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## Prevalence Of Carbapenemase Genes in *Klebsiella Pneumoniae* Isolated from Urine Samples in Iraq

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### Abstract

*Klebsiella pneumoniae* is a leading uropathogen and has become an increasing global threat with the rise of carbapenem-resistant strains. The spread of MBL genes, such as *bla<sub>VIM</sub>* and *bla<sub>NDM</sub>*, also considerably supports multidrug resistance and failure of treatment. The aim of this study was to investigate the frequency distribution of *bla<sub>VIM</sub>* and *bla<sub>NDM</sub>* genes for *K. pneumoniae* strains isolated from urine specimens and their association with resistance to commonly used antibiotics. Methods: 80 *K. pneumoniae* isolates were recovered from urine specimens of patients referred to Al-Sadr Medical City, Iraq during April 2024 –March 2025. Susceptibility patterns to antibiotics (imipenem, meropenem, ceftriaxone, ciprofloxacin and gentamicin) were also tested by the disc diffusion method. The molecular detection of *bla<sub>VIM</sub>* and *bla<sub>NDM</sub>* genes was done by polymerase chain reaction (PCR). Associations of gene presence with antibiotic susceptibility profiles were statistically analyzed. Results: There was a higher resistance rate to ceftriaxone (77.5%), ciprofloxacin (67.5%) imipenem and meropenem (57.5 %; 61.2% respectively) whereas gentamicin showed low resistance (38.7%). The *bla<sub>NDM</sub>* and *bla<sub>VIM</sub>* genes were found in 58.7% and 37.5% of isolates, respectively. The existence of either gene was found to be associated with the resistance to imipenem, meropenem, ceftriaxone and ciprofloxacin ( $p < 0.05$ ). Conclusions: The high occurrence of *bla<sub>VIM</sub>* and *bla<sub>NDM</sub>* in urinary *K. pneumoniae* isolates indicates the increasing menace of carbapenem resistance in Iraq. The high correlation between gene carriage and MDR emphasizes the necessity for ongoing molecular surveillance and stringent antimicrobial stewardship to limit dissemination of these resistant pathogens.

**Keywords:** *bla<sub>VIM</sub>* and *bla<sub>NDM</sub>*, *Klebsiella pneumoniae*, UTIs, CRKP.

### Introduction

Antimicrobial resistance (AMR) represents a major global public health challenge of the 21st century, making antibiotics useless and rendering once treatable infectious diseases difficult to treat (Tesfa et al., 2022). Of Gram-negative bacteria, *K. pneumoniae* has recently been recognized as the most frequently isolated cause of both hospital and community-acquired infections and is an especially concerning organism due to its ability to gain and extend resistant genes (Tesfa et al., 2022). Carbapenems have been regarded as the last-resort  $\beta$ -lactam agents against severe infections induced by

Enterobacteriaceae resistant to drugs, and hence, the emergence and dissemination of carbapenem-resistant *K. pneumoniae* (CRKP) has compromised patient outcomes and imposed significant burden on health care systems worldwide (Lin et al., 2024).

For *K. pneumoniae*, for example, the development of carbapenem resistance is often due to the use of carbapenemase enzymes  $\beta$ -lactamases that hydrolyze carbapenems encoded by mobile genetic elements (plasmids and transposons) which promote their rapid dissemination within/between bacterial populations (Aghamohammad et al., 2023). The most prevalent

carbapenemase classes worldwide are the class A KPC, class B metallo- $\beta$ -lactamases such as NDM and VIM, plus the class D enzymes, which comprise OXA-48 like variants; these genes demonstrate some geotemporal variation in prevalence with certain (e.g., NDM) more common in Asia and others (e.g., KPC) historically predominant on the American continent and parts of Europe (Aghamohammad et al., 2023). The presence of multiple carbapenemase genes on conjugative plasmids exacerbates the risk and results in high level resistance and multi-drug phenotypes, that limit therapeutic alternatives (Aghamohammad et al., 2023).

Urinary tract infections (UTIs) are a common clinical manifestation of *K. pneumoniae* infection in the community and hospital setting, and bloodstream and urosepsis complications of CRKP have high morbidity and mortality (Lee et al., 2023). In LMICs, surveillance has detected increasing carbapenem resistance among *K. pneumoniae*, often due to dissemination of plasmid-acquired carbapenemase genes (Tesfa and Gedif, 2022; Lin et al., 2024). Regional meta-analyses and multicenter studies have reported a rising pooled prevalence of carbapenemase-encoding genes in recent years, indicating temporal spread and regional variability in the frequency of these genes (Sisay et al., 2025; Tesfa et al., 2022).

Iraq, located in an area with high AMR pressures, has revealed increasing rates of carbapenem-resistant *K. pneumoniae* and a changing molecular epidemiology which points out for the dominance of certain carbapenemase genes. Surveillance studies on clinical isolates, particularly those causing urinary tract infections in local settings have shown a variety of carbapenemase genes (*bla*NDM, *bla*OXA-48 (and other OXA type), *bla*VIM, *bla*IMP and less frequently, with less occurrences and distributions in the CPS, also *bla*KPC) often coexist within an isolate (Hamad & Ghaim, 2022; Mohanna & Al-Yasseen, 2024). A case in point is the Baghdad based study was that reported high levels of carbapenem resistance in *K. pneumoniae* isolated from urine samples, with *bla*IMP, *bla*OXA-48 and *bla*VIM being among the most common genes detected (Hamad & Ghaim, 2022). The same as well a high rate of *bla*NDM and OXA gene frequencies were reported among carbapenem resistant isolates by the multicenter study in Najaf where many isolates carried more than one Carbapenemase genes which is an important finding

concerning infection transmission and therapy (Mohanna & Al-Yasseen, 2024).

Notwithstanding the local reports, the situation in Iraq is still unclear: sample sizes, clinical origin, detection techniques (phenotypic versus molecular) and geographical coverage vary among studies making comparisons and national estimations difficult. Furthermore, the highly dynamic nature of the plasmid-borne gene transmission and possibility of entry of a global high-risk international clones require continuous methodologically standardized surveillance (Aghamohammad et al., 2023). A targeted synthesis of *K. pneumoniae* isolated from urine in Iraq is merited due to the burden of UTIs as a major clinical infection, and as isolates from urine may represent community and/or hospital reservoirs of resistant strains.

The current study was conducted to detect *bla*VIM and *bla*NDM genes in the *K. pneumoniae* isolates recovered from urine samples of patients in Iraq. It is also an attempt to estimate a correlation between these genes and antimicrobial resistance profiles.

## Patients and Methods

### Study Design and Setting

This study is a cross-sectional one carried out at Al-Sadr Medical City hospital, Najaf, Iraq on summer of 2024 till the beginning of winter 2025. All 80 patients with presumed UTI by clinical FT were enrolled in the study. The subjects were 32 males and 48 females, ranging in age from 18 to 70 years. Only patients with clinical and microbiological diagnosis of *K. pneumoniae* infection were considered. Patients taking any antibiotics in the last 3-days before urine sampling and with underlying chronic illnesses were excluded from the study.

### Sample collection and bacterial isolation

The mid-stream urine specimens were gathered in sterile containers aseptically and sent directly to the microbiology laboratory at Al-Sadr Medical City for examination. Samples were planted on MacConkey agar (Oxoid Ltd.), and blood agar plates (Oxoid Ltd.) and incubated aerobically at 37 °C for 24 h. Colonies with typical appearance of *K. pneumoniae* were selected and used for standard biochemical tests which include: colony morphology on agar plate, Gram staining, Triple Sugar Iron (TSI), citrate utilization test, indole production test, urease test and motility test (Cheesbrough 2017). The positive isolates were stored in tryptic soy broth

(TSB) with 20% glycerol at  $-80^{\circ}\text{C}$  for further molecular analysis. The quality control *K. pneumoniae* ATCC<sup>®</sup> 13883<sup>™</sup> strain was used for comparison.

### Antimicrobial susceptibility testing (AST)

Antibiotic susceptibility testing was performed using the Kirby–Bauer disc diffusion method on Mueller–Hinton agar (Oxoid Ltd., UK), following the Clinical and Laboratory Standards Institute (CLSI, 2024) guidelines. The following five antibiotics representing different antimicrobial classes were tested:

1. Imipenem (10  $\mu\text{g}$ ) – Bioanalyse, Turkey
2. Meropenem (10  $\mu\text{g}$ ) – Oxoid, UK
3. Ciprofloxacin (5  $\mu\text{g}$ ) – Mast Diagnostics, UK
4. Gentamicin (10  $\mu\text{g}$ ) – Bio-Rad, France
5. Ceftriaxone (30  $\mu\text{g}$ ) – HiMedia Laboratories, India

The diameter of inhibition zones was measured, and isolates were classified as sensitive, intermediate, or resistant according to CLSI criteria. Isolates showing resistance to at least three antibiotic classes were categorized as multidrug-resistant (MDR). *Escherichia coli* ATCC<sup>®</sup> 25922<sup>™</sup> and *K. pneumoniae* ATCC<sup>®</sup> 13883<sup>™</sup> were used as control strains for antibiotic susceptibility testing.

### Detection of carbapenemase genes *bla*<sub>VIM</sub> and *bla*<sub>NDM</sub>

Genomic DNA was extracted from all carbapenem-resistant *K. pneumoniae* isolates using the GeneJET Genomic DNA Purification Kit (Thermo Fisher Scientific, Lithuania) according to the manufacturer's protocol. Detection of carbapenemase-encoding genes *bla*<sub>VIM</sub> and *bla*<sub>NDM</sub> was performed by conventional PCR using gene-specific primers as previously described (Poirel et al., 2011; Nordmann et al., 2012).

Each PCR reaction mixture contained a total volume of 25  $\mu\text{L}$ , including 12.5  $\mu\text{L}$  of 2 $\times$  PCR Master Mix (Promega, USA), 1  $\mu\text{L}$  (10 pmol/ $\mu\text{L}$ ) of each primer, 3  $\mu\text{L}$  of DNA template, and nuclease-free water up to the final volume. Amplification was carried out in a thermal cycler (Eppendorf Mastercycler, Germany) under the following conditions: initial denaturation at  $94^{\circ}\text{C}$  for 5 min, followed by 35 cycles of denaturation at  $94^{\circ}\text{C}$  for 30 s, annealing at  $55^{\circ}\text{C}$  for 40 s, and extension at  $72^{\circ}\text{C}$  for 45 s, with a final elongation at  $72^{\circ}\text{C}$  for 7 min. PCR products were analyzed by agarose gel electrophoresis (1.5%) stained with ethidium bromide (0.5  $\mu\text{g}/\text{mL}$ ) and visualized under UV transillumination using a gel documentation system (Bio-Rad, USA). Positive control strains *K. pneumoniae* ATCC<sup>®</sup> BAA-1705<sup>™</sup> (*bla*<sub>NDM</sub>) and *K. pneumoniae* ATCC<sup>®</sup> BAA-2146<sup>™</sup> (for *bla*<sub>VIM</sub>) were used, while sterile distilled water served as a negative control (table 1).

**Table1: List of primers applied for amplification of carbapenemase genes**

| Gene                            | Amplicon size (bp) | Annealing temp ( $^{\circ}\text{C}$ ) | Primer sequence (5'–3')                         |
|---------------------------------|--------------------|---------------------------------------|-------------------------------------------------|
| <b><i>bla</i><sub>VIM</sub></b> | 390                | 54                                    | F: GTTTGGTCGCATATCGCAAC R: AATGCGCAGCACCAGGATAG |
| <b><i>bla</i><sub>NDM</sub></b> | 621                | 57                                    | F: GGTTCGGCGATCTGGTTTTC R: CGGAATGGCTCATCACGATC |

### Results

The resistance profile of *Klebsiella pneumoniae* isolates revealed a high prevalence of resistance to  $\beta$ -lactam and fluoroquinolone antibiotics, particularly ceftriaxone (77.5%) and ciprofloxacin (67.5%). Carbapenem

resistance was also notable, with 57.5% and 61.2% of isolates resistant to imipenem and meropenem, respectively, indicating the possible dissemination of carbapenemase-producing strains. Gentamicin displayed comparatively better activity, though nearly half of the isolates remained resistant (47.5%) (Table 2).

**Table 2. Rates of antibiotic resistance recorded in the isolates of *Klebsiella pneumoniae***

| Groups        | Resistant Isolates No. (%) | Susceptible Isolates No. (%) |
|---------------|----------------------------|------------------------------|
| Imipenem      | 46 (57.5%)                 | 34 (42.5%)                   |
| Meropenem     | 49 (61.2%)                 | 31 (38.8%)                   |
| Ciprofloxacin | 54 (67.5%)                 | 26 (32.5%)                   |
| Gentamicin    | 38 (47.5%)                 | 42 (52.5%)                   |
| Ceftriaxone   | 62 (77.5%)                 | 18 (22.5%)                   |

Among the carbapenem-resistant *Klebsiella pneumoniae* isolates, the **bla<sub>NDM</sub>** gene was detected in 58.7% of strains, while **bla<sub>VIM</sub>** was identified in 43.5%. The

coexistence of both genes in some isolates may further enhance resistance to  $\beta$ -lactam antibiotics and limit therapeutic options. (Table 2).

**Table 3. Presence of carbapenemase genes in the carbapenem-resistant *Klebsiella pneumoniae* isolates**

| Groups                   | Positive No. (%) | Negative No. (%) |
|--------------------------|------------------|------------------|
| <b>bla<sub>VIM</sub></b> | 20 (43.5%)       | 26 (56.5%)       |
| <b>bla<sub>NDM</sub></b> | 27 (58.7%)       | 19 (41.3%)       |

Table 4 presents the association between antibiotic resistance patterns and the presence **bla<sub>VIM</sub>** and **bla<sub>NDM</sub>** genes among *Klebsiella pneumoniae* isolates. The table includes Chi-square ( $\chi^2$ ) and p-values for each antibiotic tested, indicating the statistical relationship between

gene occurrence and resistance profiles. The results show that isolates carrying **bla<sub>VIM</sub>** or **bla<sub>NDM</sub>** genes were distributed across all antibiotic groups, with variations in resistance frequency and corresponding statistical significance levels.

**Table 4. Association between antibiotic resistance and Presence of carbapenemase genes in the isolates of *Klebsiella pneumoniae***

| Groups        | <b>bla<sub>VIM</sub></b><br>Chi Square (P value) | <b>bla<sub>NDM</sub></b><br>Chi Square (P value) |
|---------------|--------------------------------------------------|--------------------------------------------------|
| Imipenem      | 7.82 (p = 0.005)**                               | 9.63 (p = 0.002)**                               |
| Meropenem     | 6.45 (p = 0.011)*                                | 8.57 (p = 0.004)**                               |
| Ciprofloxacin | 4.16 (p = 0.041)*                                | 6.92 (p = 0.008)**                               |
| Gentamicin    | 2.24 (p = 0.134)                                 | 3.87 (p = 0.049)*                                |
| Ceftriaxone   | 5.75 (p = 0.017)*                                | 7.11 (p = 0.007)**                               |

\* Significant at P<0.05 ; \*\* High significant at P<0.001

## Discussion

These results are consistent with local studies which reported the rise in carbapenem resistance among K.

*pneumoniae* urinary isolates, in Iraq and in other surrounding countries due to dissemination of **bla<sub>VIM</sub>** and **bla<sub>NDM</sub>** (Al-Khafaji et al., 2023 ; Kar et al., 2024). These

data suggest that NDM-type metallo- $\beta$ -lactamases are prevalent among urinary isolates from Iraq, in agreement with recent national and regional reports on the growing spread of bla<sub>NDM</sub>-containing plasmids within clinical environments (Kar et al., 2024; Al-Khafaji et al., 2023). The finding of bla<sub>VIM</sub> also further supports the genetic diversity and contribution of carbapenem resistance determinants into *K. pneumoniae* strains in this context.

Resistance to main classes of antibiotics was high amongst urinary *K. pneumoniae* isolates in the present study conducted in Al-Sadr Medical City, Iraq and a strong correlation was observed between the presence of bla<sub>VIM</sub> and bla<sub>NDM</sub> carbapenemase genes and resistance to many drugs. Resistance to imipenem, meropenem, ceftriaxone and ciprofloxacin was significantly associated with gene carriage, whereas resistance to gentamicin showed a borderline association. The abundance of bla<sub>VIM</sub> and bla<sub>NDM</sub> among the carbapenem-resistant isolates is consistent with recent regional studies reporting on increased emergence of NDM-type metallo- $\beta$ -lactamases in *K. pneumoniae* (Neupane et al., 2025; Tesfa et al., 2022). These results emphasise that, in Iraq and neighbouring countries the molecular epidemiology is evolving over time as NDM producers *K. pneumoniae* are emerging species that predominate over other bacterial types.

Our gene frequency findings are in concordance with previous Iraqi studies. For example, Hamad et al. (2022) detected bla<sub>VIM</sub> in 28.75% and bla<sub>NDM</sub> in 11.25% of urinary *K. pneumoniae* strains in Baghdad. The high prevalence of bla<sub>NDM</sub> in our group supports the hypothesis that NDM carrying plasmids have widely spread over the last years. The pattern was also similar in Najaf where bla<sub>NDM</sub> was found in 70.4% of CRKP while only 11.1% isolates carried VIM (Aboud, 2024). This trend reflects findings from regional meta-analyses, which have highlighted NDM as being often the main carbapenemase gene in the Middle East and North Africa (Alshahrani et al., 2022; Tesfa et al., 2022). From a clinical point of view, the genetic dominance of NDM is important as NDM-positive strains usually co-harbour also other resistance determinants making so complex multidrug resistant phenotype (Lin et al., 2024).

Comparatively, Jafari-Sales et al. (2023) on clinical *K. pneumoniae* isolates in the northwest of Iran and they showed that bla<sub>VIM</sub> as infrequent but bla<sub>NDM</sub> was not

detected at all among carbapenem - resistant strains (Jafari-Sales et al., 2023). The significantly higher prevalence of both genes in our study (43.5% for bla<sub>VIM</sub> and 58.7% for bla<sub>NDM</sub>) indicates that geography, time and source of clinical specimens can dramatically affect the gene circulation. Our emphasis on urinary isolates from a tertiary hospital may in part account for the higher gene prevalence seen when compared with the mixed-site data of Jafari-Sales et al. The contrast highlights both how fast local epidemiology can change, and why circuit-based data is necessary. The high resistance rates of ceftriaxone (77.5%) and ciprofloxacin (67.5%) in our isolates were indicative of the common co-resistances among carbapenemase-producing *K. pneumoniae*. Indeed, plasmids carrying bla<sub>NDM</sub> frequently also carry other genes for resistance to cephalosporins, fluoroquinolones and aminoglycosides (Taha et al., 2020b). In fact, the relevant  $\chi^2$  association found between the existence of carbapenemase genes and resistance to cephalosporins/fluoroquinolones supported the idea of plasmid rainbow expression. In contrast, a relatively low resistance to gentamicin and weak association with gene carriage might suggest that AG resistance is mediated by different mechanisms (e.g., AG modifying enzymes) rather than being directly linked to carbapenemase-encoding plasmids in this setting cohort as well (Taha et al., 2023). Saudi Arabia exhibited comparable results, as with high-level resistance to gentamicin, which showed no consistent relationship with carbapenemase gene presence (Hafiz et al., 2023).

In the same line, a national surveillance report demonstrated that the prevalence of CRKP has been increasing, with NDM genes being predominant (Hussein et al., 2025). Prior research in Iraq also documented a lower imipenem-resistance (e.g., 9.3% in the Najaf burns/urinary survey) (Al-Mustansiriyah study, 2018), and these new data now indicate for an alarming rise particularly with UTI consecutively to our findings presented here (Kar et al., 2024). At the international level, meta-analysis also showed that CRKP prevalence in *K. pneumoniae* is still on the rise worldwide and the Middle East region predominates in NDM propagation (Tesfa et al., 2022; Vaez et al., 2018). The presence of bla<sub>NDM</sub> among a high percentage of our isolates shows that possibility.

Clinically and from a public-health perspective, the predominance of bla<sub>NDM</sub> among urinary *K. pneumoniae*



should rise as very concerning since therapeutic options are restricted (e.g., colistin, tigecycline or novel combinations). Colistin was reported as one of the last choice drugs among others in Iraq and surrounding areas against carbapenemase-producing *K. pneumoniae* (Hamad & Ghaim, 2022). The molecular results also emphasise the importance for regular laboratory screening of carbapenemase genes and inclusion of molecular diagnostics in stewardship programmes. In light of the potential for horizontal gene transfer, particularly in densely populated hospital environments with free cytoplasmic plasmids, prevention and control measures including cohorting, environmental decontamination and antimicrobial stewardship need to be emphasised. Systematic reviews on the region state that the high variety of resistance genes and plasmid types indicates the importance of surveillance tailored to each location and intervention plans (Idrees et al., 2025).

The study has some limitations. It is performed in a single centre and isolates of urinary origin only; bloodstream or respiratory isolates could exhibit different genetic/resistance patterns. Molecular screening was restricted to only blaVIM and blaNDM, thus presence of other carbapenemase (such as: bla<sub>OXA</sub>, or bla<sub>NPC</sub>) could be missed and underestimate the overall burden (Alshahrani et al., 2022). In addition, no whole genome sequencing or plasmid tracking were performed so information on clonal spread or plasmid architecture was limited. However, the results offer important local epidemiological information on urinary CRKP in Iraq and advocated more strict antimicrobial policy.

### Conclusion:

In summary, the results here depict a high prevalence of MDR and an evolving molecular epidemiology, mainly driven by bla<sub>NDM</sub> in urinary *K. pneumoniae* isolates from Iraq. The strong association between gene carriage and antibiotic resistance showed the importance of molecular surveillance, antimicrobial stewardship and infection-control interventions to prevent onward spread of NDM-VP or NDM and VIM strains.

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