*IOSR Journal of Dental and Medical Sciences (IOSR-JDMS)*
*e-ISSN: 2279-0853, p-ISSN: 2279-0861.Volume 16, Issue 12 Ver. X (Dec. 2017), PP 90-93*
*www.iosrjournals.org*

# A Comparison of Rapid Phenotypic Tests for The Identification of Carbapenamase Production in Gram Negative Isolates

Maitreyi Bandyopadhyay[1,] *Simit Kumar[2*], Abhisekh Sengupta[3], Mohiruddin Sk[4], Prabir Kumar Mukhopadhyay[5], Manas Kumar Bandyopadhyay[6], Anindita Chatterjee[7], Mitali Chatterjee[8]

[12345678]*(Department of Microbiology, R.G.Kar Medical College, Kolkata / The West Bengal University of Health Sciences, India)*

**Corresponding Author: Dr. Simit Kumar*

**Abstract**

***Introduction:*** *CLSI recommends that until laboratories can implement the revised carbapenem MIC interpretive criteria, the Carba NP test (or an alternative confirmatory test for carbapenemases) should be performed when isolates of Enterobacteriaceae are suspicious for carbapenemase production based on imipenem or meropenem MICs of 2–4 μg/mL or ertapenem MIC of 2 μg/mL. CLSI recommends that for isolates that are Carba NP positive, report all carbapenems as resistant, regardless of MIC. If the Carba NP test is negative, interpret the carbapenem MICs using CLSI interpretive criteria as listed in Table 2A in M100-S20 (January 2010).*

***Aim and objectives :*** *To compare different phenotypic methods of carabapenemase detection namely, the CNPt-direct, the Blue-Carba test, the Carbapenem Inactivation Method in comparison to the Modified Hodge test.*

***Material and Methods:*** *The CNPt-direct, the Blue-Carba test, the Carbapenem Inactivation Method in comparison to the Modified Hodge test were performed to detect Carbapenemase production.*

***Results and Analysis:*** *70 isolates of Klebsiella spp., 26 isolates of E.coli, 52 Pseudomonas spp. and 56 Acinetobacter spp. were compared for carbapenamase production by the various phenotypic methods as described before.*

*Among all the phenotypic tests, the least detection rate for carbapenamase production in comparison with MHT was for the BCT. The rest of the tests showed good correlation with MHT results.*

***Discussion:*** *CNPt-direct and BCT could also be used to quickly identify carbapenem-resistant isolates from critically ill patients, despite their limitation to detect OXA expressing isolates, while the CIM can be used routinely for detection in other clinical isolates in resource limited settings.*

---

Date of Submission: 15- 12-2017 Date of acceptance: 22-2-2017

---

## I. Introduction

The proportion of members of the family *Enterobacteriaceae* and non-fermenters resistant to multiple antimicrobial classes has grown. Carbapenems, the last line of therapy, are now frequently needed to treat nosocomial infections, and increasing resistance to this class of β-lactams leaves the health care system with almost no effective drugs [1]. However, reports of carbapenem-resistant *Enterobacteriaceae* have increased [1].The mechanisms underlying carbapenem resistance in *Enterobacteriaceae* are complex and include both the production of carbapenem-hydrolyzing β-lactamases (carbapenemase-producing CRE [CP-CRE]) and resistance due to the presence of a combination of other factors (non-CP-CRE), such as hyperproduction of AmpC β-lactamases or extended-spectrum β -lactamases (ESBLs) combined with altered membrane permeability [2]. Spread of carbapenemase producers is a relevant clinical issue because carbapenemases confer resistance to most β-lactams [2]. In addition, carbapenemase producers are usually associated with many other non–β-lactam resistance determinants, which give rise to multidrug- and pandrug-resistant isolates [1].Accordingly, the emergence and spread of carbapenem-resistant *Enterobacteriaceae* (CRE) and carbapenem resistant non-fermenters is an issue of great clinical and public health concern [2].Characterization of the mechanism of carbapenem resistance is currently not recommended for the guidance of therapeutic decisions [2] and is not routine in most clinical laboratories; however, this distinction between CP-CRE and non-CP-CRE is important for infection control and epidemiologic purposes because many carbapenemases are carried on mobile genetic elements that facilitate horizontal transfer of resistance between Gram-negative organisms. CP-CRE can spread rapidly, and their detection may warrant implementation of more-intensive infection control interventions than would be employed for non-CP-CRE [2]. Additionally, as novel antimicrobial agents with activity against CP-

CRE are introduced, distinguishing CP-CRE from non-CP-CRE will be increasingly important for antimicrobial stewardship programs seeking to rationally prioritize the use of these new drugs [2]. Furthermore, a recent report suggested that CP-CRE might be more virulent than non-CP-CRE [2]; if this finding is confirmed, routine delineation of resistance mechanisms in CRE may become important for clinical care. Unfortunately, the phenotypic antimicrobial susceptibility testing (AST) profiles of CP-CRE and non-CP-CRE overlap [2]. Therefore, the Centers for Disease Control and Prevention (CDC) currently recommend that clinical laboratories consider actively screening for carbapenemase production in isolates that meet the CDC surveillance definition for CRE [2].

Potential carbapenemase producers are currently screened first by susceptibility testing, using breakpoint values for carbapenems [1]. However, this technique is time-consuming, and many carbapenemase producers do not confer obvious resistance levels to carbapenems. There
is a need for laboratories to search for carbapenemase producers [1]. To rapidly identify carbapenemase producers in Enterobacteriaceae, Nordmann and Poirel developed the Carba NP test. The test uses isolated bacterial colonies and is based on in vitro hydrolysis of a carbapenem, imipenem [3]. Pasteran et al developed a novel modified protocol (CNPt-direct) designed for carbapenemase detection directly from bacterial cultures (instead of bacterial extracts required by the CLSI tests). The specificities were comparable (100%), but the CNPt-direct was more sensitive (98% versus 84%). The CNPt-direct was easier to perform due to the direct use of colonies and offered a more robust detection of carbapenemase producers [4]. Another test, the Blue-Carba test (BCT) is a biochemical test for rapid (<2 h) detection of carbapenemase production in Gram-negative bacilli directly from bacterial culture [5]. It is based on the in vitro hydrolysis of imipenem by bacterial colonies (direct inoculation without prior lysis), which is detected by changes in pH values revealed by the indicator bromothymol blue (blue to green/yellow or green to yellow). It was reported to be 100% sensitive and specific for Enterobacteriaceae, Pseudomonas spp., and Acinetobacter spp. harboring carbapenemases [5]. The overall sensitivity and negative predictive value of the BCT were 97 and 96%, respectively. The BCT is an accurate and inexpensive way to unequivocally identify class A and B carbapenemases. However, the Carba NP test and BCT have shown limitations in the detection of OXA-48-like enzyme-producing Enterobacteriaceae[5].A new phenotypic test, called the Carbapenem Inactivation Method (CIM), was developed to detect carbapenemase activity in Gram-negative rods within eight hours. This method showed high concordance with results obtained by PCR to detect genes coding for the carbapenemases along with OXA-23. It allows reliable detection of carbapenemase activity encoded by various genes in species of Enterobacteriaceae (e.g., Klebsiella pneumoniae, Escherichia coli and Enterobacter cloacae), but also in non-fermenters Pseudomonas aeruginosa and Acinetobacter baumannii. The CIM was shown to be a cost-effective and highly robust phenotypic screening method that can reliably detect carbapenemase activity [6]. The sensitivity of the mCIM for the detection of NDM carbapenemases and OXA-48-type carbapenemases was found to be higher than that reported in some evaluations of the MHT and Carba NP test, respectively [6]. In addition, very few false-positive mCIM results have been observed, including among Enterobacter spp. expressing AmpC β-lactamases, in contrast to what has been reported for the MHT [2].

## II. Aim And Objectives

To compare different phenotypic methods of carabapenemase detection namely, the CNPt-direct, the Blue-Carba test, the Carbapenem Inactivation Method in comparison to the Modified Hodge test.

## III. Material And Methods

A modified protocol was attempted for the direct use of colonies (instead of bacterial extracts) (CNPt-direct), as follows: 0.1% (vol/vol) of Triton X-100 (Mallinckrodt, St. Louis, MO) was added to the aqueous indicator mix referred to above (0.05% phenol red-0.1 mmol/liter $ZnSO_4$) before the pH adjustment. This Triton X-100 concentration used was 0.2% (vol/vol). A full 1-μl loop of a pure bacterial culture recovered from Mueller-Hinton agar was directly suspended in 1.5-ml Eppendorf tubes containing 100 μl of CNPt-direct mix, supplemented with 12 mg/ml imipenem-cilastatin injectable form (reaction tube) or without antibiotic (control tube). Tubes were vigorously mixed during 5 to 10 s using a vortex device before incubation (foaming does not affect the test). Finally, tubes were incubated at 35°C and monitored throughout 2 h for color change from red to orange/yellow in the antibiotic-containing tube, which was interpreted as a positive result. Test solutions were supplemented with imipenem (standard powder or the injectable form) immediately before being used [4].

The BCT was performed by using a modified protocol as follows. Strains were grown on Mueller-Hinton agar plates. Subsequently, a 5-μl loop of a pure bacterial culture was resuspended in the test mixture, which consisted of an aqueous solution of bromothymol blue at 0.04% (Sigma) and 0.1 mmol/liter $ZnSO_4$ (Sigma) with (reaction tube) or without (control tube) supplementation with 6 mg of imipenem-cilastatin (equivalent to 3 mg of imipenem) and adjusted to a final pH of 7.0. Unsupplemented mixture solution was stored at 4°C (pH adjustments were needed every 4 to 6 weeks). The test solution was supplemented with

imipenem-cilastatin immediately before being used. *In vitro* hydrolysis of imipenem by bacterial colonies (direct inoculation without prior lysis), were detected by changes in pH values revealed by the indicator bromothymol blue (blue to green/yellow or green to yellow) [5].

To perform the CIM, a suspension was made by suspending a full 10 µl inoculation loop of culture, taken from a Mueller-Hinton or blood agar plate in 400 µl water. Subsequently, a susceptibility-testing disk containing 10 µg meropenem (Oxoid Ltd, Hampshire, United Kingdom) was immersed in the suspension and incubated for a minimum of two hours at 35°C. After incubation, the disk was removed from the suspension using an inoculation loop, placed on a Mueller-Hinton agar plate inoculated with a susceptible *E. coli* indicator strain (ATCC 29522) and subsequently incubated at 35°C. Inoculation of the Mueller-Hinton agar plate with the indicator strain was done with a suspension of $OD_{595}$ 1.25 (correlates with a McFarland value of 0.5) streaked in three directions using a sterile cotton swab. If the bacterial isolate produced carbapenemase, the meropenem in the susceptibility disk was inactivated allowing uninhibited growth of the susceptible indicator strain. Disks incubated in suspensions that do not contain carbapenemases yielded a clear inhibition zone. If results are required within the same day, they can be read after six hours, but within the setting of our laboratory, we prefer reading results after overnight incubation [6].

## IV. Results And Analysis

70 isolates of Klebsiella spp., 26 isolates of E.coli, 52 Pseudomonas spp. and 56 Acinetobacter spp. were compared for carbapenamase production by the various phenotypic methods as described before.
Among all the phenotypic tests, the least detection rate for carbapenamase production in comparison with MHT was for the BCT. The rest of the tests showed good correlation with MHT results.

**Table No. 1:** Table showing detection rates of the

| GNB | CIM +VE No.(%) | Carba NP +VE No.(%) | Blue-Carba +VE No.(%) | MHT |
|---|---|---|---|---|
| *Klebsiella spp. (n=70)* | 68 (97.1) | 68 (97.1) | 66 (94.2) | 70 (100) |
| *Escherichia coli (n=26)* | 24 (92.3) | 24 (92.3) | 24 (92.3) | 26 (100) |
| *Pseudomonas spp. (n=52)* | 52 (100) | 52 (100) | 50 (96.15) | 52 (100) |
| *Acinetobacter spp. (n=56)* | 52 (92.9) | 50 (89.3) | 50 (89.3) | 56 (100) |

various isolates by the phenotypic methods

## V. Discussion And Conclusion

Phenotype-based techniques for identifying in vitro production of carbapenemase, such as the modified Hodge test, are not highly sensitive and specific [1]. Both the phenotype based techniques and molecular tests are time-consuming (at least 12–24 hours) and are poorly adapted to the clinicalneed for isolating patients rapidly to prevent nosocomial outbreaks. Furthermore, genotypic assays (such as PCR and DNA microarray tests) for the detection of carbapenemase genes are limited in their scope because only known targets are detected and mutations within targets could compromise assay performance. In addition, these molecular methods are expensive, require special equipment and expertise to perform, and are not in widespread use [2]. CNPt-direct has been shown to be 100% sensitive and specific compared with molecular-based techniques. This rapid (<2 hours), inexpensive technique may be implemented in any laboratory. CLSI recommends that until laboratories can implement the revised carbapenem MIC interpretive criteria, this test (or an alternative confirmatory test for carbapenemases) should be performed when isolates of *Enterobacteriaceae* are suspicious for carbapenemase production based on imipenem or meropenem MICs of 2–4 µg/mL or ertapenem MIC of 2 µg/mL.CLSI recommends that for isolates that are Carba NP positive, report all carbapenems as resistant, regardless of MIC. If the Carba NP test is negative, interpret the carbapenem MICs using CLSI interpretive criteria as listed in Table 2A in M100-S20 (January 2010) [3]. .

The advantages and disadvantages of the different phenotypic methods for detection of carbapenamase production have been depicted below:

| **Test** | **MHT** | **CNPt-direct** | **BCT** | **CIM** |
|---|---|---|---|---|
| **Advantages** | Simple to perform<br>No special reagents or media necessary | Rapid<br>Less expensive | Rapid<br>Less expensive | • Sensitivity of the mCIM for the detection of NDM carbapenemases and OXA-48-type carbapenemases was found to be higher than that reported in some evaluations of the MHT and Carba NP test, respectively.<br>• very few false-positive mCIM results have been observed, including among Enterobacter spp. expressing AmpC β- |

| | | | | |
|---|---|---|---|---|
| | | | | lactamases, in contrast to what has been reported for the MHT |
| **Disadvantages** | • False-positive results can occur in isolates that produce ESBL or AmpC enzymes coupled with porin loss.<br>• False-negative results are occasionally noted (eg, some isolates producing NDM carbapenemase).<br>• Only applies to *Enterobacteriaceae.*<br><br>Requires overnight incubation | Limitations in the detection of OXA-48-like enzyme-producing Enterobacteriaceae | Limitations in the detection of OXA-48-like enzyme-producing Enterobacteriaceae | Requires overnight incubation |

These tests are inexpensive, rapid, reproducible, and highly sensitive and specific. It eliminates the need for using other techniques to identify carbapenemase producers that are time-consuming and less sensitive or specific. Using these tests would improve detection of patients infected or colonized with carbapenemase producers [1]. These tests could be used, for example, for directly testing 1) bacteria obtained from antibiograms of blood culture or 2) bacterial colonies grown on culture media before antimicrobial drug susceptibility testing. Further studies will evaluate their clinical values for antimicrobial drug stewardship on bacteria isolated directly from clinical samples. CNPt-direct and BCT could also be used to quickly identify carbapenem-resistant isolates from critically ill patients, despite their limitation to detect OXA expressing isolates, while the CIM can be used routinely for detection in other clinical isolates in resource limited settings [1]. These tests can efficiently indicate the strains to be further tested by PCR or submitted to sequencing for a detailed identification of the carbapenemase genes. Last, they could be used in low-income countries that are large reservoirs for carbapenemase producers [7]. It offers a practical solution for detecting a main component of multidrug resistance. Use of these tests will contribute to a better stewardship of carbapenems by changing the paradigm of controlling carbapenemase producers worldwide [1].Further studies can be performed to validate these tests from clinical specimens collected from sterile sites.

## References


[1]. Nordmann P, Poirel L, Dortet L. Rapid Detection of Carbapenemase-producing Enterobacteriaceae. Emerg Infect Dis. 2012;18(9):1503-1507.

[2]. Virginia M. Pierce, Patricia J. Simner, David R. Lonsway, Darcie E. Roe-Carpenter, J. Kristie ohnson, William B. Brasso,et al. Modified Carbapenem Inactivation Method for Phenotypic Detection of Carbapenemase Production among Enterobacteriaceae. J. Clin. Microbiol. August 2017 vol. 55 no. 8 2321-2333

[3]. Clinical and Laboratory Standards Institute. 2016. Performance standards for antimicrobial susceptibility testing, 26th ed. CLSI supplement M100S. Clinical and Laboratory Standards Institute, Wayne, PA.

[4]. Fernando Pasteran, Nathalie Tijet, Roberto G. Melano, Alejandra Corso. Simplified Protocol for Carba NP Test for EnhancedDetection of Carbapenemase Producers Directly from Bacterial Cultures. *J. Clin. Microbiol.* December 2015 vol. 53 no. 12 3908-11.

[5]. Pasteran F, Veliz O, Ceriana P, Lucero C, Rapoport M, Albornoz E, Gomez S, Corso A. Evaluation of the Blue-Carba test for rapid detection of carbapenemases in gram-negative bacilli**.** J Clin Microbiol. 2015 Jun;53(6):1996-8.

[6]. van der Zwaluw K, de Haan A, Pluister GN, Bootsma HJ, de Neeling AJ, et al. (2015) The Carbapenem Inactivation Method (CIM), a Simple and Low-Cost Alternative for the Carba NP Test to Assess Phenotypic Carbapenemase Activity in Gram-Negative Rods. PLOS ONE 10(3): e0123690.

[7]. Tijet N, Boyd D, Patel SN, Mulvey MR, Melano RG. Evaluation of the Carba NP test for rapid detection of carbapenemase-producing Enterobacteriaceae and Pseudomonas aeruginosa. Antimicrob Agents Chemother. 2013 Sep;57(9):4578-80.


Dr. Simit Kumar. "A Comparison of Rapid Phenotypic Tests For The Identification of Carbapenamase Production in Gram Negative Isolates." IOSR Journal of Dental and Medical Sciences (IOSR-JDMS) 16.12 (2017): 90-93