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New Recorded of Metallo-β-Lactamase (*blaGIM*) Genes Among *Klebsiella pneumoniae* Clinical Isolates in Al-Najaf, Iraq

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ABSTRACT

Background: *Klebsiella pneumoniae is significant pathogen causing nosocomial and community opportunistic infections. It has become a challenge because many strains are resistant to all β-lactam antibiotics through the production of carbapenemases. Objectives: The study aims to detect the presence of blaGIM, blaVIM, and blaNDM genes encoding metallo-β-lactamases (MBL) in K. pneumoniae isolates using polymerase chain reaction (PCR) analysis.* ***Methodology****: A total of 120 clinical samples were collected, among which 100(83.33%) specimens exhibited bacterial growth of the cases. Thirty-seven (37%) isolates were identified as Klebsiella pneumoniae, and antimicrobial susceptibility testing was conducted using the Vitek-2 system. Phenotypic detection of metallo-β-lactamase (MBL) production was performed using modified carbapenem inactivation method (mCIM). Furthermore, PCR analysis was employed to detect the presence of blaGIM, blaVIM, and blaNDM genes encoding MBL in the K. pneumoniae isolates.* ***Results****: Among the 37 K. pneumoniae isolates, a high level of resistance to multiple antibiotics was observed. (97.3%) of isolates were resistance to ceftriaxone, cefixime, ciprofloxacin, and levofloxacin, (94.6%) for ceftazidime, (81.08%) for trimethoprim/sulfamethoxazole, (89.19%) for tobramycin, (86.5%) for cefepime and gentamicin, (81.08%) for piperacillin/tazobactam, (78.4%) for amikacin, (75.7%) for ertapenem, (72.9%) for minocycline, (70.27%) for meropenem and imipenem Phenotypic detection of MBL using mCIM identified 27(72.97%) isolates as MBL producers. PCR results showed that7(19%) of isolates carried the blaGIM while 19(51.4%) contained the blaVIM gene and (36)97.3% carried the blaNDM gene.* ***Conclusions****: The significant increase in multidrug-resistant (MDR) K. pneumoniae and high resistance to most antibiotics was due to the predominance of non-traditional resistance genes, such as blaGIM blaVIM, in the local environment.*

Keywords: Metallo-β-Lactamase , (*blaGIM*) Genes , and *Klebsiella pneumoniae* .

Article Information

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INTRODUCTION

Klebsiella pneumoniae is a clinically, Gram-negative bacilli belonging to the Enterobacteriaceae family, which is an important opportunistic bacterial pathogen. It is associated with both community- and hospital-acquired infections, including septicemia, respiratory tract infections, urinary tract infections (UTIs), bacteremia, and surgical site infections worldwide[1]. Infections caused by *K. pneumoniae* are associated with elevated rates of morbidity and mortality, particularly among hospitalized patients, due to widespread dissemination and the rapid spread of multidrug-resistant Enterobacteriaceae, especially in *K. pneumoniae*, dependence on carbapenems[2]. Carbapenems are often considered “antibiotics of last resort” to treat resistant Gram-negative pathogens, and the bacterium uses a variety of

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strategies to protect itself against antibiotics, one of which is the production of β-lactamase enzymes[3]. Treatment of diseases caused by *K. pneumoniae* has become a challenge because many strains are resistant to all β-lactam antibiotics. The bacteria may have multiple resistance mechanisms to carbapenems, but the most common is carbapenemase enzyme production[4].

Carbapenemase enzymes represent a complicated health issue worldwide because of their prevalence clinical settings[5]. Additionally, *K.pneumoniae* has genes encoding carbapenemases, such as Ambler class A KPC (*Klebsiella pneumoniae* carbapenemase), NDM-1 (New Delhi Metallo-β-lactamase), Imipenemase (IMP), Verona imipenemase (VIM), and class D oxacillinase-48 (OXA-48)[6]. Furthermore, they undermine last-resort treatments by conferring resistance to carbapenems, which have the broadest spectrum of all β-lactam antibiotics and are increasingly used to treat infections caused by multidrug-resistant Gram-negative bacteria. Consequently, emerging resistance to carbapenems is a major public health concern. Carbapenem-resistant isolates are difficult to control because they spread easily within and between hospitals[7,8].

METHODOLOGY

A. Sample collection and identification

One hundred and twenty samples were collected from different sources (urine, sputum, wound and burn swabs, blood, and body fluids) from various hospitals in Al-Najaf Governorate between September 2025 and December 2025. The specimens were streaked on blood and MacConkey agars and then incubated for 18-24 hours at 37°C. Of these, 100 (83.33%) showed bacterial growth, and 20 (16.67%) showed no growth. Among them, 37 (37%) bacterial isolates were identified as *K. pneumoniae* according to the biochemical tests and VITEC-2 Compact system**.**

B. Antimicrobial Susceptibility Test

The antimicrobial susceptibility and minimal inhibitory concentration (MIC) of *K. pneumoniae* isolates tested according to CLSI (2025), using the VITEK-2 Compact system.

C. Screening of some Metallo B-lactamase (MBL)

The phenotypic detection of carbapenemase production in K. pneumoniae isolates was performed using the modified carbapenem inactivation method (mCIM) according to CLSI guidelines[9,10]. A full loop (10 µl) of pure bacterial growth was suspended in 2 mL of Brain Heart Infusion (BHI) broth, and a 10µg meropenem disk was added. The suspension was incubated for 4 hours at 35±2°C. Subsequently, the disk was transferred onto Mueller-Hinton Agar (MHA) plates previously inoculated with the susceptible indicator strain E. coli ATCC 25922 (adjusted to the 0.5 McFarland standard) and incubated for 18–24 hours. The results were considered positive (carbapenemase-producing) if the zone of inhibition was 6–15 mm or if pinpoint colonies were present within a 16–18 mm zone. A zone of inhibition ≥19 mm was interpreted as a negative result, and carbapenemase was inconclusive in the absence of pinpoint colonies within a 16–18 mm zone[11].

D. Extractions of DNA

Genomic DNA was manually extracted using a modified boiling method described by[12]. DNA purity and concentration were estimated using a spectrophotometer (Thermo Fisher Scientific) at 260/280 nm. The purity values ranged between 1.7 and 1.9.

PCR detection of *blaGIM*, *blaVIM*, and *blaNDM*

Polymerase chain reaction was used to amplify the entire sequence of the *blaGIM*, *blaVIM*, and *blaNDM* genes, with specific primers (Macrogen) Table 1. PCR was performed in a final reaction volume of 25

PCR tube, and the thermocycler conditions for PCR Table 2.

Table 1: The primer sequences of *blaGIM*, *blaVIM*, and *blaNDM* genes.

primer	Sequence	Product size, bp	Reference
NDM /F	GGGCAGTCGCTTCCAACGGT	476	[13]
NDM /R	GTAGTGCTCAGTGTCGGCAT		
VIM /F	GATGGTGTTTGGTCGCATA	390	[14]
VIM /R	CGAATGCGCAGCACCAG		
GIM /F	AGAACCTTGACCGAACGCAG	477	[15]
GIM /R	ACTCATGACTCCTCACGAGG		

Table 2: The conditions of polymerase chain reaction in *K. pneumoniae*.

Primer name	cycle	Initial Denaturation	cycling	Final Extension
NDM	35	95ºC 5 min	95ºC 15 s, 55ºC 20 s, 72ºC 40 sec	72ºC 5min
VIM	35	95ºC 5 min	95ºC 15 s, 55ºC 20 s, 72ºC 40 sec	72ºC 5min
GIM	35	95ºC 5 min	95ºC 15 s, 57ºC 30 s, 72ºC 45 sec	72ºC 5min

RESULTS

During the study period, a total of 120 specimens were collected from various infections and cultured on MacConkey and blood agar. Following an incubation period of 18 to 24 hours, 100 specimens (83.33%) exhibited bacterial growth, while 20 specimens (16.67%) did not show any growth (Figure 1).

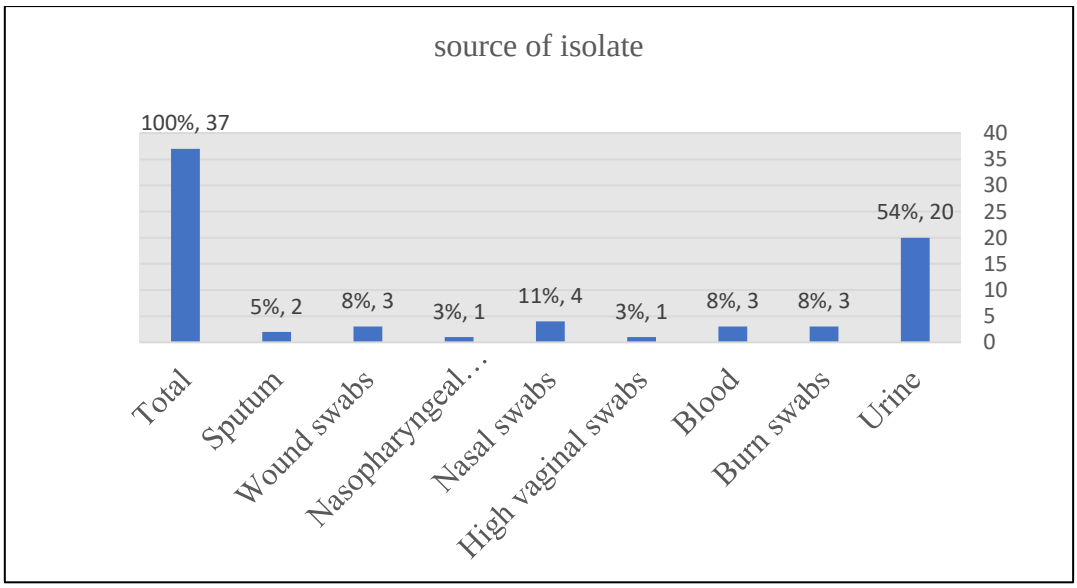

Figure 1: Distribution of *Klebsiella pneumoniae* isolates according to clinical sources.

Antibiotics Susceptibility Test:

The susceptibility of *K. pneumoniae* isolates to common antibiotics used in the treatment of most bacterial infections was tested. The majority of the isolates were MDR, as they exhibited high resistance to more than one of the tested antibiotics. The resistance to β-lactamase was represented by (97.3%) resistance for each of, Ceftriaxone and Cefixime, (94.6%) resistance to ceftazidime, (89.19%) resistance to amoxicillin + clavulanic acid, (86.5%) resistance to cefepime and (81.8%) resistance to piperacillin/tazobactam Figure (2). The high resistance of isolates was attributed to the different methods of resistance, including production of β-lactamases and carbapenemase enzymes[6].

Phenotypic detection of metallo β-lactamases:

Phenotypic detection of MBLs was carried out using the modified carbapenem inactivation method (mCIM). The results showed that (73%) of the isolates were positive (figure-2). The mCIM method is a superior phenotypic screening tool for MBLs compared with traditional disk methods used in earlier regional studies, providing 100% interpretive clarity for the isolate's studies [16,17].

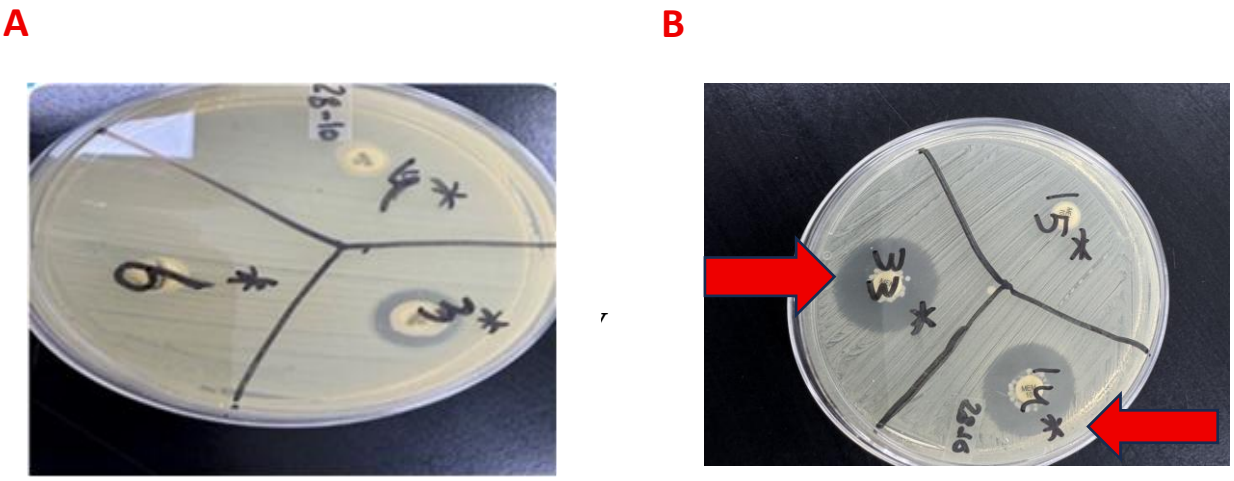

Figure 2: (A) positive mCIM. Carbapenemase production: meropenem zone 6-15 mm, or 16-18 mm, with internal pinpoint colonies (B) negative mCIM Carbapenemase production: the zone of inhibition was ≥19 mm.

***TIM*, and *blaNDM* Genes**

The PCR technique was used to detect the presence of *blaGIM*, *blaVIM*, and *blaNDM* genes among multidrug-resistant *K. pneumoniae* with specific primers. The results showed 7 (19%), 19 (51.4%), and 35 (94.6%), respectively of isolates were carried *blaGIM*, *blaVIM*, and *blaNDM* genes (Figs. 3, 4, and 5).

No previous studies recorded the presence of the *blaGIM* gene in *K. pneumoniae* isolates, but other metallo-β-lactamase genes such as *blaNDM-1* and *blaVIM* genes were more commonly documented in Iraq according to[18,19]. The emergence of the *blaGIM* (19%) gene represents a significant finding in the region.

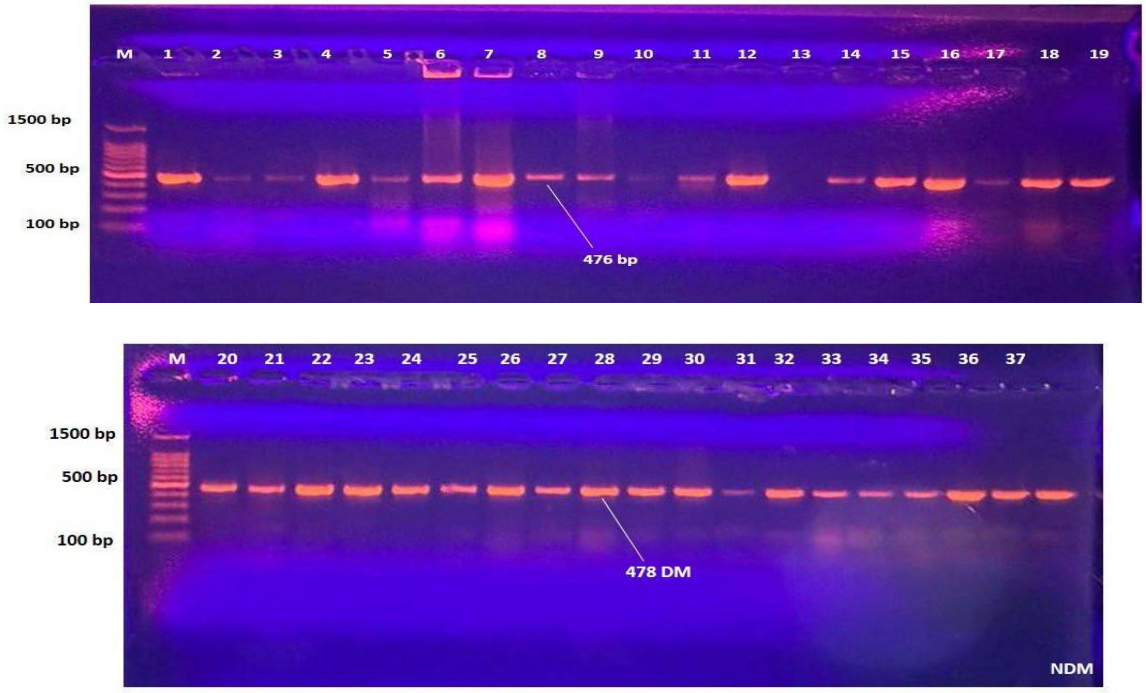

Figure 3: agarose gel with ethidium bromide-stained mono-plex PCR amplified product from extract DNA of *K. pneumoniae* isolates with the *blaNDM* gene, Lane DNA molecular size marker (100 bp ladders), only Lane (10,13) shows a negative result band with (570 bp).

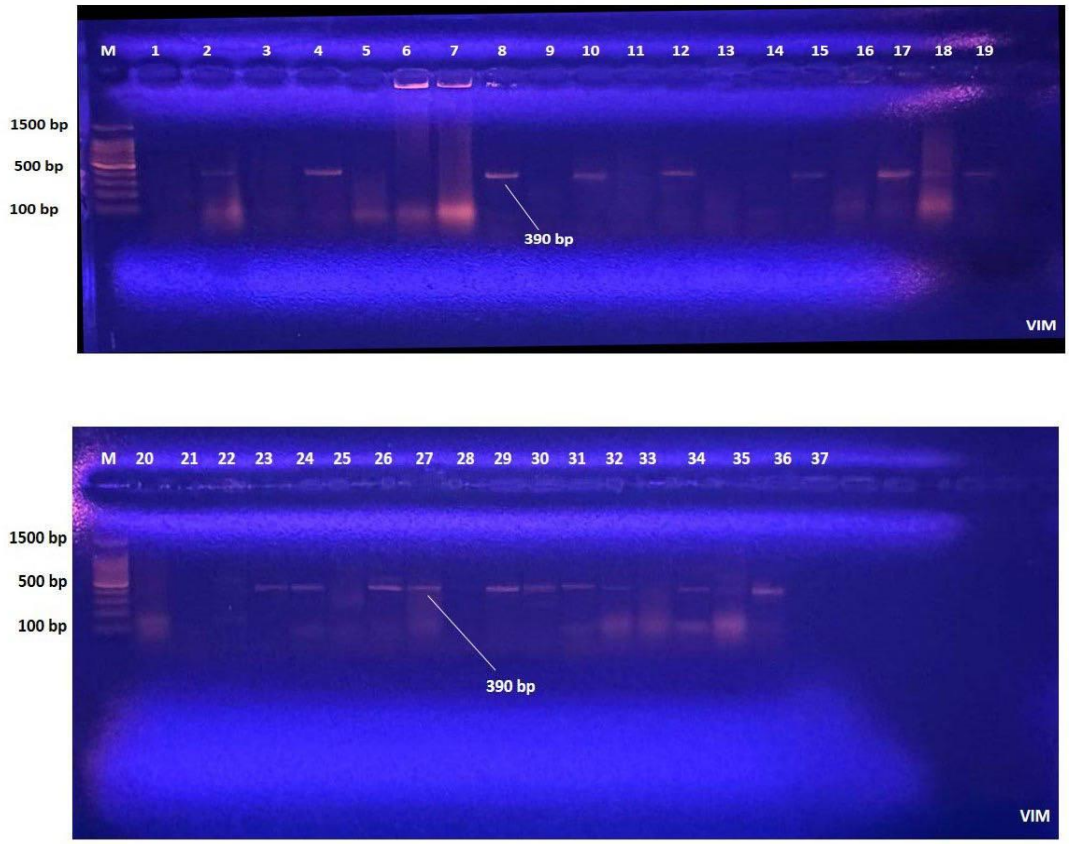

Figure 4: agarose gel with ethidium bromide-stained mono-plex PCR amplified product from extract DNA of *K. pneumoniae* isolates with *blaVIM* gene, Lane DNA molecular size Marker (100 bp ladders), Lane (2,4,8,10,12,15,17,18,19,23,24,26,27,29,30,31,32,34,36) show positive results band with (570bp).

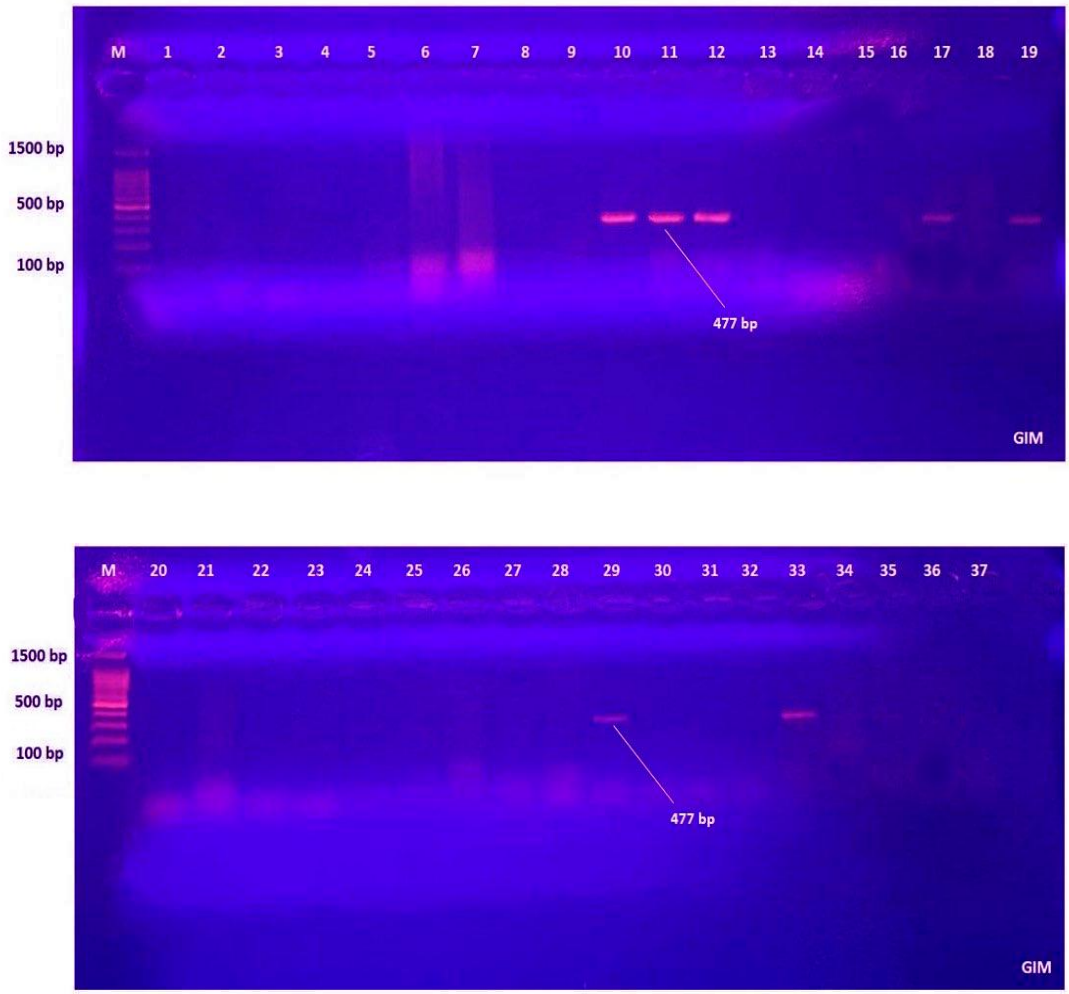

Figure 5: agarose gel with ethidium bromide-stained mono-plex PCR amplified product from extract DNA of *K. pneumoniae* isolates with *blaGIM* gene, Lane DNA molecular size Marker (100 bp ladders), Lane (10,11,12,17,19,29,33) show positive results band with (570bp).

DISCUSSION

Many previous studies recorded that *Klebsiella pneumoniae* was the second agent causing opportunistic infections in most clinical sites[20]. The results of this study underscore a critical epidemiological situation regarding *Klebsiella pneumoniae* in Al-Najaf Province. AMR issues are becoming worse, even with carbapenems, the final line of defense[21]. The study reported that the isolates exhibited extremely high resistance levels, reaching 97.3% against cephalosporins, which indicates a significant contraction of effective therapeutic options in Iraq due to the widespread dissemination of multidrug-resistant (MDR) strains. Molecular analysis identified a significant dominance of metallo-β-lactamase (MBL) genes: the *blaNDM*, *blaGIM*, and *blaVIM* genes. A comparison has been made between the results of this study and similar studies19 at the Baquba Teaching Hospital, which reported that the prevalence of the *blaNDM* and *blaVIM* genes was 92.85% and 47.5%, respectively. This finding aligns closely with our study, which recorded 94.6% and 51.4%[22], identifying *blaNDM* as the dominant genotype and the primary driver of this resistance in Iraq. Other study results align closely with our findings; [23]in Erbil, a prevalence of the *blaVIM* gene was reported at 47.5% but in Pseudomonas aeruginosa. Furthermore, our results are higher than those reported in Thi-Qar[24], where a lower prevalence of 30% was observed.

Additionally[25], a relatively lower prevalence of 17.5% was observed in adjacent Iran, suggesting a rapid evolution in the local genetic resistance landscape and an increase in horizontal gene transfer among isolates in Iran. The most significant finding recorded in this study was the first once identification of the *blaGIM* gene among 19% of *Klebsiella pneumoniae* isolates. According to the documented literature (2021–2026) in the provided review, there has been no recent evidence to support the presence of *blaGIM* in Iraqi *K. pneumoniae* isolates during that period. Thus, our results provide the first molecular identification of this gene in the Al-Najaf clinical setting. This development marks an "epidemiological shift" that necessitates an investigation into the methods currently used for infection control and surveillance. Furthermore, a large diagnostic gap was identified between phenotypic data (72.97%) and genotypic findings (94.6%); this variation supports the existence of "silent" or poorly expressed genes, reinforcing previous research concerns that phenotypic testing is insufficient to determine the actual size of the genetic resistance reservoir. This variation demonstrates that germs that appear to be "susceptible" or "negative" in traditional testing may actually possess a genetic arsenal that can spread and lead to treatment failure[26]. Therefore, it is now essential to incorporate PCR-based methods into Al-Najaf's health surveillance protocols.

CONCLUSIONS

The significant increase in multidrug-resistant (MDR) K. pneumoniae and high resistance to most antibiotics was due to the predominance of non-traditional resistance genes, such as blaGIM blaVIM, in the local environment.

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