Phenotypic and Molecular Detection of Efflux Pumps in Klebsiella Pneumoniae Isolated from Sinus Infections

Maisoon Suhail Ahmed and Zainab Hussein Mahdi
Department of Biology, College of Education for Pure Sciences, University of Diyala, 32001 Diyala, Iraq
maisoonsuhail@gmail.com, zainabhusseinmahdyi@gmail.com

Keywords: Sinus Infections, *acrAB*, *mdtK* and *oqxA*, Efflux Pump Resistance.

Abstract: *Klebsiella pneumoniae* is an opportunistic Gram-negative pathogen associated with severe hospital- and community-acquired infections. Its clinical significance has increased because of its growing multidrug resistance (MDR) and virulence potential. This study aimed to phenotypically and molecularly detect efflux pumps in K. pneumoniae isolated from patients with sinus infections and to identify the presence of the mdtK, acrAB, and oqxA genes. A total of 203 clinical swab samples were collected between August 2025 and February 2026 from patients attending the outpatient ENT clinic at Baquba Teaching Hospital, Diyala Governorate, Iraq. Among these, 110 samples were culture-positive, yielding 25 K. pneumoniae isolates that were identified by conventional methods and confirmed using the Vitek 2 system. Antibiotic susceptibility testing was performed according to Clinical and Laboratory Standards Institute (CLSI) guidelines. The highest susceptibility rates were observed for imipenem (76%), chloramphenicol (76%), and doxycycline (76%), whereas lower susceptibility rates were recorded for amikacin (56%) and cefepime (56%). Twelve isolates (48%) were classified as MDR. PCR analysis showed that the acrAB and oqxA genes were detected in 100% of the MDR isolates, whereas the mdtK gene was detected in 91.6%. The high prevalence of these efflux pump genes suggests that they contribute substantially to the multidrug-resistant phenotype by promoting active antibiotic efflux. These findings highlight the importance of molecular detection of efflux pump-mediated resistance for improving antimicrobial surveillance, guiding appropriate therapy, and supporting effective infection control strategies.

1 INTRODUCTION

Klebsiella pneumoniae is a Gram-negative, non-motile, encapsulated bacillus [1], and an opportunistic pathogen responsible for a range of infections including pneumonia, urinary tract infections, bacteremia, meningitis, and liver abscesses [2]. It commonly colonizes the human gastrointestinal tract and nasopharynx, as well as animal mucosa and environmental sources such as water and soil [3]. Immunocompromised individuals, newborns, and the elderly are particularly at risk [1], [4]. In recent years, *K. pneumoniae* has drawn significant attention due to its increasing multidrug resistance (MDR), especially to carbapenems antibiotics often used as a last resort [5]. Resistance mechanisms include the production of extended-spectrum β-lactamases (ESBLs) and carbapenemases (e.g., *KPC, VIM, OXA-48, IMP*), along with the acquisition of mobile genetic elements such as integrons and transposons [6].Virulence in *K. pneumoniae* is mediated by several factors including the capsule, lipopolysaccharides, iron acquisition systems, serum resistance, fimbrial adhesins (Type 1 and Type 3 fimbriae), and biofilm formation [7]. Sinusitis infection (inflammation of sinuses) is a most common health problem, also in patients of all age groups usually due to secondary bacterial infection. *K. pneumoniae* has been implicated in these cases, particularly among patients who are immunocompromised or who wait too long to start antibiotics [8]. The bacterium can adapt to the conditions in its local environment through biofilm formation and has multiple virulence factors that promote its dissemination within the host [9]. Efflux pumps activity is one of the critical mechanisms underlying K. pneumoniae antibiotic resistance[10]. Efflux pumps are membrane proteins that actively pump out a wide variety of toxic compounds, such as antibiotics, from within the bacterial cell. Such a phenomenon lowers the intracellular concentration of these agents, reducing their efficacy and allowing the bacteria to survive in presence of antibiotics [11]. The AcrAB-TolC system is one of

the most well-known efflux pump systems in *K. pneumoniae* and belongs to the RND family. This system is important for mediating resistance to a large array of antibiotic classes, including the fluoroquinolones, β-lactams, tetracyclines and chloramphenicol [12]. The genes *acrA* and *acrB* code for major parts of this efflux system, whose overexpression has been strongly correlated with a rising level of resistance in clinical isolates [13]. Other genes, such as *oqxA*, have also been identified as being associated with antibiotic resistance, particularly in clinical strains, in addition to the *mdtK* gene, which belongs to the MATE family and contributes to resistance to some drug compounds through an active transport mechanism. The distribution and expression of efflux pump genes in clinical isolates is crucial for developing effective therapeutic strategies to combat antibiotic resistance [14]. Furthermore, the use of efflux pump inhibitors to enhance antibiotic efficacy and activity is a promising area of research. Therefore, this work was designed to study the excretory pumps in *K. pneumoniae* isolates from sinusitis patients, both phenotypically and molecularly, with particular emphasis on the *mdtK, acrAB*, and *oqxA* genes, which plays an important role in promoting antibiotic resistance.

2 MATERIALS AND METHODS

2.1 Collection of Samples

A total of 203 samples were collected from patients attending the outpatient ENT clinic, along with 20 samples from healthy individuals of different ages at Baquba Teaching Hospital, Diyala Governorate, during the period from August 1 to December 1, 2025. According to ethics book numbered (CEPEC/31) Samples were obtained with the assistance of specialist physicians using sterile cotton swabs. Nasal swabs were placed in transport media and immediately transferred to the laboratory for further processing. Morphological identifications of growth isolates by Gram staining and Colony features. [15]. Phenotypic characterization involved observing colony morphology on selective and differential media, including blood agar, and MacConkey agar. The VITEK 2 compact system was employed to bacteria diagnostics with a high level of precision [16].

2.2 Antibiotic Susceptibility Test

The antibiotic susceptibility of *Klebsiella pneumoniae* isolates was determined using the Kirby–Bauer disk diffusion method according to Cockerill (2010). Bacterial suspensions were prepared and adjusted to match the 0.5 McFarland standard (1.5×10^8 CFU/ml). The standardized inoculum was then spread uniformly onto Mueller–Hinton agar plates using sterile cotton swabs. After allowing the surface to dry for a few minutes, 5–6 antibiotic discs were placed on each plate at appropriate distances. (piperacillin-tazobactam, Amikacin, Gentamicin, Ciprofloxacin, Levofloxacin, Imipenem, Aztreonam, Cefepime, Chloramphenicol, Doxycycline, Tetracycline, Cefotaxime, Amoxicillin_clavulanic acid, The plates were incubated at 37°C for 24 hours, after which the inhibition zones were measured in millimeters and interpreted according to the guidelines of the Clinical and Laboratory Standards Institute (CLSI, 2025).

2.3 Detection of Efflux Pump Activity

The Ethidium bromide-agar cartwheel method was used to detect efflux pump activity in bacterial isolates. (2013). In summary, Trypticase Soy Agar plates with various concentrations of EtBr (0.0–2.5 mg/L) were prepared and used in this experiment. A bacterial suspension of 0.5 McFarland was used to inoculate the plates using a radial (cartwheel) inoculation pattern. Plates were visualized using UV light after 16 hr incubation at 37°C. The lowest EtBr concentration that produced visual fluorescence was noted for each isolate. This indicated that isolates which required higher concentrations of EtBr had increased efflux pump activity.

2.4 Molecular Study

2.4.1 DNA Extraction

Genomic DNA was isolated from bacterial isolates using the boiling method (Ali et al., [17]). The DNA was purified and its purity assessed using a nanodrop spectrometer. Subsequently, the genomic DNA was quantified and stored at -20°C. The polymerase chain reaction (PCR) program included a 5-minute DNA separation step at 94°C, followed by one annealing cycle at 54°C and 58°C for the *acrAB, mdtK* and *oqxA* genes, respectively, and at 59°C for the algD gene for 30 seconds according to the primer design, followed by a final extension at 72°C for 10 minutes in one cycle. PCR products were examined using electrophoresis on a 1.5% agarose gel, stained with ethidium bromide and visualized under ultraviolet light. (Table 1).

Table 1: Primer used in present study.

Gene	Primer Sequence (5' → 3')	Tm (°C)	Product Size (bp)	Reference
mdtK	GCGCTTAACTTCAGCTCA GATGATAAATCCACACCAGAA	43	453	[18]
acrAB	GAAGCAGGAGCTAGCCGAC GATTGAGCCGGTGGTCTGAT	60	145	This study
oqxA	GGTGCTGTTCACGATAGATG GAGACGAGGTTGGTATGGAC	57	144	[19]

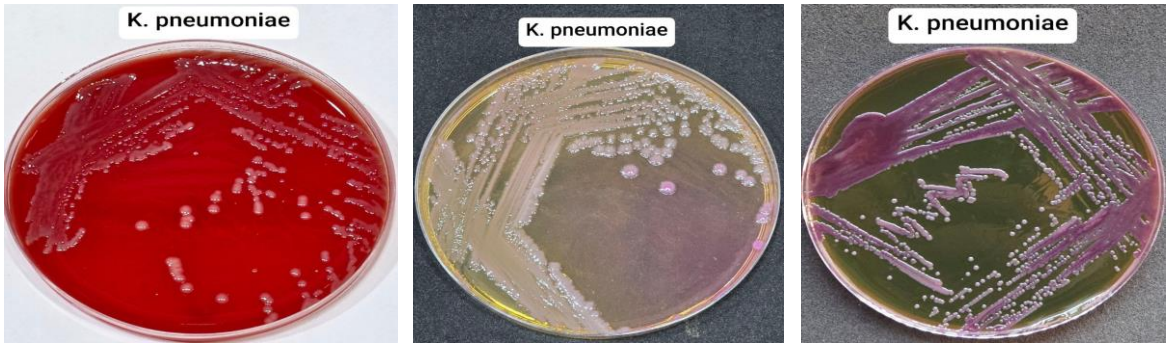

Figure 1: Growth of *K. pneumoniae* after incubation at 37 ◦C for 24 hours *(*A): on Blood agar; (B) on MacConkey agar, and (C): on EMB agar.

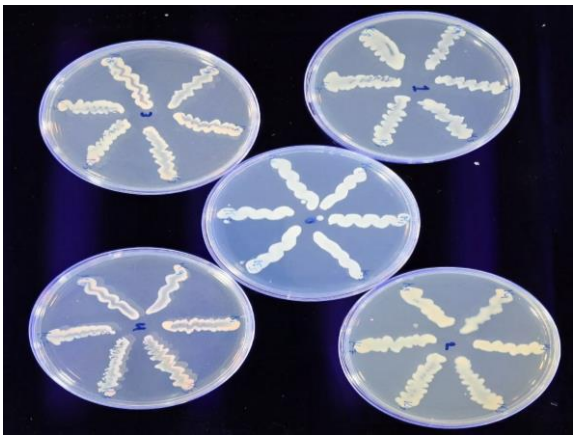

Figure 2: Positive results of *K. pneumoniae* isolates for EtBrCW based method.

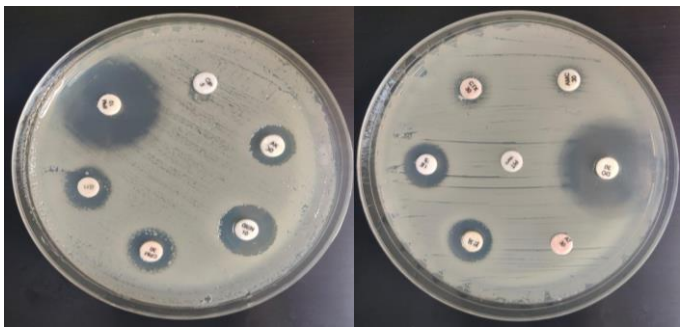

Figure 3: Antibiotic susceptibility pattern of bacterial isolate on Mueller–Hinton agar.

3 RESULTS AND DISCUSSION

3.1 Isolation of K. Pneumonia

Of 203 sinusitis samples collected from Baquba Teaching Hospital and Al-Batoul Hospital in Diyala Governorate, Iraq, 25 samples (12.3%) were found to contain *K. pneumoniae*. Initial identification of the bacteria was based on culture of the samples on Blood agar, and MacConkey agar. This was confirmed using the Vitek 2-compact system [20].

3.2 Morphological and Cultural Identification

For identification of *K. pneumoniae* isolates, conventional morphological characteristics were studied. Colonies of *K. pneumonia* isolates on MacConkey were mucoid, convex, circular organism, and a pink color as a result of the fermentation of lactose sugar. On blood agar, *K. pneumoniae* isolates appear as mucoid, non-hemolytic and grey to white in color. These findings are similar to those obtained by Shaghati and Jassim [21]. On blood agar they were identified as non- hemolytic and greyish-white in color. On MacConkey agar, 25 isolates were considered to be *K. pneumoniae* were they appeared to be lactose-fermented and pink in colour as shown in Figure 1. These findings are similar to Basil *et al.* [22]. The distinctive color of the bacterial colonies was caused by the degradation of synthetic substrates in the medium, which broke down by bacterial enzymes they produce during metabolism.

The positive result of K. pneumoniae isolates on Ethidium Bromide agar (EtBr-CW method) indicates active efflux pump systems. The bacteria were able to expel EtBr outside the cell, resulting in reduced intracellular accumulation and confirming the presence of functional efflux mechanisms associated with antibiotic resistance Figure 2.

3.3 Antibiotic Susceptibility of K. Pneumoniae Isolates

Table 2 shows the relative distribution of susceptibility and resistance test results for 25 *K.pneumoniae* isolates against 13 antibiotics using the disc diffusion assay (Kirby-Bauer technique), as illustrated in Figure 3, in compliance with (CLSI) criteria (2026). The results showed a clear variation in antibiotic response patterns among the studied isolates. The highest resistance rates were recorded against Amikacin (AK), Cefepime (CPM), Tetracycline (TE), and Amoxicillin-clavulanic acid (AMC), with a resistance rate of 44.0% for each. Piperacillin/tazobactam (PIT), Ciprofloxacin (CIP), and Aztreonam (AT) each recorded a resistance rate of 40.0% (Fig. 3). Gentamicin (GEN) had a resistance rate of 36.0%, while Levofloxacin (LE) and Cefotaxime (CTX) each recorded a resistance rate of 32.0%, which are intermediate rates compared to the other antibiotics. In contrast, the isolates showed the lowest rates of resistance to Imipenem (IMP), Chloramphenicol (C), and Doxycycline (DO), at 24.0% each.

Table 2: antibacterial susceptibility test of klebsiella pneumoniae.

Antibiotic	Sensitive, No. (%)	Resistant, No. (%)
PIT	15 (60.0)	10 (40.0)
AK	14 (56.0)	11 (44.0)
GEN	16 (64.0)	9 (36.0)
CIP	15 (60.0)	10 (40.0)
LE	17 (68.0)	8 (32.0)
IMP	19 (76.0)	6 (24.0)
AT	15 (60.0)	10 (40.0)
CPM	14 (56.0)	11 (44.0)
C	19 (76.0)	6 (24.0)
DO	19 (76.0)	6 (24.0)
TE	14 (56.0)	11 (44.0)
CTX	17 (68.0)	8 (32.0)
AMC	14 (56.0)	11 (44.0)

Results also indicate that among the 25 *K. pneumoniae* isolates, only 12 (48%) were Multi-Drug Resistant (MDR) as shown in Figure 4. The escalating resistance to antibiotics for bacteria, particularly *K.pneumoniae*, has resulted in significant challenges in the management of infectious illnesses [23]-[25]. Resistance genes to antibiotics can be disseminated, with integrons playing a crucial part in this process [26]. Where Different isolates were resistant to more than 3 classes of selected antibiotics that resist to at least one type of antibiotics from different classes. These results are compatible with those obtained by [27] and [28], meaning that the treatment options were limited. *K. pneumoniae* play an important role in spreading antibiotic resistance in the environment to clinically important bacteria. The increase of antibiotic resistance is involving many factors such as the use of antibiotics in the hospitals, in community and environment [29].

3.4 PCR Detection of Efflux Pump Genes

PCR amplification confirmed the presence of the *acrAB* , *mdtK*, and *oqxA* genes in 100% of *K. pneumoniae* isolates (25/25); more specifically, acrAB and oqxA were detected in 100% (25/25) of isolates, whereas mdtK was detected in 91.6% (23/25) of isolates. Among the total isolates (n = 25), 12 isolates (48%) were classified as multidrug-resistant (MDR). This result could be related to these efflux pump genes, *acrAB* which actively transports antimicrobial agents out of the bacterial cytoplasm contributing to antibiotic resistance (Fig. 5-7).

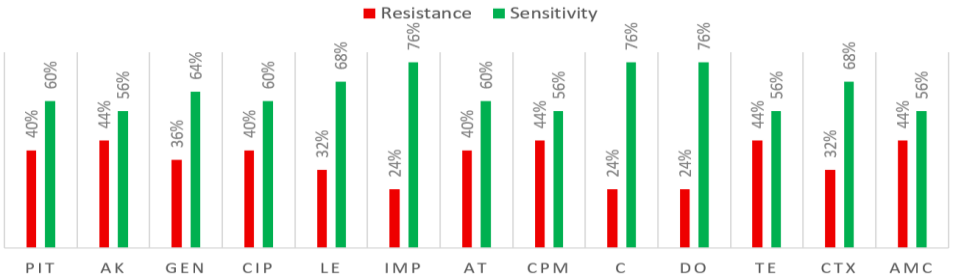

Figure 4: Antibacterial susceptibility test of *Klebsiella pneumoniae.*

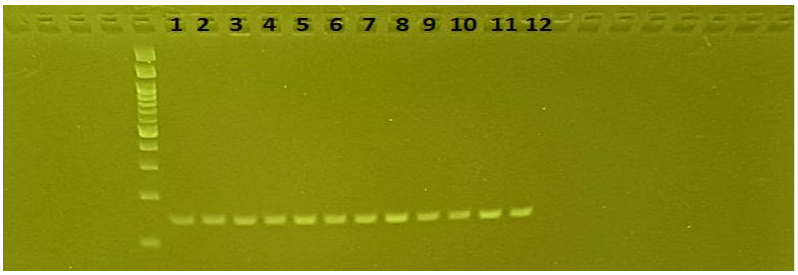

Figure 5: Agarose gel electrophoresis (1.5% agarose) for *acrAB* gene (145 bp amplicon), M lane 100 bp DNA Ladder.

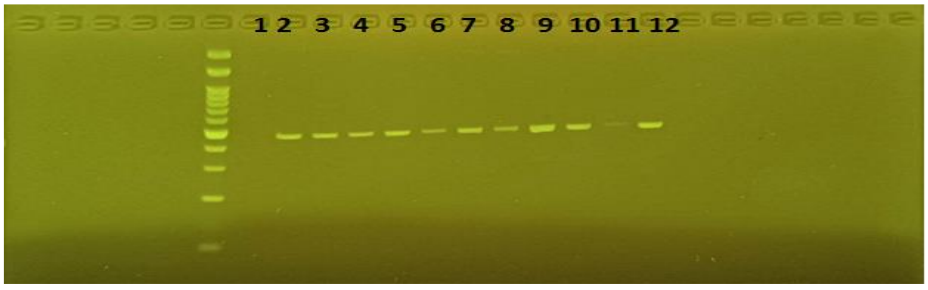

Figure 6: Agarose gel electrophoresis (1.5% agarose,7v/cm^2 for 60 min) for *mdtK* gene (435 bp amplicon), lane 100 bp DNA Ladder.

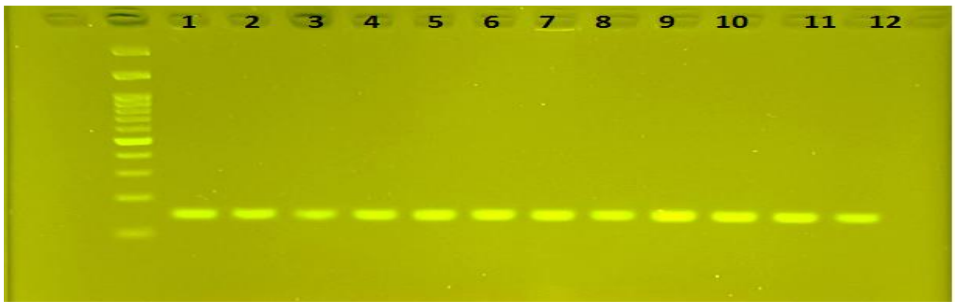

Figure 7: Agarose gel electrophoresis (1.5% agarose) for *oqxA* gene (144 bp amplicon), lane 100 bp DNA Ladder.

The Efflux pump system is one of this disease's most prevalent antibiotic resistance mechanisms.

Numerous investigations conducted across the globe have established the critical role of these pumps in building *K. pneumoniae* strain resistance to several antibiotic families, *AcrAB* and *oqxAB* genes have been extensively studied in the Enterobacteriaceae family, particularly *K. pneumoniae* species, and have been implicated in antibiotic resistance [18], [30]. Efflux pumps, particularly the AcrAB-TolC system, are considered one of the most important mechanisms contributing to antibiotic resistance in Gram-negative bacteria, including K. pneumoniae [31]. These systems actively expel a wide range of antimicrobial agents from the bacterial cell, thereby decreasing susceptibility to multiple antibiotic classes [32]. The coexistence of different efflux pump genes within the same bacterial isolate may enhance the overall resistance level through additive or synergistic effects. The findings of this study are consistent with previous reports that documented a high prevalence of efflux pump genes in *K. pneumoniae*. For instance, a study reported that both *acrAB* and *mdtK* genes were widely distributed among clinical isolates and were significantly associated with MDR phenotypes [33]. Previous studies have shown that deletion of the *acrAB* gene results in increased antibiotic susceptibility, validating its direct function in resistance. Moreover, *acrAB* overexpression is at least partially responsible for resistance to a variety of antibiotics including β-lactams and fluoroquinolones, thus it has clinical significance. This is consistent with the result of the present study indicating that different efflux pump genes could be responsible for the identified MDR phenotype among isolates. The findings of this study are consistent with previous research conducted by [34], in Jordan, which reported high prevalence rates of efflux pump genes, including *acrAB* and *mdtK*, in *K. pneumoniae* isolates, reaching 96.4% and 90.4%, respectively, alongside a high MDR rate of 75.4% . Similarly, another Iraqi study demonstrated a significant correlation between the presence of the acrAB efflux pump gene and antibiotic resistance, as well as biofilm formation, suggesting that these genes contribute not only to resistance but also to bacterial persistence [35]. Despite the fact that the MDR rates in our study (48%) were below those described elsewhere, with variability in sample size, regions targeted, antibiotic susceptibility and sources of infection. This variation shows that environmental and clinical factors influence the distribution of resistance genes.

4 CONCLUSIONS

In summary, the current study showed that *Klebsiella pneumoniae* isolates from sinus infections have a high level (48%) of multidrug resistance and a high prevalence of efflux pump genes (*acrAB, mdtK* and *oqxA*) with (100%, 100%, 91.6) frequency underlying their major role in antibiotic resistance. This sensitivity testing data also shows variability in antibiotic susceptibility patterns. Such findings underline that molecular approaches may be used to identify mechanisms of resistance and, when utilized appropriately, inform better strategies. Explore the expression levels of these genes and their direct correlation with resistance phenotypes in future studies. Further studies could involve efflux pump inhibitors or the use of alternative therapeutic agents like plant derived compounds that can be utilized against multidrug-resistant strains.

ACKNOWLEDGMENTS

The authors would like to express their sincere gratitude to the Department of Biology, College of Education, University of Diyala, for providing the laboratory facilities and scientific support necessary to accomplish this study.

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