS* and 13.7 % for *qnrA* in *K. pneumoniae* isolates [47]. Tahou et al. (2017) stated a prevalence of 2.17 % for *qnrA* and 26.08 % for *qnrS* [62]. Correspondingly, in a study conducted by Salah et al. (2019) in Togo, the prevalence of *qnrA* and *qnrS* genes among *Klebsiella* spp. were 7.2 % and 45.45 %, respectively [48]. Additionally, in Morocco (2010), the *qnrA* gene was detected in 10 % of *Klebsiella* species [55]. Few studies have been conducted on the *qepA* gene in Iran. Malek Jamshidi et al. (2019) and Shams et al. (2015) found no evidence of the *qepA* gene among ESBL-producing *K. pneumoniae* isolates, while our study demonstrated a high frequency (67.3 %) of this gene [13,59]. Notably, the *qepA* gene was only found in our ESBL-Kp isolates in Iran. Similar observations were made by researchers in France, Japan, and China, who noted the presence of the *qepA* gene in clinical isolates. However, studies in Thailand, Korea, and Mexico reported no *qepA* gene in *K. pneumoniae* isolates [49,67–69].

Our findings suggest that the prevalence of PMQR can vary across different regions and hospitals. These variations in PMQR gene prevalence among studies may reflect regional disparities in quinolone and other antibiotic usage, emphasizing the ongoing need for surveillance, antimicrobial stewardship, and appropriate antibiotic utilization. Plasmid isolation examination and conjugation experiments in various studies have shown that PMQR genes are carried by high molecular weight conjugative plasmids, enabling their transfer between different bacteria. This understanding elucidates the epidemic spread of quinolone resistance via horizontal gene transfer [48,69–71]. The coexistence of ESBL and certain PMQR encoding genes within the same mobile genetic elements could account for co-resistance to beta-lactams and FQs [48]. Given that these genes are often carried on plasmid and can be easily disseminated among members of Enterobacterales through gene transfer mechanisms, further research is essential for establishing

appropriate antibiotic treatments and preventing the dissemination of resistance determinants among pathogenic species, including *K. pneumoniae*. It's important to note that in addition to transferable plasmid genes, other mechanisms such as chromosomal point mutations at the *gyrA/B* and *parC/E* genes in the QRDR, as well as alterations in the outer membrane and efflux pumps, also contribute to FQ resistance [8,9]. Therefore, to validate our results, it seems necessary to conduct comprehensive epidemiological studies to widely and simultaneously identify all resistance mechanisms. While such extensive studies are costly and time-consuming, their results can significantly impact the healthcare system by preventing the spread of resistant strains, particularly within hospital settings.

The most important limitations of this study are as follows: First, the study was limited by a relatively small sample size (samples collected from three teaching hospitals affiliated with Babol University of Medical Sciences, Babol, north of Iran). A larger, multi-center study would be necessary to improve generalizability of the findings. Other limitation is survey of alternative FQs-resistance mechanisms, such as mutation types. Finally, the study did not assess the genetic relationships between the resistant strains. This information could be valuable for elucidating potential transmission patterns and emergence of clonal outbreaks.

5. Conclusion

Our study demonstrates a worrying prevalence of PMQR genes in QR-*K.p* isolates from Iran, especially among those producing ESBLs and multidrug-resistant strains. Efflux pumps mechanism might be the main factors for quinolone resistance and MDR; it enhanced efflux transporters to reduce intracellular drug concentrations. We showed that the bla_{TEM} ESBL gene is the main factor responsible for *K. pneumoniae* with ESBL phenotype. The co-occurrence of PMQR and ESBL genes signifies a significant threat, contributing to the emergence of MDR bacteria and hampering effective treatment options. To combat this growing challenge, it is imperative to adhere to key programs, such as antimicrobial stewardship initiatives and the vigilant oversight of judicious antibiotic prescriptions. Furthermore, rigorous epidemiological studies are essential to examine the prevalence of drug-resistant strains across diverse geographic regions. Employing highly sensitive and specific diagnostic tests for early detection and screening of resistance determinants, along with practical monitoring and control strategies, is crucial to thwart the dissemination of pathogenic resistant strains. Moreover, further investigation is necessary to comprehend the fundamental principles underlying antibiotic resistance, the mechanisms of drug resistance in pathogenic bacteria, and to develop innovative approaches to address this evolving challenge.

Funding and financial support

This study was financially supported by the Babol University of Medical Sciences (grant no. 5422).

Data availability statement

Data available within the article or its supplementary materials.

CRediT authorship contribution statement

Maedeh Hoseinzadeh: Writing – review & editing, Funding acquisition, Data curation, Conceptualization. **Mansour Sedighi:** Writing – review & editing, Writing – original draft, Validation, Methodology, Investigation. **Yousef Yahyapour:** Software, Methodology, Formal analysis, Data curation. **Mostafa Javanian:** Writing – original draft, Visualization, Data curation, Conceptualization. **Maryam Beiranvand:** Writing – review & editing, Visualization, Validation, Methodology, Investigation. **Mohsen Mohammadi:** Writing – review & editing, Writing – original draft, Resources, Methodology. **Sepide Zarei:** Writing – original draft, Visualization, Validation, Resources, Investigation. **Abazar Pournajaf:** Supervision, Software, Project administration, Methodology, Investigation, Formal analysis, Conceptualization. **Amirmorteza Ebrahimzadeh Namvar:** Visualization, Validation, Supervision, Software, Resources, Project administration, Funding acquisition, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e37534.

References

[1] Ş. Nigiz, G. Hazırolan, Ö. Köseoglu Eser, D. Gür, First detection of Klebsiella pneumoniae isolate Co-harboring fosfomycin resistance gene fosA3 and bla ctx-m among gram negative urine isolates in a Turkish hospital, Microb. Drug Resist. 28 (3) (2022) 317–321.

[2] N.A. Mohd Asri, S. Ahmad, R. Mohamud, N. Mohd Hanafi, N.F. Mohd Zaidi, A.A. Irekeola, et al., Global prevalence of nosocomial multidrug-resistant Klebsiella pneumoniae: a systematic review and meta-analysis, Antibiotics 10 (12) (2021) 1508.
[3] N.A. Mohd Asri, S. Ahmad, R. Mohamud, N. Mohd Hanafi, N.F. Mohd Zaidi, A.A. Irekeola, et al., Global prevalence of nosocomial multidrug-resistant Klebsiella pneumoniae: a systematic review and meta-analysis, Antibiotics 10 (12) (2021).
[4] Z. Jafari, A.A. Harati, M. Haeili, J. Kardan-Yamchi, S. Jafari, F. Jabalameli, et al., Molecular epidemiology and drug resistance pattern of carbapenem-resistant Klebsiella pneumoniae isolates from Iran, Microb. Drug Resist. 25 (3) (2019) 336–343.
[5] S. Shadkam, H.R. Goli, B. Mirzaei, M. Gholami, M. Ahanjan, Correlation between antimicrobial resistance and biofilm formation capability among Klebsiella pneumoniae strains isolated from hospitalized patients in Iran, Ann. Clin. Microbiol. Antimicrob. 20 (1) (2021) 13.
[6] M. Farhadi, M. Ahanjan, H.R. Goli, M.R. Haghshenas, M. Gholami, High frequency of multidrug-resistant (MDR) Klebsiella pneumoniae harboring several β-lactamase and integron genes collected from several hospitals in the north of Iran, Ann. Clin. Microbiol. Antimicrob. 20 (1) (2021) 70.
[7] J.C. Ontong, N.F. Ozioma, S.P. Voravuthikunchai, S. Chusri, Synergistic antibacterial effects of colistin in combination with aminoglycoside, carbapenems, cephalosporins, fluoroquinolones, tetracyclines, fosfomycin, and piperacillin on multidrug resistant Klebsiella pneumoniae isolates, PLoS One 16 (1) (2021) e0244673.
[8] P.V. Geetha, K.V.L. Aishwarya, S. Mariappan, U. Sekar, Fluoroquinolone resistance in clinical isolates of Klebsiella pneumonia e, J. Lab. Phys. 12 (2) (2020) 121–125.
[9] S.M. Kareem, I.M. Al-Kadmy, S.S. Kazaal, A.N. Mohammed Ali, S.N. Aziz, R.R. Makharita, et al., Detection of gyrA and parC mutations and prevalence of plasmid-mediated quinolone resistance genes in Klebsiella pneumoniae, Infect. Drug Resist. (2021) 555–563.
[10] Q. Zhan, Y. Xu, B. Wang, J. Yu, X. Shen, L. Liu, et al., Distribution of fluoroquinolone resistance determinants in Carbapenem-resistant Klebsiella pneumoniae clinical isolates associated with bloodstream infections in China, BMC Microbiol. 21 (1) (2021) 1–8.
[11] M. Goudarzi, M. Azad, S.S. Seyedjavadi, Prevalence of plasmid-mediated quinolone resistance determinants and OqxAB efflux pumps among extended-spectrum-lactamase producing Klebsiella pneumoniae isolated from patients with nosocomial urinary tract infection in Tehran, Iran, Sci. Tech. Rep. (2015) 2015.
[12] N. Jomehzadeh, K. Ahmadi, M.A. Bahmanshiri, Investigation of plasmid-mediated quinolone resistance genes among clinical isolates of Klebsiella pneumoniae in southwest Iran, J. Clin. Lab. Anal. 36 (7) (2022) e24342.
[13] E. Shams, F. Firoozeh, R. Moniri, M. Zibaei, Prevalence of plasmid-mediated quinolone resistance genes among extended-spectrum β-Lactamase-producing Klebsiella pneumoniae human isolates in Iran, Journal of pathogens 2015 (2015).
[14] M. Ghane, L. Babaeekhou, M. Asgharifard, Prevalence of plasmid-mediated quinolone resistance genes and molecular typing of Klebsiella pneumoniae isolates from blood cultures in Milad hospital, Tehran, Iran, within 2018 - 2019, Jundishapur J. Microbiol. 15 (7) (2022) e124054.
[15] L. Asadpour, M. Moradi Bazghaleh, Frequency of plasmid-located quinolone resistance genes in clinical isolates of Klebsiella pneumoniae in northern Iran, Medical Laboratory Journal 17 (3) (2023) 32–37.
[16] M. Heidary, M.J. Nasiri, H. Dabiri, S. Tarashi, Prevalence of drug-resistant Klebsiella pneumoniae in Iran: a review article, Iran. J. Public Health 47 (3) (2018) 317.
[17] M. Nourozi, S. Mirkalantari, S. Omidi, Frequency of plasmid-mediated quinolone resistance genes qnrA, qnrB, and qnrS among clinical isolates of Klebsiella pneumoniae, Journal of Applied Biotechnology Reports 7 (4) (2020) 203–207.
[18] G.S. Sani, M. Ghane, L. Babaeekhou, Fluoroquinolone-resistance mechanisms and molecular epidemiology of ciprofloxacin-resistant Klebsiella pneumoniae isolates in Iran, Folia Microbiol. 68 (4) (2023) 633–644.
[19] R. Onishi, K. Shigemura, K. Osawa, Y.-M. Yang, K. Maeda, H. Tanimoto, et al., Impact on quinolone resistance of plasmid-mediated quinolone resistance gene and mutations in quinolone resistance-determining regions in extended spectrum beta lactamase-producing Klebsiella pneumoniae isolated from urinary tract infection patients, Pathogens and Disease 80 (1) (2022) ftac030.
[20] G.S. Sani, M. Ghane, L. Babaeekhou, Fluoroquinolone-resistance mechanisms and molecular epidemiology of ciprofloxacin-resistant Klebsiella pneumoniae isolates in Iran, Folia Microbiol. (2023) 1–12.
[21] M.W. Vetting, C.H. Park, S.S. Hegde, G.A. Jacoby, D.C. Hooper, J.S. Blanchard, Mechanistic and structural analysis of aminoglycoside N-acetyltransferase AAC (6′)-Ib and its bifunctional, fluoroquinolone-active AAC (6′)-Ib-cr variant, Biochemistry 47 (37) (2008) 9825–9835.
[22] C.H. Park, A. Robicsek, G.A. Jacoby, D. Sahm, D.C. Hooper, Prevalence in the United States of aac (6′)-Ib-cr encoding a ciprofloxacin-modifying enzyme, Antimicrob. Agents Chemother. 50 (11) (2006) 3953–3955.
[23] M. Goudarzi, M. Fazeli, Quinolone resistance determinants qnr, qep, and aac (6')-Ib-cr in extended-spectrum B-lactamase producing Escherichia coli isolated from urinary tract infections in Tehran, Iran, Shiraz E-Medical Journal. 18 (5) (2017).
[24] S. Shaikh, J. Fatima, S. Shakil, S.M.D. Rizvi, M.A. Kamal, Antibiotic resistance and extended spectrum beta-lactamases: types, epidemiology and treatment, Saudi J. Biol. Sci. 22 (1) (2015) 90–101.
[25] S. Ghafourian, Z. Bin Sekawi, N. Sadeghifard, R. Mohebi, V. Kumari Neela, A. Maleki, et al., The prevalence of ESBLs producing Klebsiella pneumoniae isolates in some major hospitals, Iran, Open Microbiol. J. 5 (2011) 91–95.
[26] T. Ramatla, T. Mafokwane, K. Lekota, M. Monyama, G. Khasapane, N. Serage, et al., "One Health" perspective on prevalence of co-existing extended-spectrum β-lactamase (ESBL)-producing Escherichia coli and Klebsiella pneumoniae: a comprehensive systematic review and meta-analysis, Ann. Clin. Microbiol. Antimicrob. 22 (1) (2023) 88.
[27] R. Beigverdi, L. Jabalameli, F. Jabalameli, M. Emaneini, Prevalence of extended-spectrum β-lactamase-producing Klebsiella pneumoniae: first systematic review and meta-analysis from Iran, Journal of Global Antimicrobial Resistance 18 (2019) 12–21.
[28] S. Davoudabadi, M. Goudarzi, A. Hashemi, Detection of virulence factors and antibiotic resistance among *Klebsiella pneumoniae* isolates from Iran, BioMed Res. Int. 2023 (2023) 3624497.
[29] E. Haghighifar, F. Norouzi, R.K. Dolatabadi, Molecular detection of Extended-Spectrum β-lactamases (ESBLs) and biofilm formation in uropathogen Klebsiella pneumoniae in Iran, Med. J. Islam. Repub. Iran 35 (2021) 72.
[30] M. Shyaula, C. Khadka, P. Dawadi, M.R. Banjara, Systematic review and meta-analysis on extended-spectrum β-lactamases producing Klebsiella pneumoniae in Nepal, Microbiol. Insights 16 (2023) 11786361221145179.
[31] E. Ugbo, C. Anyamene, I. Moses, I. Iroha, O. Babalola, E. Ukpai, et al., Prevalence of blaTEM, blaSHV, and blaCTX-M genes among extended spectrum beta-lactamase-producing Escherichia coli and Klebsiella pneumoniae of clinical origin, Gene Reports 21 (2020) 100909.
[32] M. Castanheira, P.J. Simner, P.A. Bradford, Extended-spectrum β-lactamases: an update on their characteristics, epidemiology and detection, JAC Antimicrob Resist 3 (3) (2021) dlab092.
[33] M. Salihu, A. Yarima, H. Atta, Methods for the phenotypic detection of extended spectrum beta lactamase (ESBL)-Producing bacteria, Nigerian Journal of Biotechnology 37 (2) (2020) 113–125.
[34] G. Odewale, M.Y. Jibola-Shittu, O. Ojurongbe, R.A. Olowe, O.A. Olowe, Genotypic determination of extended spectrum β-lactamases and carbapenemase production in clinical isolates of Klebsiella pneumoniae in southwest Nigeria, Infect. Dis. Rep. 15 (3) (2023) 339–353.
[35] CLSI standard M100, Performance Standards for Antimicrobial Susceptibility Testing, 34th ed., Clinical and Laboratory Standards Institute, 2023.
[36] A. Abdeta, A. Bitew, S. Fentaw, E. Tsige, D. Assefa, E. Tigabu, et al., The diagnostic capacity of three phenotypic techniques of extended-spectrum β-lactamase detection, Avicenna Journal of Clinical Microbiology and Infection 9 (1) (2022) 1–7.
[37] H.B. Kim, M. Wang, C.H. Park, E.C. Kim, G.A. Jacoby, D.C. Hooper, oqxAB encoding a multidrug efflux pump in human clinical isolates of Enterobacteriaceae, Antimicrob. Agents Chemother. 53 (8) (2009) 3582–3584.
[38] W-p Chen, T.-T. Kuo, A simple and rapid method for the preparation of gram-negative bacterial genomic DNA, Nucleic Acids Res. 21 (9) (1993) 2260.
[39] K.S. Ikuta, L.R. Swetschinski, G.R. Aguilar, F. Sharara, T. Mestrovic, A.P. Gray, et al., Global mortality associated with 33 bacterial pathogens in 2019: a systematic analysis for the Global Burden of Disease Study 2019, Lancet 400 (10369) (2022) 2221–2248.

[40] M. Sedighi, M. Halajzadeh, R. Ramazanzadeh, N. Amirmozafari, M. Heidary, S. Pirouzi, Molecular detection of β-lactamase and integron genes in clinical strains of Klebsiella pneumoniae by multiplex polymerase chain reaction, Rev. Soc. Bras. Med. Trop. 50 (2017) 321–328.
[41] M.M. Abdulkareem, M.A. Abdulrahman, N.A. Yassin, Molecular detection of plasmid-mediated quinolone resistance genes among clinical isolates of Klebsiella pneumoniae during Covid-19 pandemic, Pharmacia 70 (1) (2023) 225–231.
[42] M.B. Amin, S.R. Saha, M.R. Islam, S.A. Haider, M.I. Hossain, A.H.K. Chowdhury, et al., High prevalence of plasmid-mediated quinolone resistance (PMQR) among E. coli from aquatic environments in Bangladesh, PLoS One 16 (12) (2021) e0261970.
[43] S.H. Abdel-Rhman, Characterization of β-lactam resistance in K. pneumoniae associated with ready-to-eat processed meat in Egypt, PLoS One 15 (9) (2020) e0238747.
[44] M. Mirzaii, S. Jamshidi, M. Zamanzadeh, M. Marashifard, S.A.A.M. Hosseini, M. Haeili, et al., Determination of gyrA and parC mutations and prevalence of plasmid-mediated quinolone resistance genes in Escherichia coli and Klebsiella pneumoniae isolated from patients with urinary tract infection in Iran, Journal of Global Antimicrobial Resistance 13 (2018) 197–200.
[45] C. Shankar, K. Vasudevan, J.J. Jacob, S. Baker, B.J. Isaac, A.R. Neeravi, et al., Hybrid plasmids encoding antimicrobial resistance and virulence traits among hypervirulent Klebsiella pneumoniae ST2096 in India, Front. Cell. Infect. Microbiol. (2022) 435.
[46] P.E.L. Fils, P. Cholley, H. Gbaguidi-Haore, D. Hocquet, M. Sauget, X. Bertrand, ESBL-producing Klebsiella pneumoniae in a University hospital: molecular features, diffusion of epidemic clones and evaluation of cross-transmission, PLoS One 16 (3) (2021) e0247875.
[47] R. Abossedgh, M. Ghane, L. Babaeekhou, High Frequency of qnr genes in urinary isolates of extended-spectrum β-lactamase (ESBL)-producing Klebsiella pneumoniae in Tehran, Iran, Shiraz E-Medical Journal. 21 (3) (2020).
[48] F.D. Salah, S.T. Soubeiga, A.K. Ouattara, A.Y. Sadji, A. Metuor-Dabire, D. Obiri-Yeboah, et al., Distribution of quinolone resistance gene (qnr) in ESBL-producing Escherichia coli and Klebsiella spp, in: Togo Lomé (Ed.), Antimicrobial Resistance & Infection Control, vol. 8, 2019, pp. 1–8.
[49] K.S. Park, M.H. Kim, T.S. Park, Y.S. Nam, H.J. Lee, J.T. Suh, Prevalence of the plasmid-mediated quinolone resistance genes, aac (6′)-Ib-cr, qepA, and oqxAB in clinical isolates of extended-spectrum β-lactamase (ESBL)-producing Escherichia coli and Klebsiella pneumoniae in Korea, Ann. Clin. Lab. Sci. 42 (2) (2012) 191–197.
[50] G.A. Jacoby, K.E. Walsh, D.M. Mills, V.J. Walker, H. Oh, A. Robicsek, D.C. Hooper, qnrB, another plasmid-mediated gene for quinolone resistance, Antimicrob. Agents Chemother. 50 (4) (2006) 1178–1182.
[51] H.S. Jeong, I.K. Bae, J.H. Shin, H.J. Jung, S.H. Kim, J.Y. Lee, et al., Prevalence of plasmid-mediated quinolone resistance and its association with extended-spectrum beta-lactamase and AmpC beta-lactamase in Enterobacteriaceae, The Korean journal of laboratory medicine 31 (4) (2011) 257.
[52] S. Vaziri, M. Afsharian, F. Mansouri, M. Azizi, F. Nouri, N. Madadi-Goli, et al., Frequency of qnr and aac (6') Ib-cr Genes among ESBL-producing Klebsiella pneumoniae strains isolated from burn patients in Kermanshah, Iran, Jundishapur J. Microbiol. 13 (7) (2020).
[53] R. Yashavanth, N. Narendra, Detection of extended spectrum beta-lactamase production and multidrug resistance in clinical isolates of E. coli and K. pneumoniae in, Mangalore 4 (2010) 2442–2445.
[54] M.M. Feizabadi, S. Delfani, N. Raji, A. Majnooni, M. Aligholi, F. Shahcheraghi, et al., Distribution of bla TEM, bla SHV, bla CTX-M genes among clinical isolates of Klebsiella pneumoniae at Labbafinejad Hospital, Tehran, Iran, Microb. Drug Resist. 16 (1) (2010) 49–53.
[55] M. Bouchakour, K. Zerouali, JD. Perrier Gros Claude, H. Amarouch, N. El Mdaghri, P. Courvalin, M. Timinouni, Plasmid-mediated quinolone resistance in expanded spectrum beta lactamase producing enterobacteriaceae in Morocco, The Journal of Infection in Developing Countries 4 (12) (2010) 779–803.
[56] H. Yang, H. Chen, Q. Yang, M. Chen, H. Wang, High prevalence of plasmid-mediated quinolone resistance genes qnr and aac (6′)-Ib-cr in clinical isolates of Enterobacteriaceae from nine teaching hospitals in China, Antimicrob. Agents Chemother. 52 (12) (2008) 4268–4273.
[57] H.-F. Yang, J. Cheng, L.-F. Hu, Y. Ye, J.-B. Li, Plasmid-mediated quinolone resistance in extended-spectrum-β-lactamase-and AmpC β-lactamase-producing Serratia marcescens in China, Antimicrob. Agents Chemother. 56 (8) (2012) 4529–4531.
[58] F. Eftekhar, S.M. Seyedpour, Prevalence of qnr and aac (6')-Ib-cr genes in clinical isolates of Klebsiella pneumoniae from Imam Hussein hospital in Tehran, Iran. J. Med. Sci. 40 (6) (2015) 515.
[59] M.R.M. Jamshidi, H. Zandi, F. Eftekhar, Correlation of quinolone-resistance, qnr genes and integron carriage in multidrug-resistant community isolates of Klebsiella spp, Iranian Journal of Basic Medical Sciences 22 (12) (2019) 1387.
[60] F. Amereh, M.R. Arabestani, S.M. Hosseini, L. Shokoohizadeh, Association of qnr genes and OqxAB efflux pump in fluoroquinolone-resistant Klebsiella pneumoniae strains, International Journal of Microbiology 2023 (2023).
[61] A. Dehghan Banadkouki, G. Eslami, H. Zandi, A. Dehghan Banadkouki, Prevalence of qnr genes in extended-spectrum β-lactamase producing Klebsiella pneumoniae isolated from clinical urine specimens in university teaching hospitals, Iran, International Journal of Medical Laboratory 4 (1) (2017) 25–33.
[62] J.E. Tahou, N.K. Guessennd, P.D. Sokouri, V. Gbonon, F. Konan, J. Kouadio, et al., Antimicrobial Resistance of Klebsiella pneumoniae-ESBL producing strains isolated from clinical specimens in Abidjan (cote de Ivoire), Microbiol Res J Int 20 (2) (2017) 1–7.
[63] J. Li, H. Zhang, J. Ning, A. Sajid, G. Cheng, Z. Yuan, H. Hao, The nature and epidemiology of OqxAB, a multidrug efflux pump, Antimicrob. Resist. Infect. Control 8 (1) (2019) 1–13.
[64] J. Rodríguez-Martínez, P. Díaz de Alba, A. Briales, J. Machuca, M. Lossa, F. Fernández-Cuenca, et al., Contribution of OqxAB efflux pumps to quinolone resistance in extended-spectrum-β-lactamase-producing Klebsiella pneumoniae, J. Antimicrob. Chemother. 68 (1) (2013) 68–73.
[65] L. Crémet, N. Caroff, S. Dauvergne, A. Reynaud, D. Lepelletier, S. Corvec, Prevalence of plasmid-mediated quinolone resistance determinants in ESBL Enterobacteriaceae clinical isolates over a 1-year period in a French hospital, Pathol. Biol. 59 (3) (2011) 151–156.
[66] S. Ferjani, M. Saidani, F.S. Amine, I. Boutiba-Ben Boubaker, Prevalence and characterization of plasmid-mediated quinolone resistance genes in extended-spectrum β-lactamase-producing Enterobacteriaceae in a Tunisian hospital, Microb. Drug Resist. 21 (2) (2015) 158–166.
[67] M.H. Wong, E.W. Chan, L.Z. Liu, S. Chen, PMQR genes oqxAB and aac (6′) Ib-cr accelerate the development of fluoroquinolone resistance in Salmonella typhimurium, Front. Microbiol. 5 (2014) 521.
[68] W. Pasom, A. Chanawong, A. Lulitanond, C. Wilailuckana, S. Kenprom, P. Puang-Ngern, Plasmid-mediated quinolone resistance genes, aac (6′)-Ib-cr, qnrS, qnrB, and qnrA, in urinary isolates of Escherichia coli and Klebsiella pneumoniae at a teaching hospital, Thailand, Jpn. J. Infect. Dis. 66 (5) (2013) 428–432.
[69] J. Silva-Sánchez, E. Cruz-Trujillo, H. Barrios, F. Reyna-Flores, A. Sánchez-Pérez, B.R. Consortium, U. Garza-Ramos, Characterization of plasmid-mediated quinolone resistance (PMQR) genes in extended-spectrum β-lactamase-producing Enterobacteriaceae pediatric clinical isolates in Mexico, PLoS One 8 (10) (2013) e77968.
[70] J. Silva-Sanchez, H. Barrios, F. Reyna-Flores, M. Bello-Diaz, A. Sanchez-Perez, T. Rojas, et al., Prevalence and characterization of plasmid-mediated quinolone resistance genes in extended-spectrum β-lactamase–producing Enterobacteriaceae isolates in Mexico, Microb. Drug Resist. 17 (4) (2011) 497–505.
[71] L. Jamali, F. Haouzane, M. Bouchakour, S. Oufrid, Z. Ghazlane, N. El Mdaghri, et al., Prévalence des gènes de résistance plasmidique aux quinolones chez des entérobactéries communautaires isolées au Maroc [Prevalence of plasmid mediated quinolone resistance genes among enterobacteria isolates in Moroccan community], Int J Innov Sci Res. 11 (2) (2014) 387–399.
[72] H. Yang, G. Duan, J. Zhu, W. Zhang, Y. Xi, Q. Fan, Prevalence and characterisation of plasmid-mediated quinolone resistance and mutations in the gyrase and topoisomerase IV genes among Shigella isolates from Henan, China, between 2001 and 2008, Int. J. Antimicrob. Agents 42 (2) (2013) 173–177.
[73] S. Mahmud, K. Nazir, M.T. Rahman, Prevalence and molecular detection of fluoroquinolone-resistant genes (qnrA and qnrS) in Escherichia coli isolated from healthy broiler chickens, Vet. World 11 (12) (2018) 1720–1724.
[74] J.H. Liu, Y.T. Deng, Z.L. Zeng, J.H. Gao, L. Chen, Y. Arakawa, Z.L. Chen, Coprevalence of plasmid-mediated quinolone resistance determinants QepA, Qnr, and AAC(6')-Ib-cr among 16S rRNA methylase RmtB-producing Escherichia coli isolates from pigs, Antimicrob. Agents Chemother. 52 (8) (2008) 2992–2993.
[75] A. Robicsek, J. Strahilevitz, D.F. Sahm, G.A. Jacoby, D.C. Hooper, qnr prevalence in ceftazidime-resistant Enterobacteriaceae isolates from the United States, Antimicrob. Agents Chemother. 50 (8) (2006) 2872–2874.
[76] M. Halaji, S. Shahidi, A. Atapour, B. Ataei, A. Feizi, S.A. Havaei, Characterization of extended-spectrum β-lactamase-producing uropathogenic Escherichia coli among Iranian kidney transplant patients, Infect. Drug Resist. 13 (2020) 1429–1437.