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In vitro methods of CLSI to detect Carbapenemases in Carbapenem-resistant Enterobacteriaceae

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Aims: Detection of Carbapenemases production in Carbapenem Resistant Enterobacteriaceae(CRE) using CarbaNP and its comparison with the newer CLSI recommended modified Carbapenemases Inactivation Method(mCIM). **Materials and Methods:** 100 isolates of CRE were selected in a period of 3 months from June-August 2018 for the tests, based on MIC values of >= 2mg/ml given by vitek2compact systems. CarbaNP test was performed as per M100, CLSI 2018 guidelines. CarbaNP solutions A and B were prepared and Imipenem-Cilastatin powder was replaced for standard imipenem powder. The reading was taken at the end of 10 mins,30 mins, and 2 hours. mCIM was performed as per CLSI M100,2018. The presence of carbapenemase was indicated by an inhibition zone ≤15mm in diameter. eCIM was also performed in the same way, with 0.1M EDTA added in TSB broth. Descriptive analysis. **Results:** Among the 100 CRE isolates, Carba NP detected 71 CP-CRE while mCIM detected 80(80%) of the same. mCIM detected 8 more positives than the carbaNP test. 1 isolate tested positive CarbaNP but tested negative in mCIM. **Conclusion:** CarbaNP is a novel protocol recommended by CLSI for detecting CP-CREs and studies prove that it is a very good test in detecting KPC and OXA mediated resistance, while mCIM is said to detect all 5 genes i.e. KPC,NDM,OXA,IMP, and VIM. Carba NP is easy to perform and gives quick results once the reagents are prepared. mCIM on the contrary as a newly developed test detects more CP-CREs but takes more time for the interpretation.

Keywords: Carbapenemases, Enterobacteriaceae, CarbaNP, mCIM, CP-CRE

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Introduction

The emerging spread of carbapenemase-producing Gram-negative bacilli is a worldwide emerging public health threat. Carbapenemase-producing bacteria are a cause of increased morbidity and mortality in hospital settings [1]. To prevent the spread of carbapenemase producers, rapid detection of these in bacteria has become imperative. The enzymes carbapenemases are carried on mobile genetic elements that facilitate the horizontal transfer of resistance between gram-negative organisms [2].

Other mechanisms of acquired resistance being followed by chromosomal-mediated porin loss and efflux pump overexpression [3].

Carbapenemase genes have been described that are either plasmid or chromosomally encoded, including *bla*KPC, *bla*SME, *bla*IMI, *bla*NDM, *bla*VIM, *bla*IMP, and *bla*OXA-48 type [4]. CP-CRE can spread rapidly, and their prevention may need implementation of more intensive infection control interventions than that employed for non-CP-CRE.

Routine delineation of resistance mechanisms in CRE becomes important for clinical care. Detection of carbapenemases will help to cut the CP-CRE spread.

Detection of CP-CRE in clinical laboratories is not easy, as isolates mostly have reduced susceptibilities to carbapenems, or resistance may be mediated by other mechanisms, such as overproduction of extended-spectrum-beta-lactamase (ESBL) and/or AmpC beta-lactamase producers with decreased membrane permeability[5].

Several tests have been described for the screening of carbapenemases, but it is time-consuming, requires expertise and a well-established laboratory to perform these assays[3]. For these reasons, Nordmann P et al. developed an acidometric assay Carba NP, which can detect the presence of carbapenemase in <2 hours of time [6]. Thereafter, Van der Zuwel et al described the carbapenem inactivation test for the detection of CP-CRE [5].

The present study was carried out to detect Carbapenemases in carbapenem-resistantEnterobacteriaceae(CRE) using CLSI recommended CarbaNP test and the newer CLSI recommended modified Carbapenemases Inactivation Method (mCIM).

Methodology

The study was carried out in Victoria hospital, Department of microbiology, BMCRI for a period of 6 months from June-November 2018. A total of 100 isolates of Carbapenem-Resistant Enterobacteriaceae were included in the study. These isolates were obtained from various clinical samples like blood, pus, urine, body fluids and others (stool, conjunctival swabs, vaginal swabs, tissue bits, etc) received at the laboratory for culture and antimicrobial susceptibility testing. The samples were processed using standard laboratory protocols. The identification and AST weredone using Vitek2ms systems. The Enterobacteriaceae isolates which showed MIC value of >= 2µg/mL for all three carbapenem drugs tested (Imp, Mem, Etp) were selected for further processing for detection of carbapenemase production. Isolates sensitive for any of the carbapenems were excluded from the study. Institutional ethical clearance was obtained from the institute.

The CarbaNP test was performed as performed by Rudresh SM et al. [4], and the mCIM test was performed according to M100 28th edition CLSI. In brief Carba NP test, mCIM, and eCIM test were performed as given below.

CarbaNP test: This test was performed for all 100 Carbapenem resistance Enterobacteriaceae. A modified protocol was attempted for the direct use of colonies (instead of bacterial extracts). In house preparation of **Carba NP solution, A** was done using $ZnSO_4.7H_2O$ and Phenol red powder, adjusting the pH to around 7.0. 0.1% Triton X was added to the solution. **Carba NP solution B** was prepared by adding 12mg/ml of imipenem-cilastatin powder which contains 6mg/ml of imipenem powder. In Two sterile Eppendorf tubes, 100µl of inocula was prepared in distilled water, 100µl of solution A to tube 1, and solution B to tube 2. The tubes were incubated at 37oC in ambient air. The interpretation was done at the end of 10 mins, 30 mins, and 2 hours. Figure 1 shows the CarbaNP test setup with positive and negative controls, with each test control.

MCIM: The test was performed according to CLSI M100,2018. In brief, a 10-µl inoculum of the test organism was suspended in 2 ml of TSB (Trypticase soy broth) .to the suspension 10-µg meropenem disc was added and incubated in ambient air at 37oC for 4 hours. The discs with the help of a loop

Were then transferred onto Muller Hinton agar lawned with *Escherichia coli* ATCC 25922. The plates were incubated in ambient air at 37°C for 18 to 24 h. The presence of carbapenemase was indicated by a zone of inhibition ≤15mm in diameter. Figure 2 shows the mCIM test plate (MH agar) after 18 hours of incubation.

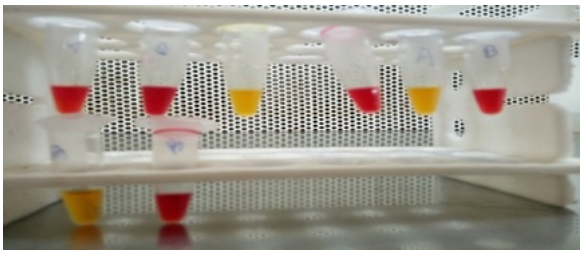

Fig-1: Carba NP showing the color change in the positive test.

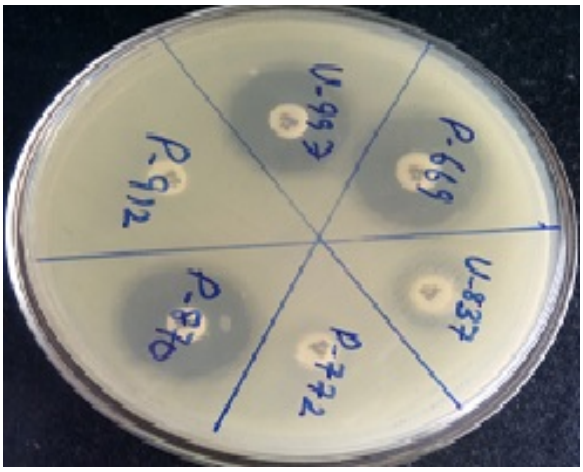

Fig-2: Three 10µg meropenem discs inactivated by mCIM test and three not inactivated.

ECIM: The test was performed similar to mCIM but 0.5 M EDTA was added to TSB. This test is valid only when performed with mCIM. A descriptive analysis was done for the study.

Results

A total of 100 CREs were subjected to carbapenemase detection by CarbaNP and mCIM. All isolates were resistant to Imipenem, meropenem, and ertapenem (MIC >=2 µg/L).

Among the 100 CRE isolates, the different genus that reflected were as Klebsiella SPS 53%, Escherichia coli 31%, Enterobacter SPS 4%, Proteus SPS 6%, and others 6%, also shown seen in Figure 3.

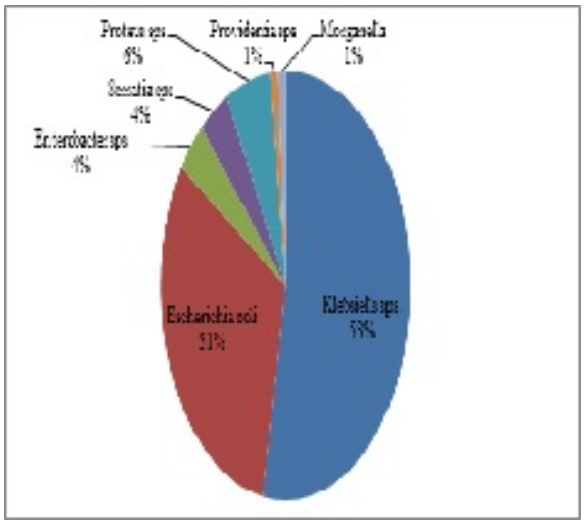

Fig-3: Genus detected in the CREs.

80% isolates were detected to be carbapenemase producers by mCIM method while 71% were detected by CarbaNP. The CarbaNP test results were ready in 2 hours, while the mCIM test and eCIM took around 24 hours. The results have been represented in Figure 4.

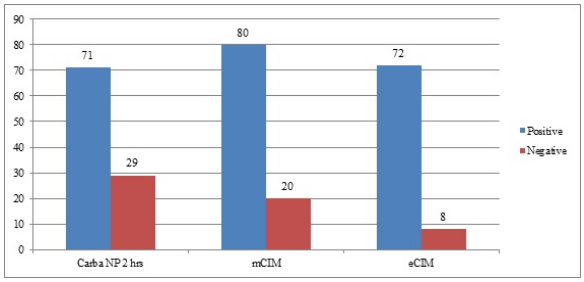

Fig-4: Comparison of CarbaNP, mCIM, and eCIM.

52% (37) of the interpreted color change was noted to be within 10 min and 47.8% (34) within 2 hours. Out of 80 positives, 72 were detected by both methods. 07 isolates found to be positive for carbapenemase production only by the mCIM method. 01 isolate tested positive for CarbaNP but tested negative in mCIM. 87% (72) of the mCIM positives were found eCIM positive, which indicated the presence of Metallo-beta-lactamases.

Discussion

The concept of demonstrating enzymatic hydrolysis of beta-lactam antibiotics by incubating them with bacterial suspensions dates back to the late 70s (9). CIM is the first test that uses antibiotic discs as substrates for carbapenemase detection [4].

Genotypic tests are the gold standard for carbapenemase detection, they cannot effortlessly be conducted on all *Enterobacteriaceae* clinical isolates, due to cost factors related to these tests. Genotyping needs infrastructure, technical manpower, and other requirements, which makes it less feasible in small setups and for a large number of isolates [2].

Also, results are limited by the targets included in assays. Thus, it has become customary for the present clinical laboratories to implement phenotypic methods to broadly categorize CRE into CP-CRE and non-CP-CRE.

CarbaNP described by Nordman and Poiral is easy to perform, with a few reagents available [6]. These reagents are also simple to prepare and can be stored at room temperature for a week and for a year in the refrigerator, which makes it less cumbersome.

The test gives results in 2 hours and the color change is easily noted. However, it lacks the sensitivity for detecting *bla*Oxa-48 like producers and some enzymes possessing weak carbapenemase activity such as *bla*SME and *bla*GES [3].

The newer recommended mCIM test in CLSI M100, 2018 edition is a simpler test, can be performed in all laboratories. It does not need any special reagents. This detects all carbapenemases including oxa-48 like enzymes with weak carbapenemase activity.

In the present study, the findings were in concordance with other studies published previously as shown in Table 1. Pragasam et al., did a similar study while reviewing other tests for carbapenemase detection and they found mCIM to be 99 % sensitive [3]. Kim van der Zwaluw et al. did genotypic detection for KPC, NDM, OXA-48, IMP, VIM genes and then they performed CIM and CarbaNP tests for the positive isolates [5].

They found CIM to be 100% sensitive while 4 (3.7%) isolates giving false-negative results in the CarbaNP test. Previously the CarbaNP results have been reported showing 90% sensitivity and specificity, but weak carbapenemase producers show less sensitivity in the CarbaNP test like the oxa-48 enzyme producers [7-10].

However, in the present study genotypic detection of carbapenemase genes was not done.

Table-1: Comparison of CarbaNP and mCIM.

	CarbaNP	Mcim
Pragrasam AK et al[3]	89%	98%
Zwaluw K V [5]	94.44%	100%
Mc Mullen A R et al [11]	79.9%	95%
Present study	71%	80%

Limitation of the study: Genotypic verification was not done in the present study. Also,the comparison was done between only 2 methods of carbapenemase production.

Other methods were not taken into consideration. The sensitivity and specificity calculated with these into consideration would have given more accurate results

Conclusion

To conclude, it was observed that mCIM provides a low-cost alternative for the Carba NPtest. It allows easy and rapid identification of carbapenemase activity.

What does the study add to the existing knowledge?

This provides a tool toidentify carbapenem-resistant Enterobacteriaceae bacteria that may easily spread in healthcare settings. mCIM method can be easily used in small settings to detect carbapenemases production and predict CP-CRE. This would help to change antibiotics early and save hospital costs for patients.

Author's contribution

Dr. Deepa Sinha: Concept and manuscript writing

Dr.Shantala G B.: Concept

Dr.Ambica R.: Study design and manuscript writing

Mrs. Kusuma C.R.: Manuscript writing

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