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CARBA NP - EVALUATING ITS PERFORMANCE USING THREE DIFFERENT INDICATORS

Microbiology

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ABSTRACT

INTRODUCTION: The greatest threat to antimicrobial treatment of infections caused by Gram-negative bacteria is the production of carbapenemases. The CLSI has recommended CarbaNP (CNP) test based on the principle of acidimetry for the detection of these carbapenemases.
AIMS & OBJECTIVES: To evaluate the performance of CNP test for the detection of carbapenemases using three different indicators – phenol red, cresol red and bromothymol blue.
METHODOLOGY: The CarbaNP test was done according to CLSI guidelines using three different indicators 0.05% phenol red / 0.05% cresol red / 0.05% bromothymol blue on 72 well characterised *E. coli* isolates.
RESULTS: CarbaNP test with phenol red as the indicator could detect a total of 52/53 MBL producing E coli. The sensitivity of CNP test with cresol red and bromothymol blue was 92.5% and 96.2% respectively. The specificity was 100% with all the three indicators.
CONCLUSION: The CLSI described CNP test using phenol red was more sensitive for detection of carbapenemases. But faster results were obtained using cresol red, although weak carbapenemase activity could be better visualized using bromothymol blue indicator.

KEYWORDS

carbaNP, carbapenemases, rapid test, Enterobacteriaceae, low cost

INTRODUCTION

Multidrug resistant gram-negative bacteria (GNB) is one of the greatest threats faced by healthcare systems nowadays. Carbapenems are sorted as the last line of resort to treat such life-threatening infections. The widespread use of carbapenems in clinical practice has led to the development of resistance to these antibiotics. In the present scenario, production of carbapenamase enzymes is an important cause for resistance. The genes coding for carbapenemase enzymes are located on bacterial chromosomes or on mobile genetic elements. These genes are horizontally transferred from one organism to another easily and also at an alarming rate. Intra and intercontinental movement of patients has led to the worldwide spread of carbapenem resistant GNB.[1]

The gold standard method to detect carbapenamases remains to be molecular techniques. These methods are time consuming and delay the onset of right treatment. This poses a serious threat to the public and they are associated with increased morbidity and mortality. Hence, a rapid, easy and reliable method to detect carbapenemases will help in containment of the spread and also improve the patient outcome.[2]

A novel carbapenemase detection test (Carba NP [CNP] test) based on the principle of acidimetry has been developed by Nordmann et al.[3] In the acidimetric method, hydrolysis of beta-lactam ring results in a drop in pH, causing a color change of phenol red indicator from red to yellow. The Clinical and Laboratory Standards Institute (CLSI) with a few modifications recommended the CNP test as a confirmatory test for carbapenemase production.[4]

The present study aimed to evaluate performance of CNP test for the detection of carbapenemases using two different indicators i.e., cresol red and bromothymol blue.

AIMS AND OBJECTIVES:

To evaluate the performance of CNP test for the detection of carbapenemases using three different indicators – phenol red, cresol red and bromothymol blue.

MATERIALS AND METHODS:

A total of 72 non repetitive clinical isolates of E. coli from various clinical samples received in the Department of Microbiology, ESIC MC & PGIMSR were identified using standard biochemical reactions and subjected to antibiotic susceptibility test using Kirby Bauer disk diffusion method using Imipenem/Meropenem (10µg) discs. E strip based (Imipenem & Imipenem+EDTA Ezy MICTM strip) MIC detection and MBL confirmation was done for these N=72 isolates. Further these isolates were used for the evaluation of performance of CarbaNP test using the three different indicators.

Carba test using three different indicators:

The CarbaNP test was done according to CLSI guidelines using three different indicators 0.05% phenol red / 0.05% cresol red / 0.05% bromothymol blue.[4]

Solution A was prepared by adding indicator (0.05% phenol red / 0.05% cresol red / 0.05% bromothymol blue) and ZnSO4.7H2O (0.1 mmol/L) to Clinical Laboratory Reagent Water; pH was adjusted to 7.8 ± 0.1. The solution B was freshly prepared by adding 12 mg/ml imipenem-cilastatin injectable form to solution A. The test strain was grown in peptone water (pH 7) for 2 h, 100 µl of which was used as inoculum and added to each of the two microcentrifuge tubes labelled "a" and "b." Solutions A and B were added to tubes a and b, respectively, incubated at 37°C and readings were taken at 10 min, 30 min, and 120 min. by two different observers. Quality control was achieved using Klebsiella pneumoniae ATCC BAA 1705 (positive control), K. pneumoniae ATCC BAA 1706 (negative control).

INTERPRETATION:

Phenol red indicator (CarbaNP):

- Positive: tube "a" was red and tube "b" was orange/yellow (Figure 1)
- Negative: both tubes remained red (Figure 2)
- Invalid: tube "a" turns orange/yellow

Cresol red indicator (Red carba test):

- Positive: tube "a" was red and tube "b" was orange/yellow (Figure 3)
- Negative: both tubes remained red (Figure 4)
- Invalid: tube "a" turns orange/yellow

Bromothymol blue indicator (Blue carba test):

- Positive: tube "a" was blue/green and tube "b" was yellow (Figure 5)
- Negative: both tubes remained blue/green (Figure 6)
- Invalid: tube "a" turns yellow

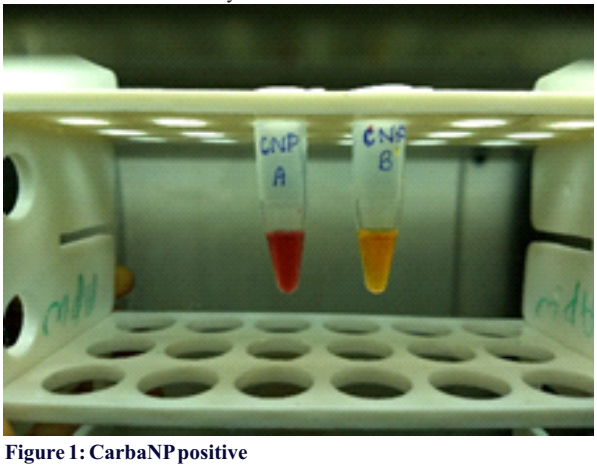

Figure 1: CarbaNP positive

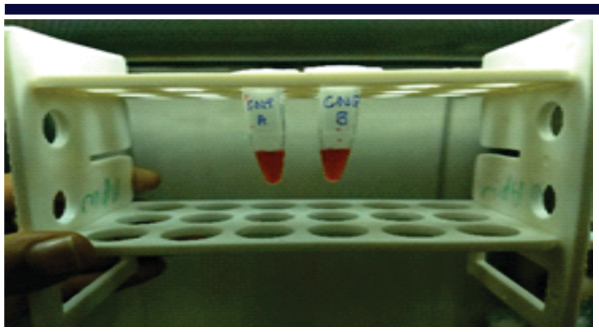

Figure 2: CarbaNP negative

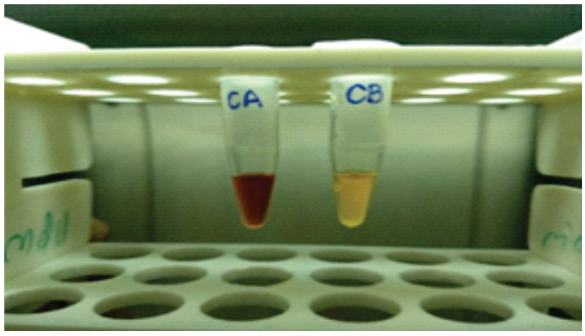

Figure 3: Red carba positive

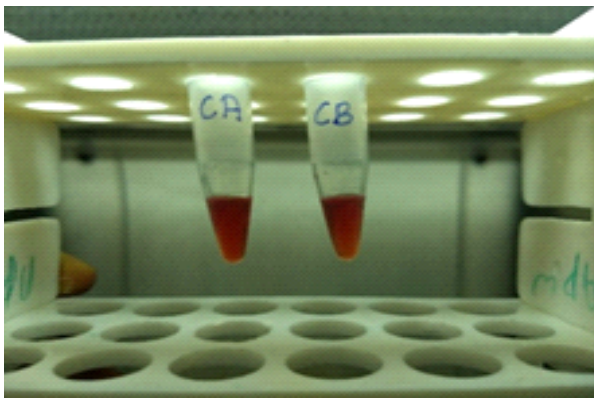

Figure 4: Red carba negative

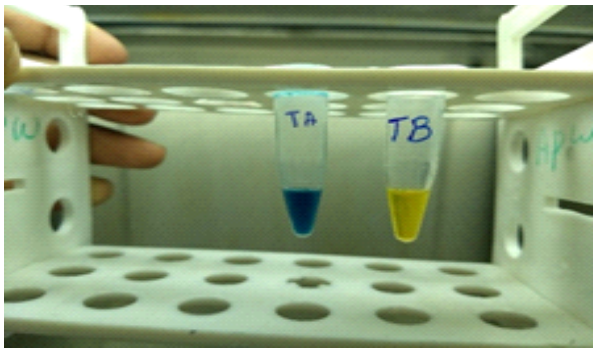

Figure 5: Blue carba positive

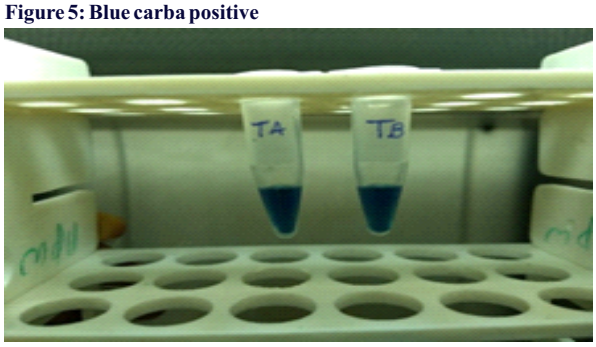

Figure 6: Blue carba negative

RESULTS AND DISCUSSION:

In this study, 56/72 isolates were Imipenem resistant according to disc diffusion method and E strip-based MIC for Imipenem. 53/56 isolates were found to be MBL positive according to E strip-based method (Imipenem & Imipenem+EDTA Ezy MICTM strip) and 3/56 did not show any carbapenemase activity and assumed to be having other mechanisms of carbapenem resistance. The CLSI recommended carba NP test could pick up 52/53 MBL positive isolates giving a sensitivity of 98%. The red carba test gave positive results for N=49/53 MBL positive isolates showing sensitivity of 92.5%. In this study the blue carba test could pick up N=51/53 MBL positive isolates with a sensitivity of 96.2%. (Table 1)

With the CLSI recommended CarbaNP majority of the positive results were obtained between 2nd and 3rd readings, though few positive isolates showed change in colour between 1st and 2nd readings. The red carba test which was done using cresol red as the indicator gave most of the positive results within 1st and 2nd readings, but the sensitivity of this indicator was lesser compared to the other two. With bromothymol blue as the indicator, the test took longer time to change colour when compared to phenol red and cresol red. But, the colour change from blue to yellow made the visual interpretation very easy and accurate with respect to others. The blue carba also showed good sensitivity comparable to the CLSI recommended method.

The carba test with all the three indicators did not give any false positive results with the imipenem sensitive isolates showing a specificity of 100%.

TABLE – 1: Performance of CarbaNP test with three different indicators.

	MBL (N= 53)	Non MBL(N=3)	Imipenem sensitive(N=16)	Sensitivity (%)	Specificity (%)
Carba NP	52	0	0	98	100
Red carba	49	0	0	92.5	100
Blue carba	51	0	0	96.2	100

CONCLUSION:

The sensitivity and specificity of the carba test with three different indicators were comparable. The added advantage of using cresol red indicator was that the test results were appreciated faster compared to CLSI recommended carba NP test, whereas by using bromothymol blue as the indicator the test results were better and easily visualized when compared to the other two indicators. Hence, carbaNP test can be performed using cresol red and bromothymol blue as indicators in routine laboratory practice with few considerations and after standardising it.

REFERENCES:

[1] Nordmann P, Naas T, Poirel L. Global spread of carbapenemase producing Enterobacteriaceae. Emerg Infect Dis 2011;17:1791-8.
[2] Rudresh SM, Ravi GS, Sunitha L, Sadiya NH, Kalairasan E, Harish BN. Simple, rapid, and cost effective modified Carba NP test for carbapenemase detection among Gram negative bacteria. J Lab Physicians. 2017;9(4):303-307.
[3] Nordmann P, Poirel L, Dortet L. Rapid detection of carbapenemase producing Enterobacteriaceae. Emerg Infect Dis 2012;18:1503-7.
[4] Clinical and Laboratory Standards Institute. M100-S25. Performance standards for antimicrobial susceptibility testing; 25th informational supplement. Wayne, PA: CLSI; 2015.