International Journal of Microbiology Research
ISSN: 0975-5276 & E-ISSN: 0975-9174, Volume 11, Issue 11, 2019, pp.-1736-1738.
Available online at https://www.bioinfopublication.org/jouarchive.php?opt=&jouid=BPJ0000234

Research Article

PHENOTYPIC DETECTION OF CARBAPENEMASE PRODUCTION AMONG GRAM NEGATIVE BACILLI BY MODIFIED CARBAPENEM INACTIVATION METHOD

JINSHA K.M.[1], JOSE R.*[2], VALSAN C.[2] AND SATHIAVATHY K.A.[2]
[1]School of Health Sciences, University of Calicut, Malappuram, 673 635, Kerala, India
[2]Department of Microbiology, Jubilee Mission Medical College & Research Centre, Thrissur, 680005, Kerala, India
*Corresponding Author: Email - rosdinmi@gmail.com

Received: November 02, 2019; Revised: November 24, 2019; Accepted: November 26, 2019; Published: November 30, 2019

Abstract- Several phenotypic methods for detection of carbapenemase producing isolates have been developed and used in clinical microbiology laboratories and all existing methods have limitations. Modified Carbapenem Inactivation (mCIM) method is a new growth-based assay recommended by CLSI for the detection of carbapenemases. In this study we performed mCIM for 129 meropenem resistant Gram negative bacilli (*E.coli* =20, *K. pneumoniae*= 55, *P.aeruginosa* = 13 and *A.baumannii* = 41). Hundred and one (78.3%) isolates were positve, 23 (17.8%) were negative and five isolates gave indeterminate results in mCIM. We found out that mCIM is an easy and inexpensive method for detection of carbapenemase production among Gram negative bacilli.

Keywords- *mCIM, CLSI*

Citation: Jinsha K.M., *et al*., (2019) Phenotypic Detection of Carbapenemase Production among Gram negative Bacilli by Modified Carbapenem Inactivation Method. International Journal of Microbiology Research, ISSN: 0975-5276 & E-ISSN: 0975-9174, Volume 11, Issue 11, pp.-1736-1738.

Academic Editor / Reviewer: Abdel Raheem M.A., Palaksha M.N., Dey S., Sharanya K., Sodani Sadhna

Introduction

Carbapenems are a class of beta-lactam antibiotics with broad spectrum of activity and are stable against most beta-lactamase enzymes produced by bacteria. Therefore, carbapenems are usually reserved for multidrug resistant bacterial infections. However, the emergence and spread of carbapenem resistant Gram negative bacilli is a worldwide emerging public health threat [1-3]. The mechanisms underlying carbapenem resistance is complex. Carbapenem resistance is mostly mediated by production of carbapenemase enzymes, followed by chromosomal mediated porin loss and efflux pump over expression. Many carbapenemases are carried on mobile genetic elements that facilitate horizontal transfer of resistance between the Gram-negative organisms. So, the distinction between carbapenemase producing Carbapenem resistant organisms (CP-CRO) and non carbapenemase producing Carbapenem resistant organisms (non-CP-CRO) is important for the infection control and epidemiological purposes. Several phenotypic methods for detection of carbapenemase producing isolates have been developed and used in clinical microbiology laboratories. In 2017, CLSI recommended the modified Carbapenem Inactivation method (mCIM), which is effective in detecting a variety of carbapenemase in most routine microbiology laboratories [4]. Hence, this study was conducted to detect the carbapenemase production among Gram negative bacilli using mCIM.

Materials and Methods

This study was conducted in the Microbiology Department of an 1800 bedded tertiary care teaching hospital of central Kerala. Study period was two months (May 2019 to June 2019). This study included pure growth of *Klebsiella pneumoniae, Escherichia coli, Pseudomonas aeruginosa* and *Acinetobacter baumannii* isolated from blood, urine, pus and respiratory samples. Identification and antibiotic susceptibility testing of all these isolates were done in vitek 2 compact system (bio Meriux). All meropenem resistant (*E.coli* and *K. pneumoniae* MIC≥ 4µg/ml, *P.aeruginosa* and *A.baumannii* MIC ≥8 µg/ml) isolates further subjected to mCIM for the detection of cabapenemase production.

Modified Carbapenem Inactivation method

1ul loop full of bacteria for Enterobacteriaceae or 10µl loop full of bacteria for *P. aeruginosa* and *A. baumannii* from an overnight culture was emulsified in 2ml of Trypticase soy broth (TSB).
Vortexed for 10-15 seconds.
10µg of meropenem disc is then added to each tube using sterile forceps.
Incubated at 37°C in ambient air for 4hours.
0.5 McFarland suspension of *E.coli* ATCC 25922 prepared and inoculated on MHA plate as for routine disc diffusion procedure. Allowed the plates to dry for 3-10 minutes.
Removed the meropenem disc from the TSB using 10µl loop and placed on the MHA plate inoculated with *E.coli* ATCC 25922 strain.
Incubated the MHA plates at 37°C in ambient air for 18-24hours.
After incubation measure the zone of inhibition around meropenem disc.
In house Strain of *E. coli* (Carbapenemase positive) was used as positive control and ATCC 25922 was used as negative control.
Interpretation done based on CLSI criteria
Carbapenemase positive: zone diameter of 6-15 mm or pinpoint colonies within 16-18mm [Fig-3]
Carbapenemase negative: zone diameter of ≥19mm (clear zone) [Fig-4]
Indeterminate: zone diameter of 16-18mm or zone diameter of >19mm and presence of pinpoint colonies within the zone [Fig-5]

Results

A total of 722 Gram negative bacilli were isolated from 3859 clinical samples received for bacteriological culture during the study period. From these non-repetitive isolates of *E.coli* (n=269) *K. pneumoniae* (n=197), *P. aeruginosa* (n=88) and *A baumannii* (n=80) were included in the study. Hundred and twenty nine out of these 634 (20.3%) isolates were meropenem resistant. Meropenem resistance among the isolates were as follows *E. coli* 7.4%, *K. pneumoniae* 27.9%, *P. aeruginosa* 14.8% and *A. baumannii* 51.3% [Fig-1].

All 129 Meropenem resistant isolates were further subjected to mCIM for detection of carbapenemase production. Results of the mCIM is given in [Table-2]. Carbapenemase production among carbapenem resistant isolates were as follows *E.coli* 95%, *K. pneumoniae* 81.81%, *P. aeruginosa* 69.23% and *A. baumannii* 68.29% [Fig-2]. Two isolates of *K. pneumoniae* and three of *A. baumannii* showed indeterminate results.

Table-1 *Sample wise distribution of CROs*

Clinical Samples	Total	No of CR isolates
Pus	652	35(5.4%)
Respiratory	500	40(8.0%)
Blood	1214	17(1.4%)
Urine	1493	37(2.4%)
Total	3859	129(3.3%)

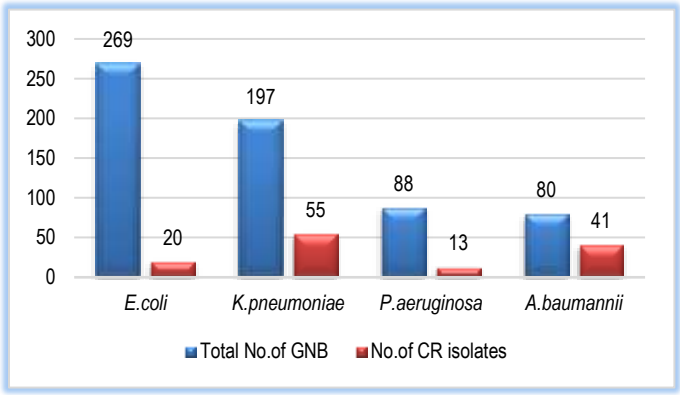

Fig-1 Organism wise distribution of Carbapenem resistance

Table-2 *Results of the mCIM*

Results	N=129 n(%)
positive	101(78.3%)
Negative	23 (17.8%)
Indeterminate	5(3.9%)

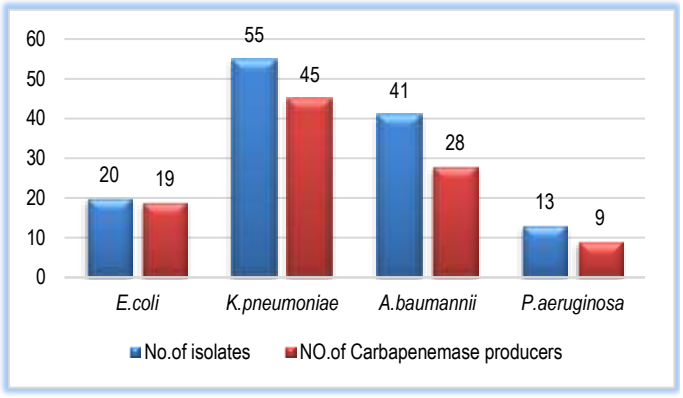

Fig-2 Distribution of Carbapenemase production among CR isolates

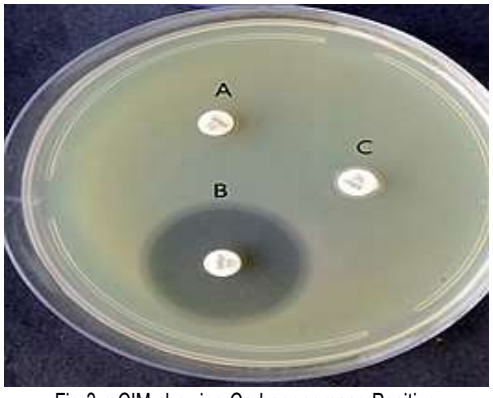

Fig-3 mCIM showing Carbapenemase Positive
A: Positive Control, B: Negative Control, C: Carbapenemase Positive

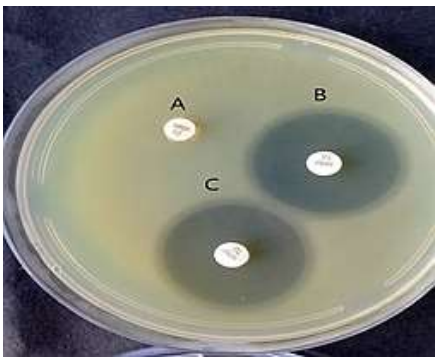

Fig-4 mCIM showing Carbapenemase negative
A: Positive Control, B: Negative control C: Carbapenemase negative

Fig-5 mCIM showing indeterminate result (zone diameter of 23mm and pinpoint colonies within the zone)

Discussion

The production of carbapenemase among Gram negative bacilli varies greatly from country to country and different institutions within the country. Phenotypic assays which currently used in clinical practice to detect carbapenemase production include growth-based assays, hydrolysis methods and lateral flow immunoassay. Modified Hodge test is the most well-known growth-based approach for carbapenemase detection. This assay demonstrates acceptable sensitivity for most carbapenemases particularly KPC enzymes, but low sensitivity for MBLs [5-7]. Carba NP test is a hydrolysis method which detect carbapenem degradation products. Even though this is a rapid method for carbapenemase detection, low sensitivity for OXA-48 like enzymes and cost of the kit were found to be major limitations [8,9] Carbapenem Inactivation method (CIM) was first described in 2015 [10]. This test is based on the promise that when 10µg meropenem disc is incubated for 2 hours in water with 10µl loop of carbapenemase producing isolates, meropenem will be hydrolysed. Initial investigations suggested that the CIM have limitations with detection of OXA type carbapenemases and MBL enzymes. Modified Carbapenem Inactivation method is a new phenotypic method recommended by CLSI in 2017 for detection of carbapenemase production among Gram negative bacilli. Carbapenem resistance in the present study was 20.3% and was highest among *A. baumanni* 51.2 % followed by *K. pneumoniae* 27.9%. Carbapenem resistance was high when compared to a previous study from same part of the country which reported 11% Carbapenem resistance among multidrug resistant Gram negative bacilli [11]. Studies from other parts of India have reported that carbapenem resistance ranges from 9 to 22% among Gram negative bacilli [12-14]. In a community-based study from south India Sekar *et al* documented three percent carbapenem resistance in members of *Enterobacteriaceae* [15], however in the treatment guidelines document released by the Indian Council of Medical Research surveillance data a high meropenem resistance of 42, 47 and 62 percent was reported among members of *Enterobacteriaceae, P. aeruginosa and A. baumannii*, respectively [16]. Gupta *et al* reported that maximum carbapenem resistance seen among *P.aeruginosa* 37.6%. Datta *et al* reported 52% carbapenem resistance among *K. pneumoniae* isolates from blood stream infections in the year 2009. In our study isolation rate of CROs were maximum from respiratory samples (8%) and least from blood culture isolates (1.4%). This may be due to the high rate of colonisation of respiratory tract with CROs among hospitalised patients.

In our study carbapenemase production among CROs were found to be 78.3%. Carbapenemase production among CRE was 85.3% (*E. coli* 95%, *K. pneumoniae* 81.8%). Carbapenemase production among meropenem resistant *P aeruginosa* and *A. baumannii* in our study was 69%and 68% respectively. We got indeterminate results for five isolates which included three isolates of *A. baumannii* and two isolates of *Klebsiella pneumoniae*. Anjali *et al* reported that 74% of the CROs were carbapenemase producers both by phenotypic and genotypic methods.

A multicentre study conducted showed that mCIM has got a mean sensitivity and mean specificity of 97%and 99% respectively for detection of Carbapenemase among CRE [17]. Another ten-site study reported a mean sensitivity and specificity of 98% and 95% for the detection of carbapenemase-producing *P. aeruginosa* [18]. The mean sensitivity and specificity for *A. baumannii* were 80% and 53% respectively. It is also recognized that a larger inoculum is required for reliable carbapenemase detection for non-glucose-fermenting organisms, compared with the Enterobacteriaceae. However, the increased sensitivity (60% to 93%) was at the expense of specificity, as was observed with *A. baumannii* isolates, where the specificity decreased from 100% using a 1-µl loop to 63% using a 10-µl loop [18]. Pierce et al reported indeterminate results for two isolates among 61 isolates tested which included one IMP positive *K. pneumoniae* and one carbapenemase negative *E. coli*. Major limitation of our study was that we could not do genotypic characterisation of the carbapenem resistant isolates.

Conclusion

Our study concluded that carbapenem resistance is on the rise and more than three fourth of the carbapenem resistance is due to carbapenemase production.

Application of research: Modified Carbapenem Inactivation method is found to be a simple and inexpensive method for detection carbapenemase producers which can be adopted in every clinical microbiology laboratories.

Research Category: Medical Microbiology

Acknowledgement / Funding: Authors are thankful to Department of Microbiology, Jubilee Mission Medical College & Research Centre, Thrissur, 680005, Kerala, India

****Principal Investigator or Chairperson of research: Dr Rosmi Jose**
University: Jubilee Mission Medical College & Research Centre, Thrissur, 680005
Research project name or number: Clinical research study

Author Contributions: All authors equally contributed

Author statement: All authors read, reviewed, agreed and approved the final manuscript. Note-All authors agreed that- Written informed consent was obtained from all participants prior to publish / enrolment

Study area / Sample Collection: Microbiology Department, Tertiary Care Teaching Hospital of Central Kerala

Cultivar / Variety / Breed name: Nil

Conflict of Interest: None declared

Ethical approval: This article does not contain any studies with human participants or animals performed by any of the authors.
Ethical Committee Approval Number: Nil

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