

# Utility of Carba NP test (Inhouse/RAPIDEC Commercial Kit) in the Identification of Carbapenemase Producing Clinical Isolates in a Tertiary Care Hospital

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## ABSTRACT

**Introduction:** Carbapenems are the drugs of choice for the treatment of many multidrug resistant hospital acquired infections. Resistance to carbapenems also is not uncommon and is increasingly being reported now-a-days. Though various tests like modified hodge test are available for the detection of carbapenemases, they lack sensitivity and specificity. Recently, the Carba NP test has been introduced for carbapenemase detection which has been approved and included in CLSI guidelines.

**Aim:** To identify the prevalence of carbapenemase producing isolates and the utility of Carba NP in house as well as commercial RAPIDEC Carba NP test in the identification of these carbapenemases.

**Materials and Methods:** All the carbapenem resistant organisms isolated during the study period from July 2018 to December 2018 were included in the study. A total of 91 isolates were identified during the study period which were further tested for carbapenemase production by both in House Carba NP test as well as commercially available RAPIDEC Carba NP (Biomérieux) as per the existing protocols. Twenty five carbapenem sensitive isolates were also tested. *Klebsiella pneumoniae* BAA ATCC-1705 and BAA ATCC-1706 were used as controls.

**Results:** Out of 91, carbapenem resistant strains tested, 72 were identified as carbapenemase producers. Out of them, *Klebsiella pneumoniae* accounted for 59.7% of the total carbapenemase producing organisms followed by *Pseudomonas aeruginosa* (25%) and *E.coli* (6.9%) followed by *Acinetobacter* and *Enterobacter* which constituted less than 5%. Among enterobacteriaceae, 43 out of 59 carbapenem resistant *Klebsiella* were carbapenemase producers whereas all the 5 carbapenem resistant *E.coli* were positive for Carba NP test. Among *Pseudomonas* 18 out of 21 were carbapenemase producers. All the positive isolates by in-house Carba NP test were also positive by commercial RAPIDEC test and vice-versa and none of the 25 carbapenem sensitive strains tested were positive for Carba NP test by either method indicating 100% correlation between the two methods studied.

**Conclusion:** Carba NP in house as well as commercial RAPIDEC Carba NP test were equally effective in the identification of the carbapenemase producing gram negative bacilli. Wide adaptability of these tests by various laboratories will help in the early identification of these potentially spreading carbapenemase producers, which in association with appropriate treatment and infection control practices will prevent the emergence of these strains and will decrease the mortality and morbidity.

**Keywords:** Enterobacteriaceae, *Klebsiella pneumoniae*, Multidrug resistance, *Pseudomonas aeruginosa*

## INTRODUCTION

Carbapenems are the drugs of choice for the treatment of many multidrug resistant hospital acquired infections. With the increasing emergence of carbapenem resistant bacteria in the recent past, it has become extremely difficult to treat these infections as only very few alternatives exist to treat such infections. This results in increased mortality and morbidity in hospitals and also imparts great economic burden on the health care system. There is a higher likelihood for the spread of these carbapenemase producers in the hospital settings which further adds to this complication. Rapid identification of these carbapenemase producers in association with appropriate treatment and infection control practices will help in the containment of these infections [1,2].

There are various mechanisms that lead to carbapenem resistance in bacteria among which carbapenemase production is a major contributor and is widespread among the Gram negative organisms [2,3]. Other mechanisms like efflux pumps, altered function of porins or binding proteins also contribute to carbapenem resistance to some extent. Though various tests like modified hodge test are available for the detection of carbapenemases, they lack sensitivity and specificity. On the other side, molecular techniques like polymerase chain reaction have good sensitivity and specificity, yet

needs expert handling and infrastructure. In this scenario, there is a need for a rapid, cheap and easily performable test for the detection of carbapenemase production in clinical strains. Recently, the Carba NP test has been introduced for carbapenemase detection which has been evaluated, approved and included in CLSI guidelines [4]. The test is based on the change in the pH using indicator phenol red (change in colour from red to yellow or orange) caused by the hydrolysis of imipenem by a bacterial lysate. The report shows Enterobacteriaceae to be 100% sensitive and specific, whereas it is 94.4% sensitive for *Pseudomonas* in screening carbapenemases [5,6]. CLSI also states that the rapid carbapenemase detection methods are having more than 90% of sensitivity and specificity with regards to enterobacteriaceae members like *Klebsiella pneumoniae* where NDM, VIM and IMP type of resistance is more common where as their sensitivity and specificity are low for non-fermenting organisms like *Acinetobacter* where OXA48 carbapenemases are common mechanisms involved [4].

Many researchers have identified the importance of rapid carbapenemase detection methods both in India and abroad and the sensitivity and specificity varied between different studies. Overall the sensitivity of RAPIDEC carba NP tests was between 90% to 100% and specificity varied from 96% to

100% [7-10]. Though many authors in India have evaluated these rapid carbapenemase detection methods in their clinical set up. Prevalence of carbapenemase producing organisms in our Telangana state have not been studied well by using these rapid methods like RAPIDEC carba NP tests. As the prevalence of these carbapenemase producers varies from place to place, it is important that they should be identified promptly for both epidemiological and therapeutic purposes. Thus, the present study aims to identify the prevalence of carbapenemase producing isolates and compare the utility of an in-house Carba NP test to the commercial RAPIDEC Carba NP test in the detection of these carbapenemase producing isolates in and around Mahabubnagar district, Telangana, India.

## MATERIALS AND METHODS

This was a prospective study done in the Department of Microbiology, SVS Medical College from July 2018-December 2018. Ethical clearance certificate (SVSMC/IEC Approval/No.05/2018-623) was taken from the institutional ethical committee. All gram negative bacterial isolates from patients admitted in the hospital were included and all the gram positive isolates and samples isolates from outpatient were excluded from the study. A total of 612 Gram negative organisms were isolated during the study period. Clinical samples such as pus, blood, sputum, stool, urine, endotracheal aspirates and other body fluids were taken-up for the study. Samples were identified up to the species level and antibiotic sensitivity testing was done by Vitek 2 systems as per standard protocols which has usual sensitivity of 99% and specificity of 96% [11]. All the carbapenem resistant organisms isolated were further tested for carbapenemase production. The carbapenemase production was tested using both commercially available RAPIDEC Carba NP test (Biomerieux) as per the standard protocols and an in-house Carba NP test.

For the in-house carba NP test, two 1.5 mL Eppendorf tubes were taken for each strain and labeled as A and B. 200  $\mu$ L of sterile water was added to tube A and emulsified with 2-3 loop full of bacterial colony to make a heavy suspension. From this, 100  $\mu$ L of the inoculum was transferred to tube B. Separately, Solution A (0.05% phenol red, 10 mM ZnSO<sub>4</sub>, pH 7.8) and Solution B (1 mL sol A, 6 mg imipenem powder) were prepared. Now, 100  $\mu$ L of sol A was added to tube marked A and 100  $\mu$ L of sol B was added to tube B and both the tubes were incubated at 37°C for 2 hours. A change in colour from red to yellow was considered as positive Carba NP test indicating carbapenemase production [10].

Twenty five carbapenem sensitive isolates were also included for the analysis. The positive and negative controls used were *Klebsiella pneumoniae* ATCC BAA-1705 and *Klebsiella pneumoniae* ATCC BAA-1706.

### Procedure Flow chart

Culture of the clinical samples → identification of the organism → detection of carbapenem resistance → detection of carbapenemase production by RAPIDEC carba NP + in-house Carba NP test → result obtained in 2 hours.

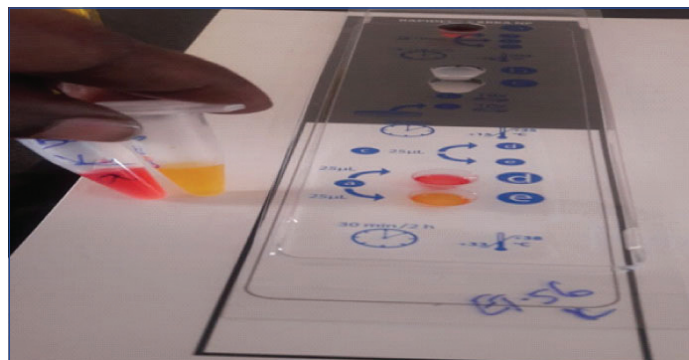
## STATISTICAL ANALYSIS

Statistical analysis was performed by SPSS VERSION 20 and graph pad prism software version 6.0. The collected data was entered in to MS excel and tabulated. Sensitivity, specificity, positive predictive and negative predictive values were detected.

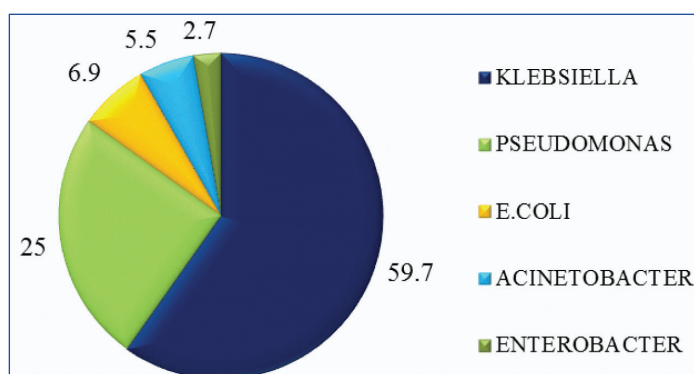
## RESULTS

A total 612 Gram negative organisms were isolated during the study period, out of which 91 isolates were identified as carbapenem resistant and were further tested for carbapenemase production by both our in-house Carba NP test as well as RAPIDEC Carba NP test [Table/Fig-1] Out of the 91 carbapenem resistant strains tested,

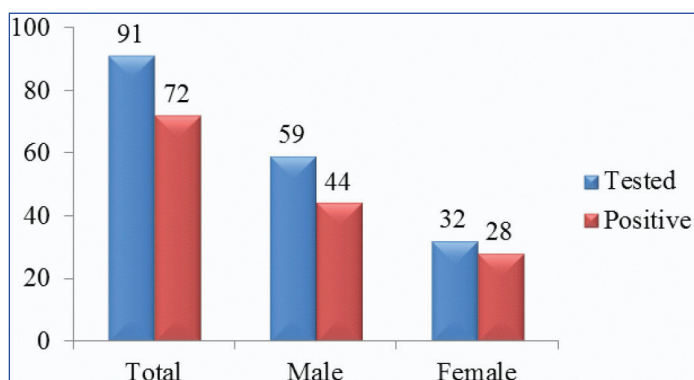
72 were identified as carbapenemase producers in both the methods. Out of them, *Klebsiella pneumoniae* accounted for 59.7% of the total carbapenemase producing organisms followed by *Pseudomonas aeruginosa* (25%) and *Escherichia coli* (6.9%). *Acinetobacter* and *Enterobacter* constituted less than 5% of the strains showing carbapenemase production [Table/Fig-2]. Among Enterobacteriaceae, 43 out of 59 carbapenem resistant *Klebsiella* were carbapenemase producers whereas all the 5 carbapenem resistant *E. coli* were positive for Carba NP test. Among *Pseudomonas*, 18 out of 21 isolates were carbapenemase producers. The performance of the test in detecting carbapenemase production was high when considering the enterobacteriaceae members like *E. coli* and *Klebsiella* where as it was low for non-fermenters like *Acinetobacter*. Out of 72 carbapenemase producing organisms, 44 positive cases are from males and 28 are from females as shown in [Table/Fig-3].



**Table/Fig-1:** Results from both in house Carba NP test (left side) and RAPIDEC carba NP (Biomerieux) (right side) showing positive for carbapenemase production.



**Table/Fig-2:** Distribution of carbapenemase producing bacterial isolates in the present study.



**Table/Fig-3:** Gender wise distribution of the isolates obtained and those that tested positive for carbapenemase production in the present study.

All the positive isolates identified by our in-house Carba NP test were also positive by commercial RAPIDEC test and none of the 25 carbapenem sensitive strains tested were positive for carbapenemase production by either method indicating 100% correlation between the two methods studied.

The overall sensitivity of the test was 79.12% (CI:69.33% to 86.94%) and specificity was 82.79% (CI: 71.48% to 85.14%). The positive predictive value PPV was 82.91% (CI:71.48% to 85.14%). and

negative predictive value NPV was 79.38% (CI:71.65% to 94.08%). [Table/Fig-4] shows the distribution of carbapenemase positive Gram negative bacteria from various clinical samples. *Klebsiella pneumoniae* was the predominant carbapenemase producer and most of the carbapenemase producing GNB were isolated from endotracheal secretions, followed by blood and others.

Bacterial genus	Sputum and BAL	Blood	Pus	Urine	ET	Stool	Total
<i>Klebsiella</i>	7	9	6	3	18	0	43
<i>Pseudomonas</i>	2	8	4	0	4	0	18
<i>E. coli</i>	1	0	0	0	2	2	5
<i>Acinetobacter</i>	0	1	0	1	2	0	4
<i>Enterobacter</i>	0	1	0	0	1	0	2
Total organisms	10	19	10	4	27	2	72

**[Table/Fig-4]:** Distribution of carbapenemase positive Gram negative bacteria from clinical samples (n=72).

## DISCUSSION

Multidrug resistance is increasingly being observed both in community acquired and hospital acquired pathogens. Carbapenems are the drugs of choice in majority of the serious nosocomial infections. With the wide spread emergence of carbapenem resistance especially in enterobacteriaceae members like *Klebsiella*, it has become extremely difficult for the clinicians to treat such cases as only few alternatives like tigecycline and colistin are available for the treatment [12]. As the antibiotic pipeline is almost empty, it is mandatory that the emergence of resistance to carbapenems is contained by early identification and adherence to appropriate infection control measures along with the best clinical practices. Sound knowledge about the mechanisms of carbapenem resistance and identification methods will help in achieving this goal. As carbapenemase production is the predominant mechanism for resistance to carbapenems, early detection of carbapenemase producing organisms is extremely important in preventing the spread of these infections.

Nordmann P et al., developed a novel method for rapid detection of these carbapenemase producers [5]. The basic principle of this test was the hydrolysis of carbapenem by the bacterial enzyme carbapenemase which detects the pH changes by measuring the change of the colour of phenol red indicator. Based on this principle, many commercial kits have been developed among which RAPIDEC Carba NP test developed by Biomerieux is widely used with good sensitivity and specificity [10,13,14]. Our in-house Carba NP test was also based on the same principle.

In the present study, both in-house Carba NP and commercial RAPIDEC Carba NP tests were evaluated in their ability to detect carbapenemase producing isolates. Out of the total 91 carbapenem resistant isolates tested, 72 were identified to be positive for carbapenemase production by both the tests. In addition, none of the carbapenem sensitive isolates tested were positive by either test. *Klebsiella* was the most common isolate showing carbapenemase production. Gupta V et al., tested 75 bacterial strains and found all strains to be positive for carbapenemase production by both Carba NP and RAPIDEC Carba NP tests [10]. They also observed *Klebsiella* as the most common bacterial isolate showing carbapenemase production in their study. But they have also used other methodologies like Modified Carba NP (MCNP) test, Carbapenem Inactivation Method (CIM) test. Similarly, Dortet L et al., compared RAPIDEC Carba NP, the Rapid CARB Screen and the Carba NP test and found that sensitivity and specificity were 99% and 100%, respectively for the RAPIDEC Carba NP test, 96.8% and 100% for the Carba NP test [15]. They have stated that RAPIDEC Carba NP possesses the best performance for rapid and efficient detection of carbapenemase-producing Enterobacteriaceae and suggested it as a first-line screen of carbapenemase-producing

Enterobacteriaceae in clinical settings. Comparatively, limited literature is available in India against western countries with regards to the utilisation of rapid carbapenemase detection methods [16-18]. Wide utilisation of these rapid detection methods in a country like India will help not only in decreasing the mortality and morbidity but also the health care economical burden.

RAPIDEC carba NP test was extremely useful in the early detection of carbapenemase producing isolates in our hospital because of the rapid result which can be obtained within 2 hours and also doesn't need any technical expertise. Because of this early detection, appropriate infection control practices could be placed which further decreased the spread of these infections. The high cost of RAPIDEC carba NP test may limit its use routinely in clinical laboratories in low settings but the inhouse Carba NP test is a viable option for its routine use in such settings as it is quite cheap and easy to perform and interpret.

Though various methodologies are available for the detection of carbapenemases, the methods should be individualised in accordance with the needs, work load and economical constraints of the hospitals. Nevertheless, rapid detection of these infections plays a major role in the appropriate management of the patients and initiation of infection control measures thus preventing the spread of these infections.

## LIMITATION

The limitation of this study, is it has less sensitivity towards OXA-48 carbapenemases. No genotyping of these isolates was done to identify any resistance mechanisms underlying.

## CONCLUSION

Both the Carba NP in-house as well as commercial RAPIDEC Carba NP test were equally effective in the identification of the carbapenemase producers among the Gram negative bacilli. Wide adaptability of these tests by various laboratories will help in the early identification of these potentially spreading carbapenemase producers, which in association with appropriate treatment and infection control practices will prevent the emergence and decreases the mortality and morbidity associated with these bacterial infections.

**Declaration:** Author had presented the Abstract (Oral Presentation) in International Conference held at Manipal, Karnataka, India.

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## REFERENCES

- [1] Garg A, Garg J, Swaroop A, Kala S, Rao YK, Kumar A, et al. Emerging carbapenemases: Bringing a step closer to extremely drug resistant bacteria. Int J Curr Res. 2011;3:35-38.
- [2] Bush K, Fisher JF. Epidemiological expansion, structural studies, and clinical challenges of new  $\beta$ -lactamases from gram-negative bacteria. Annu Rev Microbiol. 2011;65:455-78.
- [3] Nordmann P, Dortet L, Poirel L. Carbapenem resistance in Enterobacteriaceae: here is the storm! Trends Mol Med. 2012;18:263-72.
- [4] CLSI. "Performance standards for antimicrobial susceptibility testing," Twentieth informational supplement, Clinical and Laboratory Standards Institute Doc. M100ED28-2018.
- [5] Nordmann P, Poirel L, Dortet L. Rapid detection of carbapenemase producing Enterobacteriaceae. Emerg Infect Dis. 2012;18:1503-07.
- [6] Dortet L, Poirel L, Nordmann P. Rapid detection of carbapenemase-producing *Pseudomonas* spp. J Clin Microbiol. 2012;50:3773-76.
- [7] Ho PL, Wang Y, Wing-Sze Tse C, Fung KS, Cheng VC, Lee R, et al. Rapid detection of carbapenemase production in enterobacteriaceae by use of a modified paper strip Carba NP method. J Clin Microbiol. 2018;56(1):pii: e01110-17.
- [8] Vanstone G, Woodhead S, Roulston K, Sharma H, Wey E, Smith ER, et al. Improving the detection of carbapenemase-producing organisms (CPO)

- in a low-prevalence setting: Evaluation of four commercial methods and implementation of an algorithm of testing. *Journal of Medical Microbiology*. 2018;67:208-14.
- [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;59:7870-72.
- [10] Gupta V, Soni R, Jain N, Chander J. In vitro cost-effective methods to detect carbapenemases in Enterobacteriaceae. *J Lab Physicians*. 2018;10:101-05.
- [11] Nonhoff C, Rottiers S, Struelens MJ. Evaluation of the Vitek 2 system for identification and antimicrobial susceptibility testing of *Staphylococcus* spp. *Clin Microbiol Infect*. 2005;11:150-53.
- [12] Nordmann P, Cuzon G, Nass T. The real threat of *Klebsiella pneumoniae* carbapenemase-producing bacteria. *Lancet Infect Dis*. 2009;9:228-36.
- [13] Mancini S, Kieffer N, Poirel L, Nordmann P. Evaluation of the Rapidec Carba NP and beta-Carba tests for rapid detection of carbapenemase-producing Enterobacteriaceae. *Diagn Microbiol Infect Dis*. 2017;88:293-97.
- [14] Poirel L, Nordmann P. Rapidec Carba NP test for rapid detection of carbapenemase producers. *J Clin Microbiol*. 2015;53:3003-08.
- [15] Dortet L, 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;70:3014-22.
- [16] Tejasvi K, Anuradha B. Detection of carbapenemase production by rapid carba NP test among Enterobacteriaceae isolates in tertiary care hospital. *Indian J Microbiol Res*. 2019;6(3):272-76.
- [17] Rao MR, Chandrasekhar P. detection of carbapenemase production in enterobacteriaceae and *Pseudomonas* species by carbapenemase Nordmann-Poirel test. *J Lab Physicians*. 2019;11:107-10.
- [18] Mangayarkarasi V, Moses SP, Swarna SR, Kalaiselvi K, Fathima S. I In-House standardisation of Carba NP test for carbapenemase detection in gram negative bacteria. *Int J Curr Microbiol App Sci*. 2018;7(01):2876-81.

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