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ORIGINAL ARTICLE

Acr AB and Oxq AB Efflux Pump Genes among Resistant *Klebsiella pneumoniae* Isolated from Zagazig University Hospitals

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ABSTRACT

Background*: Overexpression of Acr AB and Oxq AB efflux pump genes in Klebsiella pneumoniae could increase its resistance to antimicrobials.* ***Objectives****: to explore the prevalence of Acr AB and Oxq AB efflux pump genes in resistant isolates of K. pneumoniae and to detect their role in conferring the resistance to ciprofloxacin.* ***Methodology:*** *Isolation of K. pneumoniae was followed by detection of its antimicrobial susceptibility. Then, the ciprofloxacin minimum inhibitory concentrations (MIC) were analyzed by broth microdilution method before and after treatment with the efflux pump inhibitor, carbonyl cyanide 3-chlorophenylhydrazone. The presence of Acr AB and Oxq AB efflux pump genes was investigated by polymerase chain reaction.* ***Results****: One hundred K. pneumonia strains were isolated and 65 % of these isolates were multidrug-resistant with high resistance for sulphamethoxazole (77%), amikacin (66%), and ciprofloxacin (63%). 60.3% of ciprofloxacin-resistant K. pneumoniae had a significant 4-fold decrease or more reduction in MIC for ciprofloxacin after CCCP addition (p value<0.05). There was a significant difference concerning the frequency of efflux pump genes in ciprofloxacin-resistant isolates as 82.5% were positive and acrA, acrB, oqxA, and oqxB were detected in 77.7, 71.4, 62 and 65% of isolates, respectively.* ***Conclusion****: Acr AB and Oxq AB efflux pump genes were prevalent among K. pneumoniae isolates and they had an essential role in explaining their resistance to ciprofloxacin.*

INTRODUCTION

Klebsiella pneumoniae (*K. pneumoniae)* is considered a significant multidrug-resistant (MDR) bacterium causing several infections in human beings associated with high morbidity and mortality rates[1].

It belongs to the *Enterobacteriaceae* family. Its natural environment is the gastrointestinal tract. Numerous hospital and community-acquired diseases such as, meningitis, pneumonia, septicemia, urinary tract infections, and bacteremia, are caused by this pathogen[2,3]. It harbors several virulent agents enabling it to combat the host's immune response and promote the spreading of infection in human hosts[4].

Antimicrobial resistance is an increasing issue in healthcare worldwide. Antimicrobial resistance-related mortality is expected to climb from 700,000 to 10 million per year by 2050, increasing the global economic burdens. *K. pneumoniae* represents one of the most frequent pathogens that cause current challenges in healthcare due to its natural resistance to antibiotics, capability to defeat other bacteria, capacity to resist starvation, ability to readily exchange genetic material with other members of the human microbiome, and presence of its mobile genetic elements that encode several antibiotic-resistant and virulence genes[5,6].

Because of the extensive use of broad-spectrum antibiotics, particularly carbapenems, multidrug-resistant *Klebsiella* has developed as a significant barrier in treating infections recently[7]**.** Fluoroquinolones have been suggested as a potential therapeutic alternative, but studies have revealed that a significant portion of *K. pneumoniae* strains are resistant to these antibiotics[8].

The efflux pumps are one of the resistance mechanisms used by *K. pneumoniae*, and it has been discovered that they promote β-lactams, chloramphenicol and quinolones resistance [9]. These inner membrane transporters export various antibiotics and other unrelated soluble substances from the interior of bacterial cells to the outside[10].

Drug efflux pumps are categorized into six distinct groups based on structural variations and coupling

energies. *AcrAB* and *OqxAB*, members of the resistance-nodulation division (RND) family of efflux pumps, have a significant role in antibiotic resistance in Gram-negative bacteria, particularly *K. pneumoniae*[11]. Besides their incrimination in developing resistance, they are found to have an essential role in bacterial pathogenicity by allowing their colonization and promoting their survival in appropriate host media[12].

The operon *acrRAB* encodes *AcrAB* pump. In *acrRAB* operon, a lipoprotein that spans the inner and outer cell membranes is encoded by *acrA*; an integral membrane protein which is located in the cytoplasmic membrane is encoded by *acrB*, and the *AcrAB* repressor is encoded by *acrR*[13]. One of the major pumps that contribute to the intrinsic resistance of *K. pneumoniae* isolates to fluoroquinolones, particularly ciprofloxacin, is the *AcrAB* efflux pump. Additionally, this pump results in resistance to other drugs such as β-lactams tetracycline, chloramphenicol, trimethoprim, and macrolides[14].

The *OqxAB* pump is made up of two primary domains: *OqxA*, which is a periplasmic component, and *OqxB*, a transmembrane protein. The genes of both domains are found on both the chromosome and the plasmid[15]. Reduced sensitivity or increased resistance to fluoroquinolones including ciprofloxacin, flumequine, and norfloxacin are encountered by the *OqxAB* multidrug efflux pump[16]**.** Along with quinolones, they also make bacteria resistant to other antibiotics, detergents, disinfectants, and antiseptics[17]**.**

The efflux pump inhibitor (EPI), carbonyl cyanide 3-chlorophenylhydrazone (CCCP) acts by chemically disrupting RND-efflux pumps through an oxidative phosphorylation process. After treatment with CCCP, the accumulation of antimicrobial agents increases with a remarkable reduction in the MIC in the isolates harboring active efflux pumps[14].

Consequently, multiple pieces of research have been conducted globally to explore the importance of the *AcrAB* and *OqxAB* systems in emerging antimicrobial resistance, although an extensive study on *K. pneumoniae* clinical isolates has yet to be completed. As a result, this research explores the potential contribution of the *AcrAB* and *OqxAB* efflux pumps to ciprofloxacin resistance in isolates of *K. pneumoniae*.

METHODOLOGY

Sample Collection

This cross-sectional study was performed in the Medical Microbiology and Immunology Department, Faculty of Medicine, Zagazig University. A total of 253 clinical specimens (blood, urine, pus, and endotracheal aspirate) were collected from intensive care units admitted patients, Zagazig University Hospitals. The infections caused by any pathogen other than *K. pneumoniae* were excluded from this study.

Ethical approval and consent to participate: This study got approval from the Institution Review Board of the Faculty of Medicine, Zagazig University (ZU-IRB # 10282/2-1-2023). The Code of Ethics of the World Medical Association (Declaration of Helsinki) was followed in this study. A written informed consent was collected from patients or their relatives.

Bacterial Identification

All collected samples were inoculated on MacConkey agar (Merck Co., Germany) and incubated at 37°C for 24 hours. Conventional bacteriological and biochemical tests such as colonial morphology, Gram stain, citrate utilization, triple sugar iron agar, motility, urease, oxidase, Methyl Red-Voges Proskauer (MR-VP), and indole test were used to identify the grown colonies as *K. pneumoniae*, further confirmation of the isolates was performed by MALDI-TOF/MS using the VITEK MS system, (bioMérieux. Inc. Durham. USA).

Antibacterial Susceptibility Tests

Kirby-Bauer disc diffusion technique:

It was utilized to analyze antibiotic susceptibility patterns of different isolates on Mueller-Hinton Agar medium (Oxoid, UK) following the Clinical and Laboratory Standards Institute guidelines[18]. Amoxicillin/Clavulanic acid (AMC: 20/10 µg), ciprofloxacin (CIP: 5 µg), amikacin (AK: 30 µg), trimethoprim-sulfamethoxazole (TS: 2.5 µg), cefotaxime (CTX: 30 µg), imipenem (IPM: 10 µg), gentamicin (GEN: 10 µg), ceftazidime (CAZ: 30 µg), ceftriaxone (CRO: 30 µg), meropenem (MER: 10 µg), and levofloxacin (LEV: 5 µg) (Oxoid, UK) were tested. The used quality control was *K. pneumoniae* ATCC 700603. Multi-drug-resistant *K. pneumoniae* was recorded when it had been resistant to more than one antibiotic in 3 or more groups of antimicrobials [19].

Minimal inhibitory concentration (MIC) for ciprofloxacin was analyzed by the broth microdilution assay.

Overnight incubation of bacterial strains was done on MacConkey agar at 37°C, then preparation of bacterial suspension in sterile distilled water equal to half McFarland was done and then diluted 100 times with cation-adjusted Mueller-Hinton broth (CAMHB). Aliquots of 50 µL were poured into the wells of a microtiter plate containing 50 µL of ciprofloxacin at concentrations prepared in two-fold serial dilution in CAMHB. The plates were incubated at 37°C and the MIC results were recorded after 16−18 hours. Analysis of the results was executed following CLSI guidelines and MIC ≥1µg/mL was considered ciprofloxacin-resistant[18].

Phenotypic detection of the efflux pump:

Assessment for the efflux pump role in ciprofloxacin resistance among the *K. pneumoniae* isolates was evaluated by determining the minimum inhibitory concentrations (MICs) for ciprofloxacin after treatment of Mueller-Hinton broth wells containing 0.5-512 µg/

mL ciprofloxacin with CCCP (Sigma-Aldrich, Dorset, UK) at a final concentration of 12.5 µg/mL. The control well contained only CCCP without antibiotics. A fourfold or more decline in MIC after CCCP addition was considered positive[14].

PCR for the detection of efflux pump genes:

Isolation of the total genomic DNA from bacterial colonies was performed using (G-spin ™ Total DNA Extraction Kit, iNtron, Korea) following the manufacturer's instructions.

PCR was carried out by the thermal cycler (Veriti ®96-Well Thermal Cycler, Applied Biosystems, Singapore). The reactions included 10 µL of master mix, 1 µL of the DNA, 0.5 µL (25 pmol) of each primer, and sterile distilled water up to 20 µL volume was added to each PCR bead **(iNtron, Certified Company, Korea).** The sequences of the used primers and cycling conditions were listed in Table 1 and 2. The PCR products were detected by 1.5% agarose gel electrophoresis.

Table 1: Primer sequence for the *k. pneumoniae* efflux pump genes

Gene	Primer sequence	Amplicon size (bp)	Reference
oqxA	5′-CTCGGCGCGATGATGCT-3′	392	19
	5′-CTCGGCGCGATGATGCT-3′		
oqxB	5′-TTCTCCCCCGGCGGGAAGTAC-3	512	
	5′-CTCGGCCATTTTGGCGCGTA-3′		
acrA	5′-TCTGATCGACGGTGACATCC 3′	157	20
	5′-TCGAGCAATGATTTCCTGCG3′		
acrB	5′-CAATACGGAAGAGTTTGGCA3′	64	
	5′-CAGACGAACCTGGGAACC3′		

Table 2: Conditions of PCR reactions [20, 21]

Genes	Initial denaturation	Denaturation	Annealing	Extension	Final extension	Cycles no
oqxA	94°C for 2 min	94°C for 15 sec	56°C for 30 sec	72°C for 1 min	72°C for 7 min	25
oqxB	94°C for 2 min	94°C for 30 sec	55°C for 30 sec	72°C for 1 min	72°C for 10 min	32
acrA	94°C for 5 min	94°C for 45 sec	57°C for 45 sec	72°C for 45 sec	72°C for 5 min	36
acrB	94°C for 5 min	94°C for 45 sec	52°C for 45 sec	72°C for 45 sec	72°C for 5 min	36

Statistical analysis:

All data were analyzed using SPSS 22.0 for Windows (SPSS Inc., Chicago, IL, USA). The mean, standard deviation, and range were used to represent Quantitative data. The Chi-square test was used to compare two groups for qualitative data. Kappa was used to compare the similarity between the two tests. P < 0.05 was considered statistically significant at a 95% confidence interval.

RESULTS

The clinical isolates were from patients with a mean age of 45.7 ±7.2 and the male/female distribution was 45/55. There was a statistically significant difference as regards the type of clinical specimens where more than half of the isolates were obtained from urine samples (p-value= 0.002, Table 3).

Table 3: Distribution of the *K. pneumoniae* isolates according to the type of specimen.

Specimen	No of samples	*K. pneumoniae* Isolates		X^2 test	P value
		No.	%		
Endotracheal aspirate	86	23	26.7	14.553	0.002*
Pus	38	14	36.8		
Blood	18	5	27.8		
Urine	111	58	52.3		
Total	253	100	39.5		

X^2 = Chi-square test * =Significant

Concerning the antibiotic susceptibility, about 2/3 of isolates were multidrug-resistant where the highest resistance was for amoxicillin/clavulanic acid (88%), sulphamethexazone (77%), amikacin (66%) and ciprofloxacin (63%) (Table 4, Figure 1).

Table 4: Antibiotic susceptibility by the disc diffusion method

Antibiotic	Resistant%
Amoxicillin/Clavulanic acid	88%
Gentamicin	57%
Amikacin	66%
Imipenem	43%
Meropenem	41%
Ceftazidime	47%
Levofloxacin	55%
Ciprofloxacin	63%
Ceftriaxone	52%
Cefotaxime	56%
Sulphamethexazone	77%
MDR	65%

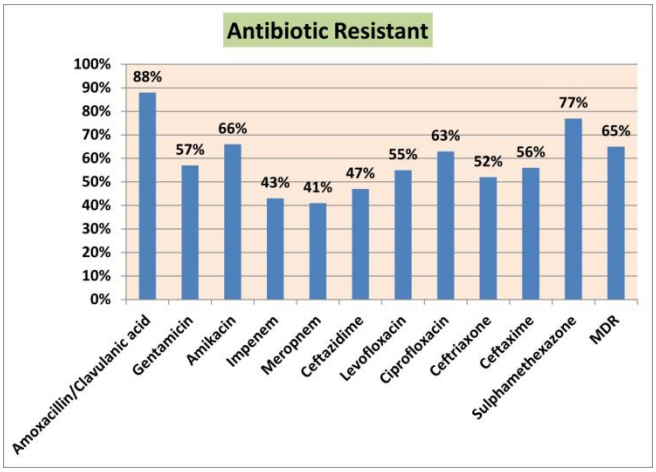

Fig. 1: Antibiotic resistance of the isolates by the disc diffusion method

Regarding the phenotypic detection of efflux pump, out of 63 ciprofloxacin-resistant *K. pneumoniae*, 60.3% of the isolates showed an efflux pump-overexpressing phenotype (4-fold decline or more) while the negative results were detected for the other isolates with a statistically significant difference (p-value < 0.001) as shown in Table 5.

Table 5: Patterns of reduction in MIC after CCCP addition

Reduction in MIC after CCCP addition	No.	%	X^2 test	P value
▪ Phenotypic positive efflux pump (4-fold decrease or more)	38	60.3	33.857	<0.001**
▪ Phenotypic negative efflux pump	25	39.7		
(2-fold decrease)	17	27		
(No change)	8	12.7		
Total	63	100		

X^2 = Chi-square test ** =Highly Significant

The frequency of the efflux pump genes among ciprofloxacin-resistant isolates showed that, out of 63 ciprofloxacin-resistant isolates, 82.5% of isolates were carrying efflux pump genes where acrA, acrB, oqxA and, oqxB were detected in 77.7%, 71.4%, 62% and 65% of isolates, respectively and 17.5% of isolates were negative and this was statistically significant (p-value < 0.001) (Table 6).

Table 6: The frequency of the efflux pump genes among the ciprofloxacin-resistant isolates

Efflux pumb genes	No.	%	X^2 test	P value
Efflux pumb gene positive	**52**	**82.5**		
▪ *acrA*	49	77.7	59.202	<0.001**
▪ *acrB*	45	71.4		
▪ *oqxA*	39	62		
▪ *oqxB*	41	65		
Efflux pumb gene negative	**11**	**17.5**		
Total	63	100		

X^2 = Chi-square test ** =Highly Significant

There was a statistically significant good agreement between the phenotypic and molecular methods for the detection of efflux pump (kappa= 0.487, p-value < 0.001) as shown in Table 7.

Table 7: Agreement between the phenotypic and genotypic methods for detection of efflux pump

Variables		Genotypic methods		kappa	P value
		Negative (n=11, 17.5%)	**Positive** (n=52, 82.5%)		
Phenotypic methods	▪ **Phenotypic negative** (n=25, 39.7%)	11(44%)	14(56%)	0.487	<0.001**
	▪ **Phenotypic positive** (n=38, 60.3%)	0 (0%)	38(100.0%)		

** =Highly Significant

DISCUSSION

Klebseilla pneumoniae has been incriminated in several infections occurring in healthcare settings with a subsequent increase in the mortality rates among patients, prolonging the duration of hospital stay and raising economic burden. Several antimicrobial agents were extensively used to treat these illnesses, which resulted in emerging resistance among *K. pneumoniae* isolates[23].

Widespread resistance of *K. pneumoniae* to aminoglycosides, fluoroquinolones, cephalosporins, and carbapenems has been documented[24]**.** It probably occurs due to drug inactivation, or changes in binding to the target site. Also, efflux pumps were revealed as one of the factors implicated in development of the antimicrobial resistance [25].

Hence, this study highlighted the importance of the *AcrAB* and *OqxAB* efflux pumps in developing antibiotic resistance in clinical isolates of *K. pneumoniae*.

In this study, a total of 100 (39.5%) *K. pneumoniae* isolates were obtained from 253 different clinical samples with the highest percentage (52.3%) isolated from urine followed by pus (36.8%) and the lowest percentage (26.7%) from endotracheal aspirates. This finding was following other studies conducted in two different Egyptian university hospitals[26,27]**.** Furthermore, additional studies in Uganda and Iran observed that most *K. pneumoniae* isolates were isolated from urine [8,28]. However, research by Parrott et al. and Palmeiro et al. reported that blood cultures yielded the majority of *K. pneumoniae* isolates[29,30]. However, Gandor et al. found in their study that the isolation rate of *K. pneumoniae* was 31.6% and they were isolated mostly from sputum samples[31]. Variations in sample type, case count, sampling circumstances, timing, country, and patient overall health may all contribute to this disparity in results.

The rising prevalence of *K. pneumoniae* strains that are resistant to several antimicrobials is an urgent concern in different units at Zagazig University hospitals, including Internal Medicine, ICU and General Surgery Departments. Unfortunately, in this study about 2/3(65%) of isolates were MDR where the resistance to amoxicillin/clavulanic acid, sulphamethexazone, amikacin and ciprofloxacin was (88%), (77%), (66%) and (63%), respectively and the lowest level of resistance was toward meropenem (41%) and imipenem (43%). This was in line with another work performed at Cairo University Hospital, Egypt where the resistance rates of *K. pneumoniae* to amoxicillin-clavulanate, co-trimoxazole, ciprofloxacin, imipenem, and meropenem were (95.7%), (87.0%), (52.2%), (43.5%) and (56.5%), respectively[32]. Following this finding also, an Egyptian study conducted by Negm et al., 2021[33], revealed low-level sensitivity to sulphamethexazone, ciprofloxacin, amikacin, and their resistance rate were 81.4%, 88% and 67.9%, respectively. On the other hand, low level sensitivity appeared toward imipenem (19.5%) and meropenem (19%). Another Egyptian study investigated the prevalence of *K. pneumoniae* capsular serotypes, virulence factors, and antimicrobial susceptibility in Mansoura University Hospitals, reported that 72.6% of isolates were MDR with high resistance to amoxicillin-clavulanic acid (97.26%) and trimethoprim/ sulfamethoxazole (71.23%). However, most of their isolates were susceptible to imipenem (94.52%)[34].

In a trial to monitor changes in antimicrobial susceptibility, an Egyptian study analyzed the cumulative antibiogram results for multiple intensive care units. They found low-level sensitivity rates to ampicillin/sulbactam, sulphamethexazone, ciprofloxacin, amikacin, and imipenem and they were (2%, 3.5%, 11.7%, 29%, 20%, and 22%), respectively[35]. Unfortunately, a higher resistance rate was detected in an Egyptian multi-center pilot study where MDR strains represented 97.3% [36]. Moreover, Gandor et al. stated that 100 % of their isolates were MDR and that 78.2%, 98.3%, 100%, and 100% of them were resistant to sulfamethoxazole/trimethoprim, ciprofloxacin, imipenem, and meropenem, respectively[31]. This highlights the importance of the implementation of infection and prevention control measures and antimicrobial stewardship in Egyptian hospitals. However, Namratha et al. found *K. pneumoniae* isolates that were 100% sensitive to imipenem while, resistance rates to amoxicillin/clavulanic acid, sulphamethexazone, ciprofloxacin, Amikacin, and meropenem were (48.10%, 30.37%, 27.8%, 8.8%, and 46.8%), respectively [37]. The discrepancy in antibiogram among different studies may be due to different sample sizes

and types, epidemiological factors, or acquired resistance due to misuse of antibiotics.

One of the most important mechanisms explaining the resistance to antimicrobials in *K. pneumoniae* isolates is antibiotic efflux pumps that causes a decrease in the antibiotic intracellular concentration with subsequent enhancement of bacterial survival[38]. To evaluate the efflux pump's role in conferring ciprofloxacin resistance, the CCCP inhibitor was used to determine the pump activity. In the current study, out of 63 ciprofloxacin-resistant *K. pneumoniae*, 60.3% of isolates showed an efflux pump-overexpressing phenotype (4-fold decline or more) while negative results were detected for other isolates with a statistically significant difference. Abdelbary et al. agreed with this finding and stated that a significant decline in ciprofloxacin MIC after the addition of CCCP in *K. pneumoniae* isolates was recorded[39]. Similar to this finding, Pakzad et al.[14] reported that 47.5% of the ciprofloxacin-resistant *K. pneumoniae* strains showed a 2-32-fold drop in MIC when treated with CCCP as an inhibitor, which might support the findings of this work. Also, a reduction in MIC after applying CCCP was reported in a previous study and about 95% of their ciprofloxacin-resistant isolates showed an efflux pump-overexpressing phenotype[8]. Moreover, a previous study approved our result and stated that MIC of ciprofloxacin were reduced by 2 to 64-fold in (93%) of MDR-*K. pneumoniae* isolates after using CCCP as an efflux pump inhibitor [40]. These findings suggest the major role played by the efflux pump in conferring ciprofloxacin resistance.

Regarding the frequency of efflux pump genes among ciprofloxacin-resistant isolates in our work, out of 63 ciprofloxacin-resistant isolates, 82.5% of isolates were carrying efflux pump genes where *acrA, acrB, oqxA,* and *oqxB* were detected in 77.7%, 71.4%, 62% and 65% of isolates, respectively and 17.5% of isolates were negative. Similar results were found by Razavi et al[8], who reported that the *acrA, acrB, oqxA,* and *oqxB* genes prevalences were 52.72%, 52.72%, 47.27%, and 47.27%, respectively. Also, another study showed that all of the tested *K. pneumoniae* isolates had efflux pump genes from the two systems *acrAB and oqxAB* [41]. Also, Albarri et al. in their study, by using quantitative real-time PCR, showed that *acrA, acrB, oqxA, and oqxB* genes were overexpressed in 63%, 52%, 63%, 52%, and 37% of *K. pneumoniae isolates*, respectively[40]. In line with these findings, research by Swick et al. reported that 241 strains of *E. coli* were examined for assessment of the relationship between efflux pumps and resistance to fluoroquinolones. They found that overexpression of *acrA* and *acrB* genes is associated with fluoroquinolone resistance and that with the elimination of these genes, the fluoroquinolone MIC was decreased [42]**.** A significant relationship between ciprofloxacin resistance and the presence of *OqxAB* efflux pumps was detected where *oqx*A and *oqx*B genes were present in 95% and 98% of *K. pneumoniae* isolates, respectively[22]. Khoshnood et al. reported that 110 (94%) and 102 (87%) were positive for *acrA and acrB* genes with an increased expression detected by real-time PCR assays[43]. Contrary to our results, efflux pump activity was determined only in 22.3% of ciprofloxacin-resistant *K. pneumoniae* isolated from cancer patients suggesting that it had a little role in contributing to ciprofloxacin resistance[44]. At this point, other mechanisms of ciprofloxacin resistance might be accused such as alteration in DNA gyrase, decreased outer membrane permeability, or due to another efflux pump that might not be inhibited by CCCP.

In the current work, a statistically significant good agreement between phenotypic and molecular methods for the analysis of efflux pump (kappa=0.487, p-value <0.001) was found as efflux pump genes were detected in 100% of ciprofloxacin-resistant isolates. Following this finding, Razavi et al., reported that all their 20 ciprofloxacin-resistant isolates were carriers of *acrA/acrB*, and *oqxA/oqxB* genes [8]. Additionally, 52 isolates of *K. pneumoniae* from burned patients were examined for detection of the *AcrAB* efflux pump using PCR assay, and it was found that all ciprofloxacin-resistant bacteria carry the *acrA* gene[14]. On the other hand, this study showed that 14 (56%) of clinical *K. pneumoniae* isolates carried efflux pump genes, but were not resistant to ciprofloxacin phenotypically. Failure of efflux pump gene expression in these isolates could be a cause for this finding. Various factors can affect the expression and efficacy of efflux pumps; the expression of different efflux pumps changes according to different antibiotic stress. Also, the uptake of an EPI in the outer membrane and periplasmic concentration affects its activity and finally, the selectivity of some EPIs can be more for a specific efflux pump[45].

Actually, the high level of resistance seen in our study could be related to the presence of genes of both *OqxAB* and *AcrAB* efflux pumps. The expression of the efflux pump genes in resistant strains needs to be examined in more studies to prove this relation. Microorganisms that contain these pumps increase the pathogenicity in the patients and increase the drug resistance which is a main problem to their health. Therefore, it is crucial to make the right diagnosis of infections, identify resistance mechanisms in microorganisms, and choose the right antibiotics to prevent treatment failure[46].

CONCLUSION

AcrAB and OxqAB efflux pump genes were widely detected in *K. pneumoniae* isolates and these isolates have high levels of resistance to ciprofloxacin. Consequently, further research is required to assess the possibility of using efflux pump inhibitors as adjuvant

therapies that would increase the efficacy of used antimicrobials such as ciprofloxacin and decrease the emergence of MDR bacteria. A high level of resistance of *K. pneumoniae* isolates to antimicrobials was detected in this study suggesting the necessity to implement infection control policy and antimicrobial stewardship.

Declarations:
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