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Detection of OqxAB and QepA Efflux Pumps and Their Association with Antibiotic Resistance in *Klebsiella pneumoniae* Isolated From Urinary Tract Infection

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Abstract

Background: The emergence and spread of drug resistance among *Klebsiella pneumoniae* clinical isolates have limited the treatment options for these bacteria. Efflux pumps are considered as one of the key mechanisms of antibiotic resistance in *K. pneumoniae* isolates.

Objectives: The present study aimed to detect *oqxA*, *oqxB*, and *qepA* efflux genes in *K. pneumoniae* isolated from urinary tract infection (UTI) and survey their association with antibiotic resistance.

Methods: In total, 100 *K. pneumoniae* isolates were obtained from urine samples, and an antimicrobial susceptibility test was conducted using the disk diffusion method according to the Clinical and Laboratory Standards Institute (CLSI) instructions. Polymerase chain reaction (PCR) was done for the detection of efflux pump genes including, *oqxA*, *oqxB*, and *qepA*, and their association was statistically analyzed with resistance to antibiotics.

Results: The highest rate of resistance was obtained against trimethoprim-sulfamethoxazole (72%), amikacin (70%), levofloxacin (68%), gentamicin (56%), ceftazidime (56%), and ceftriaxone (51%), and the lowest resistance was against imipenem (10%). Thirty one percent of isolates were multidrug resistant (MDR). Molecular distribution test showed that 57% and 56% of isolates carried the *oqxA* and *oqxB* genes, respectively. Also, the frequency of *qepA* genes was 21%. The presence of *oqxA*/*oqxB* and *qepA* efflux genes were significantly associated with fluoroquinolone and beta-lactam resistance phenotypes ($P < 0.05$).

Conclusions: The high frequency of efflux genes showed that this resistance mechanism is the main way, along with other resistance mechanisms in *K. pneumoniae* isolates. It is necessary to adopt appropriate treatment to reduce the incidence of resistance.

Keywords: *Klebsiella pneumoniae*, Antibacterial Agents, Ciprofloxacin

1. Background

Klebsiella pneumoniae is one of the most important causative agents both in the form of community and hospital-acquired infections such as urinary tract infections, septicemia, pneumonia, and meningitis (1). Multidrug resistance has clinically developed anxiety, mainly for the elderly and immunocompromised patients. Also, infants with immature body physiology have the least ability against infection from multi drug-resistant (MDR) pathogens (2).

Antimicrobial resistance can happen due to a variety of mechanisms, including drug target alteration, enzymatic inactivation or modification of drug, and the prevention of drug penetration or accumulation. Efflux pumps are essential in drug resistance among Gram-negative bacteria as they actively pump antibiotics out of the cell and cause the accumulation of drugs inside the bacterial cell to decrease. Efflux pumps are a major concern as they can generate a concurrent resistance to several drugs (3).

Among the efflux pumps, the resistance-nodulation-division (RND) family, including OqxAB and AcrAB, are important in creating antibiotic resistance in Gram-negative bacteria, especially *K. pneumoniae*. The OqxAB pump consists of two main domains: OqxA, a periplasmic part, and OqxB, a transmembrane protein, whose genes are located on both the chromosome and the plasmid (4). The OqxAB multidrug efflux pump is responsible for developing the reduced susceptibility or resistance to fluoroquinolones, such as flumequine, ciprofloxacin, and norfloxacin (2). In addition to quinolones, they confer resistance to other antibiotics, disinfectants, detergents, and antiseptics (5). A high prevalence of chromosomal or plasmid-mediated

OqxAB has been reported in *K. pneumonia*, indicating a potential pool of this resistance determinant (2, 6, 7).

Another quinolone-specific efflux pump is QepA, which belongs to the major facilitator subfamily (MSF). QepA is a 14-transmembrane-segment MFS transporter encoded by the *qepA* gene and causes a decreased accumulation of norfloxacin (3). Minimum inhibitory concentrations (MICs) of ciprofloxacin, enrofloxacin, and norfloxacin were 32- to 64-fold higher in QepA-expressing test strains compared to host strains (8).

2. Objectives

Given the importance of efflux pumps in creating drug resistance, this study aimed to detect OqxAB and Qep efflux pumps in clinical isolates of *K. pneumoniae*. The antibiotic resistance profile of the isolates and, phenotypic and genotypic association of resistance to antibiotics were also investigated.

3. Methods

3.1. Clinical Samples and Identification of Bacteria

In this cross-sectional study, directed from March 2018 to April 2019, 100 non-repetitive urine isolates of *K. pneumoniae* were obtained from Milad Hospital in Tehran, Iran. Each urine sample was cultured on the Mac Conkey agar, and blood agar (Merck, Germany) followed by incubation at 37°C for 18 - 24 h. The isolates were then identified as *K. pneumoniae* by common microbiological and biochemical tests, including Gram-staining, catalase, oxidase, MR-VP, urease production, citrate utilization, and motility (9).

3.2. Antimicrobial Susceptibility Testing

Antibiotic susceptibility test was conducted via disc diffusion assay agreeing with Clinical and Laboratory Standards Institute (CLSI) instruction with the following antibiotics: ciprofloxacin (CIP: 5 μg), amikacin (AK: 30 μg), trimethoprim-sulfamethoxazole (TS, 2.5 μg), cefotaxime (CTX: 30 μg), nalidixic acid (NA: 10 μg), imipenem (IPM: 10 μg), gentamicin (GEN: 10 μg), ceftazidime (CAZ: 30 μg), ceftriaxone(CRO: 30 μg), meropenem (MER: 10μg), and levofloxacin (LEV: 5 μg) (Mast, UK). In short, a bacterial suspension (equivalent to 0.5 McFarland) was prepared from overnight cultures and spread onto Mueller-Hinton agar (Oxoid, UK) plates. After the incubation at 37°C for 24 h, the diameter of inhibition zones around the discs was measured, and the results were expressed as resistant, susceptible, and intermediate. The standard strain of *Escherichia coli* ATCC 25922 was applied as control (10).

3.3. Detection of oqxA, oqxB, and qep Efflux Genes

Total genomic DNA was acquired via a DNA extraction kit (CinnaGen, Iran) based on the instructions of the kit manufacturer. The primer sequences used in this study are listed in Table 1. PCR was conducted in a volume of 25 μL PCR reaction mixture including 1 μL DNA template, 1 μL of each primer, 12.5 μL of PCR master mix (Amplicon Co., Denmark), and 10.5 μL of ddH_2O. The PCR reaction was conducted with the following PCR procedure: initial denaturation at 95°C for 1 min, denaturation at 95°C for 45 s, annealing at 60°C for 45 s, extension at 72°C for 1 min (35 cycles), and the final extension at 72°C for 5 min. In the end, 10 μL of the amplicons were detected on 1% agarose gel electrophoresis.

3.4. Statistical Analysis

The association between the resistance to antibiotics and the presence of efflux genes was analyzed by SPSS version 20 using the chi-square and Spearman correlation coefficient tests, and a P value less than 0.05 was considered significant.

4. Results

4.1. Study Population

The mean age of the population studied was 52 $\pm$ 1.8 years, ranging from 13 to 81 years. The isolates were collected from patients with different age groups: 10 - 25 years no.; 18, 26 - 40 years no.; 29, 41 - 55 years no.; 36, 56 - 60 years no.; 10 and 60 - 81 years no.; 7. Seventy (70%) patients were female, and thirty (30%) patients were male.

4.2. Antibiotic Susceptibility Profiles

Table 2 shows the antibiotic susceptibility testing results. Accordingly, the highest resistance rate was observed against trimethoprim-sulfamethoxazole (72%), amikacin (70%), levofloxacin (68%), and the lowest resistance rate was observed against imipenem (10%). The frequency of MDR strains was 31%.

4.3. Molecular Distribution of Resistance Efflux Pump Encoded Genes

The results of the PCR test for *oqxA* and *oqxB* genes showed that 57% and 56% of strains were the carriers of these genes, respectively. In addition, 52% of isolates carried both genes simultaneously. The results of the molecular test also showed that 21 isolates were positive for the *qepA* gene on the electrophoresis test, and 15 isolates harbored all three genes. Figure 1 depicts the electrophoretic pattern of studied genes.

Table 1. The Sequences of Used Primers

Target Gene	Primer Sequences (5' → 3')	Amplicon Size, bp	References
oqxA	F: 5'-CTCGGCGCGATGATGCT-3'	392	(11)
	R: 5'-CCACTCTTCACGGGAGACGA-3'		
oqxB	F: 5'-TTCTCCCCCGGCGGGAAGTAC-3'	512	(11)
	R: 5'-CTCGGCCATTTTGGCGCGTA-3'		
qepA	F: 5'- GCAGGTCCAGCAGCGGGTAG-3'	218	(12)
	R: 5'- CTTCCTGCCCGAGTATCGTG-3'		

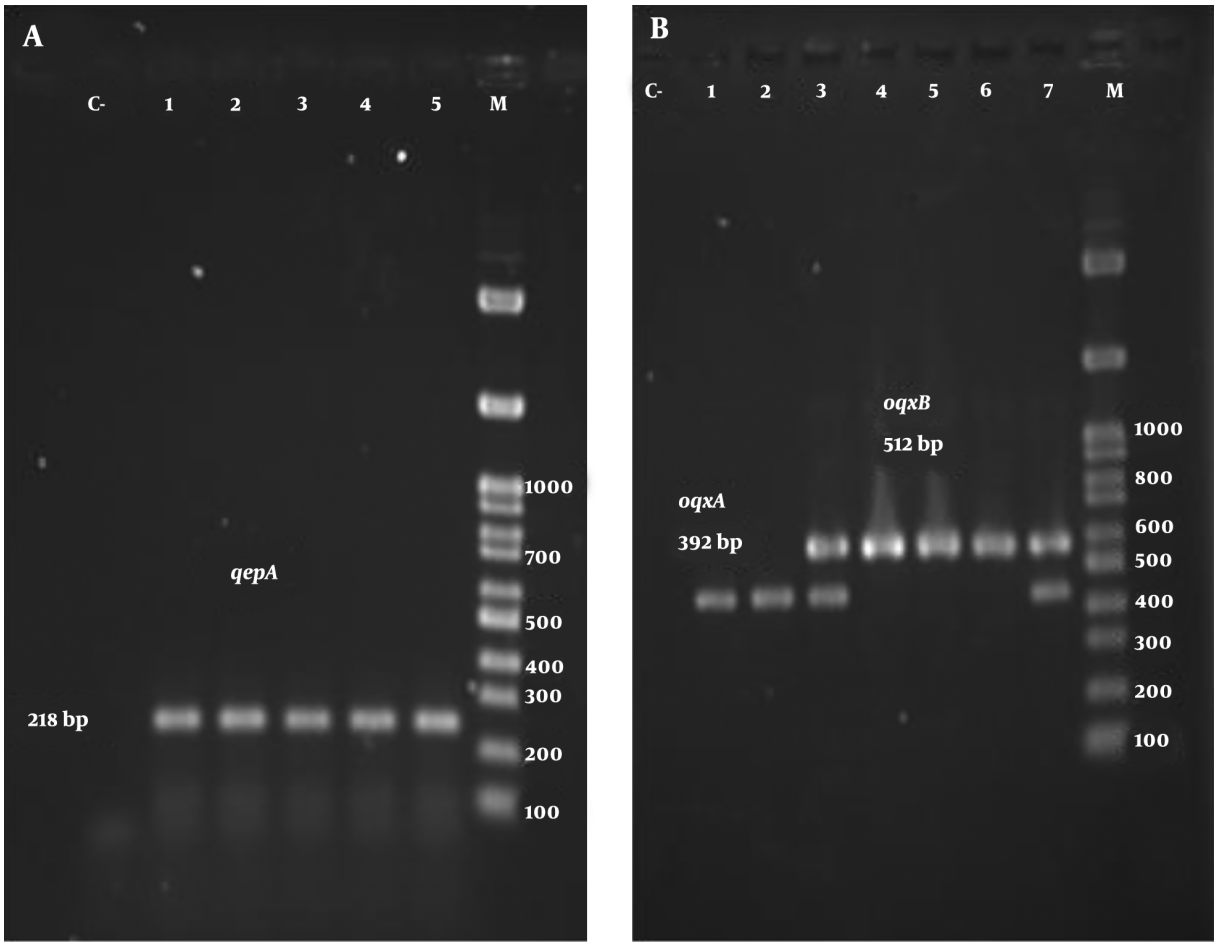

Figure 1. The results of PCR on 1% agarose gel electrophoresis. A, amplification of *qepA*; B, *oqxA* and *oqxB*; M; DNA size marker (100 bp).

Table 3 demonstrates the association between antibiotic resistance and the presence of efflux genes. As shown, resistance to fluoroquinolones and beta-lactams was associated with the presence of *oqxA*/*oqxB* genes. *qep*A gene was also associated with resistance to levofloxacin, imipenem, meropenem, and ceftriaxone ($P < 0.05$).

5. Discussion

K. pneumoniae is one of the most important causative agents of community and hospital-acquired infections worldwide. Awareness of antimicrobial susceptibility and genetic determinants that contribute to resistance in this bacterium can help control infections and reduce mortality and morbidity. The high resistance rate was related

Table 2. Antibiotic Susceptibility Profile of *K. pneumoniae* Isolates[a]

Antimicrobial agents	Susceptible	Intermediate	Resistant
Gentamicin	41 (41)	3 (3)	56 (56)
Amikacin	30 (30)	0	70 (70)
Imipenem	78 (78)	12 (12)	10 (10)
Meropenem	56 (56)	10 (10)	34 (34)
Ceftazidime	38 (38)	6 (6)	56 (56)
Ciprofloxacin	36 (39)	15 (15)	49 (49)
Levofloxacin	27 (27)	5 (5)	68 (68)
Ceftriaxone	39 (39)	10 (10)	51 (51)
Nalidixic acid	62 (62)	12 (12)	26 (26)
cefotaxime	54 (54)	8 (8)	38 (38)
trimethoprim-sulfamethoxazole	26 (26)	2 (2)	72 (72)

[a]Values are expressed as No. (%).

to ciprofloxacin (49%), amikacin (70%), trimethoprim-sulfamethoxazole (72%), gentamicin (56%), ceftazidime (56%), and levofloxacin (68%). In contrast with our data, Amiri et al. (13), posited that resistance to cefotaxime, ceftriaxone, and ceftazidime was 59%, 65%, and 67%, respectively. This conflict may be related to the year of study. Resistance to some antibiotics has increased significantly over the years. For instance, in the present study, 70% of the isolates were resistant to amikacin, while in studies conducted in 2014, 26% (14) in 2015, 35% (15), and in 2019, 65% (16) of isolates were resistant to amikacin. In the present study, resistance to ciprofloxacin increased from 30% in 2013 (17), 33% in 2015 (15), and 43% in 2019 (18), to 49%. This increasing resistance to antibiotics indicates the indiscriminate and uncontrolled use of antibiotics, as well as the acquisition of numerous antibiotic resistance mechanisms in the *K. pneumoniae*. One of the most worrisome findings in this study was the resistance of *K. pneumoniae* strains to imipenem. Since carbapenems are used as the first-line drugs, the treatment of antimicrobial-resistant infections (19), resistance to imipenem can lead to serious problems in the treatment of *K. pneumoniae* infections.

The results of the PCR test for *oqxA* and *oqxB* genes showed that 57% and 56% of isolates were carriers of these genes, respectively. Also, 52% of isolates carried both genes simultaneously. The prevalence of *oqxA* and *oqxB* genes in the study conducted by Zomorrodi et al. (18) was 69.7% and 72.1%, respectively. Higher prevalence of *oqxA* and *oqxB* were obtained among *K. pneumoniae* strains isolated in Spain (76% and 75%, respectively) (7). In our study, 21% of *K. pneumoniae* isolates harbored the *qepA* gene, which was higher than those reported by Goudarzi et al. (20), (2%) and Heidary et al. (1), (4%). The genetic diversity of strains, geographical distance, type of samples, and the number of samples are among the main reasons for the discrepancy between the prevalence of efflux genes in the present study and other studies.

Numerous studies have shown that efflux pump systems are involved in the resistance to the fluoroquinolones (7, 21, 22). In this study, 83.7% of ciprofloxacin-resistant strains had the *oqxA*/*oqxB* efflux genes. The frequency of these genes was significantly higher in the ciprofloxacin-resistant isolates than ciprofloxacin sensitive isolates ($P = 0.001$). In addition, 77.8% of levofloxacin-resistant isolates harbored *oqxA*/*oqxB*, and a significant association was observed between levofloxacin resistance phenotype and the presence of *oqxA*/*oqxB* efflux genes. Levofloxacin resistance was also associated with the presence of the *qepA* efflux gene ($P < 0.05$); however, no association was observed between ciprofloxacin resistance and the presence of the *qepA* gene. Interestingly, the presence of *oqxA*/*oqxB* efflux genes was significantly associated with resistance to imipenem, meropenem, ceftriaxone, and ceftazidime, which has not been reported earlier. To date, there have been no reports regarding the association of the *qepA* efflux gene with beta-lactam resistance. Further studies are needed to investigate the role of these pumps in beta-lactam resistance in *K. pneumoniae* isolates.

5.1. Conclusions

The high frequency of efflux genes in this study is a serious concern, so we require a careful control of drug administration and identification of resistant isolates to control infection and prevent the spread of drug-resistant bacteria. We found a significant association between the presence of the efflux genes and resistance to fluoroquinolone and beta-lactam antibiotics, which signifies the need for novel treatment strategies to reduce the incidence of resistance.

Footnotes

Authors' Contribution: Concept: MG. Data collection and laboratory analysis; MG, LB, and MD. Interpretation of results: MG and LB. Writing the main manuscript text: MD and MG. All authors have read and approved the manuscript.

Conflict of Interests: The authors declare that there are no conflicts of interest.

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Table 3. Significant Relationships Between the Efflux Genes and Antibiotic Resistance

Efflux Genes	Ciprofloxacin	Levofloxacin	Imipenem	Meropenem	Ceftriaxone	Ceftazidime
oqxA/oqxB	0.001	0.001	0.025	0.001	0.001	0.001
qepA	0.243	0.036	0.032	0.025	0.001	0.07

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