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Original Article

Involvement of AcrAB and OqxAB Efflux Pumps in Antimicrobial Resistance of Clinical Isolates of *Klebsiella pneumonia*

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Abstract

Introduction: Infections caused by multidrug-resistant *Klebsiella pneumoniae* characterize a major warning throughout the world owing to enhanced mortality and treatment limitations. Efflux pumps have an important role as a mechanism of antibiotic resistance in *K. pneumoniae*. In the current study, the role of AcrAB and OqxAB efflux pumps to antibiotic resistance was investigated in clinical isolates of *K. pneumonia*.

Materials and Methods: During August 2017-October 2018, 110 clinical strains of *K. pneumoniae* were obtained from patients referred to the hospitals in Tehran. After microbiological/biochemical identification, and antimicrobial susceptibility test was performed using the disc diffusion method. Then, the minimum inhibitory concentration of ciprofloxacin-resistant *K. pneumoniae* strains was measured by the broth microdilution method. For investigating the efflux pump mediated drug resistance in *K. pneumoniae*, the presence, and prevalence of efflux genes (*acrA*/*acrB* and *oqxA*/*oqxB*) were examined by the polymerase chain reaction (PCR) technique.

Results: The results showed that resistance to ciprofloxacin, norfloxacin, gentamicin, kanamycin, cefotaxime, trimethoprim, chloramphenicol, and colistin was 19.09%, 21.81%, 10.0%, 9.09%, 44.54%, 25.45%, 11.81%, and 61.81%, respectively, in *K. pneumoniae* clinical isolates. The PCR technique demonstrated that the prevalence of *acrA*/*acr*B and oqxA/*oqxB* genes is 58 (52.72%) and 52 (47.27%), respectively.

Conclusions: The results of this study reveal that the AcrAB and OqxAB efflux pumps have a major role in the antibiotic resistance of multidrug resistance *K. pneumoniae* isolates. Therefore, due to the easy transfer of antimicrobial resistance genes, the accurate detection of resistance genes by molecular methods is essential to control the spread of resistant strains.

Keywords: *Klebsiella pneumonia*, Efflux Pumps, AcrAB, OqxAB, Antimicrobial Resistance

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Introduction

Klebsiella pneumoniae, which belongs to the Enterobacteriaceae family, is one of the most widespread serious pathogens with a high percentage of morbidity and mortality. The microorganism is responsible for a wide range of nosocomial and community-acquired infections such as urinary tract infections, septicemia, bacteremia, meningitis, and pneumonia.[1,2] Concerns regarding this pathogen are growing worldwide due to the increasing incidence of severe infections, resistance to most antibiotics (e.g. β-lactams, aminoglycosides, quinolones, and several other antibiotics), and reduced treatment efficacy.[3-5] With the widespread application of broad-spectrum antibiotics, especially carbapenems, an increasing number of multidrug-resistant *K. pneumoniae* (MDR-Kp) strains are being isolated from hospitalized patients.[5,6] Gram-negative bacteria have achieved multidrug resistance through the vertical or horizontal transmission of resistance genes by transportable genetic elements such as integrons, plasmids, transposons (Tn), and insertion sequences (IS).[2,7] Fluoroquinolones have been raised as a suitable treatment choice; however, researchers have shown that a high level of *K. pneumoniae* strains is resistant to these antibiotics.[1] Tigecycline also showed strong antimicrobial activity against MDR-Kp in vitro, so this antibiotic can be the last option for treatment against clinical infections of MDR-Kp.[8] Nevertheless, cases of tigecycline non-susceptible *K. pneumoniae* have occurred in hospitalized patients with the extensive use of tigecycline.[9,10]

Among the numerous resistance mechanisms exhibited by *K. pneumoniae*, the efflux pumps have been revealed to stimulate antibiotic resistance to β-lactams and other antibiotics families, such as chloramphenicol and quinolones.[11] Several efflux pumps have been reported in gram-negative bacteria. These systems diffuse soluble materials, including antibiotics

out of the bacterial cell.[12] There is a strong confirmation that the pathogenicity of the bacterium is associated with efflux pumps activity that confer clinically related to antimicrobial resistance.[13] Among the efflux pumps, the resistance/nodulation/division (RND) family is more important for antibiotic resistance in gram-negative pathogens, such as *Pseudomonas aeruginosa*, and species belonging to the Enterobacteriaceae family, especially *K. pneumonia*.[12] The most comprehensively studied efflux pumps in *K. pneumonia*, related to antibiotic resistance, are pump AcrAB and more recently described efflux pump OqxAB.[14-18] In the *K. pneumoniae* efflux system, AcrAB is encoded by the operon acrRAB. In *acr*RAB operon, *acrA* encodes a lipoprotein in bacterial periplasmic space (40 kDa) that bridges the inner and outer cell membranes, and *acrB* encode an integral membrane protein (113.5 kDa) with 12 membrane-spanning α-helices which are placed in the cytoplasmic membrane, while *acrR* encodes the AcrAB repressor. The AcrB links with an outer membrane protein called TolC (belongs to a family of envelope proteins) which is found in all gram-negative bacteria and is necessary for the exclusion of an overabundance of materials.[1] The AcrAB efflux pump is one of the main pumps that create the intrinsic resistance of the *K. pneumoniae* isolates to the fluoroquinolones, especially ciprofloxacin. Also, this pump causes resistance to tetracycline, chloramphenicol, trimethoprim, macrolides, and β-lactams.[19]

The *oqx*AB operon (quinolone/olaquindox efflux pump) was initially explained on plasmid pOLA52 in *Escherichia coli* strain obtained from swine manure.[20] The OqxAB has been exposed to encode an efflux pump consulting resistance to olaquindox, chloramphenicol, and quinolones.[21,22] The OqxAB efflux pump is assembled by two chief domains called OqxA and OqxB. The OqxA is known as a periplasmic portion, but OqxB is a transmembrane protein. Its efflux activity is characterized in a TolC independent system; but, an outer membrane protein enriches its influence.[20] It is confirmed that efflux pump systems are responsible for resistance to fluoroquinolones and biocides, such as trichlorosin, chlorohexidine, and ethidium bromide. Since then, several studies have investigated the presence of AcrAB and OqxAB systems in gram-negative bacteria around the world, however, comprehensive studies have not yet been carried out in this regard on *K. pneumoniae* clinical isolates. So, in the current study we aimed to phenotypically and molecularly investigate the possible role of AcrAB and OqxAB efflux pumps in the antibiotic resistance in the multidrug resistance clinical isolates of *Klebsiella pneumonia* and also, acquire the prevalence of *acrA*/*acrB* and *oqxA*/*oqxB* efflux pumps genes in the studied strains.

Materials and Methods

Study Design and Sample Collection

In this descriptive-sectional study, a total of 296 non-repetitive samples were obtained from different clinical specimens (including blood, urine, and sputum) of hospitalized patients that referred to four hospitals in Tehran over 14 months (August 2017-October 2018).

Bacterial Identification

All clinical samples were inoculum on the Blood agar and MacConkey agar (Merck Co., Germany) and incubated at 37°C for 24 hours. Samples with over 100.000 cfu/mL *K. pneumoniae* count were defined as a positive infection. Suspected grown colonies were recognized as *K. pneumonia* by standard bacteriological and biochemical tests such as colony morphology on the medium, citrate utilization, Triple Sugar Iron (TSI) agar, motility, urease, oxidase, Methyl Red-Voges Proskauer (MR-VP), and Sulfide Indole Motility (SIM), as well as were additionally approved by the API 20E identification kit (Analytab, Inc., New York) according to the manufacturer´s instruction.

Antibacterial Susceptibility Testing

The antimicrobial susceptibility pattern of *K. pneumoniae* strains was determined by the Kirby-Bauer disc diffusion technique on Mueller-Hinton Agar (Merck Co., Germany) medium according to the Clinical and Laboratory Standards Institute (CLSI) strategies for the following antibiotics (Mast, Merseyside, UK): ciprofloxacin 5 μg (CIP; 5 μg), norfloxacin 10 μg (NOR; 10 μg), gentamicin 10 μg (GEN; 10 μg), kanamycin 30 μg (KAN; 30 μg), cefotaxime 30 μg (CTX; 30 μg), trimethoprim 5 μg (TMP; 5 μg), chloramphenicol 30 μg (CHL; 30 μg) and colistin 10 μg (CST; 10 μg). Briefly, a bacterial suspension was obtained from fresh cultures. The turbidity of each bacterial suspension was adjusted to a value equivalent to the no. 0.5 McFarland turbidity standard and then cultured on Mueller-Hinton agar (Oxoid, UK). The zone of inhibition diameter was measured following incubation at 37°C for 18-24 hours. The results were reported as susceptible, intermediate, and resistant. *K. pneumonia* ATCC 700603 was used as quality control.

Minimum Inhibitory Concentrations Test

Phenotypic evaluation of the efflux pump in ciprofloxacin-resistant *K. pneumoniae* was assessed by measuring the minimum inhibitory concentrations (MICs) for ciprofloxacin before and after exposure to the efflux pump inhibitor (EPI), carbonyl cyanide 3-chlorophenylhydrazone (CCCP) (Sigma-Aldrich, Dorset, UK). The CCCP is an uncoupler of oxidative phosphorylation, which disrupts RND-type pumps. As a result, adding CCCP to Mueller-Hinton broth leads to increased gathering accumulation of antibiotics and, consequently, and decreases the MIC in isolates that carry active efflux pumps. Briefly, CCCP was added to all of the Mueller-Hinton broth wells containing 0.5-512 μg/mL ciprofloxacin at a final concentration of 12.5 μg/mL.[19] The well with CCCP that did not contain antibiotic was used as a control. The effects of the EPI were determined by detecting a 4-fold or greater increment in susceptibility after augmentation of CCCP.[19]

The MIC for ciprofloxacin were determined using the reference broth microdilution method in 96-well microtiter plates. Bacterial strains were incubated overnight on MacConkey agar broth at 37°C. Bacterial cultures were diluted in sterilized distilled water to a McFarland 0.5 turbidity

standard and were then diluted 100 times with nutrient broth. Aliquots of 0.05 mL were transferred to each well of a 96-well plate that contained 0.05 ml of each compound at concentrations prepared in two-fold serial dilutions in nutrient broth medium. The plates were incubated at 37°C and the MIC results registered after 16–18 hours. All assays were carried out three times. The obtained results of the tests were interpreted according to the guidelines of the CLSI.[23]

DNA Preparation

For molecular diagnosis, the total genomic DNA was extracted from *K. pneumoniae* colonies grown on LB broth (Merck Co., Germany) by the QIAprep Spin Miniprep kit (Qiagen Retsch GmbH, Hannover, Germany) according to the manufacturer's protocol. The quality of DNA and concentrations were determined by Nanodrop spectrophotometer (Nanodrop Technologies, Wilmington, DE, USA) and agarose gel electrophoresis. The extracted DNAs were immediately stored at -70°C.

PCR Assay for Detection of Efflux Pump Genes

Detection of four efflux pump genes (*acrA*, *acrB*, *oqxA,* and *oqxB*) in *K. pneumoniae* clinical isolates was performed using the PCR amplification technique. The primers sequences used in the present research were designed and handled specifically for the first time. Oligo software (version 6), AlleleID software (version 7.5), and NCBI (National Center for biotechnology information) site were used in several stages for primers sequence design. The desired genes and their primer sequences used in this study have been demonstrated in Table 1. The total volume of reaction per each PCR test was 25 µL in a PCR tube. The reaction mixtures for detection of efflux pump genes contained 1.0 µL DNA sample, 0.5 µL (25 pmol) of each primer, 2.5 mM $MgCl_2$, 1.5 µL (1.5 U) of Taq DNA polymerase, 1 µL dNTPs (200 µM), 2 µL 10x PCR buffer (75 mM Tris-HCl, pH 9.0, 2 mM $MgCl_2$, 50 mM KCl, 20 mM $(NH4)_2SO_4$ and sterile distilled water up to 25 µL volumes. Thermal cycling conditions for gene amplification were performed as follows: initial denaturation at 94°C for 5 minutes, followed by 32 cycles of application with denaturation at 94°C for 1 minute, annealing (*acrA*: at 58°C for 1 minute, *acrB*: at 59°C for 1 minute, *oqxA*: at 57°C for 1 minute and *oqxB*: at 58°C for 1 minute), extension at 72°C for 1 minute and a final extension at 72°C for 5 minutes in the BioRad thermal cycler (MJ Mini, BioRad, USA). The PCR products were analyzed in 1.0% agarose gels including 1 µg/mL of power safe stain and exposed to electrophoresis in a 0.5X TBE buffer for 1hour at 95 V and 30 mA. DNA fragments were deposited on the gel under UV light and recognized using Bio-imaging document systems (VisiDoc-It™ system). A molecular weight ladder with 100 bp increments was applied as a DNA standard. *K. pneumonia* ATCC 700603 was used as a positive control strain and *P. aeruginosa* ATCC 27853 and *E. coli* ATCC 25922 were used as negative control strains.

Genome Sequencing

Afterward, among fluoroquinolone-resistant *E. coli* strains, 13 isolates were randomly selected for the genetic characterization of the efflux pump genes by the sequencing process (Bioneer Inc., Seoul, Korea). *E. coli* ATCC25922 was used as quality control for all sequencing reactions. Sequences were compared with the nucleotide sequence of the *acrA*/*acrB*, and *oqxA*/*oqxB* genes in the GenBank database.

Statistical Analysis

The patients' information (raw data), was entered in the Excel 2010 (Microsoft Office, Microsoft, Washington D.C, USA) and statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS) version 16.0 software (SPSS Inc., Chicago, IL, USA). The MIC50 and MIC90 values were subjected to descriptive statistical analysis. *The P* value 0<0.05 was considered as statistically significant.

Results

Clinical samples were collected from patients admitted to specialist intensive care units, special care of neurosurgery, and neurological care from several hospitals in Tehran, Iran. Overall, 110 *K. pneumoniae* strains were isolated from 296 (37.16%) various clinical samples. Samples included blood (n=15, 13.63%), urine (n= 64, 58.18%) and sputum (n= 31, 28.18%). The highest numbers of *K. pneumoniae* isolates (58.18%) were acquired from urine and the lowest quantity (13.63%) was obtained from blood specimens.

Antibiogram tests by the disc diffusion method presented that the rate of resistance to ciprofloxacin, norfloxacin, gentamicin, kanamycin, cefotaxime, trimethoprim, chloramphenicol, and colistin was 19.09%, 21.81%, 10.0%, 9.09%, 44.54%, 25.45%, 11.81%, and 61.81%, respectively (Table 2). The antibiotic susceptibility profile shows that the highest and lowest rate of antibiotic resistance was associated

Table 1. Specific Primer Sequences for Detection of *Klebsiella pneumonia* Efflux Pump Genes

Target Genes	Primers	Primer Sequences (5' – 3')	Size (bp)	Product Size (bp)	Ref.
oqxA	Primer F	GGTGCTGTTCACGATAGATG	20	144	This study
	Primer R	GAGACGAGGTTGGTATGGAC	20		
oqxB	Primer F	CGGCCAGTTCTACAAACAGT	20	136	This study
	Primer R	GGTAGGGAGGTCTTTCTTCG	20		
acrA	Primer F	TGATGCTCTCAGGCAGCTTA	20	226	This study
	Primer R	GCCTGGATATCGCTACCTTC	20		
acrB	Primer F	CGTCTCCATCAGCGACATTAAC	22	219	This study
	Primer R	GAACCGTATTCCCAACGCGA	20		

with colistin (R: 61.81%) and kanamycin (R: 9.09%), respectively.

After analyzing the antibiotic resistance pattern of the *K. pneumoniae* samples, 22 multi-drug resistant strains that were resistant to more than three antibiotics and also to fluoroquinolones, were identified and isolated. These isolates were suitable candidates for testing OqxAB and AcrAB pumps as a large family of efflux pumps in *K. pneumoniae* because they were resistant to various antibiotic families. The results of the MIC of ciprofloxacin in 22 *K. pneumoniae* isolates in the presence and absence of CCCP (as efflux pumps chemical inhibitor) were observed in five different patterns (Table 3). The MIC of ciprofloxacin in the presence and absence of CCCP is also displayed in Figure 1.

Amplification and detection of the target efflux pump genes by PCR reaction in 110 isolates of *K. pneumoniae* revealed that the prevalence of the *acrA*, *acrB*, *oqxA*, and *oqxB* genes was 52.72%, 52.72%, 47.27%, and 47.27%, respectively (Table 4). The results of gel electrophoresis of *K. pneumoniae* efflux pump genes amplified by PCR are shown in Figure 2 and Figure 3.

Table 2. Antibiotic Susceptibility Profile of Clinical Isolates of *Klebsiella pneumoniae*

Antibiotics	Susceptible No. (%)	Intermediate No. (%)	Resistant No. (%)
Ciprofloxacin	82 (74.54)	7 (6.36)	21 (19.09)
Norfloxacin	75 (68.18)	11 (10.0)	24 (21.81)
Gentamicin	99 (90.0)	0 (0.0)	11 (10.0)
Kanamycin	58 (52.72)	42 (38.18)	10 (9.09)
Cefotaxime	46 (41.81)	15 (13.63)	49 (44.54)
Trimethoprim	81 (73.63)	1 (0.9)	28 (25.45)
Chloramphenicol	96 (87.27)	1 (0.9)	13 (11.81)
Colistin	19 (17.27)	23 (20.9)	68 (61.81)

Table 3. Minimum Inhibitory Concentration of Ciprofloxacin in the Presence and Absence of CCCP Inhibitor in *Klebsiella pneumoniae* Strains

Patterns	MIC (Without CCCP) (µg/mL)	MIC (With CCCP) (µg/mL)	No. of Isolates
Pattern 1	512	32	9
Pattern 2	256	32	5
Pattern 3	256	16	3
Pattern 4	32	4	3
Pattern 5	2	2	2

CCCP: Carbonyl cyanide m-chlorophenyl hydrazone.

Table 4. The Results of PCR Reaction for *acrA*, *acrB*, *oqxA*, and *oqxB* Genes in Clinical Strains of *Klebsiella pneumoniae*

Efflux Pump Genes	Positive isolates	
	No.	(%)
acrA	58	52.72
acrB	58	52.72
oqxA	52	47.27
oqxB	52	47.27

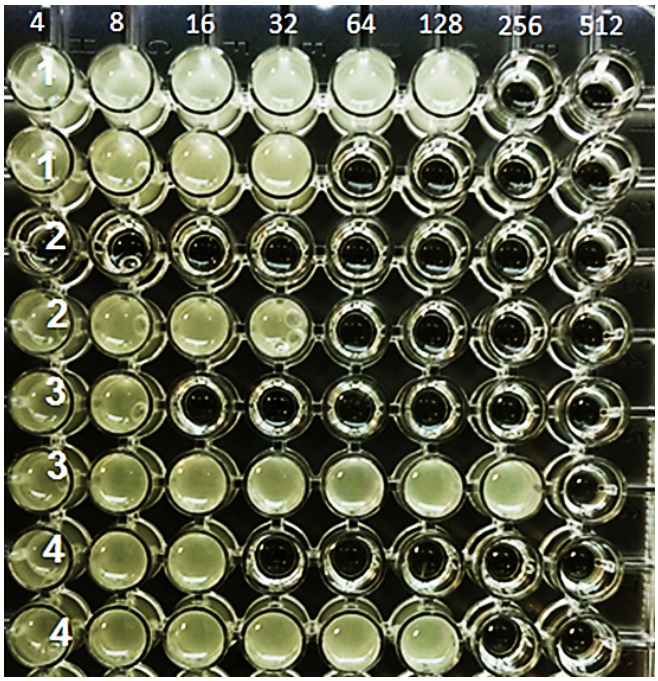

Figure 1. Minimum Inhibitory Concentration of Ciprofloxacin in the Presence and Absence of an Efflux Pump Inhibitor (CCCP). 1-4: clinical *K. pneumoniae* isolates.

Discussion

Klebsiella pneumoniae is one of the main pathogens which causes both nosocomial and community-acquired infections including meningitis, urinary tract infections, pneumonia, endophthalmitis, bacteremia, and septicemia.[2,23] Due to the increasing emergence of multi-drug resistance in the *K. pneumoniae* clinical strains, the therapeutic choices for the treatment of infections caused by this pathogen has been difficult. According to recent studies, most of the *K. pneumoniae* strains have been identified as multi-drug resistant (MDR) isolates.[24] It is confirmed that MDR strains can be relatively challenging, mainly for old people, immunosuppressive patients, and children with undeveloped physiology.[2,25] One of the most prominent antibiotic resistance mechanisms in this pathogen is the efflux pump system.[26] Different studies throughout the world have confirmed the importance of these pumps in increasing the resistance of *K. pneumoniae* strains to various antibiotic families, especially fluoroquinolones.[27-29] Fluoroquinolones are effectively used against gram-negative bacteria, especially *K. pneumoniae*. Resistance to quinolones in microorganisms occurs through chromosomal mutations. However, increased resistance to this antibiotic family implies the involvement of other mechanisms in the resistant strains of *K. pneumoniae*. Studies have shown that OqxAB and AcrAB efflux pumps have an essential role in increasing the resistance to fluoroquinolones especially ciprofloxacin, and other antibiotics including chloramphenicol, trimethoprim, tetracycline, macrolides, and β-lactams.[30] Recently, many studies have been conducted in Iran and have reported a high resistance of *K. pneumoniae* isolates to various antibiotic classes. In a study conducted by Yousefi et al,[31] the highest antibiotic resistance of *K.*

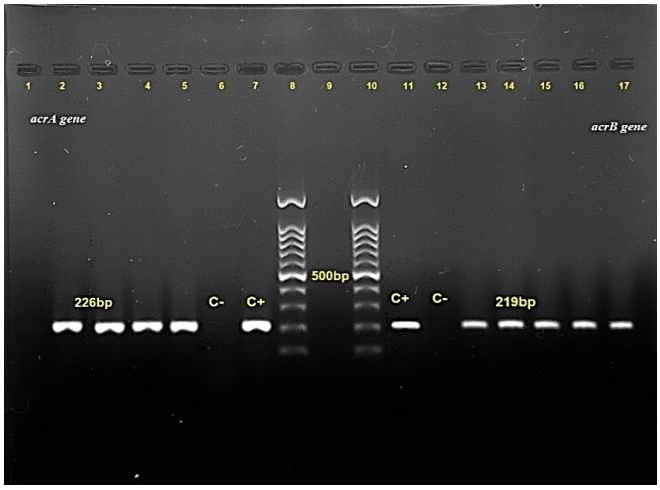

Figure 2. Amplification of *acrA* and *acrB* Efflux Pump Genes by PCR Assay. Lanes 1-5: *acrA* gene positive-clinical strains of *K. pneumonia* (band: 226 bp), Lanes 6 and 12: negative control (*Escherichia coli* ATCC25922), Lane 7: positive control (*Klebsiella pneumonia* ATCC 700603), Lanes 8 and 10: DNA Ladder 100 bp, and Lanes 13-17: *acrB* gene positive-clinical strains of *K. pneumonia* (band: 219 bp).

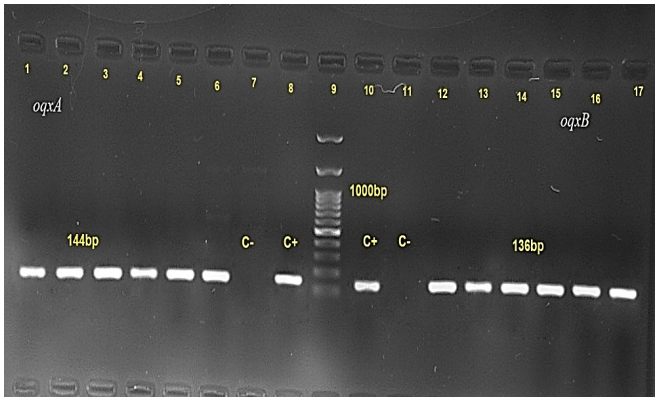

Figure 3. Amplification of *oqxA* and *oqxB* Efflux Pump Genes by PCR Assay. Lanes 1-6: *oqxA* gene positive-clinical strains of *K. pneumonia* (band: 144 bp), Lanes 7 and 11: negative control (*Escherichia coli* ATCC25922), Lanes 8 and 10: positive control (*Klebsiella pneumonia* ATCC 700603), Lane 9: DNA Ladder 100 bp, and Lanes 12-17: *oqxB* gene positive-clinical strains of *K. pneumonia* (band: 136 bp).

pneumoniae isolates was to cefotaxime (100%) and ceftazidime (100%), followed by gentamicin (63%), and the lowest level of resistance was to colistin (13%) and chloramphenicol (6%). In another study conducted on 100 isolates of *K. pneumoniae*, the antibiotic resistance was reported as follows: aztreonam (56%), meropenem (20%), gentamicin (36%), ciprofloxacin (53%), cefotaxime (56%), Colistin (0%), cefotaxime (64%) and tetracycline (50%).[27] In the current study, the resistance to colistin, cefotaxime, trimethoprim, norfloxacin, ciprofloxacin, chloramphenicol, gentamicin, and kanamycin was 61.81%, 44.54%, 25.45%, 21.81%, 19.09%, 11.81% 10.0%, and 9.09%, respectively. The results of the antibiotic-resistant rate in the current study were similar to other studies, except for colistin. However, in some cases, the resistance to the studied antibiotics was less than the present study, which can be due to the type and number of samples, the time and place of collecting samples and the geographical distribution of resistance in various countries and other factors.[31]

Contrary to the studies of Hashemi et al[27] and Yousefi et al,[31] which indicated that resistance to colistin was 0% and 13%, respectively, in the present study, high resistance (61.8%) to colistin was observed. Therefore, more studies need to be done for the recognition of the mechanisms of resistance to colistin in resistant isolates. In this study, the most susceptibility was observed to gentamicin, kanamycin and chloramphenicol. According to this study and also previous research which reported high susceptibility to chloramphenicol, this antibiotic is a good therapeutic candidate for the treatment of diseases which occur by resistant pathogen.[24,27,30] The two efflux pumps which have been most comprehensively considered in the Enterobacteriaceae family especially *K. pneumoniae* species and have been involved in antimicrobial resistance are the AcrAB and OqxAB efflux pumps.[5,32] For determining the role of efflux pumps in *K. pneumoniae* as antimicrobial resistance agents, the CCCP inhibitor was used to evaluate the efficacy of the pump. In the present study, CCCP caused a reactivity of two to 16 times the sensitivity of 21 isolates to ciprofloxacin and it is possible that these pumps could contribute to resistance to ciprofloxacin. Pakzad et al,[19] reported that while using CCCP as an inhibitor, a 2-32 fold reduction in MIC was observed in 47.5% of the ciprofloxacin-resistant *K. pneumoniae* strains, which could confirm the results of this study. In accordance with these findings, reduction in MIC of antibiotics, including ciprofloxacin, have been reported after using CCCP inhibitors in previous studies.[33-36] Pakzad et al[19] considered the role of AcrAB efflux pump in 52 isolates of *K. pneumoniae* obtained from burned patients by PCR assay and exhibited that all ciprofloxacin-resistant bacteria harbors *acrA* gene. In a study by Swick et al,[33] 241 strains of *E. coli* were studied for the evaluation of the relationship between efflux pumps and resistance to fluoroquinolones. The fluoroquinolones MIC was reduced by removing *acrA* and *acrB* efflux pump genes. They resulted that increasing the expression of *acrA* and *acrB* genes are related to fluoroquinolone resistance. The results of our study regarding the presence of the AcrAB pump in *K. pneumoniae* strains were similar to previous studies. In the present study, the prevalence of *acrA*/*acrB* genes was 52.72% among clinical strains of *K. pneumoniae*. It is interesting to note that among 20 ciprofloxacin-resistant isolates, all isolates were carriers of *acrA*/*acrB* genes. Since this efflux pump causes permeation of ciprofloxacin to out of the bacteria, the presence of this pump could justify part of the high resistance to ciprofloxacin. On the other hand, our findings revealed that 38 clinical *K. pneumoniae* isolates harbor *acrA*/*acrB* genes, but were not resistant to ciprofloxacin. One of the reasons for this issue can be the lack of expression of efflux pump genes in these isolates. However, to demonstrate and confirm this fact, the measurement of the expression of the genes encoding efflux pumps is necessary. As mentioned above, the operon *oqx*AB was firstly identified on plasmid pOLA52 which was carried by an *E. coli* strain obtained from swine manure.[32] This pump is resistant to fluoroquinolones and biocides such as triclosan, chlorhexidine and ethidium bromide.[5,33,34] In a

study conducted by Liu et al,[35] one isolate of *E. coli* had *ctxM*, *oqxA*, and *oqxB* genes simultaneously. In another study that was conducted on clinical and non-clinical *Escherichia coli* isolates, 30.3% were positive for *oqxA*.[36] Martínez-Rodríguez et al,[34] indicated that among 114 *K. pneumoniae* isolates containing broad-spectrum beta-lactamases, 76% and 75% of the strains carried *oqxA* and *oqxB* genes, respectively. Yuan et al,[29] revealed that from among 136 *E. coli* strains, 71.3% were non-susceptible to ciprofloxacin, while 66.9% of the 154 *K. pneumoniae* were non-susceptible to this quinolone. In their research, *oqxA* and *oqxB* genes were present in 6.6% of *E. coli* and in all *K. pneumoniae* strains. Using the Real-Time PCR method, the expression of *oqxA* gene in isolates of *K. pneumoniae* with decreased ciprofloxacin susceptibility was four times higher than susceptible isolates.[37] The results of the present study were confirmed by previous research, so that the prevalence of *oqxA*/*oqxB* genes was 47.27% in *K. pneumoniae* isolates. Twenty isolates with a zero growth halo relative to the antibiotic ciprofloxacin had the *oqxA*/*oqxB* genes for this pump. In fact, the presence of genes in both OqxAB and AcrAB efflux pumps could be one of the reasons for the high level of resistance observed in these 20 *K. pneumoniae* isolates. To demonstrate this relationship more studies are needed to evaluate the expression of the efflux pump genes in resistant strains. Microorganisms' harboring these pumps increase the drug resistance and, as a result, increase the pathogenicity in patients, and poses a serious health threat. Therefore, the correct diagnosis of the infections, identification of resistance pathogens, its resistant mechanisms, and the choice of appropriate antibiotics to prevent the failure of treatment is extremely important.[38]

Conclusions

The findings of the present study revealed that Gentamicin, Kanamycin, and chloramphenicol antibiotics had the best effect against the *K. pneumoniae* isolates. Resistant to colistin was elevated in the studied strains. Physicians should be careful in prescribing the medicine and send the infectious specimen to a medical laboratory for an antibiogram test to prescribe the most effective drug for treatment. Drug resistance mechanisms, including efflux pumps, should be detected by phenotypic and molecular methods in labs and then the results must be informed to the physicians. Importantly, lab staff, nurses, and those in contact with patients in the hospital should be tested for the diagnosis of the drug-resistant pathogens to prevent the spread of these microorganisms.

Authors' Contributions

All authors contributed equally to this study.

Conflict of Interest Disclosures

The authors declare that there is no conflict of interest in the current study.

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