Original Article

DOI: 10.7860/NJLM/2026/79688.2954

Microbiology Section

Evaluation of Carba NP for Rapid Detection of Carbapenem Resistance in Routine Diagnostic Laboratories: A Retrospective Validation Study

PARIJAT DAS[1], KUMAR ANAND SHRUTIRAAJ[2], NIDHI PANDEY[3]

ABSTRACT

Introduction: Resistance to carbapenems is a major concern for clinicians and infection control practitioners. Fast and simple detection methods are essential to protect our increasingly limited arsenal of antibiotics. A rapid biochemical method for detecting carbapenemase production has recently been proposed, namely the Carbapenemase Nordmann-Poirel test (Carba NP test), which is based on detecting hydrolysis of the β-lactam ring of imipenem.

Aim: The aim of the present study was to assess the performance of the RAPIDEC® Carba NP test.

Materials and Methods: The present retrospective validation study was conducted in the Department of Microbiology, Armed Forces Medical College (AFMC), Pune, India between January and April 2018. A total of 159 isolates, retrospectively collected from various specimens from patients admitted to the Intensive Care Unit (ICU) between January 2016 and December 2017, were included in the study. These isolates had previously been characterised as either carbapenemase producers (n=109) or non-producers (n=50), based on meropenem disc susceptibility testing and the presence or absence of carbapenemase genes. The carbapenemase genes screened included *Klebsiella pneumoniae* carbapenemase (KPC), New Delhi Metallo-β-lactamase-1 (NDM-1), Oxacillinase-48 (OXA-48), Imipenemase (IMP), and Verona Integron-encoded Metallo-β-lactamase (VIM), using Polymerase Chain Reaction (PCR) assays. The RAPIDEC® CARBA NP test was performed and interpreted according to the manufacturer's instructions to phenotypically detect carbapenemase production based on imipenem hydrolysis. Sensitivity, specificity, Positive Predictive Value (PPV), and Negative Predictive Value (NPV) of RAPIDEC® CARBA NP were calculated using a 2×2 contingency table, taking PCR as the gold standard. Microsoft Excel was used for data analysis.

Results: Among the 109 carbapenemase producers, the RAPIDEC® CARBA NP test was positive in 106 cases and negative in 3 cases. All 50 carbapenemase non-producers were negative by the assay. RAPIDEC® CARBA NP demonstrated a sensitivity of 97.25% {95% Confidence Interval (CI): 92.17%-99.43%}, specificity of 100% (95% CI: 92.89%-100.00%), PPV of 100% (95% CI: 96.58%-100.00%), and NPV of 94.34% (95% CI: 84.52%-98.07%).

Conclusion: The RAPIDEC® CARBA NP test is simple, easy to interpret, and cost-effective, providing a practical solution for early detection of carbapenemase-producing Gram-negative bacteria in microbiological laboratories, and is helpful in infection prevention and control measures.

Keywords: Antimicrobial stewardship, Carbapenemase Nordmann-Poirel test, Gram negative bacteria, Infection, Multidrug resistant organisms

INTRODUCTION

Carbapenems are last-line antibiotics used to treat infections caused by Multidrug-Resistant Organisms (MDROs). The emergence of resistance due to production of carbapenem-hydrolysing β-lactamases renders microorganisms resistant to all β-lactam agents, including penicillins, cephalosporins, monobactams, and carbapenems [1]. Other mechanisms of resistance to carbapenems, such as porin loss, may coexist in organisms demonstrating low-level carbapenemase resistance [2]. The presence of carbapenemase-encoding genes on conjugative plasmids significantly enhances their ability to spread rapidly, leading to severe, potentially life-threatening infections that may be untreatable with existing therapies.

Given the limited therapeutic options for combating carbapenemase-producing microorganisms, timely and accurate detection of such organisms is crucial for controlling their spread and ensuring favourable clinical outcomes [3]. Detection methods can be broadly divided into phenotypic and genotypic categories. A few techniques are available for the rapid identification of carbapenemase producers [4], including Ultraviolet (UV) spectrophotometry [5], Matrix-Assisted Laser Desorption Ionisation-Time of Flight (MALDI-TOF) technology [6], and molecular techniques [7]. These techniques have excellent sensitivity and specificity, but their limitations include the requirement for trained personnel and specialised, costly equipment. Some techniques involve laborious processing and are therefore time-consuming. Another caveat of molecular tests is their inherent inability to detect previously unknown carbapenemase genes or genes not included in the test panel.

A rapid biochemical method for carbapenemase detection, the Carba NP test, is based on detecting hydrolysis of the β-lactam ring of imipenem [7]. This test has been thoroughly validated for detecting carbapenemase-producing *Enterobacteriaceae* and *Pseudomonas* spp. [7,8]. A slightly modified version, the CarbAcineto NP test, has been developed for detecting carbapenemase-producing *Acinetobacter* spp. [9]. In the present study, the performance of the RAPIDEC® CARBA NP test (bioMérieux, La Balme-les-Grottes, France) was evaluated using isolates obtained from clinical samples at a tertiary-care center and compared with a molecular reference method.

The present retrospective validation study's novelty lies in its real-world validation of this test against PCR genotyping in a resource-conscious military tertiary-care hospital in India, which represents

a unique epidemiological setting. This approach reflects an important shift toward operational simplicity without compromising diagnostic accuracy, providing context-specific insights into diagnostic performance and practical utility for infection control and antimicrobial stewardship programs.

The rationale for the present study stems from the urgent need for rapid and reliable detection of carbapenem-resistant Gram negative bacteria to guide timely therapy and infection control. While molecular methods are accurate, they are resource-intensive and less feasible in many settings. The RAPIDEC® CARBA NP test offers a simple, rapid, equipment-free, and cost-effective alternative. Based on the available literature, the present study aimed to evaluate the diagnostic performance of the RAPIDEC® CARBA NP test for detecting carbapenemase production in Gram negative bacteria and to validate its accuracy against standard molecular methods.

The primary objective of the study was to assess the diagnostic accuracy of the RAPIDEC® CARBA NP test in detecting carbapenemase-producing Gram negative bacteria using PCR as the reference standard. The secondary objectives were to determine the distribution of carbapenemase genes among carbapenem-resistant isolates and to compare the phenotypic results of the RAPIDEC® CARBA NP test with the genotypic findings.

MATERIALS AND METHODS

The present retrospective validation study was conducted in the Department of Microbiology, Armed Forces Medical College (AFMC), Pune, Maharashtra, India, between January and March 2018, and the data were analysed in April 2018. Ethical approval was obtained from the Institutional Ethics Committee (IEC-AFMC/PD/Sept/2015). The present was a time-bound study, and all eligible samples meeting the inclusion criteria during the study period were included.

Inclusion and Exclusion criteria: A total of 159 isolates, retrospectively collected from various specimens-including urine, pus, blood, sputum, and other body fluids-from patients admitted to the ICU between January 2016 and December 2017 were included. Samples from the Outpatient Department were excluded.

These isolates had been previously characterised as either carbapenemase producers (n=109) or non-producers (n=50), based on meropenem disc susceptibility testing and the presence or absence of carbapenemase genes [10].

Study Procedure

The carbapenemase genes screened included KPC, NDM-1, OXA-48, IMP, and VIM using PCR assays. The RAPIDEC® CARBA NP test was performed and interpreted according to the manufacturer's instructions to phenotypically detect carbapenemase production based on imipenem hydrolysis. Strains positive or negative for KPC, NDM-1, OXA-48, IMP, and VIM genes were used as positive and negative controls, respectively.

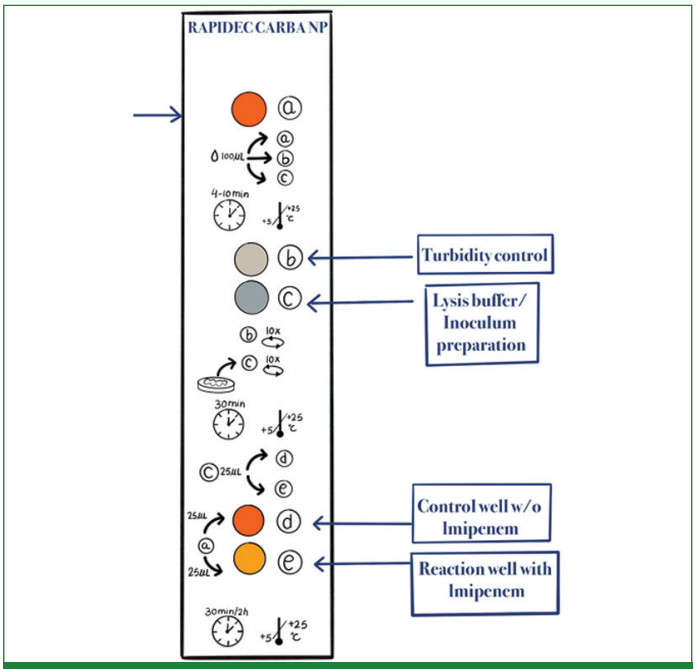
Detection of carbapenemase producers was done using RAPIDEC® CARBA NP [7,8]. All 159 genetically characterised isolates were subjected to RAPIDEC® CARBA NP for rapid detection of carbapenemases. The test was performed as per the manufacturer's instructions.

The RAPIDEC® CARBA NP test is designed to detect imipenem hydrolysis by carbapenemase-producing bacteria. This hydrolysis acidifies the medium, leading to a colour change in the pH indicator. After bacterial lysis, which enables enzyme extraction, the lysate is added to a detection solution containing:

1. Imipenem (carbapenemase substrate)
2. Phenol red (pH indicator)
3. Zinc (required for detection of metallo-enzyme–producing strains)

After incubation for a maximum of two hours, results are read visually by comparing the control well (without imipenem) with the reaction well containing imipenem.

Briefly, a 10 µL loopful of an overnight-grown bacterial colony from Mueller-Hinton Agar (MHA) plates (BioMérieux) was transferred and mixed into the suspension medium provided in the kit. After 4-5 minutes, the suspension was transferred to the wells of the test strip following the manufacturer's protocol. The test strips were then incubated at 37°C. Readings were taken at two different time intervals: after 30 minutes and after two hours. The results were interpreted based on the colour change in the wells. An illustration of the test strip template is shown in [Table/Fig-1]. A test was considered positive when there was a noticeable colour difference between the two wells: yellow-orange was interpreted as positive, and red as negative. The various possible results and their interpretation are shown in [Table/Fig-2].



[Table/Fig-1]: RAPIDEC® CARBA NP test strip template [10].

Control well	Test well	Interpretation
Red	Red	Negative
Orange	Orange	
Red	Yellow, light orange, orange, dark orange	Positive
Orange	Yellow	
Any colour other than red or orange	Not applicable	Uninterpretable
Orange	Red	

[Table/Fig-2]: Possible results and their interpretation for the RAPIDEC® CARBA NP [10].

STATISTICAL ANALYSIS

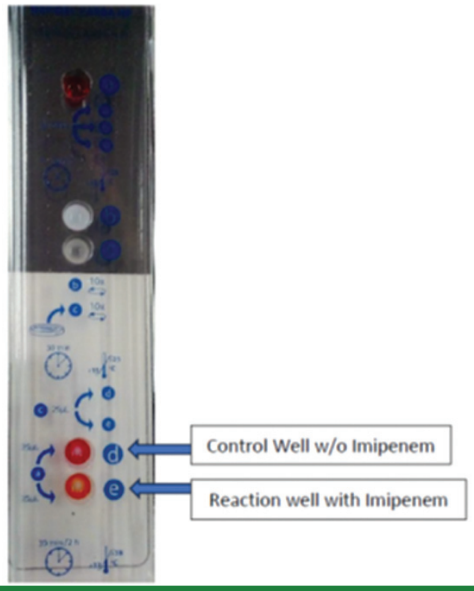
All data were entered into a Microsoft Excel spreadsheet and analysed. The sensitivity, specificity, PPV, and NPV of the RAPIDEC® CARBA NP test were calculated using a 2×2 contingency table, taking PCR as the gold standard.

RESULTS

RAPIDEC® CARBA NP results: Among the 109 carbapenemase producers, the RAPIDEC® CARBA NP test was positive for 106 isolates, while 3 tested negative. All 50 isolates that were negative for KPC, NDM-1, OXA-48, IMP, and VIM genes also tested negative. A positive Carba NP result is shown in [Table/Fig-3].

The 3 isolates that were negative by the RAPIDEC® CARBA NP test but positive by PCR were all OXA-48-positive (02 *Klebsiella pneumoniae* and 01 *Acinetobacter baumannii*). The sensitivity and specificity of the RAPIDEC® CARBA NP test were calculated as shown in [Table/Fig-4]. RAPIDEC® CARBA NP demonstrated a sensitivity of 97.25% (95% Confidence Interval (CI): 92.17%-

99.43%) and a specificity of 100% (95% CI:92.89%-100.00%). The PPV was 100% (95% CI:96.58%-100.00%) and the NPV was 94.34% (95% CI:84.52%-98.07%).



[Table/Fig-3]: A positive result in the RAPIDEC® CARBA NP test.

Rapid test	Carbapenemases				
	Present	N	Absent	N	Total
Positive	True positive(a)	106	False positive(c)	0	106 (a+c)
Negative	False negative(b)	3	True negative(d)	50	53 (b+d)
Total	a+b	109	c+d	50	

[Table/Fig-4]: Performance of RAPIDEC® CARBA NP test.

Genotypic profile of isolates: Among the 109 genetically characterised isolates, 54 (49.5%) were NDM-1 producers, 38 (34.86%) were KPC producers, 18 (16.5%) were OXA-48 producers, and 3 (2.7%) were VIM producers. No IMP producers were detected. In addition, 4 isolates (02 *Escherichia coli* and 02 *Klebsiella* spp.) were dual carbapenemase producers carrying both blaNDM-1 and blaKPC. Another 50 samples were negative for the presence of all five carbapenemase genes (KPC, NDM-1, OXA-48, IMP, and VIM). The genotypic profile of the characterised isolates is shown in [Table/Fig-5].

Meropenem resistant organisms	Carbapenemase - encoding genes				
	NDM-1 (%)	KPC (%)	OXA-48 type (%)	IMP (%)	VIM (%)
Escherichia coli (n=33)	12	22	-	-	1
Klebsiella spp. (n=22)	06	16	2	-	-
Pseudomonas spp. (n=23)	19	-	4	-	-
Acinetobacter spp. (n=21)	12	-	9	-	-
Citrobacter spp. (n=6)	3	-	2	-	1
Enterobacter spp. (n=2)	-	-	1	-	1
Miscellaneous spp. (n=2)	2	-	-	-	-
Total=109	54 (49.5%)	38 (34.8%)	18 (16.5%)	-	3 (2.7%)

[Table/Fig-5]: Genotypic profile of Isolates for various carbapenemases.
The total of last row is more than 100% as 04 isolates (02 *Escherichia coli* and 02 *Klebsiella* spp.) had dual carbapenemase genes.

DISCUSSION

Carbapenems remain the mainstay of empirical treatment for life-threatening infections. Their enzyme-mediated resistance mechanism has gained worldwide significance due to its association with MDROs, global dissemination, and nosocomial acquisition, indicating the need for continuous monitoring systems and effective infection control measures. These enzymes not only limit treatment options but are also difficult to detect with routine antibiotic susceptibility testing, often resulting in false susceptibility profiles. Inaccurate detection of carbapenemases may lead to inappropriate treatment, ultimately compromising patient outcomes [11].

In the present retrospective validation study, the performance of the RAPIDEC® CARBA NP test was evaluated and compared with PCR-based genotypic detection of five major carbapenemase genes: KPC, NDM-1, OXA-48, IMP, and VIM. The RAPIDEC® CARBA NP test exhibited high diagnostic accuracy, with a sensitivity of 97.25% and a specificity of 100%. These results are consistent with previous evaluations of Carba NP-based platforms. Nordmann P et al., (2012), the original developers of the Carba NP test, demonstrated that it could identify class A, B, and D carbapenemases in Enterobacterales with a sensitivity and specificity exceeding 98% [12]. Similarly, Garg A et al., reported a sensitivity, specificity, PPV, and NPV of 92.6%, 96.2%, 95.83%, and 92.6%, respectively [13]. In a study by Poirel L et al., rapid detection of all types of carbapenemases in *Enterobacteriaceae*, *Acinetobacter baumannii*, and *Pseudomonas aeruginosa* was achieved with a sensitivity and specificity of 96% [14].

Another study by Dortet L et al., compared the performance of two commercially available biochemical tests for the rapid detection of carbapenemase-producing *Enterobacteriaceae* with a home-made technique. The RAPIDEC® CARBA NP test performed superior to the other kits under study, with a sensitivity of 99% (95% CI: 94.3%-99.8%) and a specificity of 100% [15]. Mancini S et al., also reported superior performance of RAPIDEC® CARBA NP (sensitivity 93.7% and specificity 100%) compared to the β-CARBA® test (sensitivity 64.9% and specificity 90%) [16].

However, Vamsi KS et al., reported an overall sensitivity and specificity of the Carba NP assay of 79.12% and 82.79%, respectively, with a PPV of 82.91% and an NPV of 79.38%. These findings indicate modest diagnostic performance, particularly for non-fermenting Gram negative bacilli such as *Acinetobacter* spp., where the test showed reduced sensitivity. In contrast, excellent performance was observed for members of *Enterobacteriaceae*. Both the in-house Carba NP and the commercial RAPIDEC® test showed 100% concordance, with no discrepancies between the two methods [17].

Although no false-positive results were observed in the present study, Kabir MH et al., (2016) reported three false-positive results among 138 tested isolates [18]. Differences observed between the two studies may be explained by variations in strain distribution and differences in the reference standards used. Hombach M et al., (2015) found that the sensitivity of RAPIDEC® CARBA NP increased to 100% when doubling the inoculum of bacteria; however, this procedure was not undertaken due to concerns regarding a potential compromise in specificity and requires further investigation [19].

Three isolates that were confirmed carbapenemase producers by PCR were not detected by the test (false negatives). This limitation is consistent with the fact that OXA variants are known to lack significant carbapenemase activity, a finding that has also been highlighted in other studies [15,16]. The RAPIDEC® CARBA NP test has emerged as a dependable, rapid, and economical tool for identifying carbapenemase-producing Gram negative bacteria, showing excellent sensitivity and specificity when compared with PCR. Its straightforward protocol and quick turnaround time make it particularly useful in high-risk environments such as ICUs, where early detection is crucial for initiating appropriate antimicrobial treatment and implementing infection control strategies to limit the spread of resistant pathogens, especially in resource-constrained settings.

The findings of the present study support the expanded use of phenotypic carbapenemase detection methods within routine microbiology laboratories. Due to its simplicity, the test is well suited for use in peripheral and decentralised healthcare facilities, promoting antimicrobial stewardship. Future work could focus on incorporating this assay into national antimicrobial resistance surveillance frameworks, assessing its performance across a wider range of clinical environments, and evaluating its feasibility for direct testing from clinical samples. Furthermore, integrating the test with automated laboratory workflows and digital reporting systems could strengthen real-time tracking of resistance and enable timely public health interventions.

Limitation(s)

The present study's retrospective, single-centre design limits the broader applicability of its findings. The relatively modest sample size further constrains the robustness of the conclusions. The Carba NP test, while useful, may fail to detect specific enzyme classes- particularly OXA-type carbapenemases- resulting in possible false-negative outcomes. Moreover, interpreting the test's colour change requires subjective judgment, introducing variability among observers. Lastly, the absence of clinical outcome data limits the ability to evaluate the test's practical impact on patient treatment decisions.

CONCLUSION(S)

The RAPIDEC® CARBA NP test provides a rapid, simple, and cost-effective method for detecting carbapenemase-producing Gram negative bacteria. Its implementation in routine laboratories can facilitate early diagnosis, guide timely infection control measures, and complement molecular methods, thereby helping to curb the spread of multidrug resistance and preserve the efficacy of last-resort carbapenems. The present study highlights the practical utility of Carba NP for the rapid detection of carbapenem resistance in routine diagnostic laboratories.

Acknowledgement

The authors wish to acknowledge Mr. Rajkumar for assistance with performing the RAPIDEC® CARBA NP assay and sample processing.

Author contributions: Das P: Writing review and editing, writing-original draft, resources, methodology, investigation, formal analysis, data curation, conceptualisation. Shrutiraaj KA: Writing- review & editing, investigation, data curation. Pandey N: Writing- review & editing, literature search. All authors read and approved the final version of the manuscript. All authors had full access to all data in the study and had final responsibility for the decision to submit for publication.

REFERENCES

[1] Alba J, Ishii Y, Thomson K, Moland ES, Yamaguchi K. Kinetics study of KPC-3, a plasmid-encoded class A carbapenem-hydrolyzing beta-lactamase. Antimicrob Agents Chemother. 2005;49(11):4760-62. http://dx.doi.org/10.1128/AAC.49.11.4760-4762.2005.

[2] Smith Moland E, Hanson ND, Herrera VL, Black JA, Lockhart TJ, Hossain A, et al. Plasmid-mediated, carbapenem-hydrolysing beta-lactamase, KPC-2, in Klebsiella pneumoniae isolates. J Antimicrob Chemother. 2003;51(3):711-14. http://dx.doi.org/10.1093/jac/dkg124.

[3] Bratu S, Landman D, Haag R, Recco R, Eramo A, Alam M, et al. Rapid spread of carbapenem-resistant Klebsiella pneumoniae in New York City: A new threat to our antibiotic armamentarium: A new threat to our antibiotic armamentarium. Arch Intern Med. 2005;165(12):1430-35. http://dx.doi.org/10.1001/archinte.165.12.1430

[4] Dortet L, Bréchard L, Cuzon G, Poirel L, Nordmann P. Strategy for rapid detection of carbapenemase-producing Enterobacteriaceae. Antimicrob Agents Chemother. 2014;58(4):2441-45. http://dx.doi.org/10.1128/AAC.01239-13.

[5] Bernabeu S, Poirel L, Nordmann P. Spectrophotometry-based detection of carbapenemase producers among Enterobacteriaceae. Diagn Microbiol Infect Dis. 2012;74(1):88-90. http://dx.doi.org/10.1016/j.diagmicrobio.2012.05.021.

[6] Hrabák J, Walková R, Studentová V, Chudácková E, Bergerová T. Carbapenemase activity detection by matrix-assisted laser desorption ionization-time of flight mass spectrometry. J Clin Microbiol. 2011;49(9):3222-27. http://dx.doi.org/10.1128/JCM.00984-11.

[7] Poirel L, Walsh TR, Cuvillier V, Nordmann P. Multiplex PCR for detection of acquired carbapenemase genes. Diagn Microbiol Infect Dis. 2011;70(1):119-23. Doi: 10.1016/j.diagmicrobio.2010.12.002.

[8] Dortet L, Poirel L, Nordmann P. Rapid detection of carbapenemase-producing Pseudomonas spp. J Clin Microbiol. 2012;50(11):3773-76. http://dx.doi.org/10.1128/JCM.01597-12.

[9] Dortet L, Poirel L, Errera C, Nordmann P. CarbAcineto NP test for rapid detection of carbapenemase-producing Acinetobacter spp. J Clin Microbiol. 2014;52(7):2359-64. http://dx.doi.org/10.1128/JCM.00594-14.

[10] Rapidec® carba NP. bioMérieux Website. https://www.biomerieux.com/corp/en/our-offer/clinical-products/rapidec-carba-np.html.

[11] McGettigan SE, Andreacchio K, Edelstein PH. Specificity of ertapenem susceptibility screening for detection of Klebsiella pneumoniae carbapenemases. J Clin Microbiol. 2009;47(3):785-86. http://dx.doi.org/10.1128/JCM.02143-08.

[12] Nordmann P, Poirel L, Dortet L. Rapid detection of carbapenemase-producing Enterobacteriaceae. Emerg Infect Dis. 2012;18(9):1503-07. http://dx.doi.org/10.3201/eid1809.120355.

[13] Garg A, Garg J, Upadhyay GC, Agarwal A, Bhattacharjee A. Evaluation of the Rapidec Carba NP test kit for detection of carbapenemase-producing Gram-negative bacteria. Antimicrob Agents Chemother. 2015;59(12):7870-72. http://dx.doi.org/10.1128/AAC.01783-15.

[14] Poirel L, Nordmann P. Rapidec Carba NP test for rapid detection of carbapenemase producers. J Clin Microbiol. 2015;53(9):3003-08. http://dx.doi.org/10.1128/JCM.00977-15.

[15] Dortet L, Agathine A, Naas T, Cuzon G, Poirel L, Nordmann P. Evaluation of the RAPIDEC® CARBA NP, the Rapid CARB Screen® and the Carba NP test for biochemical detection of carbapenemase-producing Enterobacteriaceae. J Antimicrob Chemother. 2015;70(11):3014-22. http://dx.doi.org/10.1093/jac/dkv213.

[16] Mancini S, Kieffer N, Poirel L, Nordmann P. Evaluation of the RAPIDEC® CARBA NP and β-CARBA® tests for rapid detection of Carbapenemase-producing Enterobacteriaceae. Diagn Microbiol Infect Dis. 2017;88(4):293-97. http://dx.doi.org/10.1016/j.diagmicrobio.2017.05.006.

[17] Vamsi KS, Ramamoorthy S, Murali TS, Singh M, Kabra V. Utility of carba NP test (inhouse/RAPIDEC commercial kit) in the identification of carbapenemase producing clinical isolates in a tertiary care hospital. J Clin Diagn Res. 2019;13(12):DC19-DC22. http://dx.doi.org/10.7860/jcdr/2019/42780.13375.

[18] Kabir MH, Meunier D, Hopkins KL, Giske CG, Woodford N. A two-centre evaluation of RAPIDEC® CARBA NP for carbapenemase detection in Enterobacteriaceae, Pseudomonas aeruginosa and Acinetobacter spp. J Antimicrob Chemother. 2016;71(5):1213-16. http://dx.doi.org/10.1093/jac/dkv468.

[19] Hombach M, von Gunten B, Castelberg C, Bloemberg GV. Evaluation of the Rapidec Carba NP test for detection of carbapenemases in Enterobacteriaceae. J Clin Microbiol. 2015;53(12):3828-33. http://dx.doi.org/10.1128/JCM.02327-15.

PARTICULARS OF CONTRIBUTORS:
1. Clinical Microbiologist, Department of Microbiology, AFMC, Pune, Maharashtra, India.
2. Medical Specialist, Department of Medicine, AFMC, Pune, Maharashtra, India.
3. PhD, Department of Microbiology, IMS, BHU, Varanasi, Uttar Pradesh, India.

NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:
Parijat Das,
AFMC Old Sholapur Road, Near Race Course, Pune, Maharashtra, India.
E-mail: parijat5628@gmail.com

PLAGIARISM CHECKING METHODS: [Jain H et al.]
- Plagiarism X-checker: May 02, 2025
- Manual Googling: Oct 16, 2025
- iThenticate Software: Oct 20, 2025 (15%)

ETYMOLOGY: Author Origin

EMENDATIONS: 6

AUTHOR DECLARATION:
- Financial or Other Competing Interests: None
- Was Ethics Committee Approval obtained for this study? Yes
- Was informed consent obtained from the subjects involved in the study? NA
- For any images presented appropriate consent has been obtained from the subjects. Yes

Date of Submission: **Apr 03, 2025**
Date of Peer Review: **Jul 15, 2025**
Date of Acceptance: **Oct 21, 2025**
Date of Publishing: **Jan 01, 2026**