ORIGINAL ARTICLE

Detection of OqxA and OqxB Efflux Pump Genes in Multidrug Resistant Klebsiella Pneumoniae Isolated from Intensive Care Unit Patients

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Key words:
Klebsiella pneumoniae, multidrug resistance, efflux pump, OqxAB.

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ABSTRACT

Objective: *Detection of OqxA and OqxB efflux pump genes in multidrug resistant Klebsiella pneumoniae.* ***Methodology:*** *This study was done on 80 Klebsiella pneumoniae isolates from patients in Intensive Care Unit of Benha University Hospitals. The isolated Klebsiella pneumoniae was identified by conventional laboratory methods with subspecies identification by VITEK 2 system. Also, Antibiotic sensitivity tests were done by VITEK 2 method. OqxA and OqxB genes were detected by PCR.* ***Results:*** *Ninety percentages of isolates were carrying OqxA gene and 60% of isolates were carrying OqxB gene while OqxA and OqxB genes together were in 60% of isolated K. Pneumoniae. The OqxA gene was detected in 94.1% and OqxB was detected in 67.6% of isolated multidrug resistant Klebsiella pneumoniae.* ***Conclusion:*** *There is high correlation between OqxAB efflux pump genes and resistance to different antibiotic classes including quinolones and fluoroquinolone antimicrobial agents. This study shows that there is a significant association between the existence of OqxAB genes and MDR Klebsiella pneumoniae.*

INTRODUCTION

Klebsiella pneumoniae (k. pneumoniae) is a serious nosocomial infection causing wide variety of diseases and showing increase in resistance to antibiotics especially in intensive care unit (ICU). It can cause different infections in ICU patients including pulmonary infection, urinary tract infection, bloodstream infection and meningitis [1].

Multidrug resistant (MDR) *K. pneumoniae* is a great health issue in the nosocomial and community settings. *K. pneumoniae* strains are mostly resistant to several important antimicrobial classes including β-lactam drugs, quinolones, fluoroquinolones and aminoglycosides. Increasing resistance occurs among polymyxin B, colistin, fosfomycin and tigecycline [2].

Multidrug efflux pump is an important mechanism for development of drug resistance. OqxAB multidrug efflux pump is concerned to the resistance nodulation cell division family. It is encoded by two genes (OqxA and OqxB) that have been increasingly detected in *K. pneumoniae* [3].

Over the past decades, multiple studies have been explaining the importance of OqxAB efflux pump in decreasing susceptibility to different antibiotic classes [4].

The aim of this study was detection of OqxA and OqxB genes in MDR *K. pneumoniae* isolated from ICU patients of Benha University Hospitals.

METHODOLOGY

This work was done in Microbiology & Immunology Department, Faculty of Medicine, Benha University in the period from May 2022 to June 2024.

The current study was performed on 80 *K. pneumoniae* isolates from 202 different clinical samples (bronchoalveolar lavage, sputum and urine) collected from ICU patients in Benha University Hospitals.

The present study was approved by Benha University Ethical Committee and written consent was taken from the patients under study **(approval code MD 3-3-2022)**.

Isolation and identification of *Klebsiella pneumoniae*

The collected samples were submitted to Gram staining for identification of gram negative bacilli. Gram negative bacilli sample were cultured on MacConkey's and CLED agar plates and incubated at 37°C for 24 hours. The growing organisms were identified as *K. pneumoniae* by conventional laboratory technique including: Gram staining, colony morphology and sugar fermentation tests. Identification of *K. pneumoniae Subspecies Pneumoniae* was done by VITEK® 2 identification cards ***(BioMerieux, France)***.

Antibiotic susceptibility by VITEK® 2 Method

The antibiotic sensitvity testing (AST) was done by VITEK® 2 using AST-GN 73 card ***(BioMerieux, France)*** according to the manufacturer's prescripts.

Detection of OqxA and OqxB genes

PCR was done for detection of OqxA and OqxB as follow:

1- **Genomic DNA extraction:** DNA extraction was done using the G-spin™ Total DNA Extraction Mini Kit ***(Intron biotechnology, Germany)*** according to the manufacturer's prescripts.
2- **DNA amplification:** The extracted DNA was amplified using Qiagen HotStar Taq Master Mix ***(Qiagen, Germany)*** and OqxA and OqxB specific primers ***(eurofins Genomics, Germany)*** (Table -1). In a PCR tube an amplification mixture of a volume 50 µl was prepared as follow: 25 µl 2x Taq Red PCR Master mix, 5 µl Template DNA, 2.5 µl of each primer and 15 µl RNase free water.

The amplification programme was run as follow: the HotStar Taq DNA polymerase was first activated at 95 °C for 15 minutes. Then 35 cycles of denaturation at 94 °C for 1 min., annealing at 53 °C for 1 min. and extension at 72 °C for 1 min. Final extension at 72 °C for 10 minutes.
3- **Electrophoresis:** The amplified products were visualized by UV- transilluminator on 1.5 % agarose gel using 100bp ladder. OqxA and OqxB genes were detected at 392 bp and 512 bp respectively.

Table 1: Primer Sequence of OqxA and OqxB genes

Gene name	Primer Sequence	Product size (bp)	References
OqxA	F: CTCGGCGCGATGATGCT R: CCACTCTTCACGGGAGACGA	392 bp	(5)
OqxB	F: TTCTCCCCCGGCGGGAAGTAC R: CTCGGCCATTTTGGCGCGTA	512 bp	(5)

Statistical analysis

The data were analysed by the computer program SPSS for windows (Statistical package for social science) version 29 **(SPSS Inc., Chicago, IL, USA).** The Normality of distribution for analysed variables was checked by Kolmogorov-Smirnov test. The data were epitomized in terms of Mean ± Standard Deviation (SD) for quantitative data when it was convenient and for qualitative data as number and percentage. Comparisons between the various study groups were done using the Chi-square (X^2) and Fisher's Exact Test to compare qualitative data. Two population proportions were compared by Z test.

RESULTS

Out of 202 collected samples, 80 (39.6%) *k. pneumoniae* strains were isolated. The isolated *K. Pneumoniae* strains were from respiratory secretions 58.7% (Broncho-Alveolar lavage 36.2%, sputum 22.5 %) followed by urine 41.3%.

The sociodemographic and clinical data of patients of the isolated *K. pneumoniae* strains shows that the rate of *K. pneumoniae* isolation was higher in males 56.3% than females 43.8%. It was high among the age group > 60 years 42.5%. The rate of *K. pneumoniae* infection was high among ventilated patients 85% and those on previous antibiotic therapy 70% followed by diabetic 38.8% and hypertensive patients 31.3%. It also increases with increase the duration of hospital stay.

The antibiotic sensitivity tests of the isolated *k. pneumoniae* showed that there was *K. pneumoniae* resistance to all the tested antibiotics from different classes. The highest percentage of resistance was to ampicillin 95% followed by cefazolin 92.5%, ceftriaxone 90%, ampicillin/sulbactam and ceftazidime 87.5%, piperacillin-tazobactam 80% and cefepime 75%. The rate of resistance was 70 to cefoxitin, meropenem and tobramycin, 67.5% to ciprofloxacin, levofloxacin and trimethoprim/ Sulfa, 62.5% to gentamycin, 60% to nitrofurantoin while the lowest rate of resistance was shown to amikacin 52.5% (figure 1).

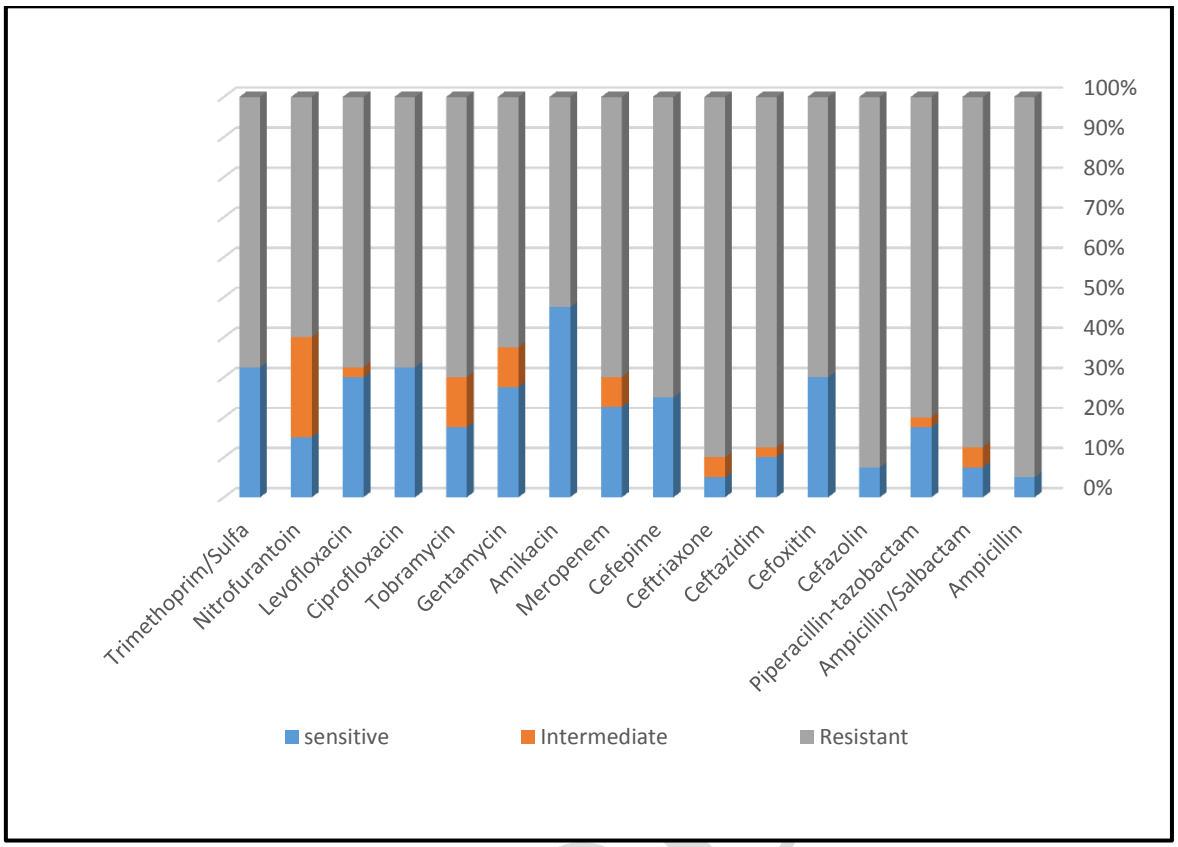

Fig. : Antibiotic sensitivity patterns of isolated *Klebsiella Pneumoniae.*

PCR revealed that 90% of isolates were carrying the OqxA gene and 60% of isolates were carrying the OqxB gene while both OqxA and OqxB genes were 60% of the isolated *K. Pneumoniae* strains (Figure 2 & 3).

The present study shows significant correlation (p value < 0.05) between OqxAB genes together and resistance to the tested B lactams, cephalosporins, meropenem, two of the studied aminoglycosides (gentamycin and tobramycin) and fluoroquinolones. The correlation between trimethoprim\ sulfa and OqxA gene is statistically non-significant. There is also no significant correlation between amikacin and nitrofurantoin and OqxB gene.

The OqxA gene was detected in 94.1% of MDR *k. pneumoniae* and OqxB was detected in 67.6% of MDR *k. pneumoniae*. There was high significant association between the both OqxAB genes and MDR *k. pneumoniae*.

Table 2: Number and Percentages of OqxA and OqxB among total k. pneumoniae and MDR *k. pneumoniae* isolates

Gene		Total *k. pneuomoniae* (80)		MDR				P Value
				No (no.= 12)		Yes (no.= 68)		
		No.	%	No.	%	No.	%	
OqxA	Positive	72	90	8	66.7	64	94.1	0.003
	Negative	8	10	4	33.3	4	5.9	
OqxB	Positive	48	60	2	16.7	46	67.6	0.001
	Negative	32	40	10	83.3	22	32.4	
OqxAB	Positive	48	60	2	16.7	46	67.6	0.001
	Negative	8	10	4	33.3	4	5.9	

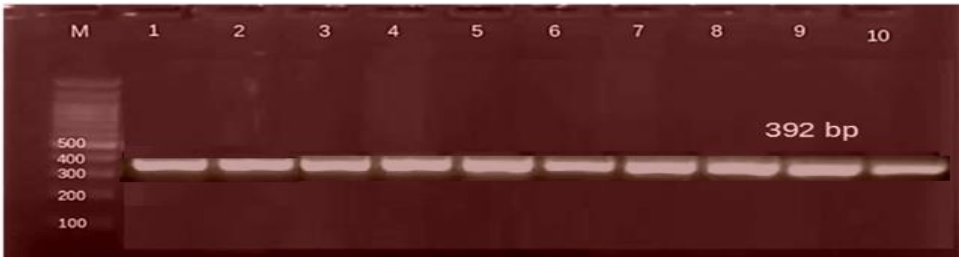

Fig. 2: Ethidium bromide stained 1.5% Gel electrophoresis of some strains of K. Pneumoniae showing OqxA gene. M (Marker): is a 100pb ladder. Lanes from 1 to 10 show the OqxA gene at 392bp.

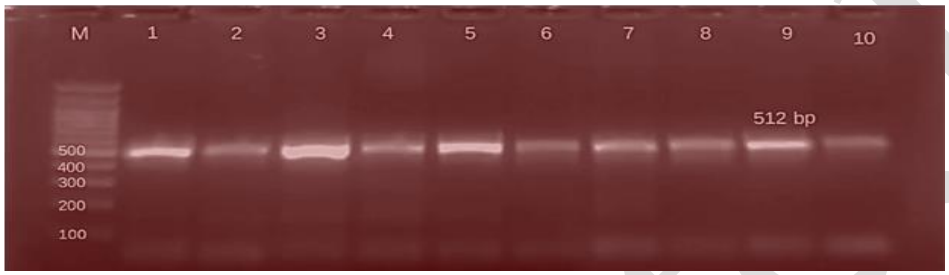

Fig. 3: Ethidium bromide stained 1.5% Gel electrophoresis of some strains of K. Pneumoniae showing OqxB gene. M (Marker) is a 100pb ladder. Lanes 1,3,5 and 9 show the OqxB gene at 512bp while lanes 2,4,6,8 and 10 were negative.

DISCUSSION

MDR *K. pneumoniae* is a great health issue in the healthcare and community settings. Drug inactivation, target transformation, decreased permeability and increased efflux activity are important mechanisms that contribute to antibiotic resistance [6]. MDR efflux pump is a mechanism responsible for development of drug resistance. It increases the resistance mechanisms that lower the amount of antibiotics inside cells and boost mutation [7].

In this study, the isolated *k. pneumoniae* were 39.6% of the overall isolates which coincides with *El-Badawy et al.,* [8] and *Engda et al.,* [9] who detected that *k. pneumoniae* was the most predominant among the isolated organisms in their study 38% and 42% respectively.

The present study showed that the percentage of *K. Pneumoniae* was 58.7% from respiratory samples (Broncho-Alveolar lavage 36.2% and sputum 22.5%) followed by urine 41.3%. Such results came in consistence with the study done by Fu et al. [10], *Karimi et al.* [11] and *Fursova et al.* [1]. On the other hand, *Shakib et al.*, [12] found that 71.4% of *K. pneumoniae* strains was isolated from urine followed by 14.3% from respiratory secretions and 12.8% from other samples.

This study detected that *K. pneumoniae* strains showed high resistance to all tested antibiotics from different classes. *MohdAsri et al.* [13] and *Maina et al.* [14] are in accordance with the present study.

In this study, MDR *k. pneumoniae was* detected 85% of studied isolated *k. pneumoniae*, this is in agreement with *Fahim* [6] and *Maina et al.* [14] who reported in their study that the MDR *k. pneumoniae* strains were 87.8% and 89.3% respectively. *El-Kholy et al.* [15] *and Awoke et al.* [16] reported that MDR phenotype among isolated *k. pneumoniae* was higher than the current study 98.5% and 95.8% respectively. While *Nakamura-Silva et al.* [17] detected that the MDR *k. pneumoniae* was lesser than this study 62%.

In current study, The OqxA and OqxB genes were found in 90% and 60% of the isolated *K. Pneumoniae* strains respectively. *Mustafa and Abdullah* [18] reported that OqxA and OqxB genes were 96% and 12% respectively. *Dehnamaki et al.* [19] showed that OqxA and OqxB genes were detected in 57% and 56% of *K. pneumoniae* isolates respectively. On the other hand, *Bahrami Chegeni and Goudarzi,* [20] found that OqxA gene was 26% and OqxB was 56.1% among their isolated *k. pneomoniae*.

The current study showed that both OqxA and OqxB genes were reported in 60% of isolated *K. Pneumoniae* strains. *Abdelmegeed et al.* [21] detected OqxAB genes in 92% of isolated *k. pneuomoniae* and *Hamed et al.* [22] reported both OqxAB genes in 92.5% of their isolated *K. pneumoniae.* The expression of OqxAB genes is a leading cause in the evolution of resistance. This expression can be realized constitutively or transiently under specific circumstances as exposure to antibiotics[5].

The present study detected significant correlation (p value < 0.05) between both OqxAB genes and resistance to B lactams (ampicillin, ampicillin/ salbactam, piperacillin-tazobactam), cephalosporins (cefazolin and ceftazidime cefepime, cefoxitin and ceftriaxone), meropenem, two of the studied aminoglycosides (gentamycin and tobramycin) and fluoroquinolones (ciprofloxacin and levofloxacin). For Trimethoprim\ sulfa, there was no significant

correlation with OqxA. While for amikacin and nitrofurantoin, there was no significant correlation with OqxB. *Dehnamaki et al.* [19] found significant association with fluoroquinolones, beta-lactam resistance and presence of both OqxA and OqxB genes (P value < 0.05). *Gabr et al.* [23] correlated the relation of OqxAB genes and fluoroquinolones and trimethoprim resistance related to the OqxAB pump.

In this study, OqxA and OqxB genes together were reported in 67.6% of MDR *k. pneumoniae*. It reveals that there is high significant association between the presence of both OqxAB genes and MDR phenotype. The studies of Mustafa *and Abdullah* [18] *and Gabr et al.* [23] are in accordance with this study. Earlier studies, *Shahbazi et al.* [24] *& Farivar et al.* [25] declared that the decreased sensitivity of the studied *K. pneumoniae* against to many antibiotics, especially fluoroquinolones, may be due to the OqxA and OqxB in their isolates.

CONCLUSION

This study investigates the correlation of existence of OqxAB efflux pump genes and resistance to different antibiotic classes including quinolones and fluoroquinolone antimicrobial agents. So, we concluded that OqxAB genes have an importance in resistance of *k. pneumoniae* isolates. Also, OqxAB efflux pump increase MDR phenotype.

Conflicts of interest:

The authors declare that they have no financial or non-financial conflicts of interest related to the work done in the manuscript.

Each author listed in the manuscript had seen and approved the submission of this version of the manuscript and takes full responsibility for it.

This article had not been published anywhere and is not currently under consideration by another journal or a publisher. **Fund:** none

Recommendation

Appropriate use of antibiotics are the best ways to decrease and prevent infections by k. *pneumoniae*. Antibiotic treatment should be based on antibiotic sensitivity tests to decrease emergence of MDR bacteria.

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