SSR Institute of International Journal of Life Sciences
ISSN (O): 2581-8740 | ISSN (P): 2581-8732
Munuswamy *et al.*, 2025
crossref doi: 10.21276/SSR-IIJLS.2025.11.1.13

Original Article

open access

Rapid Detection of Carbapenemase Production by Modified Carbapenem Inactivation Method (mCIM) among Gram-negative Bacteria at a Tertiary Care Hospital, Puducherry

Sangeetha Munuswamy[1]*, S. Umadevi[2], Joshy M Easow[3]

[1]Assistant Professor, Department of Microbiology, Karpagam Faculty of Medical Sciences and Research, Othakkalmandapam, Coimbatore, Tamil Nadu, India

[2]Professor, Department of Microbiology, Mahatma Gandhi Medical College & Research Institute, Sri Balaji Vidyapeeth University, Puducherry, India

[3]Professor & Head, Department of Microbiology, Mahatma Gandhi Medical College & Research Institute, Sri Balaji Vidyapeeth University, Puducherry, India

***Address for Correspondence:** Dr. Sangeetha Munuswamy, Assistant Professor, Department of Microbiology, Karpagam Faculty of Medical Sciences and Research, Othakkalmandapam, Coimbatore, Tamil Nadu-641032, India
E-mail: sangeetha92cool@gmail.com **& ORCID ID:** https://orcid.org/0009-0008-5519-4013

Received: 28 Aug 2024/ Revised: 25 Oct 2024/ Accepted: 17 Dec 2024

ABSTRACT

Background: Antimicrobial Resistance is increasing globally. Carbapenems are one of the effective antibiotics against a broad range of pathogenic bacteria. The development of carbapenem resistance among gram-negative bacteria is one of the crucial concerns and threatening issues which could cause increased morbidity and mortality, especially in hospitalized patients. The development of carbapenem resistance among gram-negative bacteria occurs due to various mechanisms among which carbapenemase production (carbapenem hydrolyzing enzymes) is the major mechanism. Carbapenemase-producing genes can be easily transferred from one bacterium to another, they have the potential to cause major outbreaks and contribute to high fatality. Therefore, it is essential to identify carbapenemase-producing bacteria among all carbapenem-resistant bacterial isolates. This study aims to detect carbapenemase production among carbapenem-resistant gram-negative bacteria by Modified carbapenem inactivation method.

Methods: Our study was a cross-sectional descriptive study. The bacterial isolates resistant to carbapenems from pus samples were subjected to the Modified carbapenam inactivation method (mCIM) test.

Result: Out of 70 isolates, the Modified Carbapenem Inactivation Method (mCIM) test was positive in 21(30%) isolates. Of the 30%(n=21) mCIM-positive isolates, 5(23.80%) isolates were sensitive to Meropenam and resistant to Imipenam and the remaining isolates were resistant to both Imipenam and Meropenam.

Conclusion: The modified Carbapenem Inactivation Method (mCIM) test can be used as a primary tool for early identification of carbapenemase producers among carbapenem-resistant bacteria.

Key-words: Antimicrobial Resistance, Carbapenemase, Carbapenem resistance, Gram Negative Bacteria, Modified carbapenem inactivation method(mCIM)

How to cite this article

Munuswamy S, Umadevi S, Easow JM. Rapid Detection of Carbapenemase Production by Modified Carbapenem Inactivation Method (mCIM) among Gram-negative Bacteria at a Tertiary Care Hospital, Puducherry. SSR Inst Int J Life Sci., 2025; 11(1): 6702-6706.

Access this article online
https://iijls.com/

INTRODUCTION

Antimicrobial Resistance (AMR) occurs when microorganisms change over time and become resistant to drugs, making even the common infections harder, increasing the disease spread, severe illness and death. It is estimated that bacterial AMR was directly responsible for 1.27 million Global deaths and contributed to 4.95 million deaths in a year [1]. In INDIA, there were 2,97,000 deaths attributable to AMR and 10,42,500 deaths associated with AMR [2]. WHO- Global Antimicrobial Resistance And Use Surveillance System

doi: 10.21276/SSR-IIJLS.2025.11.1.13

(GLASS) has estimated that in the year 2050, around 10 million deaths due to AMR and the economic burden of up to 3.5**%** of global GDP [1].

Carbapenems are one of the effective antibiotics against a broad range of pathogenic bacteria whose antibacterial activity is wide [3,4]. Carbapenems are potent against large groups of lactamase-producing bacteria [4]. Carbapenems are one of the preferred antibiotics in the treatment of infections caused by multidrug-resistant gram-negative bacteria (MDR–GNB) [5].

The development of carbapenem resistance among gram-negative bacteria is one of the major concerns and threatening issues. Carbapenem-resistant bacteria prevalence is on a rising trend worldwide [6]. In a study done in ASIA, the prevalence of Carbapenem-resistant isolates ranges from 0.6-0.9% and its prevalence in India is around 18-31% among culture-positive infections [6]. Development of resistance to carbapenems among gram-negative bacteria occurs due to various mechanisms among which carbapenemase production (carbapenem hydrolyzing enzymes) is the major mechanism [7]. Carbapenemase-producing genes can be easily transferred from one bacterium to another, they have the potential to cause major outbreaks and contribute to high fatality [8,9].

Therefore, it is essential to detect carbapenemase-producing bacteria in all carbapenem-resistant bacterial isolates. WHO has also mentioned to consider carbapenemase-producing Gram-negative bacteria (GNB) as critical pathogens [10]. Rapid detection of Carbapenemase-producing bacteria would help us streamline the antibiotic treatment and spread of those bacteria in healthcare and the community. At present, CLSI advises the Carba NP test (CNPt) and mCIM as methods for rapid detection of carbapenemase. This study aims to detect carbapenemase production among carbapenem-resistant gram-negative bacteria by the Modified carbapenem inactivation method (mCIM).

MATERIALS AND METHODS

Research Design- Our study was a cross-sectional descriptive study of carbapenem-resistant gram-negative bacteria isolated from pus samples received in the Microbiology department from January 2018 to July 2018 at Mahatma Gandhi Medical College & Research Institute, Puducherry, India.

Inclusion criteria- All gram-negative bacteria resistant to Carbapenems (i.e. Imipenam/ Meropenam) isolated from pus samples received in the Microbiology department during the study period were included in the study.

Exclusion criteria – There were no exclusions.

Methodology- Bacteria were identified by colony morphology, gram staining, standard biochemical test (IMViC) and sugar fermentation tests. Antibiotic susceptibility of bacteria was done in Muller Hinton agar by standard Kirby Bauer's disc diffusion test as per Clinical and Laboratory Standards Institute (CLSI) guidelines. Following antimicrobials were tested against all gram-negative bacteria: Ampicillin; Cephalosporins (Cefoxitin, Ceftazidime and Ceftriaxone); βlactam/β lactamase inhibitor (cefoperazone/ sulbactum and Piperacillin/tazobactum); fluoroquinolones (ciprofloxacin); aminoglycosides (Amikacin, gentamicin and tobramycin); carbapenems (imipenam and meropenam); cotrimoxazole, polymyxins (polymyxin B, colistin). Quality control: Batch-wise testing was done for all agar plates, biochemical tests and antibiotic discs as per CLSI 2018 Guidelines. Bacterial strains ATCC *S. aureus* 25923 and ATCC *E. coli* 25922 were used for quality control purposes.

Gram-negative bacteria which were found to be resistant to Carbapenams- Imipenam/ Meropenam as per CLSI guidelines [11] were subjected to the Modified Carbapenam Inactivation Method (mCIM) test. Bacterial Isolate to be tested is emulsified in Trypticase soy broth, 10µg Meropenam disc is added to the broth and incubated 37© for 4 hours. Meropenam disc was placed on MHA plate inoculated with ATCC *E. coli,* incubated at 37© overnight and zone sizes were recorded. mCIM test was interpreted as follows: Positive: Carbapenemase detected- zone size 6-15mm or presence of pinpoint colonies within 16-18mm zone; Negative: Carbapenamase not detected - Zone diameter >19mm as per CLSI guidelines [11].

Statistical Analysis- The results were entered into an Excel sheet and analyzed with frequency and ratio.

RESULTS

During the study period, a total of 70 gram-negative bacterial isolates were resistant to Carbapenem antibiotics. Among the 70 gram-negative isolates,

doi: 10.21276/SSR-IIJLS.2025.11.1.13

Pseudomonas sp. was found to be the majority with 43%, followed by *E. coli*, non-fermentive gram-negative Bacilli (NFGNB), *Proteus* sp. and *K. pneumoniae*. Table 1 shows the distribution of Carbapenem-resistant Gram-negative bacteria. Among the total isolates, 25 isolates (35.7%) were resistant to both Imipenam and Meropenam (IRMR), whereas the remaining 45 isolates (64.2%) were resistant to Imipenam and Sensitive to Meropenam (IRMS).

Table 1: Distribution Of Carbapenem-Resistant Gram-Negative Bacteria

GNB	mCIM Positive n (%)
Pseudomonas sp.	11(52.38)
E. coli	05 (23.80)
K. pneumoniae	03(14.28)
Acinetobacter sp.	01(4.76)
Enterobacter sp.	01 (4.76)

Out of a total of 70 isolates, mCIM test was found to be positive in 21(30%) isolates and the remaining 49(70%) isolates were negative for the mCIM test. mCIM positivity was maximum among Pseudomonas sp. followed by *E. coli*, *K. pneumoniae, Acinetobacter* sp. and *Enterobacter* sp. as shown in Table 2.

Of the 30%(n=21) mCIM positive isolates, 5(23.80%) isolates were sensitive to Meropenam and resistant to Imipenam and the remaining isolates were resistant to both Imipenam and Meropenam as depicted in Table 3.

Table 2: Distribution of mCIM-positive isolates among Carbapenem-resistant gram-negative bacteria

Bacteria	Percentage (%)
Pseudomonas sp.	43
E. coli	26
Acinetobacter sp.	16
Proteus sp.	8
K. pneumoniae	7

Table 3: Distribution of mCIM Positive Gram-Negative Bacteria (GNB)

Isolates	mCIM Test	n	%
IRMR	Positive	16	76.19
IRMS	Positive	5	23.80

IRMR-Bacterial isolates resistant to Imipenem and Meropenem
IRMS-Bacterial isolates resistant to Imipenem and Sensitive to Meropenem

DISCUSSION

In our study, mCIM test was carried out on all carbapenem-resistant gram-negative bacteria isolated from pus samples. In our study, *Pseudomonas* sp. was the maximum among the carbapenem-resistant isolates with 43%, which coincides with the study conducted by Gandra *et al.* which showed 46.8% [12]. Next to Pseudomonas, Escherichia coli showed 26% resistance to carbapenems, the study conducted at Coimbatore [13] had similar results whereas studies done at Bangalore [6] and Jordan [4] showed very low Carbapenem-resistant *E. coli. Acinetobacter* sp. which is one of the important nosocomial pathogens, showed carbapenem resistance of about 16% which coincided with the results of a study done by Himanshi Khanchandani *et al.* and a study done at Coimbatore with carbapenem-resistant Acinetobacter of about 14.3% and 13.3% respectively [13,14]. Studies done at Mizoram and Bihar showed carbapenem resistance in Acinetobacter only 8.5% and 5.8% respectively [15,16]. *Proteus* sp. showed 8% resistance to carbapenem which coincided with the study done at Coimbatore [13]. Most of the studies showed *Klebsiella* sp. showed a high rate of carbapenem resistance at 92.64%, 30.4%, and 42.7% in studies done at Bangalore, Mizoram and Jordan respectively [4,6,15]. But whereas in our study, carbapenem resistance among *Klebsiella* sp. was only about 7%. This difference can be due to the study period was only 6 months, a low sample size and GNB from pus samples were only included in the study.

The majority of mCIM test-positive bacterial isolates (76.19%, n=16) were found to be resistant to both imipenem and meropenem. The mCIM test was also found to be positive in isolates (23.80%, n=5) that were sensitive to Meropenem and Resistant to imipenem. This kind of isolates having discordant carbapenem susceptibility was also found by Harino *et al*. in Japan [17] and Ku *et al.* [18]. The mCIM test is a rapid, easy-to-perform, cost-effective and reliable test to detect Carbapenemase production [19–21]. The mCIM test has a 100% sensitivity and specificity when compared to other methods of detection of carbapenem detection such as

crossref doi: 10.21276/SSR-IIJLS.2025.11.1.13

the Modified Hodge test, and Carba NP Test which is shown in a study done by Kumudunie WGM *et al*. [21].

CONCLUSIONS

The modified Carbapenem Inactivation Method (mCIM) test can be used as a primary tool for early identification of carbapenemase producers among carbapenem-resistant bacteria which could help in planning the management of the infections and contain the spread of carbapenemase producers as they are easily transmissible so as to stop the outbreak.

ACKNOWLEDGMENTS

The authors would like to thank the Faculties and Technicians of the Department of Microbiology, Mahatma Gandhi Medical College, Sri Balaji Vidyapeeth University, Pondicherry, India for providing their support.

CONTRIBUTION OF AUTHORS

Research concept- S. Umadevi, Joshy M Easow
Research design- Sangeetha Munuswamy, S. Umadevi
Supervision- S. Umadevi
Materials- Sangeetha Munuswamy
Data collection- Sangeetha Munuswamy
Data analysis and Interpretation- Sangeetha Munuswamy, S.Umadevi, Joshy M Easow
Literature search- Sangeetha Munuswamy
Writing article- Sangeetha Munuswamy, S. Umadevi
Critical review- Sangeetha Munuswamy, S. Umadevi, Joshy M Easow
Article editing- Sangeetha Munuswamy, S. Umadevi, Joshy M Easow
Final approval- Sangeetha Munuswamy, S. Umadevi, Joshy M Easow

REFERENCES

[1] Antimicrobial resistance [Internet]. [cited 2024 Dec 24]. Available from: https://www.who.int/news-room/fact-sheets/detail/antimicrobial-resistance.
[2] India.pdf [Internet]. [cited 2024 Dec 24]. Available from: https://www.healthdata.org/sites/default/files/2023-09/India.pdf.
[3] Zagui GS, De Andrade LN, Moreira NC, Silva TV, Machado GP, et al. Gram-negative bacteria carrying β-lactamase encoding genes in hospital and urban wastewater in Brazil. Environ Monit Assess, 2020; 192(6): 376.
[4] Hammour KA, Abu-Farha R, Itani R, Karout S, Allan A, et al. The prevalence of Carbapenem Resistance Gram negative pathogens in a Tertiary Teaching Hospital in Jordan. BMC Infect Dis., 2023; 23(1): 634. doi: 10.1186/s12879-023-08610-4.
[5] Elbadawi HS, Elhag KM, Mahgoub E, Altayb HN, Ntoumi F, et al. Detection and characterization of carbapenem resistant Gram-negative bacilli isolates recovered from hospitalized patients at Soba University Hospital, Sudan. BMC Microbiol., 2021; 21(1): 136. doi: 10.1186/s12866-021-02133-1.
[6] Joshi DN, Shenoy B, Bhavana MV, Adhikary R, Shamarao S, et al. Prevalence of Carbapenem-Resistant Enterobacteriaceae and the Genes Responsible for Carbapenemase Production in a Tertiary Care Hospital in South India. EMJ, 2023; doi: 10.33590/emj/10300425.
[7] Suay-García B, Pérez-Gracia MT. Present and future of carbapenem-resistant Enterobacteriaceae infections. Adv Clin Immunol Med Microbiol COVID-19 Big Data, 2021; 435–56. doi: 10.3390/antibiotics8030122.
[8] Goodman K, Simner P, Tamma P, Milstone A. Infection control implications of heterogeneous resistance mechanisms in carbapenem-resistant Enterobacteriaceae (CRE). Expert Rev Anti Infect Ther., 2016; 14(1): 95–108. doi: 10.1586/14787210.2016.1106940.
[9] Beyrouthy R, Barets M, Marion E, Dananché C, Dauwalder O, et al. Novel Enterobacter lineage as leading cause of nosocomial outbreak involving carbapenemase-producing strains. Emerg Infect Dis. 2018; 24(8): 1505. doi: 10.3201/eid2408.180151.
[10] Jiménez A, Castro JG, Munoz-Price LS, De Pascale D, Shimose L, et al. Outbreak of Klebsiella pneumoniae carbapenemase–producing *Citrobacter freundii* at a tertiary acute care facility in Miami, Florida. Infect Control Hosp Epidemiol., 2017; 38(3): 320–26. doi: 10.1017/ice.2016.273.
[11] Clinical and Laboratory Standards Institute (CLSI). Performance Standards for Antimicrobial Susceptibility Testing. CLSI Approved Standard M100-S15. Clinical and Laboratory Standards Institute, Wayne, 2018
[12] WHO publishes list of bacteria for which new antibiotics are urgently needed. [cited 2024 Dec 24]. Available from: https://www.who.int/ news/item/27

-02-2017-who-publishes-list-of-bacteria-for-which-newantibiotics-are-urgently-needed.

[13] Gandra S, Mojica N, Klein EY, Ashok A, Nerurkar V, et al. Trends in antibiotic resistance among major bacterial pathogens isolated from blood cultures tested at a large private laboratory network in India, 2008–2014. Int J Infect Dis., 2016; 50: 75–82. doi: 10.1016/j.ijid.2016.08.002.

[14] Kumar A, VinodKumar CS. Detection of carbapenem resistance encoding genes among gram negative bacteria from urinary tract infection in patients with type 2 diabetes mellitus. J Pure Appl Microbiol., 2017; 11(2): 1061–66. doi: 10.22207/JPAM.11.2.49.

[15] Khanchandani H, Chaudhury M, Rao MS, Ramakrishna N, et al. In vitro activity of the newly approved antimicrobial agent Cefiderocol against Carbapenem resistant Gram-negative clinical isolates. Indian J Med Microbiol., 2024; 48: 100556. doi: 10.1016/j.ijmmb.2024.100556.

[16] Ralte VS, Loganathan A, Manohar P, Sailo CV, Sanga Z, et al. The emergence of carbapenem-resistant gram-negative bacteria in Mizoram, Northeast India. Microbiol Res., 2022; 13(3): 342–49. doi: 10.3390/microbiolres13030027.

[17] Kumari N, Kumar M, Katiyar A, Kumar A, Priya P, et al. Genome-wide identification of carbapenem-resistant Gram-negative bacterial (CR-GNB) isolates retrieved from hospitalized patients in Bihar, India. Sci Rep., 2022; 12(1): 8477. doi: 10.1038/s41598-022-12471-3.

[18] Harino T, Kayama S, Kuwahara R, Kashiyama S, Shigemoto N, et al. Meropenem Resistance in Imipenem-Susceptible Meropenem- Resistant *Klebsiella pneumoniae* Isolates Not Detected by Rapid Automated Testing Systems. J Clin Microbiol., 2013; 51(8): 2735–38. doi: 10.1128/JCM.02649-12.

[19] Ku PM, Hobbs DA, Gilmore M, Hobbs AL. 1234. can susceptibility to one carbapenem be conferred to another? frequency of discordance in gram-negative clinical isolates. InOpen Forum Infectious Diseases 2021 Nov 1 (Vol. 8, No. Supplement_1, pp. S706-S707). US: Oxford University Press.

[20] Butler-Wu SM, Abbott AN. Is This the Carbapenemase Test We've Been Waiting for? A Multicenter Evaluation of the Modified Carbapenem Inactivation Method. Ledeboer NA, editor. J Clin Microbiol., 2017; 55(8): 2309–12. doi: 10.1128/JCM.00538-17

[21] Gupta V, Soni R, Jain N, Chander J. *In vitro* cost-effective methods to detect Carbapenemases in Enterobacteriaceae. J Lab Phys., 2018; 10(1): 101–05. doi: 10.4103/JLP.JLP_25_17.

[22] Kumudunie WG, Wijesooriya LI, Wijayasinghe YS. Comparison of four low-cost carbapenemase detection tests and a proposal of an algorithm for early detection of carbapenemase-producing Enterobacteriaceae in resource-limited settings. PLoS One, 2021; 16(1): e0245290. doi: 10.1371/journal.pone.0245290.