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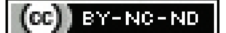
Original Article

Microbiology Section

Rapid Detection Methods for Carbapenemase Producing Gram-negative Bacilli with Reference to Phenotypic Carba M Test: A Cross-sectional Hospital-based Study

P GANESH PERUMAL[1], B DHANUSH KUMAR[2], T PRIYADHARSINI[3], B APPALARAJU[4], R SOMESHWARAN[5]



ABSTRACT

Introduction: Carbapenem-resistant Gram-negative Bacilli (CR-GNB) cause many serious infections, resulting in increased treatment costs, prolonged hospitalisation, and a high mortality rate among infected patients. The survival of carbapenemase-producing Gram-negative organisms is enhanced by mechanisms such as the presence of carbapenemases, reduced expression, and porin mutations. Accurate tests for rapid detection of bacterial antibiotic resistance are crucial for tracking, preventing, and containing the spread of resistant genes within hospitals and communities, as well as for guiding therapy.

Aim: To evaluate the performance of the 'Carba M test' compared to phenotypic Carba NP tests, Carbapenem Inactivation Method (mCIM/eCIM) tests, and genotypic Carba R tests for the phenotypic detection and characterisation of various types of carbapenemase-producing GNB.

Materials and Methods: A cross-sectional study was conducted in the Department of Microbiology at PSG Hospitals, Coimbatore, Tamil Nadu, India which has 1400 beds. The study was carried out over a period of 15 months (from June 2022 to August 2023). A total of 250 CR-GNB were included as the study population. A comparative evaluation of the Carba M test, Carba NP test, Carbapenem Inactivation Method (mCIM/eCIM), and Carba R test for the rapid detection of carbapenemase-producing GNB using standard methods was done. The data were analysed, and odds ratios and p-values were calculated for statistical significance at a 95% Confidence Interval (CI).

Results: A total of 250 CR-GNB were isolated during the study period, of which 83 (33.2%) were from the Medical Intensive Care Unit (MICU), followed by the Medicine department with 24 (9.6%). Enterobacteriaceae accounted for the majority of the isolates, with 229 (91.6%), followed by *Pseudomonas aeruginosa* with 17 (6.8%) and *Acinetobacter* species with 4 (1.6%). *Klebsiella pneumoniae* was the most common isolate among Enterobacteriaceae, accounting for 216 (86.4%). Out of the 250 CR isolates tested by the Carba R test, 100 (40%) were positive for the Oxacillinase (OXA)-48 enzyme, 35 (14%) for the New Delhi Metallobetalactamase (NDM) enzyme, 4 (1.6%) for Verona Integron-encoded Metallobetalactamase (VIM), and all were negative for Imipenemase (IMP), and Oxacillinase (OXA), and *Klebsiella pneumoniae* Carbapenemase (KPC) genotypes. The sensitivity of mCIM/eCIM observed in the study was 67.12%, and specificity was 85.71% when compared to the molecular genotype (GeneXpert® Carba R test). The modified Carba NP test detected carbapenemases in 229 isolates, yielding an overall sensitivity of 95.07% and a specificity of 74.07%. The overall sensitivity and specificity of the Carba M test for detecting carbapenemase-producing GNB were 97.31% and 70.37%, respectively. The isolates producing Class A/D (OXA-48-like) enzymes were identified with sensitivities of 98% and specificities of 73.68%, while those producing Class B (NDM, VIM) enzymes showed sensitivities of 94.87% and specificities of 87.5%.

Conclusion: The Carba M test is a cost-effective, feasible, and rapid phenotypic test for detecting and differentiating Class B from Class A/D carbapenemases. The Carba M test could serve as a supplemental test alongside the Carba R, Carba NP test, or mCIM/eCIM for diagnosing carbapenemase production in GNB.

Keywords: Carbapenem-resistant enterobacteriaceae, Clinical laboratory testing, Diagnostic techniques and procedures, *Pseudomonas aeruginosa*, Rapid diagnostic tests

INTRODUCTION

Carbapenem resistance among GNB is most commonly due to the expression of carbapenemase enzymes, followed by other mechanisms such as efflux pumps and porin loss [1]. The source of carbapenemase resistance is harboured on mobile genetic elements that are easily transferable from one bacterium to another, such as *Pseudomonas aeruginosa, Acinetobacter* spp*., Escherichia coli*, and *Klebsiella* spp. The World Health Organisation (WHO) designated these carbapenemase-producing organisms as "high priority" in 2017. Recently, in 2024, *Pseudomonas aeruginosa* carbapenemases were changed to "medium priority [2]."

The most common beta-lactamase (bla) genes include blaKlebsiella pneumoniae carbapenemase (*blaKPC*), blaOxacillin-hydrolysing enzymes-48 (bla_{OXA-48}), *bla*Verona integron-mediated metallo-β-lactamase (bla_{VIM}), *bla*-New Delhi metallo-β-lactamase (bla_{NDM}), and *bla*-active on imipenem (bla_{IMP}). These genes are found worldwide and are responsible for the spread of nosocomial infections, resulting in significant mortality. According to Ambler's molecular classification, these carbapenemases are classified into Class A, Class B, and Class D β-lactamases. Class A and D enzymes facilitate serine-based hydrolysis, while Class B enzymes, known as metallo-β-lactamases (MBL), utilise zinc in their active sites. Notable members of Class A carbapenemases include KPC (*Klebsiella pneumoniae* carbapenemase), IMI (Imipenem-hydrolysing beta-lactamase), and SME families. Among them, KPC is the most prevalent carbapenemase gene detected and reported worldwide, while the most common genotypes in developing countries like India are NDM followed by OXA-48. Class B metallo-β-lactamases include NDM, IMP, and VIM, while Class D includes OXA-48, the oxacillin-hydrolysing carbapenemase enzymes [3]. Failure to promptly

identify carbapenem resistance may result in ineffective treatment with less potent antibiotics and/or adverse patient outcomes [4].

A 5-year study conducted in 2021 reported a prevalence of 29.7% Carbapenem-Resistant Enterobacterales (CRE) in India [5]. The average prevalence of CRE was 13.95% in Southern India and has been increasing over time [6]. The prevalence of CR non fermentative GNB was 8.7%, with *Acinetobacter baumannii* and *Pseudomonas aeruginosa* being the predominant species; all isolates were obtained from pus samples [7]. In one study conducted in Eastern India, the prevalence of non fermentative GNB was found to be 411 (13.18%) [6]. In the domain of carbapenem resistance detection, timely identification is crucial for effective management [8].

Conventional antimicrobial susceptibility testing, despite its reliability, has a prolonged turnaround time of two to five days, which makes it inferior in urgent situations. Rapid molecular methods, such as the Carba R-GeneXpert, offer a more expeditious alternative, providing results within two to three hours [9]. Phenotypic methods, including Carba M, Modified Carba NP, mCIM, and eCIM, can identify carbapenemase production [10]. The genotypic method, Xpert Carba R test, exhibits 100% sensitivity and specificity, detecting the five most common carbapenemases: KPC, IMP, NDM, and OXA-48-like, within a two-hour time frame [11]. Accurate detection of carbapenem resistance is critical for timely diagnosis and treatment. The appropriate method should be guided by considerations of sensitivity, specificity, and practical feasibility in clinical settings, where the Carba M test could prove valuable because it not only detects carbapenemase production but also distinguishes between the classes of carbapenemase enzymes [12].

Multidrug-resistant or carbapenemase-producing GNB are of great concern and pose a serious threat to patients, treating physicians, microbiologists, and infection control teams. There is a need for rapid tests capable of simultaneously detecting and classifying carbapenemases among clinical isolates of GNB. Recently, class A and D carbapenemase inhibitors, such as avibactam, relabactam, and vaborbactam, have been introduced into clinical practice. However, these inhibitors do not act on class B enzymes (metallo-beta-lactamase producers). Thus, differentiating carbapenemase classes will aid clinicians in selecting the most appropriate carbapenemase inhibitor for critically ill patients.

Present study aimed to assess the role of the modified Carba M test for rapid confirmation and differentiation of class B from class A and D carbapenemases. This will strengthen diagnostic stewardship, antimicrobial stewardship, and infection control and prevention activities, facilitating the detection and containment of Multidrug-Resistant Organisms (MDROs). As a result, phenotypic methods to detect carbapenemase-producing isolates were used, which are important for choosing empirical therapy, designing effective antibiotic policies, updating local antibiotic guidelines for physicians, and determining clinical treatment [13-15].

Diagnostic stewardship plays a key role in the management of infectious diseases by enabling clinicians and clinical microbiologists to select the appropriate tests for diagnosis and prognosis.

The aim of the study was to conduct a comparative evaluation of the Carba M Test, Carba NP Test, Carbapenem inactivation method, and Carba R Test for the rapid detection of Carbapenemase-producing GNB in a tertiary care hospital.

The primary objective was to evaluate the performance of the Carba M test for the phenotypic detection, characterisation, and classification of carbapenemase-producing GNB. The secondary objectives were to identify and differentiate carbapenemase-producing GNB using the modified Carba NP test, the carbapenem inactivation method, and the genotypic Carba R test.

This study also compared the evaluation and diagnostic utility of the Carba M test, Carba NP test, Carba R test, and m-CIM and/or e-CIM in the detection and characterisation of carbapenemase-producing GNB.

MATERIALS AND METHODS

A cross-sectional study was conducted in Department of Microbiology of a 1400 bedded PSG Hospitals, Coimbatore, Tamil Nadu, India. The study was conducted for a period of 15 months (June 2022 to August 2023). Institutional Human Institutional Ethical Committee (Ref. No.: PSG/IHEC/2022/Appr/Exp/167; Project No.: 22/163. Approval number PSG/ IHEC/ 2023/ Renew/ 100) was obtained. Waiver of consent applicable.

Inclusion criteria: The study population included CR-GNB identified using the Vitek 2 compact system. The criteria for inclusion were as follows: for Enterobacterales, an imipenem or meropenem Minimum Inhibitory Concentration (MIC) ≥ 4 μg/mL or an ertapenem MIC ≥2 μg/mL; for *Pseudomonas aeruginosa*, an imipenem or meropenem MIC ≥8 μg/mL; and for *Acinetobacter baumannii* and *Stenotrophomonas maltophilia*, an imipenem or meropenem MIC ≥8 μg/mL.

Exclusion criteria: Drug-susceptible Gram-negative isolates, including carbapenem-sensitive or intermediate GNB, were identified using the Vitek 2 compact system. The criteria for exclusion were as follows: for Enterobacterales, an imipenem or meropenem MIC ≤2 μg/mL or an ertapenem MIC ≤1 μg/mL; for *Pseudomonas aeruginosa* and *Acinetobacter baumannii*, an imipenem or meropenem MIC ≤4 μg/mL. Additionally, samples that grew isolates other than GNB during the study period were excluded. Duplicate isolates from the same patients were also not included in the study.

Sample size calculation: Sample size was calculated by using this formula,

$$n=Z^2 p (1-P)/e^2$$

where 'Z' is the value for the corresponding confidence level (CI) - 1.96 for 95% CI; e is the margin of error (0.05=±5%) and p is the estimated value for the proportion of a sample that have the condition of interest.

$n=(1.96)^2\times0.297\times(1-0.297)/(0.05)^2\approx320$ sample size calculated [4], 250 Carbapenemase producing GNB as appropriate study population to finish the study within study period were included.

Study Procedure

A total of 250 non duplicate CR-GNB isolates, namely Enterobacterales, *Pseudomonas aeruginosa, Acinetobacter baumannii,* and *Stenotrophomonas maltophilia*, were included in the study population. CR-GNB isolated from specimens received from inpatients and outpatients for culture and sensitivity testing in the bacteriology section, belonging to various clinical departments during the study period, were included. Identification and antibiotic susceptibility testing were performed using routine manual conventional methods, followed by automated phenotypic identification using the Vitek-2 Compact ID and AST system (bioMérieux). Quality assurance was ensured using reference control standard strains, namely *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853 [7]. The phenotypic tests conducted were as follows: a. Carba M test [6,8], b. Carba-NP test [7], and c. Modified carbapenem inactivation method (mCIM), with or without the EDTA carbapenem inactivation method (eCIM) [7]. The genotypic test was performed using the Xpert Carba-R assay [12], which detects bla_{NDM}, bla_{KPC}, bla_{VIM}, bla_{OXA-48}, and bla_{IMP}. The results were tabulated for data analysis to evaluate the performance of these tests in detecting carbapenemase production and the type of carbapenemase, in accordance with standard operating procedures. In-house kits (Carba M, Carba NP, m-CIM, e-CIM, and Carba R) were prepared using chemicals/reagents procured from Himedia Laboratories Pvt., Ltd., India. Positive and negative controls were tested using carbapenemase-producing *Escherichia*

coli (clinical isolate) and *Escherichia coli* ATCC 29222, respectively, and were found to be satisfactory.

Carba M Test (Rapid MBL Confirmatory Test): The Carba M test was performed using direct colony isolation with in-house extraction reagents/chemicals, and the substrate used was imipenem-cilastatin, as suggested in the study by Rudresh SM et al., [9]. The Carba M test consists of three reagents in test tubes labeled 'A,' 'B,' and 'C.' All three tubes were incubated for a maximum of two hours at 37°C. The isolates were tested three times and interpreted by three different observers. Each tube's colour change was visually read and interpreted, as depicted in [Table/Fig-1].

Tube "a": Solution A (serves as internal control)	Tube "b": Solution B	Tube "c": Solution C	Interpretation
Red or red-orange	Red or red-orange	Red or red-orange	Negative No Carbapenemases detected
Red or red-orange	Light orange, dark yellow, or yellow	Red or red-orange	Positive Class B Carbapenemase detected
Red or red-orange	Light orange, dark yellow or yellow	Light orange, dark yellow or yellow	Positive Class A/D Carbapenemases detected
Red or red-orange	Dark yellow	Light orange or yellow	Positive Class A/D/B Carbapenemases detected
Red or red-orange	Orange	Orange	Invalid

[Table/Fig-1]: Interpretation of Modified Carba M test [9].

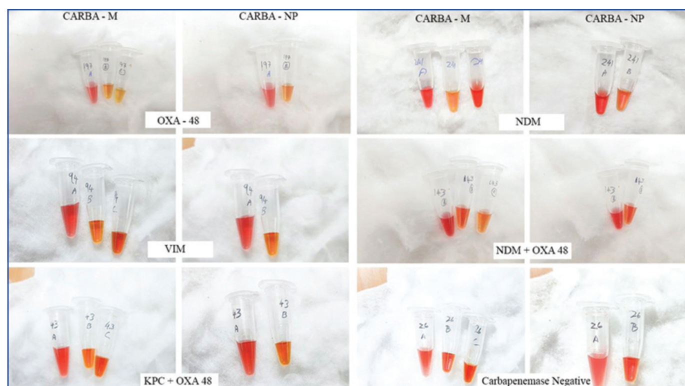
Modified CARBA NP test: The test was conducted in accordance with the description by Nordmann, Poirel, and Dordet. Approximately 5-6 colonies from a fresh culture plate were obtained and added to a 2 mL Eppendorf tube. Subsequently, 10 µL of the inoculum were introduced into two wells, one of which contained imipenem. GNB that produced MBL were treated with imipenem as the carbapenemase zinc substrate. Positive results were defined as a colour shift from red to yellow, orange, or thick orange compared to the control well. For additional analyses, organisms that tested positive for carbapenemase were used [10,11]. The isolates were tested three times and interpreted by three different observers. The results and interpretation of the Modified CARBA NP test are shown in [Table/Fig-2,3], respectively.

Tube "a": Solution A (serves as internal control)	Tube "b": Solution B	Interpretation
Red or red-orange	Red or red-orange	Negative, No Carbapenamase detected
Red or red-orange	Light orange, dark yellow, or yellow	Positive, Carbapenamase producer
Red or red-orange	Orange	Invalid
Orange, light orange, dark yellow, or yellow	Any colour	Invalid

[Table/Fig-2]: Interpretation of Modified Carba NP test as per CLSI [10].

Modified Carbapenem Inactivation Method (mCIM) and EDTA-Modified Carbapenem Inactivation Method (eCIM) Tests: The mCIM and eCIM tests were performed following the protocol recommended by the Clinical Laboratory Standards Institute (CLSI) guidelines, specifically [Table/Fig-3c] of CLSI-M31 [10]. The interpretation of these tests was conducted as illustrated in [Table/Fig-4,5], respectively.

Gene Xpert Carba-R Assay: The cartridge-based nucleic acid amplification test, run on the Cepheid GeneXpert platform, is designed for the rapid detection and differentiation of five carbapenemase genes (bla_{KPC}, bla_{IMP}, bla_{NDM}, bla_{VIM}, and $bla_{OXA-48-like}$) using the Carba-R test [9]. Less than one minute of hands-on time is needed to complete the two simple procedures required to operate the Xpert Carba-R assay within an hour. According to the



[Table/Fig-3]: Isolates tested and interpretation of Carba M and Carba NP test [6,7].

Interpretation of m-CIM	
Carbapenemase positive:	Zone diameter of 6-15 mm or presence of Pinpoint colonies within a16-18 mm zone
Carbapenemase negative:	Zone diameter ≥19 mm (clear zone)
Carbapenemase indeterminate:	Zone diameter of 16-18 mm Zone diameter of ≥19 mm and the presence of pinpoint colonies within the zone
Interpretation of e-CIM	
Interpreted only when mCIM is positive	
Metallo-β-lactamase positive:	A ≥5 mm increase in zone diameter for eCIM Vs zone diameter for mCIM
Metallo-β-lactamase negative:	A ≤1 mm increase in zone diameter for the eCIM vs zone diameter of mCIM

[Table/Fig-4]: Interpretation of mCIM with conjunction with eCIM [10].

[Table/Fig-5]: Isolates tested and interpretation of m-CIM/e-CIM test [7].

manufacturer's instructions, a 10 µL solution of a 0.5 McFarland standard suspension of the sample was added to a 5 mL Xpert Carba-R sample reagent vial and mixed for 10 seconds. The sample reagents were then added to the Xpert Carba-R kit [12] and analysed using the Cepheid GeneXpert platform. Antibiotic susceptibility testing was performed using an automated method, and the results were interpreted in accordance with CLSI guidelines, with quality assurance being satisfactory. The imipenem MIC was determined using VITEK 2. Isolates of Enterobacteriaceae with an imipenem MIC of ≥4 µg/mL and isolates of *Pseudomonas aeruginosa or Acinetobacter* spp. with an imipenem MIC of ≥8 µg/mL were interpreted as CR [10].

STATISTICAL ANALYSIS

The data were analysed using Microsoft Excel and Statistical Package for the Social Sciences (SPSS) IBM Version 28.0 statistical analysis software. The odds ratio and p-value were calculated to determine statistical significance for a 95% CI, and the data were interpreted accordingly.

RESULTS

Out of 250 CR-GNB isolated during the study period, 83 (33.2%) were from the MICU, followed by 24 (9.6%) from the Medicine department. Enterobacteriaceae accounted for 229 (91.6%) of the isolates, followed by *Pseudomonas aeruginosa* with 17 (6.8%) and *Acinetobacter* species with 4 (1.6%). *Klebsiella pneumoniae* was the most common isolate among Enterobacteriaceae, accounting for 216 (86.4%). The results of the carbapenemase resistance

(Carba R) testing in the study population revealed that 100 (40%) isolates produced the OXA-48 enzyme, 35 (14%) produced the NDM enzyme, and 4 (1.6%) produced the VIM enzyme. The remaining isolates had multiple carbapenemase genes; 89 (35.6%) were positive for both NDM and OXA-48, and one isolate (0.4%) was positive for both KPC and OXA-48. Genotypes IMP and KPC enzymes were not found among the study group. Nearly 21 (8.4%) samples of *Klebsiella pneumoniae* lacked carbapenemase enzymes and were found to be susceptible. A total of 229 carbapenemase-positive isolates and 21 carbapenemase-negative isolates were tested using the modified Carbapenem Inactivation Method (m-CIM)/ enhanced Carbapenem Inactivation Method (e-CIM).

The Carba M test detected all isolates producing carbapenemase, with the exception of two isolates of class B enzyme, two isolates of class A/D enzyme, and one isolate in which both class B and class A/D enzymes co-existed. The isolates producing class A/D (OXA-48-like) enzymes were identified with 98% sensitivity and 73.68% specificity by the Carba M test. The isolates with class B (NDM, VIM) producing enzymes were identified with a sensitivity of 94.87% and specificity of 87.5% by the Carba M test. The overall sensitivity and specificity of the Carba M test for detecting carbapenemase activity among the isolates were 97.31% and 70.37%, respectively (p-value <0.0001; odds ratio: 85.9). Additionally, the test could differentiate class B from class A and D enzymes.

The sensitivity and specificity of m-CIM/e-CIM were found to be 67.12% and 85.71%, respectively (p-value <0.0001; odds ratio: 23.46; CI: 95%), when compared to the Carba R test. Although these 21 isolates showed carbapenem resistance genotypically by GeneXpert Carba R, they were found to be negative for carbapenemase detection by the phenotypic Carba M test method, as depicted in [Table/Fig-6].

Strain (n=250)	Carbapenemase type	n (%)
Klebsiella pneumoniae (n=216)	NDM	17 (6.8)
	OXA-48	92 (36.8)
	NDM+OXA-48	85 (34)
	KPC+OXA-48	1 (0.4)
	No carbapenemase	21 (8.4)
Pseudomonas aeruginosa (n=17)	VIM	3 (1.2)
	NDM	7 (2.8)
	OXA-48	4 (1.6)
	NDM+OXA-48	3 (1.2)
Escherichia coli (n=7)	NDM	7 (2.8)
Acinetobacter baumannii (n=4)	NDM	1 (0.4)
	OXA-48	3 (1.2)
Klebsiella oxytoca (n=3)	VIM	1 (0.4)
	NDM	2 (0.8)
Citrobacter koseri (n=1)	NDM	1 (0.4)
Stenotrophomonas maltophilia (n=1)	OXA-48	1 (0.4)
Proteus mirabilis (n=1)	NDM+OXA-48	1 (0.4)

[Table/Fig-6]: Results of Carba M test among study population (N=250).

The sensitivity of the mCIM combined with eCIM for detecting different carbapenemases is shown in [Table/Fig-7].

The modified Carba NP test detected carbapenemases in 229 carbapenemase-producing isolates, with an overall sensitivity of 95.07% and a specificity of 74.07% (p-value <0.0001; odds ratio: 17.2; CI: 95%). The results of the Carba M and Carba NP tests were compared and are depicted in [Table/Fig-8].

The strains that co-produce NDM or KPC with OXA-48-like enzymes (Class B with Class A/D) were classified as Class B, Class A/D, or as having the co-existence of both enzymes. Among them, 36 isolates were interpreted as positive for Class A/D producing enzymes, while four isolates were positive for Class B producing enzymes. Out of 250 CR-GNB, 229 isolates acquired carbapenem resistance primarily due to plasmid-mediated carbapenemases, which were detected using Carba NP, mCIM, and eCIM methods. However, considering the molecular GeneXpert Carba-R assay as the gold standard, the sensitivity and specificity of the Carba-R test were compared with each of the tests, including Carba M, Carba NP, mCIM, and eCIM, as tabulated below in [Table/Fig-9].

mCIM±eCIM	Carba R test results					
	bla_{NDM}	bla_{VIM}	bla_{OXA-48}	bla_{NDM}/ bla_{OXA48}	bla_{KPC}/ bla_{OXA-48}	Total
True positive	31 (86.11%)	4 (80%)	66 (58.4%)	75 (78.9%)	1 (100%)	177 (70.8%)
False negative	4 (11.11%)	0	31 (27.43%)	14 (14.7%)	0	49 (19.6%)
True negative	-	1 (20%)	13 (11.5%)	6 (6.3%)	-	20 (8%)
False positive	1 (2.78%)	-	3 (2.6%)	-	-	4 (1.6%)
Total	36 (14.4%)	5 (2%)	113 (45.2%)	95 (38%)	1 (0.4%)	250 (100%)

[Table/Fig-7]: Results of mCIM and/or eCIM compared to Carba R test (N=250).
*If m-CIM is positive; Class A/D carbapenemase and If m-CIM and e-CIM is positive; It is Class B Metallobetalactamase (MBL). So, both are commonly referred to as Carbapenem Inactivation Methods and considered the results of this together for calculation. Thus the sensitivity and specificity of m-CIM and e-CIM are combined

Carbapenemase	β-lactamase (n)	Carba M test			Modified Carba NP	
		Class B	Class A/D	Negative	Positive	Negative
Class B enzymes (n=39)	NDM (35)	34 (13.6%)	0	1 (0.4%)	34 (13.6%)	1 (0.4%)
	VIM (4)	3 (1.2%)	0	1 (0.4%)	3 (1.2%)	1 (0.4%)
Class D enzyme (n=100)	OXA-48 like (100)	0	98 (39.2%)	2 (0.8%)	98 (39.2%)	2 (0.8%)
Co-production of Class B and Class A/D enzymes (n=90)	NDM+OXA-48 like (89)	88 (35.2%)		1 (0.4%)	88 (35.2%)	1 (0.4%)
	KPC+OXA-48 like (1)	1 (0.4%)		0	1 (0.4%)	0
No carbapenemase (n=21)	None	2 (0.8%)	5 (2%)	14 (5.6%)	7 (2.8%)	14 (5.6%)
Total	250			250		

[Table/Fig-8]: Results of Carba M test and modified Carba NP test (N-250).

Phenotypic tests	Carba R test (Genotypic test)		Odd's ratio	p-value	CI	Statistical significance
	Sensitivity (%)	Specificity (%)				
Carba M	97.31	70.37	85.9	<0.0001	95%	Significant
Modified Carba NP	95.07	74.07	17.2	<0.0001	95%	Significant
mCIM and eCIM	67.12	85.71	23.46	<0.0001	95%	Significant

[Table/Fig-9]: Comparative analysis of Carba M, Carba NP m-CIM/e-CIM with Carba R test.

DISCUSSION

The rapid and accurate detection of carbapenemases is of significant importance in addressing the threat posed by the global transmission of CR bacteria [15,16]. To effectively combat the threat of the worldwide spread of CR bacteria, it is essential to quickly and precisely detect carbapenemases. Carbapenemase detection is not only important for effective clinical chemotherapy against infections but also for the prevention and control of carbapenemase-producing strains in healthcare-associated environments [17]. Numerous

techniques have been employed to identify carbapenemases. A rapid, reliable, accurate, and cost-effective phenotypic test for detecting carbapenemase-producing organisms is necessary to aid clinicians in selecting appropriate carbapenemase inhibitor drugs and to improve infection control practices [18,19].

A study reported an overall sensitivity and specificity of 89.74% and 100% for carbapenemase detection [6]. In contrast, 100% sensitivity and specificity were reported for identifying Class B NDM and VIM enzymes using the Carba M test. The authors also highlighted that this test could effectively differentiate Class B from Class A/D enzymes. Present study results showed a sensitivity of 97.31% and a specificity of 70.37% when compared to the Carba R test, and were able to differentiate Class B from Class A/D, similar to the findings reported by Rudresh SM et al., [9]. The Carba M test is reasonably affordable, provides quick results, and requires chemicals that are readily available in the laboratory. The modified Carba NP test is an improved method for detecting carbapenemase production. In the Carba NP test, a change in the colour of the pH indicator indicates carbapenem hydrolysis caused by bacteria that produce acid, particularly carbapenemase-producing bacteria. The Carba M test not only detects carbapenemase enzymes with the same accuracy as the Carba NP test but also differentiates Class B (NDM, VIM, IMP) from Class A and D (KPC and OXA-48-like) types of carbapenemases. Therefore, the Carba M test can be considered superior to the Carba NP test.

The strains that co-produced two MBL enzymes were detected with 100% sensitivity. The test had a sensitivity of 42.85% for the detection of Oxacillinase (OXA)-48-like enzymes [9]. Another study reported 100% sensitivity and 100% specificity of the Modified Carba NP test when compared to the gold standard Carba R test [20]. The performance of the Carba NP test in present study showed a sensitivity of 95.07% and a specificity of 74.07%, which was statistically significant but lower compared to another study [11]. A study by Rudresh SM et al., reported a sensitivity of 89.74% and a specificity of 100% (n=175) [9,11]. Walthall K et al., used both the commercial kit Rapidec NP and an in-house Carba NP test for the detection of carbapenemase-producing Enterobacterales and *Pseudomonas aeruginosa*. In that study, Walthall K et al., reported that 65 out of 72 (90%) were identified as carbapenemase-producing isolates using the commercial Carba NP kit, of which about seven isolates were found to be false negatives. Overall, Walthall K et al., reported a sensitivity of 90.2% and specificity of 100% for the RAPIDEC Carba NP test, followed by 94.4% sensitivity and 100% specificity for the conventional Carba NP test, and 100% sensitivity and 81.8% specificity for mCIM. The conventional Carba NP test detected all carbapenemase producers except for four OXA-48-like–producing isolates [13].

The Carba M test showed superior results to the Carba NP test by differentiating class B from class A/D carbapenemase enzymes in our study. If OXA-48-like enzymes are highly expressed, tube “C” turns yellow due to the hydrolysis of imipenem, indicating class A and D. Conversely, if the expression of OXA-48-like enzymes is low, it is unable to act on imipenem, and tube C shows no color change, leading to the interpretation that it belongs to class B [17,18]. The emergence of Ceftazidime-Avibactam (CZA) resistance, which has recently been introduced for definitive therapy, has been reported in studies conducted by Shields RK et al., [20]. This highlights the urgent need for effective control of carbapenem resistance.

The Carba M test results would assist clinical microbiologists in advising treating physicians on the choice of antibiotics for the treatment of NDM/OXA-48/KPC-producing GNB. Once this is achieved, clinicians could initiate early and appropriate definitive therapy for these carbapenemase-producing GNB infections, enhancing patient outcomes and reducing mortality and morbidity. The Carba M test would also serve as an epidemiological tool and a supplemental test for the detection of carbapenemase-producing GNB and is an economical alternative to the Carba R test and commercial Carba NP tests. However, multicentric studies involving a larger population would prove its real value. Diagnostic stewardship through the introduction of the Carba M test would be a good option to strengthen antimicrobial stewardship practices for the treatment of CR Enterobacterales and *Pseudomonas aeruginosa* in hospital settings.

Limitation(s)

The role of other co-existing mechanisms, such as porin loss or efflux pumps, in the causation of carbapenem resistance was not evaluated. Only 3 VIM-producing strains and 1 KPC+OXA-48 producing strain were included, so the performance of these methods in the detection of VIM and KPC+OXA-48 should be further assessed in future research. Furthermore, as no IMP and isolated KPC-producing strains were included in the study, additional testing is required to determine the test’s reliability for IMP and KPC-producing strains.

CONCLUSION(S)

The Carba M test is a rapid phenotypic test and an epidemiological tool used to detect and distinguish Class B from Class A/D carbapenemases. The Carba M test can be utilised as a supplemental test in conjunction with the Carba R, Carba NP test, or m-CIM/e-CIM for the diagnosis of carbapenemase production in GNB.

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PARTICULARS OF CONTRIBUTORS:
1. Associate Professor, Department of Microbiology, PSG Institute of Medical Sciences and Research, Coimbatore, Tamil Nadu, India.
2. Medical Intern, Department of Microbiology, PSG Institute of Medical Sciences and Research, Coimbatore, Tamil Nadu, India.
3. Postgraduate Student, Department of Microbiology, PSG Institute of Medical Sciences and Research, Coimbatore, Tamil Nadu, India.
4. Professor and Head, Department of Microbiology, PSG Institute of Medical Sciences and Research, Coimbatore, Tamil Nadu, India.
5. Associate Professor, Department of Microbiology, PSG Institute of Medical Sciences and Research, Coimbatore, Tamil Nadu, India.

NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:
Dr. R Someshwaran,
1/96, IV-D, The Icon Premium Apartments, Eachanari,
Coimbatore-641021, Tamil Nadu, India.
E-mail: drsomeshwaran@gmail.com

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