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# Co-occurrence of Carbapenemase Genes *bla*$_{NDM}$, *bla*$_{VIM}$, *bla*$_{KPC}$, and *bla*$_{OXA-48}$ in *Pseudomonas aeruginosa* Clinical Isolates

Salma Mohamed[1*], Zainab Ahmed[2], Tajalseer Mubarak[2], Sara Mohamed[3], Rayan Mohamed[2], Hassan Higazi[1], Sara Ali[1]

[1]*Department of Medical Laboratory Science, Faculty of Health Sciences, Gulf Medical University, United Arab Emirates*
[2]*Department of Microbiology, Faculty of Medical Laboratory Science, University of Science and Technology, Sudan*
[3]*Deparment of Internal Medicine, Faculty of Medicine, Al Neelain University, Sudan*

## Abstract

*Pseudomonas aeruginosa*, a gram-negative bacterium, is notorious for its innate resistance to many antibiotics. Carbapenems are broad-spectrum antibiotics often used to treat severe *P. aeruginosa* infections. However, the emergence and proliferation of carbapenem-resistant *P. aeruginosa* (CRPA) strains have become a grave global health concern. This study examined the co-occurrence of four major carbapenemase genes, namely *bla*$_{NDM}$, *bla*$_{VIM}$, *bla*$_{KPC}$, and *bla*$_{OXA-48}$, in clinical isolates of *P. aeruginosa*.

Using standard microbiological methods,150 *P. aeruginosa* clinical isolates were collected and identified, and antimicrobial susceptibility testing was conducted following Clinical and Laboratory Standards Institute guidelines. Polymerase chain reaction (PCR) with gene-specific primers was used to detect the presence of carbapenemase genes.

Among the 150 P. aeruginosa clinical isolates, 62(41.3%) were found to be carbapenem-resistant. The most detected carbapenemase genes were *bla*$_{KPC}$ (49%), *bla*$_{NDM}$ (31%), *bla*$_{OXA-48}$ (22%), and *bla*$_{VIM}$ (9%). Notably 13(12.9%) isolates carried two carbapenemase genes. The combination of *bla*$_{KPC}$ and *bla*$_{NDM}$ genes was found in eight isolates, two isolates carried *bla*$_{KPC}$ and *bla*$_{VIM,}$ and three isolates carried *bla*$_{OXA-48}$ and *bla*$_{NDM}$. Four isolates (6.5%) harbored three carbapenemase genes. Co-occurrence of *bla*$_{NDM}$, *bla*$_{VIM}$, *bla*$_{KPC}$, and *bla*$_{OXA-48}$ was observed in four isolates (2.8%).

Our findings highlight the alarming prevalence of carbapenemase genes, particularly *bla*$_{NDM}$ and *bla*$_{KPC}$, in clinical isolates of *P. aeruginosa*. The co-occurrence of multiple carbapenemase genes in the same isolate raises concerns about the potential for horizontal gene transfer and dissemination of multidrug-resistant *P. aeruginosa* strains in clinical settings. Further research is needed to elucidate the molecular mechanisms underlying the co-occurrence of carbapenemase genes and their impact on the clinical outcomes of *P. aeruginosa* infections. Urgent measures, such as enhanced surveillance, infection control protocols, and antibiotic stewardship programs, are imperative to combat the emergence and spread of CRPA strains.(**International Journal of Biomedicine. 2023;13(3):123-126.**)

**Keywords:** *Pseudomonas aeruginosa* • carbapenemase • *bla*$_{NDM}$ • *bla*$_{VIM}$ • *bla*$_{KPC}$ • *bla*$_{OXA-48}$ • clinical isolates

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## Introduction

*Pseudomonas aeruginosa* is a gram-negative opportunistic pathogen that commonly causes nosocomial infections, particularly in immunocompromised patients and those with chronic illnesses.(1) *P. aeruginosa* infections are often difficult to treat due to its intrinsic resistance to many antibiotics, and carbapenems are considered as the last-resort antibiotics for treating severe *P. aeruginosa* infections. However, the emergence and spread of CRPA strains have become a global health threat, limiting treatment options, and posing challenges in infection control practices.(2

Carbapenem resistance in *P. aeruginosa* is often mediated by the acquisition of carbapenemase genes, which encode enzymes that can hydrolyze carbapenem antibiotics. Several types of carbapenemase genes have been identified in

*P. aeruginosa*, including $bla_{NDM}$, $bla_{VIM}$, $bla_{KPC}$, and $bla_{OXA-48}$, which are commonly found in other gram-negative bacteria as well.[3] These carbapenemase genes are often carried on mobile genetic elements, such as plasmids and integrons, which can facilitate their horizontal transfer among bacteria, leading to the dissemination of carbapenem resistance in clinical settings.[4]

While the presence of individual carbapenemase genes in *P. aeruginosa* has been widely reported, there are limited studies investigating the co-occurrence of multiple carbapenemase genes in the same isolate.[5] The co-occurrence of carbapenemase genes may have important clinical implications, as it can potentially lead to increased antibiotic resistance, treatment failures, and higher mortality rates. Therefore, understanding the prevalence and characteristics of co-existing carbapenemase genes in *P. aeruginosa* clinical isolates is crucial for the effective management of CRPA infections.[6,7]

In this study, we aimed to investigate the co-occurrence of four major carbapenemase genes, namely $bla_{NDM,}$ $bla_{VIM}$, $bla_{KPC}$, and $bla_{OXA-48}$, in *P. aeruginosa* clinical isolates collected from different hospitals.

## Materials and Methods

Bacterial isolates

A total of 150 *P. aeruginosa* clinical isolates were collected from different hospitals in diverse geographical regions in Khartoum state. The isolates were obtained from various clinical specimens, including blood, respiratory samples, urine, and wound swabs (Table 1). The isolates were identified as *P. aeruginosa* using standard microbiological methods.

***Table 1.***

***Samples distribution***

| Sample | Number | Prevalence (%) |
|---|---|---|
| Urine | 30 | 20 |
| Sputum | 30 | 20 |
| Blood | 10 | 6.7 |
| Wound swab | 80 | 53.3 |

Antimicrobial susceptibility testing

The antimicrobial susceptibility of *P. aeruginosa* isolates was determined by the disc diffusion method according to the Clinical and Laboratory Standards Institute (CLSI) guidelines.[8] The antibiotics evaluated included imipenem and meropenem. The interpretation of susceptibility was based on the CLSI breakpoints.

Detection of carbapenemase genes by real-time PCR

Genomic DNA was extracted from *P. aeruginosa* isolates using a commercial DNA extraction kit following the manufacturer's instructions.[9] The presence of carbapenemase genes, including $bla_{NDM}$, $bla_{VIM}$, $bla_{KPC}$, and $bla_{OXA-48}$, was detected by PCR using gene-specific primers (Table 2).[10-12] The real-time PCR amplification was performed in a thermal cycler under the following conditions: The test reaction was set to a total volume of 25 µl. 5 µl master mix, 1 µl of forward and reverse primers, the amount of Eva green dye used in the reaction varied per the amplicon size; for $bla_{KPC}$ a volume of 2 µl of the Eva green was used, 1.5 µl for $bla_{OXA-48}$ and $bla_{VIM}$ and 1 µl Eva green was used for $bla_{NDM}$ as it was the smallest amplicon size. 0.3 µl of the DNA template was used and the rest of the reaction's volume was double distilled water.

Using the SaCycler-96 system (Sacacae Biotechnologies), real-time PCR protocol was carried out as follows. Hot start 94C° for 10 min, the number of cycles 40, and the loop steps were as follows: denaturation at 94C° for 45 seconds, annealing at 52C° for 45 seconds and elongation at 72C° for 30 seconds, which included the fluorescent data at acquisition 533 nm filter, a final elongation step was set to be at 72C° for 10 min. After the end of the polymerization cycles, the melting curve step was added to start from 65C° gradually increasing by 0.1 C/s to 95C, with the fluorescence data acquisition every 1 second.

## Results

Among the 150 P. aeruginosa clinical isolates, 62(41.3%) were found to be carbapenem-resistant. The most detected carbapenemase genes were $bla_{KPC}$ (49%), $bla_{NDM}$ (31%), $bla_{OXA-48}$ (22%), and $bla_{VIM}$ (9%) (Figure 1). Notably 13(12.9%) isolates carried two carbapenemase genes. The combination of $bla_{KPC}$ and $bla_{NDM}$ genes was found in eight isolates, two isolates carried $bla_{KPC}$ and $bla_{VIM,}$ and three isolates carried $bla_{OXA-48}$ and $bla_{NDM}$. Four isolates (6.5%) harbored three carbapenem

***Table 2.***

***Details of primers used, including sequence, annealing temperature, and product size.***

| Gene | Primer | Sequence | GC% | Tm C° | M.W µg/µmol | Final Con. µM | Amp size bp |
|---|---|---|---|---|---|---|---|
| $bla_{OXA-48}$ | OXA-48-F | 5'-GCGTGGTTAAGGATGAACAC-3' | 42.1 | 50.8 | 5855.9 | 0.2 | 177 |
| | OXA-48-R | 5'-CATCAAGTTCAACCCAACCG-3' | 42.1 | 50.8 | 5865.5 | 0.2 | |
| $bla_{KPC}$ | KPC-Fm | 5'-CGTCTAGTTCTGCTGTCTTG-3' | 50 | 52.6 | 5492.6 | 0.2 | 785 |
| | KPC-Rm | 5'-CTTGTCATCCTTGTTAGGCG-3' | 58.3 | 63 | 7224.7 | 0.2 | |
| $bla_{VIM}$ | VIM-F | 5'-GATGGTGTTTGGTCGCATA-3' | 50.0 | 55.4 | 6024.0 | 0.2 | 382 |
| | VIM-R | 5'-CGAATGCGCAGCACCAG-3' | 65.0 | 57.4 | 6160.1 | 0.2 | |
| $bla_{NDM}$ | NDM-F | 5'-GGTTTGGCGATCTGGTTTTC-3' | 55.6 | 54.9 | 5463.6 | 0.2 | 82 |
| | NDM-R | 5'-CGGAATGGCTCATCACGATC-3' | 47.6 | 55.6 | 5463.6 | 0.2 | |

resistance genes. Co-occurrence of $bla_{NDM}$, $bla_{VIM}$, $bla_{KPC}$, and $bla_{OXA-48}$ was observed in four isolates (2.8%) (Table 3).

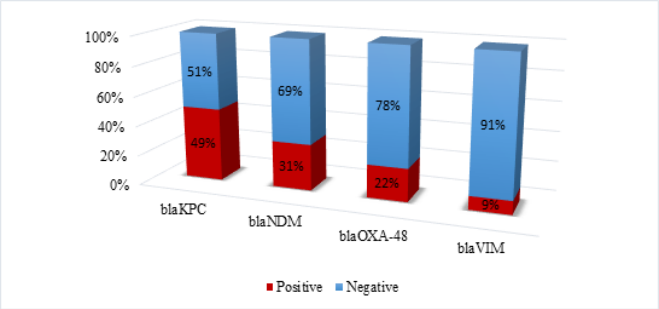


***Fig. 1.*** *Percentage of positive test results for Carbapenemase genes in selected samples: $bla_{KPC}$, $bla_{NDM}$, $bla_{OXA-48}$, and $bla_{VIM}$.*

***Table 3.***

***Carbapenemase genes prevalence: comparative analysis of isolate data***

| Carbapenemase Gene | Number of isolates | Prevalence (%) |
|---|---|---|
| $bla_{NDM}$ | 28 | 31 |
| $bla_{VIM}$ | 22 | 9 |
| $bla_{KPC}$ | 12 | 49 |
| $bla_{OXA-48}$ | 18 | 22 |
| Gene Combinations | | |
| $bla_{KPC}$ + $bla_{VIM}$ | 2 | 7 |
| $bla_{NDM}$ + $bla_{KPC}$ | 8 | 26 |
| $bla_{NDM}$ + $bla_{OXA-48}$ | 3 | 1 |

## Discussion

The study results revealed that carbapenem resistance among Pseudomonas aeruginosa clinical isolates was high, with 41.3% (62 out of 150) being resistant to carbapenems. This finding is concerning as carbapenems are often considered as the last-resort antibiotics for treating serious infections caused by multidrug-resistant bacteria.

Further analysis of the carbapenemase genes showed an interesting trend of $bla_{KPC}$ being the most detected gene, and $bla_{VIM}$ being the least detected gene. The $bla_{KPC}$ gene is commonly associated with resistance to carbapenem that is found in Enterobacteriaceae, such as *Klebsiella pneumoniae* and *Escherichia coli*.[13,14] However, it is less commonly associated with *Pseudomonas aeruginosa*, which are known to have intrinsic resistance to carbapenems due to their efflux pumps and impermeable outer membrane. Therefore, when $bla_{KPC}$ is being frequently detected in *Pseudomonas aeruginosa*, it indicates a concerning trend of horizontal gene transfer or acquisition of this resistance gene from other bacteria.[15,16]

On the other hand, $bla_{VIM}$ is a class B metallo-β-lactamase gene that confers resistance to carbapenems and is more commonly found in *Pseudomonas* species. However, the fact that it is being detected less frequently could be for several reasons, such as geographical variations in the prevalence of $bla_{VIM}$-positive *Pseudomonas* strains.

Interestingly, the study also identified isolates that carried more than one carbapenem resistance gene. Approximately 12.9% of the isolates (13 out of 101) carried two carbapenem resistance genes. The most common combination was $bla_{KPC}$ and $bla_{NDM}$, which was found in eight isolates. Other combinations included $bla_{KPC}$ and $bla_{VIM}$, as well as $bla_{OXA-48}$ and $bla_{NDM}$, which were present in 2 and 3 isolates, respectively. Additionally, 6.5% of the isolates (4 out of 62) harbored three carbapenem resistance genes. Notably, four isolates (2.8%) were found to co-exist with all four carbapenemase genes, namely $bla_{KPC}$ and $bla_{VIM}$, as well as $bla_{OXA-48}$ and $bla_{NDM}$.

The co-occurrence of resistance genes in *Pseudomonas* strains is concerning as it indicates the presence of multiple mechanisms of carbapenem resistance in the same bacterial isolate and raises concerns about the potential for horizontal gene transfer, which can contribute to the rapid spread of carbapenem resistance among bacterial populations, making treatment options more limited and increasing the risk of treatment failure.

The findings of this study have important implications for clinical practice and highlight the need for continuous surveillance of carbapenem resistance in *P. aeruginosa* isolates. Understanding the prevalence and diversity of carbapenemase genes in *P. aeruginosa* can aid in the development of appropriate treatment strategies and infection control measures to mitigate the spread of carbapenem resistance in healthcare settings. Further research is warranted to better understand the mechanisms underlying the emergence and dissemination of carbapenem resistance in *P. aeruginosa*, and to explore potential strategies to combat this growing threat to public health.

**In conclusion**, it is important to note that antibiotic resistance is a complex and dynamic phenomenon, and continuous monitoring and surveillance are crucial in understanding the trends and patterns of resistance genes in bacterial populations. This information can help guide antibiotic stewardship efforts and infection control strategies to mitigate the spread of antibiotic-resistant bacteria.

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## Competing Interests

The authors declare that they have no competing interests.

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***Corresponding author:** *Dr. Salma Elnour Rahma Mohamed, Ph.D. Department of Medical Laboratory Science, Faculty of Health Sciences, Gulf Medical University, Ajman, United Arab Emirates. E-mail: dr.sara@gmu.ac.ae*