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Original Research

Analysis of Clinical *Klebsiella pneumoniae* Isolates for Carbapenemase Genes by Molecular Method

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Abstract

Opportunistic pathogen *Klebsiella pneumoniae* is known to be resistant to many drugs, including carbapenems. Examining the genetic makeup of several carbapenemase-encoding genes in clinical *K. pneumoniae* isolates was the primary objective of this investigation. Tradition
al single-plex polymerase chain reaction (PCR) was employed to identify the *bla*NDM, *bla*OXA-48-like, and *bla*KPC genes in 35 clinical *K. pneumoniae* isolates. The results demonstrated that the isolates examined had an abundance of genes related to carbapenemase. The *bla*OXA-48 gene was detected in all 35 isolates (100%), while *bla*NDM was detected in 33 isolates (94.28%). In contrast, the *bla*KPC gene was not detected in any of the examined isolates (0%). Regarding antimicrobial resistance classification, 23 isolates (65.71%) were multidrug-resistant (MDR), 7 (20%) were extensively drug-resistant (XDR), and 14.28% were classified as non-MDR. The molecular findings demonstrated a predominance of *bla*OXA-48, followed by *bla*NDM, whereas *bla*KPC was absent among the studied isolates.

Keywords: *Klebsiella pneumoniae*; carbapenem resistance; *bla*NDM; *bla*OXA-48; *bla*KPC; Carbapenemase Genes; Antimicrobial Resistance.

Introduction

Klebsiella pneumoniae is a rod-shaped, Gram-negative, facultatively anaerobic bacterium that is known to cause a variety of human infections, such as pneumonia, bacteremia, and UTIs (Abbas et al., 2024; Chang et al., 2021; Effah et al., 2020). The multidrug-resistant phenotype and connection of carbapenem-resistant *Klebsiella pneumoniae* (CRKP) with healthcare-associated infections

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have made it a significant clinical issue in recent years (Chang et al., 2021; Wyres et al., 2020). The production of extended-spectrum β-lactamases and, more significantly, resistance to carbapenems, which is facilitated by carbapenemase enzymes like *K. pneumoniae* carbapenemase (KPC), New Delhi metallo-β-lactamase (NDM), and OXA-48 carbapenemase, are the main factors driving the rising prevalence of multidrug resistance in *Klebsiella pneumoniae* (Ding et al., 2023). An important-priority pathogen according to the World Health Organisation is carbapenem-resistant *Klebsiella pneumoniae* (CRE) (Mohammadpour et al., 2025; World Health Organisation [WHO], 2024). A method for determining the genetic factors linked to carbapenem resistance is the molecular identification of genes that encode carbapenemase. When studying carbapenem-resistant *Klebsiella pneumoniae* isolates molecularly, *bla*KPC, *bla*NDM, and *bla*OXA-48-like should be prioritised. By detecting certain carbapenemase-encoding genes, such as *bla*KPC, *bla*NDM, and *bla*OXA-48-like, this study hoped to examine the molecular features of carbapenem resistance in clinical *K. pneumoniae* isolates.

Materials and Methods

Using conventional microbiological techniques, clinical *K. pneumoniae* isolates were isolated from patient samples. After the isolates were identified, genomic DNA was extracted and tested for concentration and purity with a NanoDrop Lite UV-Visible Spectrophotometer. The carbapenemase-encoding genes *bla*NDM, blaOXA-48, and *bla*KPC were detected using conventional single-plex PCR. The final volume for each reaction was 25 µL. 1.5% agarose gel electrophoresis was used to separate the PCR products, and they were then visible under ultraviolet light.

Results

Molecular Detection of Carbapenemase-Encoding Genes

Among the 35 clinical K. pneumoniae isolates, PCR was conducted to identify three carbapenemase-encoding genes: *bla*NDM, *bla*OXA-48, and *bla*KPC. Of the 35 isolates tested, 34 (94.28%) showed evidence of the *bla*NDM gene, whereas just 2 (5.72%) did not. The blaOXA-48 gene was found in all 35 isolates, indicating a higher prevalence. The PCR amplification bands that were predicted for the target gene were present in all of the isolates. But none of the 35 isolates tested positive for the *bla*KPC gene, meaning that its prevalence was zero percent. Table 1 displays the findings of the molecular analysis, which showed that blaOXA-48 and *bla*NDM were the most common genes, whereas *bla*KPC was not.

Table 1. Distribution of carbapenemase-encoding genes among *K. pneumoniae* isolates

Gene	Positive n (%)	Negative n (%)
blaOXA-48-like	35 (100%)	0 (0%)
blaNDM	33 (94.28%)	2 (5.72%)
blaKPC	0 (0%)	35 (100%)

Antibiotic Resistance Pattern in Relation to Carbapenemase Genes

The isolates of *Klebsiella pneumoniae* that were tested showed varying degrees of sensitivity to carbapenems. Several isolates exhibited resistance to both meropenem and imipenem, but the former demonstrated somewhat superior action. Among the isolates examined, molecular analysis revealed

an abundance of *bla*NDM and *bla*OXA-48. In total, 23 of the isolates (65.71%) were found to be multidrug-resistant (MDR), 7 to be extensively drug-resistant (20%), and 14.28% to be non-MDR. The widespread resistance pattern among the isolates was in line with the high incidence of *bla*NDM and blaOXA-48.

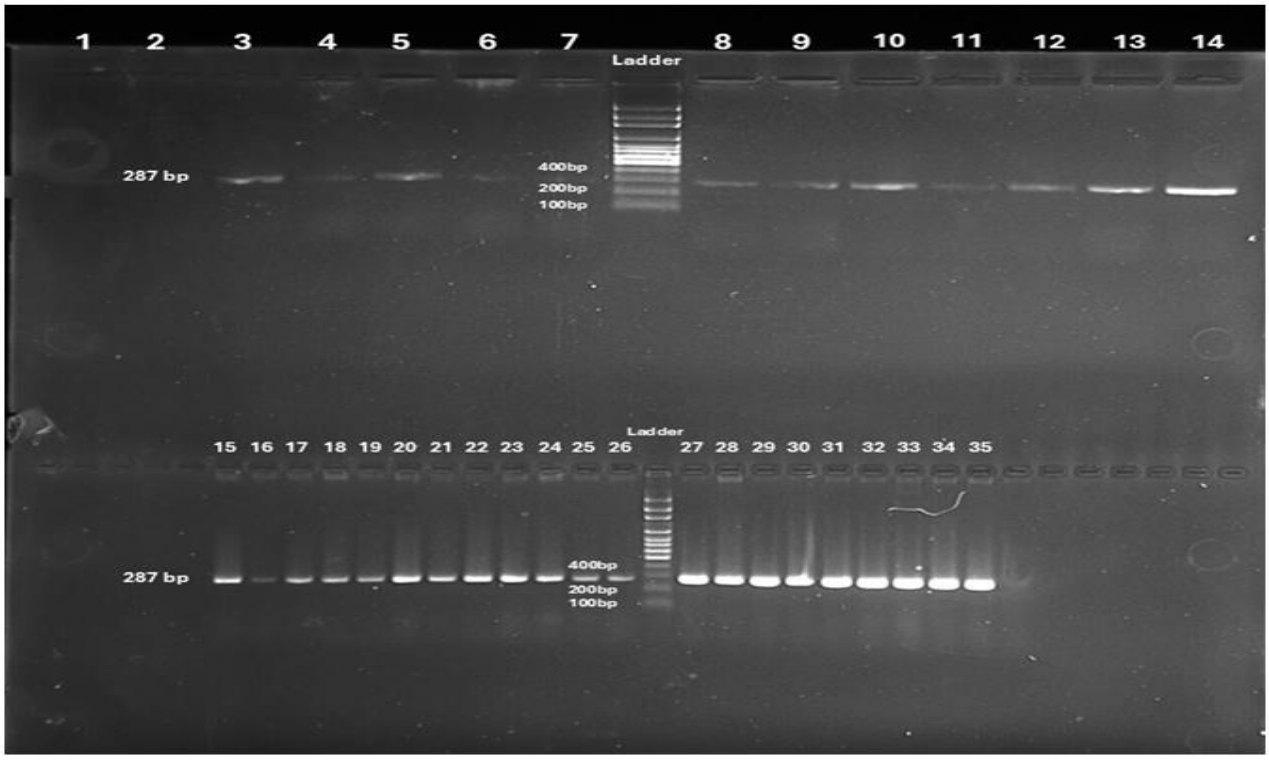

Figure (1): Agarose gel electrophoresis of *blaNDM* gene amplification. M: DNA marker (100 bp ladder); Lanes (1–35): clinical isolates of Klebsiella pneumoniae. Positive isolates showed bands at the expected size of *blaNDM* gene.

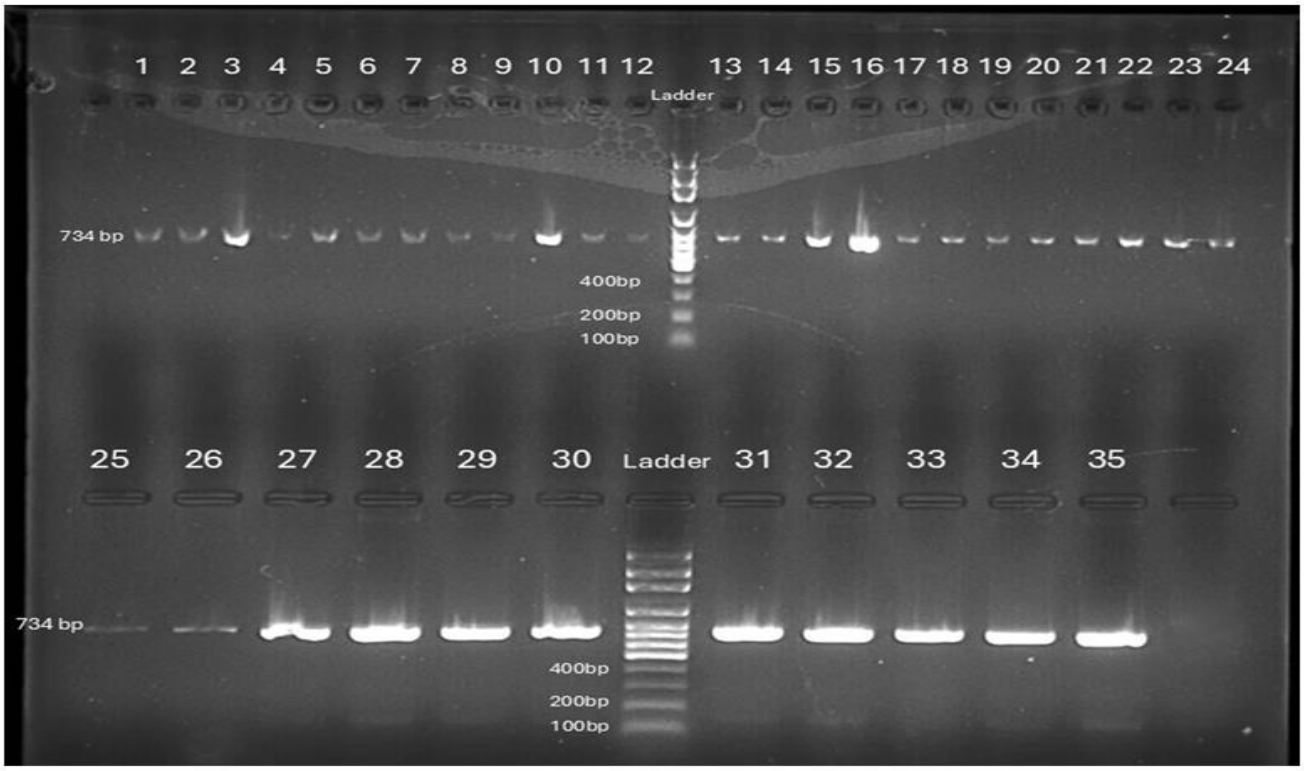

Figure (2): Agarose gel electrophoresis of *blaOXA-48* gene amplification. M: DNA marker (100 bp ladder); Lanes (1–35): clinical isolates. All isolates showed positive bands corresponding to *blaOXA-48* gene.

Discussion

One third (94.28 percent) of the 35 *K. pneumoniae* isolates found in this investigation had the *bla*NDM gene, indicating an extremely high frequency of this gene. This high frequency agrees with the findings of Baral et al. (2024), who also found *bla*NDM to be widely distributed or highly prevalent among clinical or carbapenem-resistant isolates. Both natural selection and the horizontal transfer of plasmids containing this gene could explain the high frequency seen in this study. The *bla*KPC gene, on the other hand, was present in zero percent of the isolates tested. Among the tested isolates, the lack of *bla*KPC indicates that this gene did not serve as the primary carbapenemase mechanism. This result agreed with that of Baral et al. (2024), who found that carbapenemase-producing *K. pneumoniae* had a low frequency of this gene. According to Santajit et al. (2024), all 35 *K. pneumoniae* isolates tested positive for the blaOXA-48 gene, indicating that it is highly prevalent and widely distributed among these bacteria. Plasmid transfers containing blaOXA-48-like genes and the selection pressure caused by heavy usage of beta-lactam and carbapenem antibiotics may explain the whole prevalence seen in this study (Aghamohammad et al., 2023). The molecular findings are further supported by the high incidence of XDR and MDR manifestations. The recent investigation found that 65.71 percent of the isolates were multidrug-resistant, whereas 20 percent were extensively drug-resistant. It is clear from the phenotypic and molecular data that *K. pneumoniae* isolates exhibit a high frequency of carbapenemase-associated resistance. It appears that the carbapenemase profile of the isolates under study was primarily defined by *bla*OXA-48 and *bla*NDM, while *bla*KPC was not detected. Additionally, *bla*OXA-48 was more prevalent than *bla*NDM.

Conclusion

Results showed that among clinical Klebsiella pneumoniae isolates, carbapenemase-encoding genes were very common. All isolates tested positive for the *bla*OXA-48 gene, making it the most common; next, 94.28% of isolates tested positive for the *bla*NDM gene; and finally, no isolates tested positive for the *bla*KPC gene. An essential molecular profile of carbapenem resistance in clinical K. pneumoniae isolates is provided by the preponderance of blaOXA-48 and *bla*NDM, which indicate their considerable presence among the investigated isolates.

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