Short Communication

INTERNATIONAL JOURNAL OF CURRENT MEDICAL AND PHARMACEUTICAL RESEARCH

*ISSN: 2395-6429, Impact Factor: 4.656*
*Available Online at www.journalcmpr.com*
*Volume 5; Issue 07(A); July 2019; Page No. 4325-4328*
*DOI: http://dx.doi.org/10.24327/23956429.ijcmpr201907688*


# STANDARDISATION AND EVALUATION OF INHOUSE RAPID CARBA-NP AND COMPARISON OF PHENOTYPIC METHODS FOR SCREENING OF CARBAPENEMASE PRODUCING GRAM NEGATIVE BACTERIAL ISOLATES

**Swarna .S.R*[1]., Shruthi.Y[2]., Gomathi S[2] and Radha Madhavan[2]**

[1]Department of Microbiology, JIPMER, Karaikal
[2]SRM Medical College Hospital and Research Centre, Kattankulathur

**ARTICLE INFO**

***Article History:***
Received 10th April, 2019
Received in revised form 2nd May, 2019
Accepted 26th June, 2019
Published online 28th July, 2019

***Key words:***
Carbapenemase producer, carba NP test, modified hodge test, combined disk test

**ABSTRACT**

**Background:** Identification of carbapenemase producer in routine laboratories is mandatory nowadays due to the increase in the prevalence of carbapenem resistant Gram negative bacteria. Most of the laboratories has to meet the challenge in identifying the carbapenemase producers from the clinical isolate due to non-availability of molecular diagnostics. Carba-NP test is recently added by CLSI for screening of carbapenemases is a rapid and reproducible test. Therefore, this study was done to standardize the rapid carba-NP test and to screen for carbapenemase production with other phenotypic tests in a tertiary care hospital, South India.

**Methods:** This is a prospective, cross-sectional study done with 122 nonduplicate Gram negative bacterial (GNB) isolates between February - September 2015.The isolates were identified using standard procedure and subjected to antibiotic susceptibility testing byclinical and laboratory standards institute (CLSI). The rapid Carba NP test was standardized and all the isolates were tested for carbapenemase production and screened by Modified Hodge test (MHT) and Imipenem (Imp) - Imp + EDTA combined disk test (CDT).

**Results:** Out of 122 GNB screened by disc diffusion technique, 19.67% (24/122) were carbapenem sensitive and 80.32% (98/122) were carbapenem resistant. The rapid carba-NP test showed that 21.42% (21/98) carbapenem resistant isolates were positive for rapid carba NP test and the results were compared with MHT and CDT.

**Conclusion:** The rapid detection of carbapenemase producer by Carba NP test helps to differentiate resistant pathogens from other mechanism of resistance.



## INTRODUCTION

The most urgent challenge faced in the field of healthcare is the rising antimicrobial drug resistance. Recently, the extensive use of carbapenem used against extended spectrum - β-lactamase producing Gram negative bacteria has resulted in the increased prevalence of carbapenem resistant Gram negative bacteria globally. [1]Such emergence of bacteria resistant to carbapenem are of two types: (i) Resistance may result from impermeability of cell wall of bacteria or due to efflux mechanism or porin loss and is not transmissible and (ii) Resistance may be due to the production of the enzyme carbapenemase and is transmissible (horizontal transfer of genes between two different genus and species). The infection caused by second type of carbapenem resistance i.e., carbapenemase producer is very difficult to treat. It also leads to high mortality rate and can easily spread between humans via contact, food and water leading to greater risk of outbreaks of healthcare associated infection (HAI). [2]

With recent increase in the prevalence of carbapenemase producing Gram negative bacteria, it is essential for the routine laboratories to identify and alert the clinician. This rapid identification of carbapenemase producers will help to prevent the development of nosocomial outbreaks by adaptation of antibiotic therapy and by isolation of the colonized patients. [3] In developing countries like ours, most of the laboratories are without molecular diagnostics and has to meet the challenge in identifying the carbapenemase producers from the clinical isolate. It is reported that Carba-NP test is rapid, economical and user friendly with good reproducibility for detection of carbapenemases with a sensitivity and specificity of 100% when compared to PCR. [2]Therefore, this study was designed to standardize the rapid carba-NP test and to screen for carbapenemase production among Gram negative bacterial isolates with other phenotypic tests in a tertiary care hospital, South India.

**Corresponding author:* **Swarna .S.R**
Department of Microbiology, JIPMER, Karaikal

## METHODS

A prospective, cross-sectional study was carried out with 122 nonduplicate Gram negative bacterial isolates obtained from various clinical samples of a tertiary care hospital, South India between February-September 2015. The isolates were identified using standard procedure[4] and antibiotic susceptibility testing was done according to guidelines of the Clinical and Laboratory Standards Institute. [5] Further screened by Modified Hodge test) [6] and Imipenem (Imp) - Imp + EDTA combined disk test. [7]The rapid Carba-NP (Carbapenemase Nordman Poirel) was performed. [2]One bacteriological loop of the isolate recovered from the antibiogram plate from near to the discs of imipenem, meropenem and ertapenem was taken separately and resuspended in a Tris-HCl 20mmol/L. The suspension is vortexed for 1 minute, incubated at room temperature (RT) for 30 minutes and centrifuged at 10,000xg at RT for 15 minutes. Supernatant and deposit were added separately to the 96 well microtitre plate to which 100µl of reagent is added [2ml of 0.5% w/v of phenol red + 16.6ml distilled water, adjusted the pH to 7.8 by adding drops of NaOH solution (1N) and added 500µl of ZnSo4 10mM and imipenem-cilastatin (6mg/ml)]. Incubate at 37°C for a maximum of 2 hours. The carbapenemase producer showed a colour change to orange/ yellow whereas carbapenemase non producer remained red in colour. The positive control used was *K.pneumoniae* ATCC BAA-1705 and the negative control was ATCC *Escherichia coli* 25922. All tests were performed in triplicate along with standard positive and negative control, giving identical and reproducible results.

## RESULTS

A total of 122 isolates obtained from various clinical samples were given in Table I.

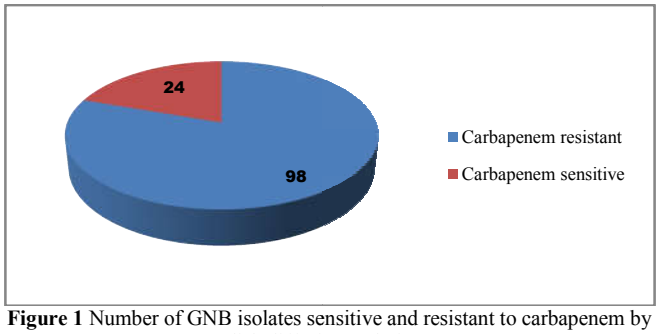


**Figure 1** Number of GNB isolates sensitive and resistant to carbapenem by disk diffusion technique

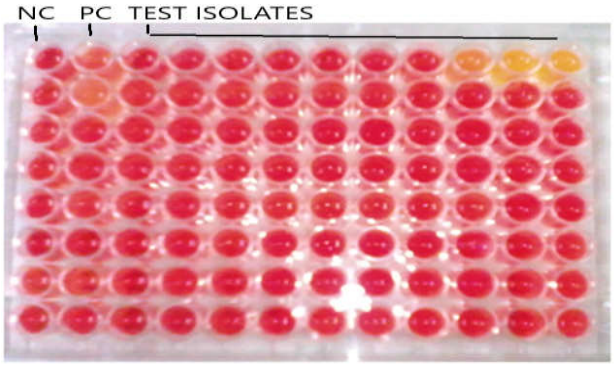


**Figure 2** Rapid Cara NP test performed on microtitre plate with positive control (*K.pneumoniae* ATCC BAA-1705), negative control (ATCC *Escherichia coli* 25922) and test isolates (Orange to Yellow – Positive for carbapenemase production ; Red – Negative for carbapenemase production)

The rapid carba-NP test showed that 21/98 carbapenem resistant isolates were positive for rapid carba NP test whereas 24 of carbapenem sensitive isolates were negative for rapid carba-NP test (Figure 2). The results of all the isolates tested with Modified Hodge test and Imipenem-Imipenem EDTA combined disk test were shown in Table II.

**Table I** Distribution of isolates

| ISOLATES (N=122) | Clinical specimens | | | | | |
|---|---|---|---|---|---|---|
| | Urine | Pus | Sputum | Blood | Tracheal aspirate | Others |
| *Escherichia coli* (n=44) | 25 | 13 | 1 | 2 | 1 | 2 |
| *Klebsiellapneumoniae*(n=24) | 6 | 6 | 3 | 2 | 3 | 4 |
| Proteus spp (n=6) | 6 | 0 | 0 | 0 | 0 | 0 |
| Citrobacter spp (n=1) | 1 | 0 | 0 | 0 | 0 | 0 |
| Pseudomonas spp (n=20) | 2 | 6 | 3 | 0 | 6 | 3 |
| Acinetobacter spp (n=20) | 2 | 10 | 4 | 0 | 4 | 0 |
| Non-fermenterGNB (n=7) | 1 | 3 | 1 | 1 | 0 | 1 |

OTHERS – Catheter tip - E.coli (2), Non fermenting GNB (1); Bronchial wash – K.pneumoniae(2); ICD fluid - K.pneumoniae(1), P.aeruginosa (1); Ear swab - K.pneumoniae(1), P.aeruginosa (1); Eye swab - P.aeruginosa (1);

All the 122 Gram negative bacteria were screened by disc diffusion technique with carbapenems namely imipenem, meropenem and ertapenem showed that 24/122 were carbapenem sensitive and 98/122 were carbapenem resistant (Figure1).

**Table II** Comparison of Modified Hodge test (MHT), Imipenem (IMP)–IMI+EDTA combined disk test and Rapid Carba-NP test

| Total No. Of Isolates (n=122) | No of Isolates Positive for | | |
|---|---|---|---|
| | MHT | IMI-IMI+EDTA | RAPID CARBA NP |
| Carbapenem Resistant (n=98) | 55 | 20 | 21 |
| Carbapenem sensitive (n=24) | 3 | 0 | 0 |

## DISCUSSION

The high prevalence of carbapenem resistance in Gram-negative bacteria from healthcare associated infections has been encountered from different parts of India. [8] There exist a different phenotypic and genotypic methods for the accurate detection of carbapenemases. [9]

In the present study, 35.24 % Gram negative bacterial isolates were from urine, followed by 31.14% from pus, 11.47% from tracheal aspirate, 9.83% from sputum, 8.19% from others, and 4.09% blood. Our results are comparable with few studies[10, 11] which reported that almost 40% of all nosocomial infections are urinary tract infection (UTI) followed by other infections.

It is important to test all the GNB isolates for susceptibility to carbapenems which serves as sensitive indicator for identification of carbapenemase producers. In the present study, the overall carbapenem resistant GNB includes 80.32% (98/122) and carbapenem sensitive includes 19.67% (24/122) by disc diffusion method. The number of isolates resistance to carbapenem includes 35/42 *Esch. Coli* (83.33%), 20/26 *K.pneumoniae* (76.92%), 3/6 Proteus spp (50%), 1/1Citrobaccter spp (100%), 19/20 Pseudomonas spp (95%), 17/20 Acinetobacter spp (85%) and 3/7 non-fermenter GNB (42.85%). It was reported that the overall prevalence of carbapenem resistance among Gram-negative bacterial isolates ranges from 7.87% - 92%%[10,12] and suggested that increased resistance is due to the unrestricted use of antibiotics.

The recent addition of Carba NP test by CLSI in routine screening for detection of carbapenemase uses phenol red indicator. [13] This phenotypic method was found to be helpful for identification of carbapenemase production in Enterobacteriaceae, *Pseudomonas aeruginosa*, and Acinetobacter species as the results were read by visible colour change from red to yellow/ orange. [2] In the present study, different isolates positive for the rapid carba NP test includes 20% (7/35) *Esch. Coli*, 25% (5/20) *K.pneumoniae*, 100% (1/1) Citrobaccter spp , 26.31% (5/19) Pseudomonas spp, 11.76% (2/17) Acinetobacter spp, 33.33% (1/3) non-fermenter GNB.

The results showed that 21.42% (21/98)carbapenem resistant GNB isolates probably produced the carbapenemase whereas 78.57% (77/98) carbapenem-resistant bacteria could have non-carbapenemase-mediated mechanisms of resistance (i.e. outer membrane permeability defect, or overproduction of cephalosporinases and/or ESBLs) as reported in one study. [2] However, in-house rapid carba NP test does not discriminate between the different types of carbapenemase as reported. [14] Low positivity (21.42%) of carba-NP test in the present study may be due to low expression of carbapenemase as reported in a study[15] and suggested that false negative is because of mucoid isolates of Proteus spp or OXA-48 producing Enterobacteriaceae having CTX-M-type ESBL (class A enzyme) leading to weak color change. [3,15] In another study, technical difficulties like poor protein extraction, species-specific traits, or the influence of the agar type are suggested to be the reason for poor sensitivity. [16]

Modified Hodge test (MHT) is one of the routine phenotypic screening test used for carbapenemase production among carbapenem resistant organisms. In the present study, MHT was positive for 57.14% *Esch. Coli* (20/35), 60% *K.pneumoniae* (12/20), 66.66% Proteus spp (2/3), 100%Citrobaccter spp (1/1), 52.63% Pseudomonas spp (10/19), 47.05% Acinetobacter spp (8/17) and 66.66% non-fermenter GNB (2/3) among 98 carbapenem resistant isolates and 12.5% (3/24) carbapenem sensitive isolates. Therefore, MHT identified 56.12% (55/98) carbapenem resistant bacterial isolates while only 21.42% (21/98) of the isolates were identified by in-house rapid carba-NP test. It was reported that MHT lacks specificity (e.g. false-positive results for high-level Amp C producers with porin deficiency in their cell walls or CTX-M-type ESBL producers, Enterobacter species) and sensitivity (ie; false negative) with non-fermenting GNB and NDM producing carbapenem-resistant Enterobacteriaceae.[1] Further, invalid results are frequently seen in Proteus spp due to swarming that obscure the cloverleaf indentation and *P.aeruginosa* due to killing of the E.coli lawn by the bacteriocins extended by the test isolates.[17] Though the MHT is simple and cheap, it is time-consuming whereas zinc supplementation leads to rapid detection of carbapenemase in-house rapid carba NP test.

Imipenem - imipenem + EDTA is the combined disk test (CDT) used for detection of metallobetalactamases (MBL) based on the inhibitory property of EDTA. In the present study, among the 98 carbapenem resistant isolates, 20 (20.40%) were positive for combined disk test and includes 20% *Esch. Coli* (7/35), 10%*K.pneumoniae* (2/20), 33.33% Proteus spp (1/3), 21.05% Pseudomonas spp (4/19), 35.29% Acinetobacter spp (6/17). The prevalence of MBL detection by imipenem-EDTA combined disc test has been reported to range from 8.0%-85.7% in various studies. [6,7]Low positivity (20.40% ; 20/98) of CDT suggests that resistance mechanisms involved could be permeability mutations via loss of porins or upregulation of efflux systems and may be missed by phenotypic tests. [18] In few studies, the results of CDT test were correlated with PCR and suggested the CDT as the superior test over MHT. [7]

Though the results of combined disk test (20.40%) was found to be similar to the inhouse rapid carba-NP test (21.42%), there exist a slight difference in the number of positive isolates between the two phenotypic tests and could be probably due to difference in the level of resistance by the carbapenemase producers to imipenem.

In the present study, among the three phenotypic test, Carba NP test and combined Imipenem/ Imipenem-EDTA showed similar results compared to MHT. The sensitivity and specificity of each test could not be determined due to lack of molecular confirmation of the carbapenem resistant isolates. However, the test was assessed with clinical isolates and not just from reference isolates alone, thus decreasing selection bias.

## CONCLUSION

Carba NP test helps to identify carbapenemase producers rapidly (< 2 hours) and reduces the time to < 24 hours for detection of carbapenem resistant when compared to other two phenotypic tests which both require at least > 24-72hours. The necessity of simple, rapid, cost effective technique makes the in-house rapid carba NP test to identify and differentiate resistant pathogens for better antibiotic stewardship and prevention of health care associated outbreaks.

## References

1. Datta S, Dey R, Dey JB, Ghosh S. A comparative study of modified Hodge test and Carba NP test for detecting

carbapenemase production in Gram-negative bacteria. Med J DY PatilUniv2017;10:365-9.
2. Nordmann P, Poirel L, Dortet. Rapid detection of Carbapenemase- producing Enterobacteriacea. Emerg Infect Dis J 2012; 18:9.
3. Dortet L, Poirel L, Nordmann P. Rapid detection of carbapenemase-producing Pseudomonas spp. *J ClinMicrobiol* 2012; 50: 3773-3776.
4. Collee JG, Miles RS, Watt B. Tests for identification of bacteria. In Collee JG, Fraser AG, Marmion BP, Simmons A. Mackie and McCartney's Practical medical microbiology, 14th ed. Churchill Livingstone, 2006.
5. Clinical Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing, Approved standard. CLSI document M100-S24, Vol 34 No1, Jean B Patel *et al*; Jan 2014.
6. Noyal MJC, Menezes GA, Harish BN, Sujatha S, Parija SC. Simple screening tests for detection of carbapenemases in clinical isolates of nonfermentative Gram-negative bacteria. Indian J Med Res 2009; 707-712.
7. Behera B, Mathur P, Das A, Kapil A, Sharma V. An Evaluation of four different phenotypic techniques for detection of metallo-ß- lactamase producing Pseudomonas aeruginosa. *Indian J Med Microbio* 2008; 26(3): 233-37.
8. Shinde S, Gupta R, Raut SS, Nataraj G, Mehta PR. Carba NP as a simpler, rapid, cost-effective, and a more sensitive alternative to other phenotypic tests for detection of carbapenem resistance in routine diagnostic laboratories. J Lab Physicians 2017; 9:100-3.
9. Kazım S, Ayse T, Sule O, Demet A, Hande Y, Aziz RD, Emine S. Evaluation of carbapenem resistance using phenotypic and genotypic techniques in *Enterobacteriaceae*isolates. Ann ClinMicrobiol Antimicrob 2015;14: 44.
10. Mate H, Sulochana D, Mamta D. Prevalence of carbapenem resistance among gram-negative bacteria in a tertiary care hospital in North-East India. *J Dent Med Sci* 2014; 13(12):56-60.
11. Jyoti D, Rajesh KV, Dharmendra PS, Amit S, Sunita K.Phenotypic detection of carbapenem resistance in gram negative bacilli from various clinical specimens of a tertiary care hospital in Western Uttar Pradesh. *Int J Res Med Sci* 2017;5(8):3511-3514.
12. Rajkumar MS, Soma S, Puranjay S, Manideepa S. Carbapenemase producing Enterobacteriaceae among urinary isolates: scenario from a tertiary care hospital in eastern India. Journal of Evolution of Medical and Dental Sciences 2014; 3(6): 1323-33.
13. Clinical and Laboratory Standards Institute. Performance Standards for Antimicrobial Susceptibility Testing; 25th Informational Supplement. CLSI Document M100‑S25. Wayne PA: Clinical and Laboratory Standards Institute; 2015.
14. Hrabak J, Chud_a_ckov_a E, Papagiannitsis CC. Detection of carbapenemases in Enterobacteriaceae: a challenge for diagnostic microbiological laboratories. ClinMicrobiol Infect 2014; 20: 839-53. Review.
15. 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: 6437-40.
16. Maurer FP, Castelberg C, Quiblier C, Bloemberg GV, Hombach M. Evaluation of carbapenemase screening and confirmation tests with Enterobacteriaceae and development of a practical diagnostic algorithm. *J ClinMicrobiol* 2015; 53:95-104.
17. Vasoo S, Cunningham SA, Kohner PC, Simner PJ, Mandrekar JN, LolansK, Hayden MK, Patel R. Comparison of a novel, rapid chromogenic biochemical assay, the CarbaNP test, with the modified Hodge test for detection of carbapenemase-producing Gram-negative bacilli. *J ClinMicrobiol* 2013; 51:3097-3101.
18. Saderi H, Lotfalipour H, Owlia P, Salimi H. Detection of Metallo-beta-lactamase producing Pseudomonas aeruginosa isolated from Burn patients in Tehran, Iran. Laboratory Medicine 2010; 41(10): 609-612.

**How to cite this article:**

Swarna .S.R *et al* (2019) 'Standardisation and Evaluation of Inhouse Rapid Carba-NP and Comparison of Phenotypic Methods for Screening of Carbapenemase Producing Gram Negative Bacterial Isolates', *International Journal of Current Medical And Pharmaceutical Research,* 05(07), pp 4325-4328.

*******