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Comparative Evaluation of Phenotypic Methods (sCIM and Carba NP) and PCR for Detection of Carbapenemase-Producing Gram-Negative Bacteria

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Abstract

Background:
Carbapenem-resistant Gram-negative bacteria have emerged as a major global health concern due to their association with limited treatment options and increased morbidity and mortality. Resistance is commonly mediated by carbapenemase enzymes such as KPC, NDM, VIM, IMP, and OXA-48, which hydrolyze carbapenem antibiotics. Rapid detection of carbapenemase-producing organisms (CPOs) is essential for timely clinical decision-making and infection control. This study aimed to evaluate and compare the diagnostic performance of phenotypic methods—Simplified Carbapenem Inactivation Method (sCIM) and Carba NP test—with polymerase chain reaction (PCR) for the detection of carbapenemase-producing Gram-negative bacteria.

Materials and Methods: This prospective laboratory-based comparative study was conducted in the Department of Microbiology of a tertiary care teaching hospital from October 2025 to February 2026. A total of 100 Gram-negative bacterial isolates showing reduced susceptibility to carbapenems were included. Clinical specimens included urine, blood, pus, respiratory samples, and wound swabs. Phenotypic detection of carbapenemase production was performed using sCIM and Carba NP tests. PCR was used as the reference method for detection of carbapenemase genes including blaNDM, blaKPC, blaVIM, blaIMP, and blaOXA-48. Diagnostic performance parameters including sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were calculated.

Results: PCR detected carbapenemase genes in 65% of isolates. Among these, blaNDM was the most frequently detected gene (32%), followed by blaOXA-48 (18%), blaVIM (9%), and blaKPC (6%). The sCIM test demonstrated a sensitivity of 92.3% and specificity of 91.4%, with a positive predictive value of 95.2% and negative predictive value of 86.5%. The Carba NP test showed a sensitivity of 86.2% and specificity of 97.1%, with a positive predictive value of 98.2% and negative predictive value of 79.1%. The sCIM test exhibited higher sensitivity, while the Carba NP test showed higher specificity when compared with PCR.

Conclusion: Both sCIM and Carba NP tests demonstrated good diagnostic performance for detecting carbapenemase-producing Gram-negative bacteria and showed strong agreement with PCR results. The sCIM method provides higher sensitivity and is suitable as an effective screening

ISSN: 0975-3583,0976-2833 VOL 17, ISSUE 3, 2026

tool, whereas the Carba NP test offers rapid results with high specificity. PCR remains the gold standard for confirmatory detection of carbapenemase genes. A combined diagnostic approach involving phenotypic screening followed by PCR confirmation can facilitate rapid detection and improve clinical management of carbapenem-resistant infections.

Keywords: Carbapenem resistance, carbapenemase, sCIM, Carba NP test, PCR, Gram-negative bacteria, antimicrobial resistance.

Introduction

Antimicrobial resistance has emerged as one of the most serious global health threats, compromising the effectiveness of many life-saving antibiotics. Among resistant pathogens, carbapenem-resistant Gram-negative bacteria, particularly members of the Enterobacterales, represent a major challenge for healthcare systems worldwide. Carbapenems are often considered antibiotics of last resort for the treatment of severe infections caused by multidrug-resistant organisms. However, the increasing emergence of carbapenem-resistant bacteria has significantly limited therapeutic options and has been associated with increased morbidity, mortality, and healthcare costs [1].

Carbapenem resistance in Gram-negative bacteria is primarily mediated by the production of carbapenemase enzymes, which are β-lactamases capable of hydrolyzing carbapenem antibiotics. These enzymes are frequently encoded by transferable genes that facilitate rapid dissemination among bacterial populations. The most clinically significant carbapenemases include Klebsiella pneumoniae carbapenemase (KPC), New Delhi metallo-β-lactamase (NDM), Verona integron-encoded metallo-β-lactamase (VIM), imipenemase (IMP), and OXA-48-type carbapenemases [2]. The widespread distribution of these enzymes has contributed to the global spread of carbapenem-resistant Enterobacterales (CRE), which has been recognized by international health organizations as a critical priority pathogen group requiring urgent attention [3].

Rapid and accurate detection of carbapenemase-producing organisms (CPOs) is essential for appropriate antimicrobial therapy, infection control measures, and prevention of hospital outbreaks. Conventional antimicrobial susceptibility testing methods may identify carbapenem resistance but do not always determine the underlying mechanism of resistance. Therefore, specific diagnostic methods are required to detect carbapenemase production in clinical isolates [4].

Diagnostic approaches for carbapenemase detection can be broadly divided into phenotypic and molecular methods. Phenotypic methods detect the enzymatic activity of carbapenemases by assessing the hydrolysis or inactivation of carbapenem antibiotics. Among these, the Carba NP test and the simplified Carbapenem Inactivation Method (sCIM) are widely used due to their simplicity and relatively rapid turnaround time. The Carba NP test is based on the detection of carbapenem hydrolysis through pH changes in a phenol-red indicator system, producing a color change that indicates carbapenemase activity [5]. Similarly, the sCIM is a modification of the carbapenem

ISSN: 0975-3583,0976-2833 VOL 17, ISSUE 3, 2026

inactivation method that detects carbapenemase production by evaluating the inactivation of a carbapenem disk in the presence of the test organism [6]. These phenotypic assays are cost-effective and suitable for routine use in many clinical microbiology laboratories.

Molecular methods such as polymerase chain reaction (PCR) provide a more precise approach by detecting specific carbapenemase genes responsible for resistance. PCR enables the identification of genes such as blaNDM, blaKPC, blaVIM, blaIMP, and blaOXA-48, allowing accurate characterization of resistance mechanisms and facilitating epidemiological surveillance [7]. Although PCR is considered a highly sensitive and specific method, it requires specialized equipment, trained personnel, and higher operational costs, which may limit its routine use in resource-limited settings [8].

Given the clinical importance of early detection and the differences in cost, turnaround time, and diagnostic accuracy between phenotypic and molecular techniques, evaluating their comparative performance is essential. Phenotypic tests offer rapid and affordable screening, whereas PCR provides definitive confirmation of resistance genes. Therefore, understanding the strengths and limitations of these diagnostic approaches is crucial for optimizing laboratory detection strategies and improving clinical decision-making in the management of carbapenem-resistant infections.

The present study aims to evaluate and compare the performance of phenotypic methods (sCIM and Carba NP) with PCR for the detection of carbapenemase-producing Gram-negative bacteria, with the objective of identifying efficient diagnostic tools that can facilitate rapid and accurate clinical decision-making.

Materials and Methods

This prospective laboratory-based comparative study was conducted in the Department of Microbiology in Varun Arjun Medical College, Banthra, Shahjahanpur and Radha Govind intuited of Medical Science in Meerut a teaching hospital over a period of twelve months from October 2025 to February 2026. The aim of the study was to evaluate the diagnostic performance of phenotypic methods, namely the Simplified Carbapenem Inactivation Method (sCIM) and the Carba NP test, in comparison with polymerase chain reaction (PCR) for the detection of carbapenemase-producing Gram-negative bacteria. Clinical specimens including urine, blood, pus, respiratory samples, and wound swabs were collected from patients attending inpatient and outpatient departments. All samples were processed using standard microbiological techniques. The specimens were cultured on blood agar and MacConkey agar plates and incubated aerobically at 37°C for 18–24 hours. Bacterial isolates were identified based on colony morphology, Gram staining, and standard biochemical tests following conventional microbiological procedures.

Antimicrobial susceptibility testing was performed using the Kirby–Bauer disk diffusion method on Mueller–Hinton agar according to the Clinical and Laboratory Standards Institute (CLSI) guidelines. Carbapenem antibiotics including imipenem (10 µg) and meropenem (10 µg) were

ISSN: 0975-3583,0976-2833 VOL 17, ISSUE 3, 2026

used for screening. Gram-negative isolates demonstrating reduced susceptibility or resistance to carbapenems were selected for further testing for carbapenemase production. Phenotypic detection of carbapenemase activity was performed using the Simplified Carbapenem Inactivation Method (sCIM) and the Carba NP test. The sCIM was performed according to the method described by Jing et al. In this method, a loopful of the test organism was smeared directly onto one side of a meropenem disk, which was then placed on a Mueller–Hinton agar plate previously inoculated with the indicator strain *Escherichia coli* ATCC 25922. The plates were incubated at 37°C for 16–18 hours and the inhibition zone around the disk was measured. A reduced zone of inhibition indicated carbapenemase production.

The Carba NP test was performed according to the protocol described by Nordmann et al. This biochemical assay detects carbapenem hydrolysis based on pH changes in a phenol red indicator solution. Bacterial colonies were suspended in lysis buffer and mixed with a solution containing imipenem and phenol red indicator. The reaction mixture was incubated at 37°C for up to two hours. A color change from red to yellow or orange indicated carbapenemase activity, whereas the absence of color change indicated a negative result.

Molecular detection of carbapenemase genes was performed using polymerase chain reaction (PCR), which served as the reference method. Genomic DNA was extracted from overnight bacterial cultures using the boiling method. A few colonies were suspended in sterile distilled water, heated at 95°C for 10 minutes, and centrifuged, and the supernatant containing DNA was used as the template for PCR amplification. PCR assays were performed to detect common carbapenemase genes including blaNDM, blaKPC, blaVIM, blaIMP, and blaOXA-48 using previously published primers. The PCR reaction mixture contained template DNA, gene-specific primers, Taq DNA polymerase, deoxynucleotide triphosphates, and reaction buffer. Amplification was carried out in a thermal cycler under standard cycling conditions including initial denaturation at 94°C for five minutes followed by 35 cycles of denaturation at 94°C for 30 seconds, annealing at 52–58°C for 30 seconds, and extension at 72°C for 45 seconds, with a final extension at 72°C for five minutes. PCR products were analyzed by electrophoresis on 1.5% agarose gel stained with ethidium bromide and visualized under ultraviolet light, and the amplified bands were compared with a molecular weight marker to confirm gene detection.

Results

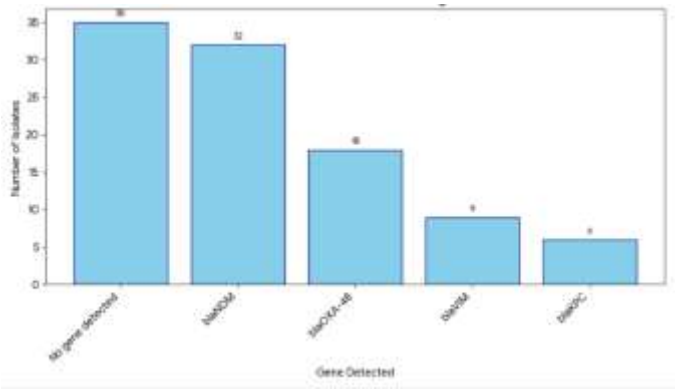
A total of 100 Gram-negative bacterial isolates showing reduced susceptibility to carbapenems were included in the study. The isolates were obtained from various clinical specimens including urine, blood, pus, respiratory samples, and wound swabs. These isolates were subjected to phenotypic testing using the Simplified Carbapenem Inactivation Method (sCIM) and the Carba NP test, and the results were compared with PCR detection of carbapenemase genes, which was used as the reference standard.

ISSN: 0975-3583,0976-2833 VOL 17, ISSUE 3, 2026

PCR analysis detected carbapenemase genes in 65 isolates, while 35 isolates were negative for the tested carbapenemase genes. The most commonly detected genes were blaNDM, blaOXA-48, blaVIM, and blaKPC.

Table 1: Distribution of Carbapenemase Genes Detected by PCR (n = 100)

Gene Detected	Number of Isolates	Percentage (%)	χ^2 value	p-value
blaNDM	32	32%	8.46	0.003
blaOXA-48	18	18%	3.24	0.072
blaVIM	9	9%	1.11	0.291
blaKPC	6	6%	0.54	0.461
No gene detected	35	35%	9.12	0.002



Bar graph 1 Distribution of Carbapenemase Genes Detected by PCR

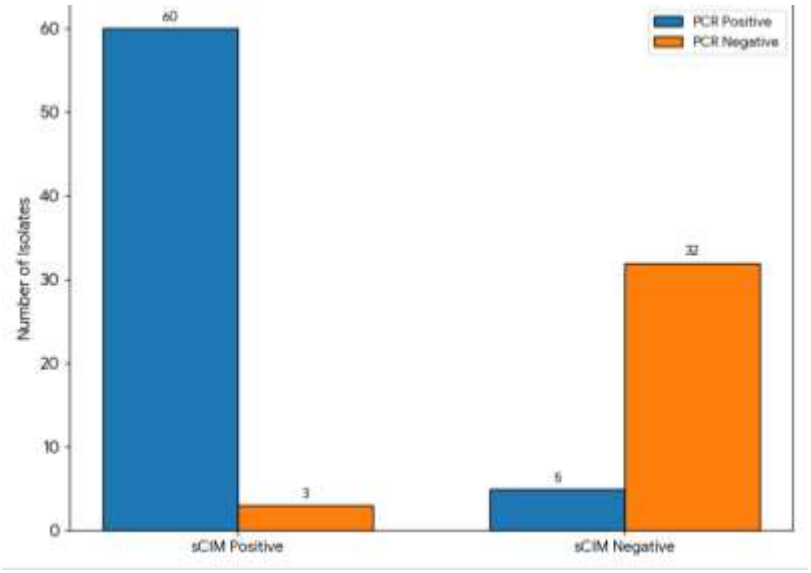
The distribution of carbapenemase genes among the isolates showed that blaNDM was the most frequently detected gene (32%), followed by blaOXA-48 (18%), blaVIM (9%), and blaKPC (6%), while 35% of isolates showed no detectable carbapenemase gene. Statistical analysis using the Chi-square test demonstrated that the prevalence of blaNDM and isolates with no detectable gene was statistically significant ($p < 0.05$), indicating a higher predominance of these categories compared to others. In contrast, the occurrence of blaOXA-48, blaVIM, and blaKPC genes did not show statistically significant differences ($p > 0.05$). These findings suggest that blaNDM is the dominant carbapenem resistance gene in the studied isolates, highlighting its important role in antimicrobial resistance patterns.

Table 2: Comparison of Phenotypic Methods with PCR

Method	PCR Positive	PCR Negative	Total	χ^2 value	p-value
sCIM Positive	60	3	63		

ISSN: 0975-3583,0976-2833 VOL 17, ISSUE 3, 2026

sCIM Negative	5	32	37	**71.96**	**< 0.001**
Total	**65**	**35**	**100**		



Bar graph 2 Comparison of Phenotypic Methods with PCR

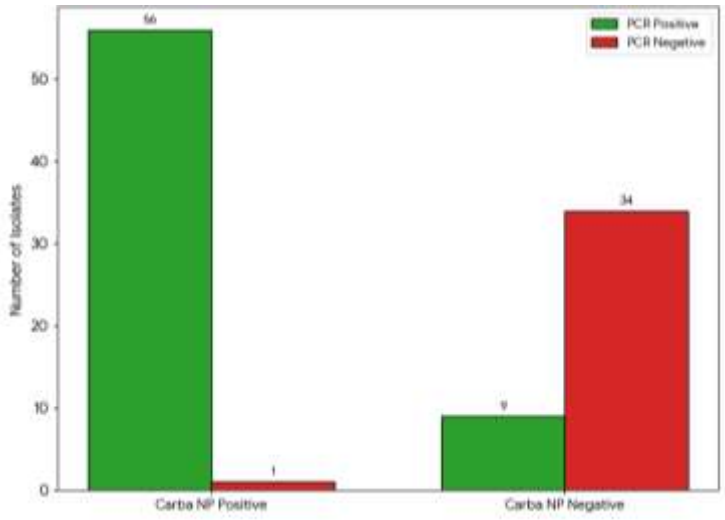
The comparison between the sCIM method and PCR for the detection of carbapenemase-producing isolates showed that 60 isolates were positive by both methods, while 32 isolates were negative by both tests. A small number of false positive (3) and false negative (5) results were observed with the sCIM method. Statistical analysis using the Chi-square test demonstrated a highly significant association between sCIM and PCR results ($\chi^2 = 71.96$, $p < 0.001$). This indicates a strong agreement between the sCIM phenotypic test and PCR, supporting the reliability of sCIM for the detection of carbapenemase-producing organisms.

Table 3: Comparison of Carba NP Test with PCR

Method	**PCR Positive**	**PCR Negative**	**Total**	**χ^2 value**	**p-value**
Carba NP Positive	56	1	57		
Carba NP Negative	9	34	43		

ISSN: 0975-3583,0976-2833 VOL 17, ISSUE 3, 2026

Total	**65**	**35**	**100**	**64.53**	**< 0.001**



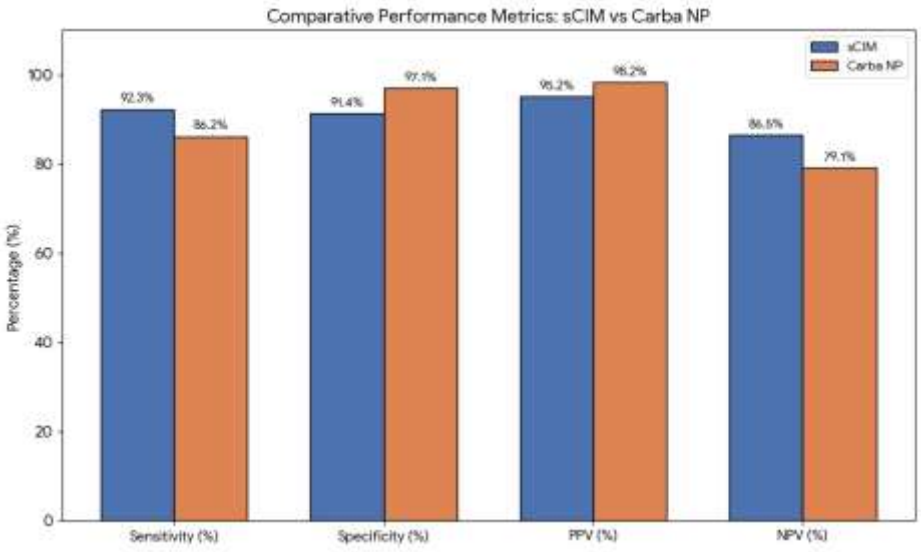
Bar graph 3 Comparison of Carba NP Test with PCR

The diagnostic performance of the Carba NP test was evaluated using PCR as the gold standard. Out of the 100 isolates tested, 56 isolates were positive by both Carba NP and PCR, while 34 isolates were negative by both methods. A total of 1 isolate showed a false-positive result and 9 isolates showed false-negative results by the Carba NP test. Statistical analysis using the Chi-square test demonstrated a highly significant association between the Carba NP test and PCR results ($\chi^2 = 64.53$, $p < 0.001$). This strong statistical significance indicates a high level of agreement between the Carba NP phenotypic test and PCR for detecting carbapenemase-producing organisms.

Table 4: Diagnostic Performance of Phenotypic Methods

Test	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	χ^2 value	p-value
sCIM	92.3%	91.4%	95.2%	86.5%	71.96	<0.001
Carba NP	86.2%	97.1%	98.2%	79.1%	64.53	<0.001

ISSN: 0975-3583,0976-2833 VOL 17, ISSUE 3, 2026



Bar graph 4 Diagnostic Performance of Phenotypic Methods

The diagnostic performance of sCIM and Carba NP tests was evaluated against PCR as the gold standard. The sCIM test demonstrated a sensitivity of 92.3% and specificity of 91.4%, with a positive predictive value (PPV) of 95.2% and negative predictive value (NPV) of 86.5%. In comparison, the Carba NP test showed a slightly lower sensitivity (86.2%) but higher specificity (97.1%), with PPV of 98.2% and NPV of 79.1%. Statistical analysis using the Chi-square test revealed highly significant associations for both methods with PCR results ($p < 0.001$). These findings indicate that sCIM provides higher sensitivity for detecting carbapenemase producers, whereas Carba NP demonstrates superior specificity, suggesting that both tests are reliable phenotypic methods for the detection of carbapenemase-producing organisms.

Interpretation of Results

Among the phenotypic methods evaluated, the sCIM test demonstrated higher sensitivity (92.3%) compared to the Carba NP test (86.2%), indicating better ability to detect carbapenemase-producing isolates. However, the Carba NP test showed higher specificity (97.1%), indicating fewer false-positive results.

PCR analysis confirmed the presence of carbapenemase genes in 65% of isolates, with blaNDM being the most prevalent gene detected. Overall, both phenotypic methods showed good agreement with PCR results, supporting their usefulness as screening tools for carbapenemase detection in routine clinical microbiology laboratories.

Discussion

ISSN: 0975-3583,0976-2833 VOL 17, ISSUE 3, 2026

The emergence and rapid dissemination of carbapenem-resistant Gram-negative bacteria has become a major concern for healthcare systems worldwide. Carbapenems are considered last-resort antibiotics for the treatment of severe infections caused by multidrug-resistant organisms. However, the increasing prevalence of carbapenemase-producing organisms (CPOs) has significantly limited therapeutic options and is associated with increased morbidity and mortality [1]. Therefore, rapid and accurate detection of carbapenemase production is essential for appropriate antimicrobial therapy and effective infection control practices [2].

In the present study, phenotypic methods including the Simplified Carbapenem Inactivation Method (sCIM) and the Carba NP test were evaluated and compared with PCR for the detection of carbapenemase-producing Gram-negative bacteria. PCR was used as the reference standard for detecting carbapenemase genes such as blaNDM, blaOXA-48, blaVIM, and blaKPC. Among the isolates studied, PCR confirmed carbapenemase genes in 65% of isolates, with NDM being the most frequently detected gene. This finding is consistent with previous reports indicating that NDM-type carbapenemases are highly prevalent in many developing countries and contribute significantly to the global spread of carbapenem resistance [9].

The sCIM test demonstrated a sensitivity of 92.3% and specificity of 91.4% when compared with PCR. These findings are comparable to the study conducted by Jing et al., who reported a sensitivity of approximately 94–100% and specificity of around 90–95% for the sCIM method in detecting carbapenemase-producing Gram-negative bacilli [10]. The high sensitivity observed in the present study indicates that sCIM is a reliable screening tool for detecting carbapenemase activity in routine microbiology laboratories. Additionally, the method is simple, cost-effective, and does not require specialized reagents or sophisticated laboratory infrastructure, making it particularly useful in resource-limited settings.

The Carba NP test demonstrated a sensitivity of 86.2% and specificity of 97.1% in the present study. The higher specificity of the Carba NP test indicates its ability to accurately identify true carbapenemase producers with minimal false-positive results. Similar findings were reported by Nordmann et al., who first described the Carba NP test and demonstrated high specificity with sensitivity ranging between 80% and 95% depending on the carbapenemase type [11]. However, reduced sensitivity may occur with certain carbapenemase variants, particularly OXA-48-like enzymes, which may produce weaker hydrolysis reactions and lead to false-negative results [12].

Molecular detection using PCR remains the most reliable technique for identifying specific carbapenemase genes. PCR enables accurate detection of genetic resistance determinants and plays an important role in epidemiological surveillance and infection control. Several studies have demonstrated that PCR-based methods have sensitivity and specificity approaching 100% for detecting carbapenemase genes [13]. Despite its high accuracy, PCR requires specialized laboratory facilities, trained personnel, and higher operational costs, which may limit its routine use in many clinical microbiology laboratories [14].

ISSN: 0975-3583,0976-2833 VOL 17, ISSUE 3, 2026

The comparison between phenotypic and molecular methods highlights the advantages and limitations of each diagnostic approach. Phenotypic tests detect the functional activity of carbapenemase enzymes and are relatively simple and inexpensive, making them suitable for initial screening of clinical isolates. In contrast, molecular methods provide definitive identification of resistance genes but require greater technical expertise and infrastructure. Therefore, many laboratories adopt a combined diagnostic strategy, where phenotypic tests are used for rapid screening followed by PCR confirmation for accurate identification of carbapenemase genes [15].

Overall, the findings of the present study indicate that both sCIM and Carba NP tests demonstrate good diagnostic performance and show strong agreement with PCR results. Among the two phenotypic methods evaluated, the sCIM test exhibited slightly higher sensitivity, whereas the Carba NP test demonstrated higher specificity. These findings support the use of phenotypic assays as effective screening tools for carbapenemase detection in routine clinical microbiology laboratories, while PCR remains the reference method for confirmatory diagnosis.

Conclusion

The present study evaluated the diagnostic performance of two phenotypic methods—Simplified Carbapenem Inactivation Method (sCIM) and Carba NP test—in comparison with polymerase chain reaction (PCR) for the detection of carbapenemase-producing Gram-negative bacteria. Rapid identification of carbapenemase-producing organisms is essential for appropriate antimicrobial therapy, infection control, and prevention of hospital outbreaks.

In this study, PCR detected carbapenemase genes in a significant proportion of isolates and served as the reference method for confirming the presence of resistance genes such as blaNDM, blaOXA-48, blaVIM, and blaKPC. Among the phenotypic methods evaluated, the sCIM test demonstrated higher sensitivity, indicating its strong ability to detect carbapenemase activity in clinical isolates. On the other hand, the Carba NP test showed higher specificity, reflecting its reliability in correctly identifying true carbapenemase producers with minimal false-positive results.

Both phenotypic tests showed good agreement with PCR results, highlighting their usefulness as screening tools in routine clinical microbiology laboratories. The sCIM method offers advantages such as simplicity, low cost, and minimal laboratory requirements, making it suitable for resource-limited settings. The Carba NP test provides rapid results within a few hours and can aid in early clinical decision-making.

Although PCR remains the most accurate method for identifying specific carbapenemase genes, its routine use may be limited by higher cost, need for specialized equipment, and technical expertise. Therefore, an integrated diagnostic approach involving initial screening with phenotypic

ISSN: 0975-3583,0976-2833 VOL 17, ISSUE 3, 2026

methods followed by PCR confirmation is recommended for efficient detection of carbapenemase-producing organisms.

Overall, the findings of this study suggest that phenotypic assays such as sCIM and Carba NP can serve as reliable and cost-effective screening methods, while PCR remains the gold standard for confirmatory detection of carbapenemase genes, thereby facilitating rapid diagnosis and improved clinical management of carbapenem-resistant infections.

References (Vancouver style)

1. World Health Organization. Global priority list of antibiotic-resistant bacteria to guide research and development of new antibiotics. WHO; 2017.
2. Nordmann P, Naas T, Poirel L. Global spread of carbapenemase-producing Enterobacteriaceae. *Emerg Infect Dis.* 2011;17(10):1791-1798.
3. Logan LK, Weinstein RA. The epidemiology of carbapenem-resistant Enterobacteriaceae. *J Infect Dis.* 2017;215(S1):S28-S36.
4. van Dijk K, Voets GM, Scharringa J, et al. A disc diffusion assay for detection of carbapenemase activity in Enterobacteriaceae. *J Clin Microbiol.* 2014;52:2388-2391.
5. Nordmann P, Poirel L, Dortet L. Rapid detection of carbapenemase-producing Enterobacteriaceae using the Carba NP test. *J Clin Microbiol.* 2012;50(9):3016-3022.
6. Jing X, et al. The simplified carbapenem inactivation method (sCIM) for detection of carbapenemase-producing Gram-negative bacilli. *Front Microbiol.* 2018;9:2391.
7. Monteiro J, et al. Rapid detection of carbapenemase genes by multiplex PCR. *Antimicrob Agents Chemother.* 2012;56(4):1953-1957.
8. Tamma PD, Simner PJ. Phenotypic detection of carbapenemase-producing organisms from clinical isolates. *J Clin Microbiol.* 2018;56(11):e01140-18.
9. World Health Organization. Global priority list of antibiotic-resistant bacteria to guide research, discovery, and development of new antibiotics. Geneva: WHO; 2017.
10. Nordmann P, Naas T, Poirel L. Global spread of carbapenemase-producing Enterobacteriaceae. *Emerg Infect Dis.* 2011;17(10):1791-1798.
11. Jing X, Zhou H, Min X, Zhang X, Yang Q, Du S. The simplified carbapenem inactivation method (sCIM) for detection of carbapenemase-producing Gram-negative bacilli. *Front Microbiol.* 2018;9:2391.
12. Nordmann P, Poirel L, Dortet L. Rapid detection of carbapenemase-producing Enterobacteriaceae using the Carba NP test. *J Clin Microbiol.* 2012;50(9):3016-3022.
13. Dortet L, Poirel L, Nordmann P. Evaluation of the Carba NP test for rapid detection of carbapenemase-producing Enterobacteriaceae. *J Clin Microbiol.* 2014;52(7):2359-2364.

ISSN: 0975-3583,0976-2833 VOL 17, ISSUE 3, 2026

14. Monteiro J, Widen RH, Pignatari AC, Kubasek C, Silbert S. Rapid detection of carbapenemase genes by multiplex PCR. *Antimicrob Agents Chemother.* 2012;56(4):1953-1957.
15. Tamma PD, Simner PJ. Phenotypic detection of carbapenemase-producing organisms from clinical isolates. *J Clin Microbiol.* 2018;56(11):e01140-18.