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Original Research Article

In-house Carba NP - II test to identify and differentiate carbapenemase-producing Gram-negative bacteria among various clinical isolates in comparison with immunochromatography assay

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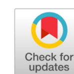

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ABSTRACT

Background: Resistance to Carbapenems has increased drastically resulting in ineffective treatment, prolonged hospital-stay, increased morbidity and mortality. A cost-effective in-house test that can differentiate carbapenemase enzymes belonging to different Amblers Class can emerge as an important tool in middle and low-income countries for guiding the clinicians in selecting the most appropriate antibacterial therapy. Cost effective commercial ICT kits are also available now for the same use.
Aims & Objectives: 1. To adapt an in-house test that can identify and differentiate Ambler class carbapenemase enzymes among the clinical isolates of multi-drug resistant Gram-negative bacteria (GNB); 2. To evaluate the commercially available immunochromatography (ICT) lateral flow assay of carbapenemase detection in comparison to in-house Carba NP – II test
Material and Methods: A prospective study conducted in the Department of Microbiology of NRI Institute of Medical Sciences, Visakhapatnam. All GNB isolates irrespective of source, age and gender, were considered in the study. A total of 565 GNB isolates were obtained, which were subsequently tested for Meropenem resistance by routine antimicrobial susceptibility test (AST), out of which 195 isolates were further tested for carbapenemase resistance by in-house Carba NP - II test and ICT (Bio-State Inc).
Result: Out of 565 isolates, 195 were Meropenem resistant, 120 belonged to Class B and 75 to Class D by in-house modified Carba NP-II test. In ICT, 117 belonged to Class B and 73 to Class D and 5 isolates showed both the enzymes of Class B and D. No class A enzyme was detected in our study by either method.
Conclusion: Carba NP - II Test is a reliable and cost-effective test with similar results in comparison to commercially available ICT.

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1. Introduction

Carbapenems are the most effective antibacterial agents available for the treatment of multidrug-resistant bacterial infections. However, the irrational and widespread use of carbapenems in unwarranted and avoidable situations has led to the emergence of certain enzymes (carbapenemase) that have the ability to hydrolyze the carbapenem ring and render the antibiotic useless. Carbapenem resistance in Gram-negative pathogens like *Enterobacteriaceae*, *Pseudomonas* species, *Acinetobacter* species has been on rise in recent years and is an ongoing public-health problem of global concern.[1]

Carbapenem resistance is mediated by transferable carbapenemase-encoding genes, hence they spread rapidly culminating in life threatening outbreaks and massively narrowing down the treatment options.[2] Carbapenem resistant Gram-negative (CR-GNB) bacteria may contain any of the following enzymes such as KPC (Ambler class

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A), Metallo-b-lactamases (MBLs) of the VIM, IMP, and NDM types (Ambler class B), and OXA-48-type enzymes (Ambler class D).[1]

The most effective carbapenemases, with respect to carbapenem hydrolysis and spread, are KPC, VIM, IMP, NDM and OXA-48 types.[2] KPCs inactivate all beta-lactam antibiotics and are only partially inhibited by beta-lactamase inhibitors like clavulanic acid, tazobactam and boronic acid. MBLs hydrolyze all beta-lactams except aztreonam and are not inhibited by the beta lactamase inhibitors like clavulanic acid, tazobactam and boronic acid. They have zinc in their active center, hence their inhibition is achieved in vitro using metal chelators, such as ethylenediaminetetraacetic acid (EDTA).[2,3] Therefore the identification of type of carbapenemase produced by CR-GNB is important for appropriate selection of antibiotic therapy.[4]

Many phenotypic tests are available for detection of carbapenem resistance; however, commonly employed methods are -

1. Modified Hodge Test (MHT)
2. Carba NP and variant
3. Modified Carbapenem inactivation method
4. Lateral Flow Immunoassay.

In resource limited middle and low-income countries, a reproducible, reliable, and cost-effective in-house test to detect and differentiate Carbapenemases belonging to Ambler's molecular Class A, B, and D can play instrumental role in guiding the treating doctor for appropriate antibiotic therapy, which will subsequently contribute to successful implementation and adoption of antimicrobial stewardship in the times of global antibiotic crisis.

2. Aims and Objectives

1. The primary aim of this study was to identify and differentiate carbapenemase producing gram negative bacteria (CR-GNB) among various clinical isolates using in-house Carba NP - II test.
2. The secondary aim of our study was to compare in-house Carba NP - II test to commercially available immunochromatography essay (ICT).

3. Materials and Methods

3.1. Study design

Prospective cross-sectional study.

3.2. Study setting

Department of Microbiology, NRIIMS, Visakhapatnam.

3.3. Study duration

May 2023 to October 2023.

3.4. Inclusion criteria

All gram-negative bacteria showing carbapenem resistance on routine AST irrespective of source, age, and gender.

3.5. Exclusion criteria

1. Bacterial Isolates from stool samples.
2. Same isolate from duplicate sample.
3. Bacterial isolates with doubtful identification.

3.6. Bacterial isolates

1. A total of 565 isolates of GNB were isolated from various clinical samples (e.g., endo-tracheal aspirates, blood, pus, urine, sputum, etc.) during the study period.
2. Out of which 195-gram negative bacteria were showing resistance to carbapenems on routine AST by Kirby Bauer disk diffusion method as per standard operating procedure (SOP).

3.7. Carba NP - II test

1. The isolates that showed resistance to carbapenems on routine AST were subsequently subjected to Carba NP - II test.
2. First Carba NP test solutions are prepared:
   (a) 0.1 N NaOH- by adding 40 mg in 10 ml distilled water,
   (b) 10% HCL,
   (c) Diluted solution of Phenol Red - prepared by using 0.5% wt/vol phenol red + 16.6ml distilled water (0.5% phenol red can be prepared by adding 50 mg of phenol red powder in 10 ml distilled water).
3. Carba NP - II test requires an additional:
   (a) 10 mM ZnSo4.7H2O - prepared by adding 28.8 mg in 10 ml distilled water.
   (b) 20 mM Tris HCL - by adding 314 mg in 10 ml distilled water.
   (c) Imipenem - Cilastatin 1 gram injectable powder of Lupin Pharmaceuticals.
   (d) Piperacillin and Tazobactam injectable powder of Intas Pharmaceuticals.
   (e) 30 mM EDTA - by adding 111.7 mg in 10 ml distilled water.
4. Using these four solutions A, B, C, and D are prepared (Figure 1)[4]
5. Carba NP - II test is performed using these four solutions (Figure 2).
6. Interpretation of Carba NP - II test results
   (a) Colour change from RED to ORANGE or YELLOW indicates positive results (Ambler Class A, or B or D). No colour change indicates negative results. (Figure 3a & b).

(b) Quality Control Strains Used.

i. *Klebsiella pneumoniae* ATCC 1705 MHT contain KPC.

ii. *Escherichia coli* ATCC BAA 2469 contain NDM.

iii. *Klebsiella pneumoniae* ATCC BAA 2524 contain OXA-48.

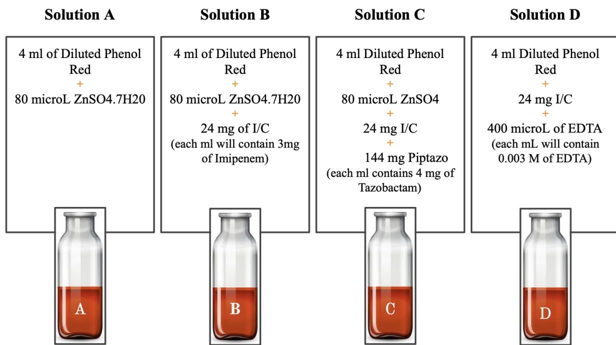

Figure 1: Preparation of four solutions using base solutions

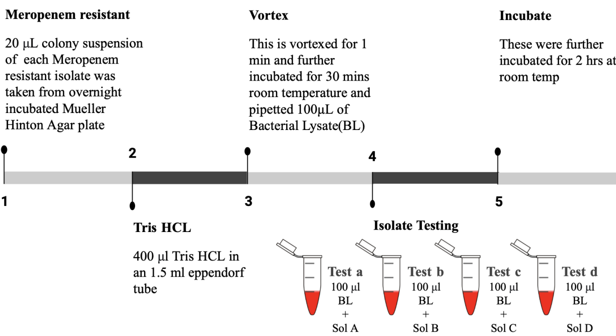

Figure 2: Timeline ofCarba NP - II test

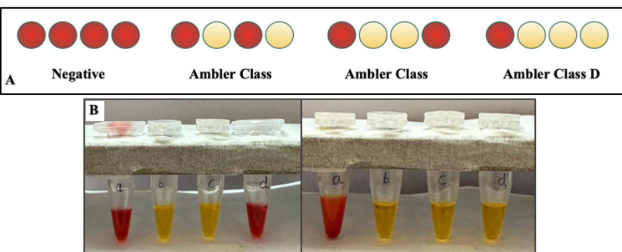

Figure 3: A: Interpretation of test results; **B:** Class B and Class D results

3.8. Immunochromatography Assay Test

1. The isolates that were subjected to Carba NP - II test were also evaluated by ICT, following the kit procedural manual (Figure 4). Positive control provided with the kit was used.
2. Interpretation of ICT test results: The presence of any red line in the test area indicates a positive result of its corresponding gene type. If the control line doesn't appear, the result is invalid, regardless of the appearance of any test line. (Figure 5)

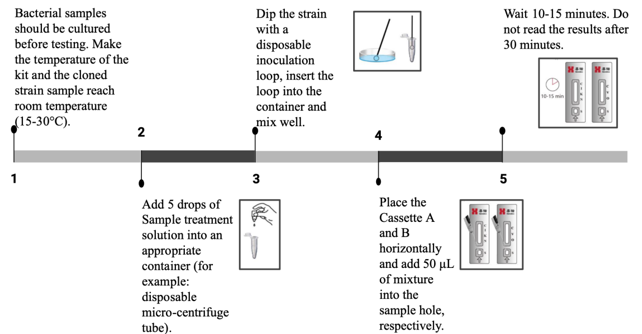

Figure 4: Timeline of ICT test

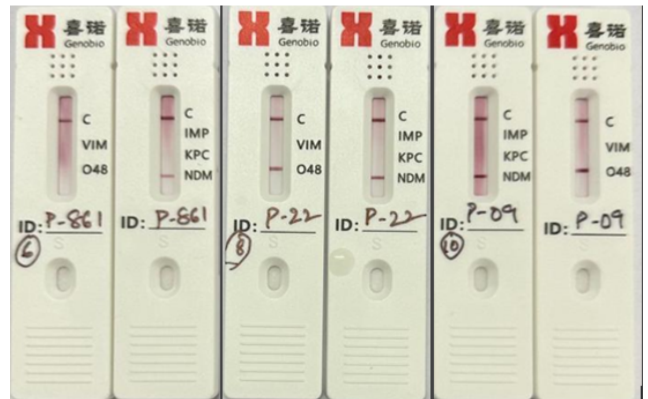

Figure 5: ICT results

4. Observations and Results

1. A total of 565 GNB were isolated during our study period. Out of which 195 met inclusion criteria.
2. Male patients in our study contributed 61.53% (n=120) and females 38.46% (n=75). Male to Female ratio in our study is 1.6:1. Age ranged from 11 to 75 years with 52.3% (n=102) belonging to 51 to 75 years (Figure 6)
3. 86% (n=167) of CR-GNB were reported from in-patients and remaining from out-patients. 36% CR-GNB were reported from the Medical Intensive Care Unit (MICU), 29.5% from Surgical Intensive Care (SICU), 12% from orthopedics, 9% from causality, and the remaining from other departments and OPD.

4. 28.2% (n=55) of CR-GNB were isolated from pus, 21.5% urine, 16.9% from endotracheal aspirates, 11.8% from wound swab and remaining from sputum, blood and Bronchial Alveolar Lavage (BAL). (Figure 7)
5. Of 195-gram negative bacilli 30.8% were identified as *Acinetobacter baumanii*, 28.7% *Klebsiella pneumoniae*, 21.5% *Pseudomonas aeruginosa*, 13.8% *Escherichia coli* and the remaining were *Enterobacter* and *Citrobacter*. (Figure 8)
6. *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* were more prevalent in pus, sputum and wound swab whereas *Acinetobacter baumannii* and *Klebsiella pneumoniae*, in Endo-treacheal aspirate (ETA) samples *Escherichia coli*, *Enterobacter* species and *Citrobacter* in urine.
7. Class A - KPC, not detected in our study by either method. However, 61.54% isolates belonged to Class B and 38.46% to Class D (OXA 48) by Carba NP -II test. ICT detected Class B in 60% isolates and Class D in 37.43% and 2.57% both.

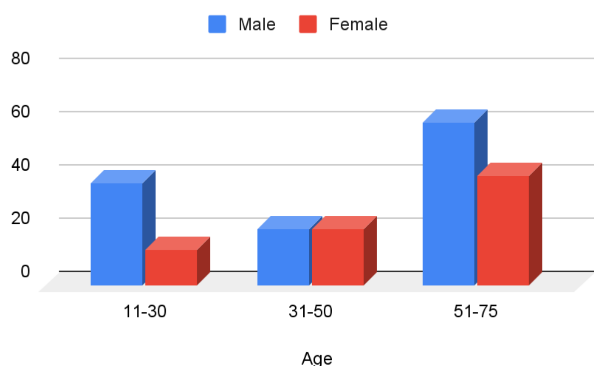

Figure 6: Barchart showing Age and Gender distribution. (X-axis: Denotes Age & Gender, Y-axis: Percentage of CR-GNB)

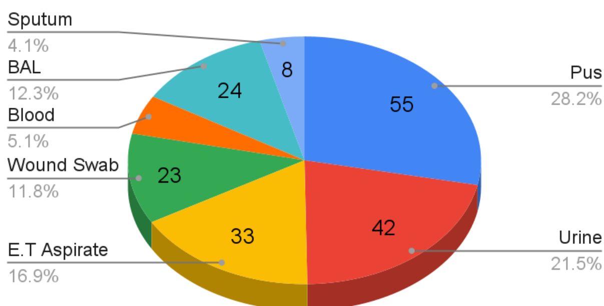

Figure 7: Pie chart showing sample distribution

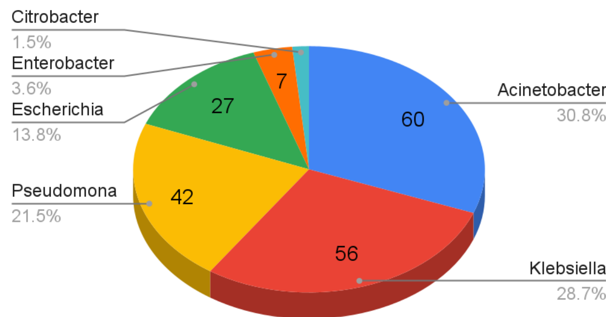

Figure 8: Pie chart showing CR-GNB distribution

Table 1: Showing carba NP - II test detected carbapenemase enzymes

CR-GNB	Class - A	Class - B	Class - D
Acinetobacter n=60		36	24
Klebsiella n=56		34	22
Pseudomonas n=42		28	14
Escherichia n=27		16	11
Enterobacter n=7		4	3
Citrobacter n=3		2	1

Table 2: Showing ICT assay detected carbapenemase enzymes

CR-GNB	KPC	NDM	VIM	IMP	OXA-48	NDM & OXA-48
Acinetobacter n=60	-	35			23	2
Klebsiella n=56	-	32		1	21	2
Pseudomonas n=42	-	24	2	1	14	1
Escherichia n=27	-	15		1	11	
Enterobacter n=7	-	4			3	
Citrobacter n=3	-	2			1	

5. Discussion

This study aimed to evaluate the performance of Carba NP - II test and immunochromatography test for the characterization and identification of carbapenemase-producing gram-negative bacteria.

In our study, 34.51% were carbapenem drug-resistant on routine AST, whereas a study by V. Mangayarkarasi et al 2018 reported 13.33% carbapenem resistance.[3] In our study 30.8% were identified as *Acinetobacter baumanii*, 28.7% *Klebsiella pneumoniae*, 21.5% *Pseudomonas aeruginosa*, 13.8% *Escherichia coli* and the remaining were *Enterobacter* and *Citrobacter* whereas in Mangayarkarasi et al 45% were identified as *Escherichia coli*, 18% identified as *Pseudomonas* spp., 16% identified as *Klebsiella pneumoniae*, 10% identified as *Acinetobacter* spp., 7% identified as *Citrobacter* spp. and 4% identified as Proteus spp.[3–6]

In-house Carba NP - II Test showed 100% sensitivity and specificity comparable to study of Laurent Dortet et al[1] whereas Nitin Kumar et al showed sensitivity and specificity for KPC detection by Modified Carba NP as 91.7% and 100% and for MBL detection as (96.7% and 100 %).[7,8] ICT in our study showed 99% sensitivity and 100 % specificity comparable to atul garg atal showed 92% and 96% sensitivity and specificity[9–11] whereas Eltahlawi RA et al study demonstrated an overall sensitivity of 69.3%.[12–15]

6. Conclusion

In-house Carba NP - II Test is an affordable and readily implementable method that can be adapted in microbiology laboratories in resource-limited countries for the identification of different classes of carbapenemases which will aid the clinician in providing appropriate antimicrobial therapy to the patients. Commercially available immunochromatographic assays are another good option for differentiation and identification of carbapenemases with similar results. However, these ICT kits are expensive.

7. Ethical Approval

Letter No. / Reference Code: IEC/NRI/68/2023, dated 08/08/2023

8. Source of Funding

None

9. Conflict of Interest

None

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References

1. Dortet L, Poirel L, Nordmann P. Rapid identification of carbapenemase types in Enterobacteriaceae and Pseudomonas spp. by using a biochemical test. *Antimicrob Agents Chemother*. 2012;56(12):6437–40.
2. Pasteran F, Tijet N, Melano R, Corso A. Simplified Protocol for Carba NP Test for Enhanced Detection of Carbapenemase Producers Directly from Bacterial Cultures. *J Clin Microbiol*. 2015;53(12):3908–11.
3. Mangayarkarasi V, Moses SP, Swarna SR, Kalaiselvi K, Fathima S. In-House Standardization of Carba NP Test for Carbapenemase Detection in Gram Negative Bacteria. *Int J Curr Microbiol App Sci*. 2018;7(1):2876–81.
4. CLSI M100 30th edition - Performance Standards for Antimicrobial Susceptibility Testing. Available from: https://webstore.ansi.org/standards/clsi/clsim100ed30.
5. Boutal H, Vogel A, Bernabeu S, Devilliers K, Creton E, Cotellon G, et al. A multiplex lateral flow immunoassay for the rapid identification of NDM-, KPC-, IMP- and VIM-type and OXA-48-like carbapenemase-producing Enterobacteriaceae. *J Antimicrob Chemother*. 2018;73(4):909–15.
6. Rudresh SM, Ravi GS, Sunitha L, Hajira SN, Kalaiarasan E, Harish BN, et al. Simple, rapid, and cost-effective modified Carba NP test for carbapenemase detection among Gram-negative bacteria. *J Lab Physicians*. 2017;9(4):303–7.
7. Kumar N, Singh VA, Beniwal V, Pottathil S. Modified Carba NP Test: Simple and rapid method to differentiate KPC- and MBL-producing Klebsiella species. *J Clin Lab Anal*. 2018;32(7):e22448. doi:10.1002/jcla.22448.
8. Campana EH, Chuster SG, Silva ID, Paschoal RP, Bonelli RR, Moreira BM, et al. Modified Carba NP test for the detection of carbapenemase production in gram-negative rods: optimized handling of multiple samples. *Braz J Microbiol*. 2017;48(2):242–5.
9. 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):7870–2.
10. Poirel L, Nordmann P. Rapidec Carba NP test for rapid detection of carbapenemase producers. *J Clin Microbiol*. 2015;53(9):3003–8.
11. Poirel L, Potron A, Nordmann P. OXA-48-like carbapenemases: the phantom menace. *J Antimicrob Chemother*. 2012;67(7):1597–606.
12. Eltahlawi RA, Jiman-Fatani A, Gad NM, Ahmed SH, Al-Rabia MW, Zakai S, et al. Detection of Carbapenem-resistance in CRE by Comparative Assessment of RAPIDEC® CARBA NP and Xpert™Carba-R Assay. *Infect Drug Resist*. 2023;16:1123–31. doi:10.2147/IDR.S393739.
13. Osterblad M, Hakanen AJ, Jalava J. Evaluation of the Carba NP test for carbapenemase detection. *Antimicrob Agents Chemother*. 2014;58(12):7553–6.
14. Hamprecht A, Vehreschild JJ, Seifert H, Saleh A. Rapid detection of NDM, KPC and OXA-48 carbapenemases directly from positive blood cultures using a new multiplex immunochromatographic assay. *PLoS One*. 2018;13(9):e0204157. doi:10.1371/journal.pone.0204157.
15. Lifshitz Z, Adler A, Carmeli Y. Comparative study of a novel biochemical assay, the Rapidec Carba NP test, for detecting carbapenemase-producing Enterobacteriaceae. *J Clin Microbiol*. 2016;54(2):453–6.

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