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Prevalence of plasmid -mediated quinolone resistance in clinical isolates of *Klebsiella pneumoniae* in Diyala province, Iraq

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Abstract

Klebsiella pneumoniae represents is a prominent opportunistic nosocomial pathogen involved in a wide range of healthcare-associated infections. Plasmid-mediated quinolone resistance (PMQR) has become a significant contributor to antimicrobial resistance, particularly among multidrug-resistant strains. Therefore, the present investigation aimed to characterize the distribution of PMQR- associated genes among extended-spectrum β-lactamase (ESBL)-producing *K. pneumoniae* isolates recovered from clinical specimens in Diyala Province, Iraq.Between October 2024 and February 2025, a total of 145 clinical samples including blood, urine, sputum, burns, and wounds swabs- were collected from hospitalized patients. Twenty isolates of *K. pneumoniae* were identified through standard microbiological procedures. Antimicrobial susceptibility testing and ESBL screening were performed using the disc diffusion method in combination with the combined disk technique. Subsequently, polymerase chain reaction (PCR) assays were conducted to detect ESBL- related genes as well as PMQR determinants among the *K. pneumoniae* isolates. Out of the 145 clinical specimens, 20 isolates of *K. pneumoniae* were identified (13.79 %). The isolates were obtained from blood (15%, 3/20), urine (25%,5/20), sputum (5%,1/20), burn swabs (25%,5/20), and wound specimens (30%,6/20). Antimicrobial sensitivity testing revealed the highest resistance rates to ciprofloxacin (100%) and nalidixic acid (90%). Of the 20 isolates,10(50%) was identified as ESBL producers. Among these ESBL-producing isolates, molecular analysis of PMQR genes showed that the *aac (6')-Ib-cr* was detected in 100% of isolates, whereas *qnrB* was found in 40%. In contrast, *qnrA*, *aac (3')-II*, and *qnrC* were not detected. The findings highlight the substantial contribution of plasmid-mediated quinolone resistance determinants to antimicrobial resistance among ESBL-producing *K. pneumoniae* isolates. These results underscore the necessity for strengthened antimicrobial stewardship programs and enhanced epidemiological

surveillance strategies to mitigate the dissemination of resistant bacterial strains within healthcare environments.

Keywords: *Klebsiella pneumoniae*, quinolones resistance genes, PMQR, ESBLs.

1. Introduction

Klebsiella pneumoniae is widely recognized as a major opportunistic pathogen responsible for infections in healthcare environments. It is frequently implicated in a variety of serious infectionsincluding bloodstream infections, lower respiratory tract infections, urinary tract infections, and wound-associated infections. A substantial proportion of infections caused by this organism originate in hospitals, where they represent an important clinical concern. These infections are often linked to elevated morbidity and mortality rates, particularly in cases involving delayed diagnosis, inappropriate antimicrobial therapy, or the emergence of multidrug-resistant strains. Nosocomial isolates commonly exhibit significant antimicrobial resistance, which complicates therapeutic management and limits available treatment options (Woodford et al.,2007) may also spread into community settings) Seyedpour and Eftekhar ,2014). Excessive antibiotic exposure among hospitalized patients has driven the development of multidrug-resistant *K. pneumoniae*, by this means limiting therapeutic effectiveness and complicating infection controlling. Multidrug-resistant *K. pneumoniae* (MDR-Kp) is commonly defined as isolates exhibiting reduced susceptibility to at least three different classes of antimicrobial agents (Mohd Asri *et al.,* 2021). Recent surveillance reports from Iraq indicate a rising prevalence of MDR-Kp strains, with resistance most frequently observed against carbapenems, aminoglycosides, cephalosporins, and and fluoroquinolones (Hadi *etal.*,2016). A number of serious clinical outcomes have been linked to MDR-Kp infections, such as treatment failure, mortality rates close to 50%, resistance spreading rapidly among Gram-negative bacteria, Infections caused by *K. pneumoniae* may lead to limited treatment options, extended hospitalisation, and significantly increased healthcare costs(Shadkam *etal.*, 2021). Resistance to quinolones commonly results from chromosomal alterations, although plasmid-encoded mechanisms can also contribute to its advance (Poirel *et al.*, 2012; Shadkam *etal.*,2021). Fluoroquinolones have been used as alternatives to conventional antibiotics due to their broad-spectrum bactericidal action and their characteristic bicyclic structure derived from a 4-quinolone core(Ontong *et al.*,2021). Resistance to Fluoroquinolones (FQs) is mediated by several mechanisms, including chromosomal point mutations in the *gyrA* and *gyrB* genes encoding DNA gyrase, as well as mutations in the *parC* and *parE* genes encoding topoisomerase IV within the quinolone resistance-determining regions (QRDRs). Additionally, modifications in outer membrane permeability and the overexpression of efflux pump systems contribute significantly to decrease intracellular drug accumulation and improved resistance(Kareem *et al.,*2021; Zhan *etal.*,2021). But other studies have shown that many *K. pneumoniae* strains exhibit resistance to these antibiotics, largely due to their frequent and uncontrolled use without medical prescription) Padilla *etal.*,2010). Plasmid-mediated quinolone resistance (PMQR) has recently has appeared as an main contributor to fluoroquinolone resistance, with its prevalence increasing worldwide (Kareem *et.al.*,2021).

The major mechanism contributing to resistance to Fluoroquinolone is chromosomal alterations, particularly point mutations occurring within the quinolone resistance-determining regions (QRDRs) of the *gyrA* and *parC* genes. These genetic alterations induce structural changes in the DNA gyrase and topoisomerase IV, that are crucial for bacterial DNA replication. As a consequence, the interaction between fluoroquinolones and their molecular targets is markedly weakened, leading to reduced drug binding efficiency and a subsequent decline in antibacterial effectiveness (Geetha *et.al.*,2020). Plasmid-mediated quinolone re-

sistance (PMQR) also plays a significant role in the development of fluoroquinolone resistance. which has been classified into three main categories. The second mechanism including the action of *aac (6')-Ib-cr*, an aminoglycoside acetyltransferase variant that can enzymatically modify ciprofloxacin and norfloxacin, thereby reducing their antimicrobial action. The third mechanism of fluoroquinolone resistance is mediated by efflux pumps, such as *qepA* and *oqxAB*, which actively expel fluoroquinolones from bacterial cells (Jamshidi *et.al.,* 2019). Extended-spectrum β-lactamases (ESBLs) are enzymes created by certain bacteria that can inactivate multiple β-lactam antibiotics through hydrolysis, which compromises the effectiveness of these agents. ESBLs were initially reported in the literature during the 1980s (Ghafourian *et. al.,*2011). Since then, ESBL-producing *Klebsiella pneumoniae* has become an important clinical challenge, particularly in hospital environments. Continued surveillance and molecular studies have revealed a large diversity of ESBL types, with hundreds of variants described worldwide. Hadi *et.al.*, 2016). Recent studies reveal a worrisome pattern, as the pooled prevalence of ESBL-Kp in Iraq reaches 43.5%, significantly higher than the 32.7% global prevalence reported from clinical samples across various countries) Alshammari & Al-Skhattat ,2015). In light of the rising threat posed by antimicrobial resistance, this study increasingly focused on elucidating the molecular mechanisms responsible for resistance in opportunistic pathogens such as *K. pneumoniae* in Diyala province, Iraq, with particular emphasis on the detection of plasmid-mediated quinolone resistance (PMQR) genes.

2. Materials and Methods

Collection of Specimens

Between October 2024 and February 2025, samples were obtained from Baqubah Teaching Hospital in Diyala city, Iraq. A total of 145 samples were obtained from different clinical sources, comprising blood (n = 25), urine (n = 38), sputum (n = 27), burn swabs (n = 35), and wound swabs (n = 20).

Identification of *Klebsiella* Species

Colonies with morphological characteristics consistent with *Klebsiella* spp. were selected and subjected to standard microbiological identification procedures and confirmed by biochemical tests. Confirmed isolates were maintained in trypticase soy broth (TSB; Merck) supplemented with 20% glycerol and stored at −70°C for subsequent analyses.

Antimicrobial Susceptibility Testing

Antimicrobial susceptibility of the isolates was determined using the agar disk diffusion method on Mueller–Hinton agar (MHA) in accordance with the guidelines of the Clinical and Laboratory Standards Institute (CLSI, 2023). Commercially prepared antibiotic disks (HiMedia Laboratories Pvt. Ltd., Mumbai, India) were employed for testing. The panel of antimicrobial agents included ceftriaxone (30 µg), tetracycline (30 µg), aztreonam (30 µg), gentamicin (10 µg), ciprofloxacin (5 µg), amikacin (30 µg), ceftazidime (30 µg), imipenem (10 µg), cefotaxime (30 µg), piperacillin (100 µg), ofloxacin (5 µg), and nalidixic acid (30 µg). Zone diameters were measured and interpreted according to CLSI (2023) criteria.

Phenotypic evidence of ESBL

ESBLs production was assessed phenotypically employing the combination disc method. In accordance with the Clinical and Laboratory Standards Institute guidelines (CLSI, 2023), ESBL production was screened via combination disc method on Mueller–Hinton agar. The antibiotic discs employed included ceftazidime (30 µg), cefotaxime (30 µg), ceftazidime–clavulanate (30/10 µg), and cefotaxime–clavulanate (30/10 µg). The incubation period for the plates

was 16–18 hours at 37C. To detect ESBL producers, indicator cephalosporins with clavulanate increased the zone diameter by 5 mm compared to indicator cephalosporins alone(Abdeta *etal.*, 2022). The researchers utilized a disc method that incorporates CTX (30 µg) and CAZ (30 µg), with or without clavulanic acid (10 µg). Inhibitory zones were measured and compared by the researchers(Kim *etal.*, 2009). The result was interpreted as positive for ESBL production when the inhibition zone diameter around the clavulanate-containing disc exceeded that of the corresponding cephalosporin disc by ≥ 5 mm.

Plasmid extraction

Plasmid DNA from the bacterial isolates was isolated and purified using a plasmid extraction mini kit (Favorgen Biotech Corp, Taiwan) following the manufacturer's guidelines.

Molecular detection of resistance determinants

Polymerase Chain Reaction (PCR) was used for the detection of target resistance genes. Genomic DNA was extracted from bacterial isolates and amplified using specific primers under standard thermocycling conditions. The PCR products were analyzed by agarose gel electrophoresis and visualized under UV illumination, as previously described by Khalaf et al. (2021). Primers employed in the polymerase chain reaction (PCR) assays are mentioned in Table (1). Amplification reactions were performed using a Techne TC-512 thermocycler (Eppendorf, Hamburg, Germany) in a total reaction volume of (25 µL), as detailed in Table (2). The PCR products were visualized under a UV transilluminator following electrophoresis for 60 min at 100 V on a 1.0% agarose gel prepared in 0.5X TBE buffer (45 mM Tris-borate, 1 mM EDTA, pH 8.0) and stained with a DNA-safe dye.

Table 1. PCR primers applied in this study for target gene amplification

Target Genes	Sequences for primer (5′→3′)	Product size (bp)	Reference
aac (6′)-Ib-cr	F: TTGGAAGCGGGGACGGA	260	(Esmaeel *et.al.*, 2020)
	R: ACACGGCTGGACCATA		
qnrA	F: AGAGGATTTCTCACGCCAGG	571	(Hadi *et.al.*,2016)
	R: GTCAAGATCTGTGCCTGGCA		
qnrB	F: ATGACGCCATTACTGTATAA	408	Alshammari, & Al-Skhattat, (2015)
	R: GATCGCAATGTGTGAAGTTT		
qnrC	F: GCAGAATTCAGGGGTGTGAT	118	(Swedan *et.al.*,2024)
	R: AACTGCTCCAAAAGCTGCTC		
aac (3′)-II	F: ATATCGCGATGCATACGCGG	877	(Swedan *et.al.*,2024)
	R: GACGGCCTCTAACCGGAAGG		
	R: TCAATTCAATTCATCAAGTTT		

Table 2. Optimum Conditions for amplifying qnr Genes using PCR

Gene	Initial denaturation	Cycling condition			Final extension	Cycles
		denaturation	Annealing	Extension		
qnrA	94°C/3min	94°C/40 sec	53°C/40sec	72°C/40sec	72°C/2min	35
qnrB	94°C/3min	94°C/40 sec	53°C/40sec	72°C/40sec	72°C/2min	
aac (6′)-Ib-cr	94°C/3min	94°C/40 sec	53°C/40sec	72°C/40sec	72°C/2min	
aac (3′)-II	94°C/3min	94°C/40 sec	60°C/40sec	72°C/40sec	72°C/2min	
qnrC	94°C/3min	94°C/40 sec	55°C/40sec	72°C/40sec	72°C/2min	

Agarose Gel Electrophoresis

Agarose gel electrophoresis (1% gel in 1× TAE buffer) was performed to analyze the PCR products, with the gel stained using 0.5 µg/mL ethidium bromide. Promega, USA) to allow

visualization and size estimation of DNA bands. Electrophoresis was performed at 100 V for 80 min. The separated PCR products were subsequently imagined under ultraviolet light at 320 nm using a UV transilluminator system (Major Science, Taiwan) (Lee *et. al.*, 2012).

3. Results

Isolation and Identification

Out of 145 clinical specimens, 20 isolates (13.79%) were identified as *K. pneumoniae*. These isolates were recovered from blood, urine, sputum, burn swabs, and wound samples. Preliminary identification of the bacterial isolates was performed based on colony morphology, microscopic characteristics, and standard biochemical assays. Isolates were processed using the VITEK® 2 Compact system (bioMérieux, France) for rapid identification and determination of antimicrobial resistance profiles (17). Twenty *K. pneumoniae* isolates were isolated from different clinical sources like blood (3 isolates, 12%), urine (5 isolates, 20%), sputum (1 isolate, 3.7%), burn infections (5 isolates, 14.28%), and wound samples (6 isolates, 30%). Detection of extended spectrum βeta lactamase-producing *K. pneumoniae* (ESBL-Kp) using the combination disk method revealed that 50% (10 isolates) were ESBL producers.

Resistance to Antibiotics

Antimicrobial susceptibility testing was performed via the agar disc diffusion method (Kirby–Bauer technique), in according to Clinical and Laboratory Standards Institute (CLSI) guidelines (2023). The susceptibility patterns of the isolates against twelve antimicrobial agents are presented in figure 1. The results demonstrated considerable variability in resistance profiles among the tested isolates. All 20 (100%) *K. pneumoniae* isolates were resistant to ciprofloxacin. The observed high resistance to ciprofloxacin is likely associated with the high prevalence of PMQR genes, suggesting a direct relationship between genotypic resistance determinants and phenotypic antimicrobial resistance. *K. pneumoniae* isolates 18 (90%) showed resistance to nalidixic acid. Although meropenem is generally considered as the most effective therapeutic options against *K. pneumoniae*, the current findings indicated a moderate level of resistance, with 50% of the isolates resistant to meropenem. An identical resistance rate (50%) was observed for tetracycline. Furthermore, all *K. pneumoniae* isolates (100%) were categorized as multidrug resistant (MDR), as they exhibited resistance to at least three classes of antibiotics included in this study.

Molecular study

Analysis of PCR revealed the presence of PMQR genes. (Figures 2 and 3) showed that all of the isolates were (100%) positive for *aac (6')-Ib-cr* gene. 40% of the isolates had *qnrB* gene. But none of the tested isolates carried *qnrA, aac (3')-II* and *qnrC* genes. These results are indicative of the occurrence of the investigated PMQR genes among isolates included in this study.

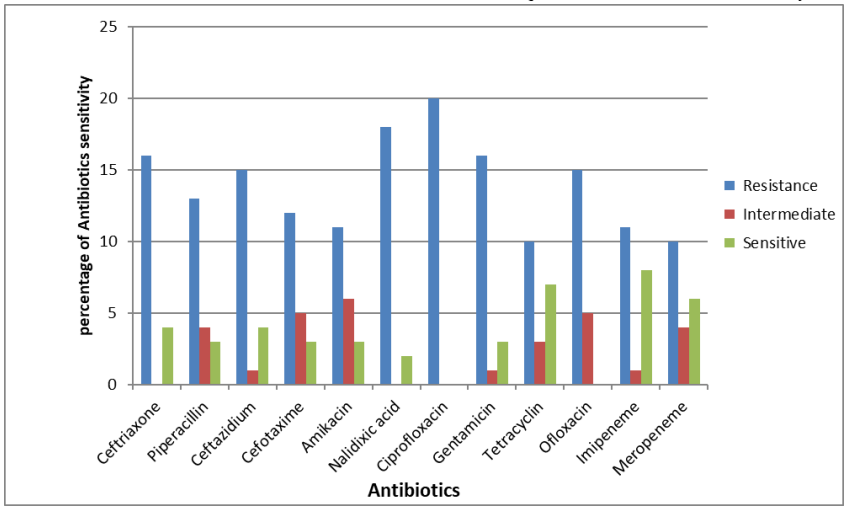

Figure 1. Percentage of Antibiotics sensitivity against *K. pneumoniae* isolates (n= 20)

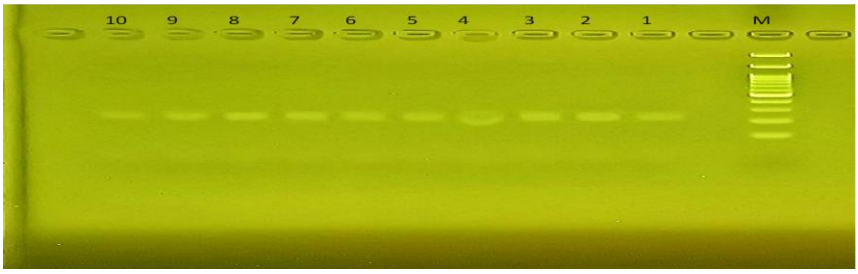

Figure 2. Agarose gel electrophoresis (1%) demonstrating PCR amplification of the *aac (6')-Ib-cr* gene, producing an expected amplicon of 260 bp. Electrophoresis was carried out at 100 V for 80 minutes. Lane M indicates the DNA molecular weight marker (100–1500 bp). Lanes 1–10 correspond to PCR-positive *Klebsiella pneumoniae* isolates.

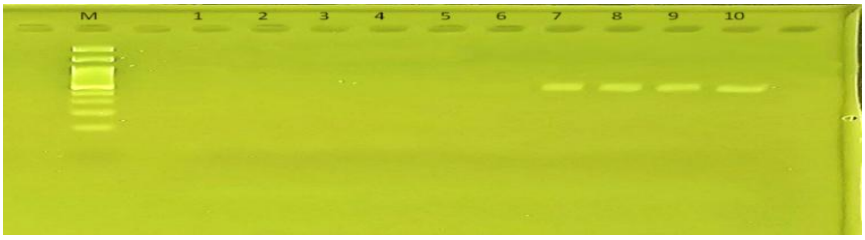

Figure 3. Agarose gel electrophoresis (1%) showing PCR amplification of the *qnrB* gene with an amplicon size of 408 bp. Electrophoresis was performed at 100 V for 80 minutes. Lane M represents the DNA ladder (100–1500 bp), whereas lanes 7, 8, 9, and 10 indicate PCR-positive *K. pneumoniae* isolates.

The molecular analysis revealed that four isolates simultaneously carried both qnrB and aac (6')-Ib-cr genes, whereas six isolates possessed only the aac (6')-Ib-cr determinant. The

distribution pattern of these plasmid-mediated quinolone resistance (PMQR) genes among ESBL-producing K. pneumoniae isolates is summarized in Table 3.

Table 3. Distribution of plasmid-mediated quinolone resistance (PMQR) genes among ESBL-producing *K. pneumoniae* isolates (n = 10).

Isolates ID	ESBLs	qnrA	qnrB	aac (6′)-Ib-cr	aac (3′)-II	qnrC
*Kp*1	+	-	-	+	-	-
*Kp*2	+	-	-	+	-	-
*Kp*3	+	-	-	+	-	-
*Kp*4	+	-	-	+	-	-
*Kp*5	+	-	-	+	-	-
*Kp*6	+	-	-	+	-	-
*Kp*7	+	-	+	+	-	-
*Kp*8	+	-	+	+	-	-
*Kp*9	+	-	+	+	-	-
*Kp*10	+	-	+	+	-	-

The distribution of plasmid-mediated quinolone resistance (PMQR) genes among the studied isolates of *K. pneumoniae*. The results revealed a high prevalence of the *aac (6')-Ib-cr* gene, which was detected in 100% of the isolates. In contrast, the *qnrB* gene was found in 40% of the isolates. However, the genes *qnrA, qnrC,* and *aac (3')-II* were not detected in any of the examined isolates.as show in Table 4.

Table 4. Frequency and percentage of plasmid-mediated quinolone resistance (PMQR) genes detected in clinical isolates of *K. pneumoniae.*

Gene name	Number of isolates	Percentage%
qnrA	0	0%
qnrB	4	40%
aac (6')-Ib-cr	10	100%
aac (3')-II	0	0%
qnrC	0	0%

4. Discussion

Klebsiella pneumoniae infections are frequently managed using β-lactam and quinolone antimicrobial agents, which constitute key therapeutic options for the treatment of infections caused by this opportunistic pathogen. Nevertheless, the indiscriminate prescription, excessive consumption, and improper administration of these antimicrobials in both clinical and community environments have substantially accelerated the evolution of antimicrobial resistant (MDR) bacterial populations. Over the past decade, resistance to β-lactam and quinolone compounds has escalated markedly. This phenomenon can largely be attributed to the acquisition and diversification of multiple resistance determinants, including the enzymatic

hydrolysis of β-lactam antibiotics via β-lactamases, structural modifications in quinolone target enzymes such as DNA gyrase and topoisomerase IV, diminished outer membrane permeability, and the upregulation of multidrug efflux transport systems. The increasing prevalence of resistant *K. pneumoniae* isolates represents a serious global public health concern, particularly in healthcare-associated infections where patients often exhibit compromised immune defenses and are exposed to prolonged antimicrobial regimens. These resistant strains are associated with limited therapeutic options, higher treatment costs, prolonged hospital stays, and increased morbidity and mortality rates. (Hassan *et al.,* 2024). The findings revealed a high prevalence of the *aac* (6′)-Ib-cr gene, which was detected in 100% of the isolates. In contrast, the *qnrB* gene was identified in 40% of the isolates. However, the genes *qnrA, qnrC*, and *aac (3′)-II* were not detected in any of the tested isolates.

The widespread presence of the *aac (6′)-Ib-cr* gene suggests that it may represent the most common plasmid-mediated mechanism of quinolone resistance among the studied isolates. This high prevalence may be attributed to continuous selective pressure resulting from the frequent use of fluoroquinolones in clinical practice, which facilitates the persistence and dissemination of resistant strains. This gene encodes an enzyme capable of modifying certain quinolone antibiotics such as Ciprofloxacin and Norfloxacin, thereby reducing their therapeutic effectiveness and contributing to the development of bacterial resistance. Previous studies have also reported that the presence of this gene is frequently associated with multidrug-resistant (MDR) strains, particularly among clinical isolates obtained from hospital settings (Bakri, 2022).

On the other hand, the *qnrB* gene was detected in 40% of the isolates. Genes of the *qnr* family function by protecting the bacterial target enzymes, *DNA gyrase* and topoisomerase IV, from the inhibitory action of quinolone antibiotics (Bateman et.al., 1998). This protective mechanism reduces bacterial susceptibility to these drugs. The detection of *qnrB* in a considerable proportion of isolates may indicate the circulation of mobile genetic elements, such as plasmids, which facilitate the horizontal transfer of resistance genes among bacterial populations (Yugendran and Harish,2016). The presence of qnrB in a considerable proportion of isolates may also indicate the role of horizontal gene transfer in accelerating the spread of resistance determinants among bacterial populations.

Conversely, the genes *qnrA, qnrC,* and *aac (3′)-II* were not detected in any of the analyzed isolates. This observation may reflect regional variation in resistance gene distribution, which is often influenced by local antimicrobial usage patterns and infection control practices.

This absence may suggest a relatively low prevalence of these resistance determinants in the studied geographic region or within the specific type of clinical samples examined. The distribution of PMQR genes is known to vary across different regions and healthcare environments, often influenced by patterns of antibiotic usage and selective pressure. Some studies have proposed a role for plasmid-mediated quinolone resistance (PMQR) determinants in the emergence and spread of quinolone resistance in clinical Enterobacteriaceae isolates. While mutational changes to the quinolone resistance-determining regions (QRDR) of topoisomerase genes are still considered the dominant method of generating resistance, PMQR can provide a mechanism for sustaining and preserving high-level resistance within bacterial communities) Goudarzi *etal.*,2015). Notably, β-lactamase-producing *K. pneumoniae* isolates have been reported in increasing numbers across many regions of the world A previous study reported that extended spectrum β-lactamase-producing *K. pneumoniae* isolates is a frequently detected pathogen in clinical environments. One such study was performed by Feizabadi *et al.*, (2010) found that 69.7% of *K. pneumoniae* isolates studied produced ESBLs. Our

findings are in accordance with more recent approvals reported by Hoseinzadeh *et al.* (2024) who found among the 68 ESBL-positive isolates, 60 were found to harbor PMQR genes. representing a prevalence percentage of 88.13 % The results here indicate a potential co-selection of ESBLs and PMQR determinants in clinical *Klebsiella pneumoniae* strains) Hoseinzadeh *et.al.*, 2024) .

Among the PMQR determinants investigated, qnrB was the most frequently detected gene within the *qnr* family. Previous studies have been reported that the *qnrB* gene is commonly linked to ciprofloxacin resistance. (Azargun *et al.*, 2019).
The prevalence of qnrB observed in the present study appears greater than that reported in some further countries, like Iran (21.7%), Korea (3.9%), and China (2.5%) (Azargun *et al.,* 2019; Yang *et al.*, 2014). Variations in the frequency of fluoroquinolone use, geographic location, and study period may contribute to variances in the distribution of point mutations affecting DNA gyrase and topoisomerase IV, as well as in the high prevalence of plasmid-mediated quinolone resistance (PMQR) determinants. A related study was conducted in Morocco; it was found that 50% of ESBL-producing *Klebsiella pneumoniae* strains carried *qnr* genes. which is comparable to the coexistence of resistance determinants observed in the current findings (Al-Sehlawi ,2011).

A previous study conducted in Malaysia reported a high prevalence of qnr genes, detected in 65.5% of clinical *K. pneumoniae* isolates(Al-Marzooq *etal.*, 2016). The presence of genes for quinolone resistance (e.g., *qnrA* and *qnrS*) was thought to be an important contributor to fluoroquinolone susceptibility. Additionally, specific clinical conditions such as diabetes mellitus, hypertension, and duration of hospitalization have all been linked with an increased likelihood for developing quinolone-resistant strains) Mashaly *etal.,* 2022). In this study, also similar to Amereh *et al.,* none of the *K. pneumoniae* isolates harbored the *qnrA* gene. (2023). In contrast, the *aac (6')-Ib-cr* gene was highly prevalent and found in 100% of the isolates. Similar results were reported in Najaf, Iraq, where 41.18% of isolates contained *aac* (6′)-Ib-cr-mediated plasmid-mediated quinolone resistance (PMQR) determinants from multidrug-resistant *K. pneumoniae* (Hadi *et.al.*, 2016).

In a study conducted in Poland, several aminoglycoside resistance genes were identified among the tested strains. The *aac* (6′)-Ib gene was detected in 26 isolates (59.2%), followed by *aph* (3′)-Ib in 16 isolates (36.2%), *aac (3″)-Ia* in 7 isolates (15.9%), and ant (2″)-Ia in 2 isolates (4.6%). Among the ESBL-non-producing isolates, four strains (26.7%) and three strains (20%) were found to carry the *aac* (6′)-Ib gene. In addition, the *aph* (3′)-Ib gene was besides detected in a proportion of these isolates, indicating that aminoglycoside resistance determinants may be present irrespective of ESBL production status (Ojdana *et.al.*, 2018). Although the present study was conducted on a relatively limited number of clinical isolates, the findings still provide valuable preliminary revealing the resistance mechanisms and patterns of *Klebsiella pneumoniae* circulating in the study area. Nevertheless, further investigations involving larger sample sizes and multiple healthcare centers are strongly recommended to obtain more comprehensive data and to better recognize the epidemiology and mechanisms of reduced susceptibility to antimicrobials in this important opportunistic pathogen.

5. Conclusion

The results of this study revealed a high prevalence of plasmid-mediated quinolone resistance (PMQR) genes among quinolone-resistant *K. pneumoniae* isolates from Diyala Province, Iraq, particularly among extended-spectrum β-lactamase (ESBL)–producing and multidrug-resistant (MDR) strains. A notably high prevalence of the *aac (6')-Ib-cr* gene (100%) and the *qnrB* gene (40%) was detected. These genes are known to confer quinolone resistance and contribute to the multidrug-resistant phenotype observed among the studied *Klebsiella pneumoniae* isolates.

Ethical approval

Ethical approval for this study was granted by the Ethics Committee of the College of Science, University of Diyala, Iraq (Approval No. 26-ASJ Sci-172). All clinical samples were obtained during routine diagnostic procedures, and patient identities were kept anonymous.

Conflict of interests

The authors declare that they have no conflicts of interest that could have influenced the conduct, analysis, or presentation of the research.

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