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Research Article

泌尿道感染患者分离的肺炎克雷伯菌流出系统 AcrAB、OqxAB 和 MarA 操纵子的基因表达

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【摘要】：
背景：全球约有 1.5 亿人患有泌尿道感染（UTI），这是最常见的细菌性疾病之一。肺炎克雷伯菌是一种常见的机会性病原体，可导致院内感染。在 AL-Dewaniyah 市两家医疗机构的专科医生监督下，确定从各种 UTI 患者中分离出的外排泵基因（AcrAB、OqxAB 和 MarA）：AL-Dewaniyah 综合教学医院和 AL-Dewaniyah 妇幼教学医院，这是本研究的目的。该研究检查了导致 UTI 的肺炎克雷伯菌的流行率。根据手工培养特征，共有 36 个分离株被鉴定为肺炎克雷伯菌，然后使用 Vitek-2 系统进行确认。使用实时 PCR 技术，表达了外排泵基因（AcrAB、OqxAB 和 MarA）。我们发现 AcrAB 基因表达率较高（17.3%），在 36 个（100%）肺炎克雷伯菌分离株中均有发现.
【关键词】：肺炎克雷伯菌、尿路感染、实时 PCR、AcrAB。

Gene Expression of The Efflux System AcrAB, OqxAB, and MarA Operons of Klebsiella Pneumoniae Isolated from Patients with Urinary Tract Infection

【Abstract】:
Approximately 150 million individuals worldwide have urinary tract infections (UTIs), which are among the most prevalent bacterial illnesses. *Klebsiella pneumoniae* is a common opportunistic pathogen that causes nosocomial infections. The efflux pump genes (*AcrAB*, *OqxAB*, and *MarA*) were isolated from a variety of UTI patients under the supervision of a specialist physician at two healthcare facilities in the city of AL-Dewaniyah: AL-Dewaniyah General Teaching Hospital and AL-Dewaniyah Maternity and Pediatrics Teaching Hospital, which is the aim of the current study. This study examined the prevalence of Klebsiella pneumoniae bacteria that cause UTIs. A total of 36 isolates were identified as *K. pneumoniae* according to manual culture characteristics and confirmed using the Vitek-2 system. Using real-time PCR, efflux pump genes (*AcrAB*, *OqxAB*, and *MarA*) were expressed. We found a high rate of AcrAB gene expression of *AcrAB* (17.3%), which was observed in 36(100%) K. pneumoniae isolates.
【Key words】: Klebsiella pneumoniae, urinary tract infections (UTIs), real-time polymerase chain reaction (PCR), AcrAB.

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1. Introduction

Klebsiella pneumoniae is a (gram-negative) opportunistic, non-motile, facultative anaerobic, rod-shaped bacterium that is singly arranged in pairs or short chains. Its range is from 0.3 to 1.0 μm in width and around 0.6 to 6.0 μm in length, and it is non-spore forming [1]-[3]. *K. pneumoniae* is an oxidase-negative and lactose-fermenting bacterium. It has a thick polysaccharide capsule that forms colonies on agar plates with a mucous appearance [4]-[5]. *K. pneumoniae* is the normal flora of the human skin, oropharynx, or gastrointestinal tract [6]. It causes more infections in humans, such as respiratory tract infections, UTIs, bloodstream infections, bacteremia, suppurative infections, cholangitis, and rarely, osteomyelitis or meningitis, especially in immunocompromised patients or those with underlying disease conditions, such as diabetes mellitus [7]-[8]. Hospital-acquired pneumonia was associated with a high percentage of contributing factors. Hospital-acquired pneumonia, which mostly affects hospitalized patients, is the fourth most prevalent infection in hospitals worldwide. This type of pneumonia is particularly prevalent among newborns, premature infants, and individuals reliant on mechanical ventilation [9]-[10]. UTIs are a common problem that can be difficult to treat owing to bacterial resistance. It is caused by bacteria and can affect people of all ages and sexes [11]. *AcrAB* and *OqxAB* are the two efflux system genes in *K. pneumoniae* that have been the most extensively examined in relation to antibiotic resistance [12]-[15]. The inherent resistance of *K. pneumoniae* isolates to fluoroquinolones, particularly ciprofloxacin, was primarily attributed to the AcrAB efflux pump. Furthermore, this pump is resistant to β-lactams, macrolides, trimethoprim, tetracycline, and chloramphenicol [16]-[17]. In *K. pneumoniae*, the OqxAB resistance determinant is frequently detected chromosomally, whereas in other *Enterobacteriaceae* species, it is usually found on plasmids [18]. Numerous factors, including antibiotics, environmental stresses, and genetic alterations, can control the expression of efflux pump genes [19]-[20].

2. Methodology

2.1. Sample Collection

Thirty-six *isolated K. pnuemoniae* isolates were collected from various patients with UTIs under the supervision of a specialist physician across different age groups and genders at two healthcare facilities: AL-Dewaniyah General Teaching Hospital and AL-Dewaniyah Maternity and Pediatrics Teachings Hospital in the city of AL-Dewaniyah. The data were collected from October 20, 2023, to January 15, 2024. Using a sterile loop, all urine samples were inoculated into specific media. Subsequently, agar plates were subjected to 24-h of aerobic incubation at 37°C. After that, the bacteria were identified by laboratory tests, and the automated Vitek®2 AST-N222 (BioMeneux, Turkey) was used to make the diagnosis. A facilitation document was issued by the College of Medical Biotechnology, Al-Qadisiyah University, Iraq. Document no. 1576,2023/9/17.

2.2. Quantitative Reveres Transcription Real-Time PCR (RT-qPCR)

Quantitative Real-Time PCR was used to quantify efflux pump gene expression in multidrug-resistant *K. pneumoniae* isolates and normalize the data using housekeeping genes. The procedure was performed in accordance with [21]-[22] and included the following steps:

-**Total RNA extraction:** Using a Total RNA Extraction Kit, total RNA was extracted from MDR *K. pneumonia* isolates in accordance with the following procedures, which were followed as directed by the manufacturer: after inoculating bacterial isolates in Luria Bertani broth with a 2μg/ml concentration of inducible ethidium bromide and incubating at 37 °C to produce bacterial cells (OD600:0.8-1.0), the bacterial cells were harvested by centrifuging them for 1 min at 10,000 rpm, and the supernatant was then removed. Then, 1 ml of TRIzol Reagent was added, and the bacterial pellets were aggressively vortexed for 10 s at room temperature. Each tube was filled with 200 μl of chloroform and shaken ferociously for a minute. The mixture was then incubated on ice for 5 min. then centrifuged for 15 min at 4C and 13,000 rpm. After transferring the supernatant into a fresh 1.5 ml microcentrifuge tube, 500μl μL isopropanol was added. Subsequently, the mixture was blended by rotating the tube four or five times and left at 4C° for 10 min. This was followed by centrifuging at 4C° and 13,000 rpm for 10 min. After discarding the supernatant, 1 ml of 80% ethanol was added and vortexed. The mixture was then centrifuged at 4°C for 15 min at 13,000 rpm. The RNA pellet was air-dried, and the supernatant was discarded. After dissolving the RNA pellet in 100 μl of free nuclease water, the extracted RNA sample was stored at 80°C.

- **Real- Time PCR primers** were created using Primer3 plus and the (NCBI-Genebank) sequence in order to detect and quantify the efflux pump genes in *K. pneumoniae* isolates. Co. Ltd. Scientific Research has provided primers from Iraq, as shown in Table 1.

表 **1**

Table 1 The qPCR detection gene primers for K. pneumoniae with their nucleotide sequences

Gene	Sequence (5'-3')	Product size	NCBI Reference code
16SrRNA gene	F: TTCGATGCAACGCGAAGAAC	123 bp	LC764401.1
	R: TTTCACAACACGAGCTGACG		
AcrAB gene	F: AAACGGCAAAGCGAAAGTGG	108 bp	OQ808798.1
	R: ATTGAGCCGGTGGTCTGATC		
OqxAB gene	F: AAAGTGACCGCCCCTATTGAC	119 bp	MN273774.1
	R: TACACCGTCTTCTGCGAGAC		
MarA gene	F: TAAGAAAGAGACCGGCCATTCC	109 bp	NC_016845.1:c2572399-2572025
	R: TTCCGCCAGGTACAGGATTG		

3. Results and Discussion

3.1. Statistical Analysis

The data were statistically analyzed using SPSS version 26. The results were subsequently released in print. Numbers and percentages were used to express the qualitative attributes. In addition, the mean ± standard deviation (SD) was used to express all normally distributed data. The chi-square test can be used to identify the relationships between any two categorical variables. An independent sample t-test was used to determine the mean difference between two normally distributed variables. The degree of significance, 95% confidence interval (CI), and odd ratio (OR) were computed at P-values of equal to or less than 0.05 for each significant and non-significant component.

***AcrAB* gene expression in *K. Pneumonia* isolates.** The mean *AcrAB* gene expression was 17.31±7.80. *K. pneumoniae* isolates are shown in Table 2. All *K. pneumoniae* isolates possessed the *AcrAB* gene (100%). In a previous study conducted by researchers [23] in Iran, the *AcrAB* gene was found to be more prevalent in *K. pneumoniae* strains because of the AcrAB efflux pump, which is thought to be one of the primary pumps causing intrinsic resistance of *K. pneumoniae* isolates to ciprofloxacin and tetracycline. The percentage of AcrAB gene expression in *K. pneumoniae* bacteria was approximately 41%. In a previous local study [22] in Baghdad, 29% of *K. pneumoniae* bacteria were present in urine isolates, and all isolates of this bacterium possessed the *AcrAB* gene (100%).

表 **2**

Table 2 AcrAB gene expression in K. Pneumoniae isolates

Gene expression	*MDR K. Pneumoniae* *n* (%)	P
AcrAB gene expression		
Mean± SE	17.31 ± 7.80	0.001 † S
Range	1.95-83.86	

***OqxAB* gene expression in *K. Pneumonia* isolates:** The mean *OqxAB* gene expression was 8.90±3.29, in the *K. pneumoniae* isolates as shown in Table 3. Also, a study was conducted in China [24], where they found *OqxAB* efflux pump gene in all isolates of *K. pneumoniae* bacterium, and noted that "the mechanisms that mediate the efflux, including high expression of the efflux pump OqxAB play a major role in tigecycline resistance."

表 **3**

Table 3 OqxAB gene expression in K. Pneumoniae isolates

Gene expression	*MDR K. Pneumoniae* *n* (%)	P
OqxAB gene expression		
Mean± SE	8.90± 3.29	0.021 † S
Range	1.62-35.26	

***MarA* gene expression in *K. Pneumonia* isolates**: Mean MarA gene expression was 6.95±3.3, in *K. pneumoniae* isolates, as shown in Table 4. In a previous study [15], it was indicated that 93% of MDR *K. pneumoniae* isolates possessed the *MarA* efflux pump gene, with a gene expression rate of approximately 37%.

表 **4**

Table 4 MarA gene expression in K. Pneumoniae isolates

Gene expression	*MDR K. Pneumoniae* *n* (%)	P
MarA gene expression		
Mean± SE	6.95 ± 3.3	0.302 † NS
Range	1.74-36.00	

3.2 The Comparison between Genes Expression in *K. Pneumoniae* Isolates

K. Pneumoniae isolates were compared, and the results are presented in Figure 1 and Table 5.

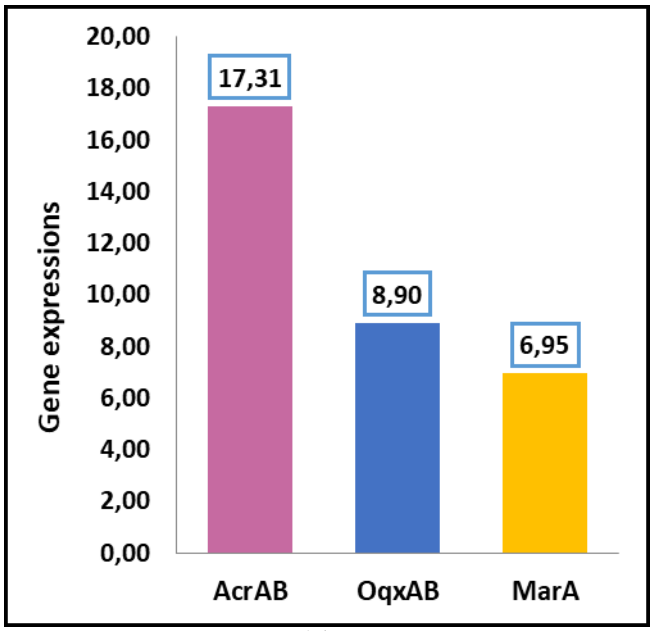

图 **1**

Fig.1 The comparison between genes expression in *K. pneumoniae.*

表 **5**

Table 5 The comparison between genes expression *in K. Pneumoniae* isolates

Characteristic	*AcrAB*	*OqxAB*	*MarA*	*P*
Gene expression				
Mean± SD	17.31 ± 7.80^{A}	8.90± 3.29^{B}	6.95 ± 3.3^{B}	0.018 † S
Range	1.95-83.86	1.62-35.26	1.74-36.00	

Different latter values denote significant differences at p< 0.05

The mean gene expressions were 17.31±7.80, 8.90± 3.29 and 6.95± 3.3, for *AcrAB*, *OqxAB*, and MarA, respectively. The mean expression was significantly higher in *AcrAB* than in the other genes (P < 0.05). However, the expression of the OqxAB and *MarA* genes was not significantly different (P < 0.05). This difference in the rates of expression of these genes may be due to the functional importance of the efflux pump gene *AcrAB* in withstanding environmental stress and resistance to antibiotics, which may lead to an increase in its gene expression in *K. pneumoniae* isolates. A previous study showed that *K. pneumoniae* may respond faster and more effectively to chemicals or environmental conditions that stimulate the expression of the *AcrAB* gene to a greater extent than the *OqxAB* and *MarA* genes [12]. A previous study showed that the gene expression rate of AcrAB is higher than that of the *OqxAB* and *MarA* genes, which may be due to differences in the population distribution of the strains that express the *AcrAB* efflux pump gene compared with the other two genes. In addition, physical environmental conditions such as temperature and humidity may be responsible for these differences, which may affect gene expression rates. It was also indicated that a genetic fusion may occur between AcrAB and another gene, leading to an increase in its gene expression rate in *K. pneumoniae* isolates [25].

As previously stated, the modern technology Real-time PCR was used in the current study to obtain the required results. Figures 2 and 3 show that the expression rate of the efflux pump gene *AcrAB* is the highest compared" to that of the other genes, *OqxAB* and *MarA*, in the *K. pneumonia* isolates.

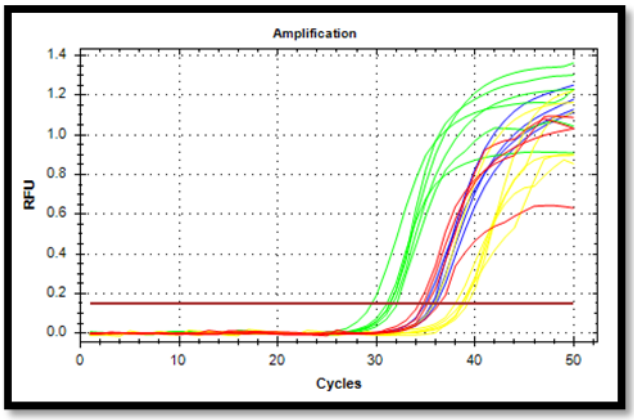

图 **2**

Fig.2 The Real-Time PCR amplification plots of efflux pump genes of MDR K. pneumoniae isolates: the blue plots (*AcrAB*), the green plots (*OqxAB*), the yellow plots (*MarA*), and the red plots (16SrRNA).

In previous studies, PCR technology was used, and this technology is considered less accurate than real-time PCR technology, as it does not accurately determine the expression rate of efflux pump genes [26]-[27]. In previous studies, real-time PCR technology has been used to express the resistance genes *AcrAB*, *OqxAB*, and *MarA* [15], [28]-[29].

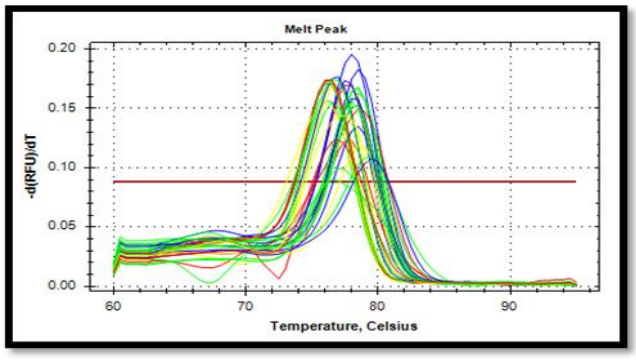

图 **3**

Fig.3 Real-time PCR melting of the efflux pump genes of the MDR *K. pneumoniae* isolate: the blue plots (*AcrAB*), the green plots (*OqxA*B), the yellow plots (*MarA*), and the red plots (16SrRNA).

5. Conclusion

All *K. pneumoniae* isolates from UTIs showed MDR, and the *K. pneumoniae* isolates showed positive results for phenotypic detection of efflux systems. The" results" of the present study also showed that all *K. pneumoniae* isolates possessed efflux pump genes. *AcrAB*, *OqxAB*, and MarA encode efflux pump proteins with ratios of 17.31%, 8.90%, and 6.95%, respectively.

Declarations

Author Contributions

Noor Sabah and Najlaa Abdullah D. AL-Oqaili contributed equally to the research conceptualization, methodology, data collection, investigation, analysis, validation, visualization, and writing.

Ethical Approval and Consent to participate

The Research Ethics Council of the Al-Qadisiyah University, Iraq, granted all necessary clearances and ethical approval.

Conflict of interest

The authors declare that they have no conflicts of interest.

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