e-ISSN: 0976-822X, p-ISSN:2961-6042

Available online on http://www.ijcpr.com/

International Journal of Current Pharmaceutical Review and Research 2025; 17(7); 2094-2098
Original Research Article

Diagnostic Efficacy of the CIMTris Phenotypic Assay for Carbapenemase Detection in Clinical Isolates of Non-Lactose Fermenting Gram-Negative Bacilli: A Tertiary Care Center Experience

Pinaki Chattopadhyay[1], Sabyasachi Saha[2], Ranadeep Ghosh[3]

[1]Associate Professor, Department of Microbiology, Midnapore Medical College & Hospital, West Bengal, India
[2]Microbiologist C&DST (under NTEP), Department of Microbiology, Midnapore Medical College & Hospital, West Bengal, India
[3]Professor, Department of Microbiology, Midnapore Medical College & Hospital, West Bengal, India

Received: 01-05-2025 / Revised: 15-06-2025 / Accepted: 27-07-2025
Corresponding Author: Dr. Pinaki Chattopadhyay
Conflict of interest: Nil

Abstract

Background: The escalating prevalence of carbapenemase-producing non-lactose fermenting (NLF) bacteria, particularly *Acinetobacterbaumannii* and *Pseudomonas aeruginosa*, represents a critical healthcare emergency. These pathogens are increasingly recalcitrant to standard antibiotic regimens, necessitating rapid and accurate diagnostic tools. Traditional phenotypic assays often lack sensitivity for certain NLF species due to membrane permeability issues.

Objectives: This study evaluated the prevalence of carbapenemase production among clinical NLF isolates in a tertiary care hospital and assessed the diagnostic utility of the CIMTris phenotypic method, a modification designed to enhance enzyme extraction.

Methods: A cross-sectional study was conducted on 351 non-lactose fermenting Gram-negative bacilli (NLFGNB) recovered from diverse clinical specimens (sputum, wound swabs, urine, blood). Isolates were identified via standard microbiological protocols. Carbapenemase activity was assessed using the CIMTris method, employing 0.5 M Tris-HCl buffer for bacterial lysis. Antibiotic susceptibility was determined via the modified Kirby-Bauer disc diffusion method.

Results: Amongst the 351 isolates, 29.3% (103/351) were confirmed as carbapenemase producers. The *Acinetobacterbaumannii* complex exhibited the highest positivity rate (49.3%), predominantly isolated from respiratory specimens in ventilator-associated pneumonia (VAP) cases. *Pseudomonas aeruginosa* showed a 16.9% positivity rate, largely associated with wound infections. Additionally, a single isolate of *Stenotrophomonasmaltophilia* was detected and tested positive for carbapenemase activity. Notably, 11.7% of isolates yielded "indeterminate" results, particularly *P. aeruginosa* from hepatobiliary sources, suggesting low-level resistance or biofilm-associated phenotypic variation.

Conclusion: The CIMTris method serves as a robust, cost-effective tool for the routine screening of carbapenemases in NLF bacteria, including *Acinetobacter* spp. and *Stenotrophomonasmaltophilia*. However, the presence of indeterminate results highlights the need for an integrated diagnostic algorithm combining phenotypic screening with molecular confirmation to prevent the silent spread of multidrug-resistant reservoirs in critical care settings.

Keywords: Antimicrobial Resistance; Carbapenemase; CIMTris; Acinetobacterbaumannii; Pseudomonas aeruginosa; Phenotypic Detection.

Introduction

The relentless progression of antimicrobial resistance (AMR) has emerged as one of the most defining public health challenges of the 21st century. The World Bank forecasts that unchecked AMR could precipitate a global economic crisis comparable to the 2008 financial meltdown, potentially causing significant GDP losses by 2050.[1] Central to this crisis are Carbapenemase-Producing Organisms (CPOs), a group of bacteria that have acquired the ability to hydrolyzecarbapenems—antibiotics traditionally reserved as agents of last resort.[2] The proliferation of CPOs leads to prolonged hospitalizations, catastrophic healthcare expenditures, and elevated mortality rates due to therapeutic failure.[3]Among CPOs, Non-Lactose Fermenting Gram-Negative Bacilli (NLFGNB), specifically *Pseudomonas aeruginosa*,

Acinetobacterbaumannii, and *Stenotrophomonasmaltophilia*, are of paramount concern.[4] These organisms possess intrinsic resistance mechanisms and a remarkable capacity to acquire exogenous resistance determinants. The World Health Organization (WHO) has categorized carbapenem-resistant *Acinetobacterbaumannii* (CRAB) as a critical priority pathogen in its 2024 Bacterial Priority Pathogens List (BPPL).[5] This designation reflects the pathogen's disproportionate impact on healthcare systems in low and middle income countries (LMICs), particularly in South Asia, where it is a leading cause of nosocomial mortality.[6]

The resilience of NLFGNBs is largely attributed to their ability to form robust biofilms on biotic and abiotic surfaces, including catheters, ventilators, and prosthetic devices. Within the hospital environment, biofilms provide a sanctuary that shields bacteria from antimicrobial agents and the host immune system.[7] The biofilm matrix facilitates the expression of efflux pumps and the exchange of plasmids carrying resistance genes (horizontal gene transfer), often resulting in multidrug-resistant (MDR) phenotypes that escape detection by conventional susceptibility testing.[8, 9]

Rapid detection of carbapenemase activity is imperative for antimicrobial stewardship and infection control. While phenotypic tests such as the Modified Carbapenem Inactivation Method (mCIM) and the Carba NP test are highly effective for *Enterobacterales* and *P. aeruginosa*, they frequently demonstrate reduced sensitivity for *A. baumannii*. This limitation stems from the low permeability of the *Acinetobacter* outer membrane and the prevalence of OXA-type carbapenemases, which exhibit weak hydrolytic activity compared to metallo-beta-lactamases (MBLs) or KPC enzymes. [10, 11, 12]To address these diagnostic gaps, this study employs the CIMTris method. This modified phenotypic assay utilizes a 0.5 M Tris-HCl buffer solution to permeabilize the bacterial cell wall, thereby facilitating the extraction of periplasmiccarbapenemases. By enhancing enzyme release, CIMTris aims to improve detection sensitivity for challenging NLFGNB isolates. This study investigates the prevalence of carbapenemase production in clinical NLFGNB isolates at a tertiary care center and evaluates the clinical applicability of the CIMTris method in a resource-limited setting.[11]

Material and Methods

Study Design and Setting: This prospective observational study was conducted at the Department of Microbiology in a tertiary care teaching hospital in West Bengal, India. The institution serves a diverse patient population, including referrals from critical care units and trauma centres.

Isolate Collection and Identification: A total of 351 consecutive non-lactose fermenting Gram-negative bacterial isolates were recovered from various clinical specimens (blood, urine, sputum, wound swabs, endotracheal aspirates, and body fluids) over the study period. Initial identification was performed using classical microbiological techniques, including colony morphology on MacConkey agar and Blood agar, Gram staining, motility testing, oxidase production, and a battery of biochemical tests assessing carbohydrate and amino acid metabolism.

Antimicrobial Susceptibility Testing: Antibiotic susceptibility profiles were determined using the modified Kirby-Bauer disc diffusion method on Mueller-Hinton Agar (MHA) plates. Commercially available antibiotic discs were applied, and zone diameters were interpreted in strict accordance with the Clinical and Laboratory Standards Institute (CLSI) guidelines.[13]

Phenotypic Carbapenemase Detection: The CIMTris Method [14, 15, 16]:

The CIMTris assay was utilized to detect carbapenemase production. The protocol was standardized as follows: Extraction: A 10-µl loopful of bacterial growth from a fresh overnight culture was suspended in 400 µl of 0.5 M Tris-HClbuffer (pH 7.6) in a sterile microcentrifuge tube. Lysis: The suspension was vortexed vigorously for 10–15 seconds to disrupt cell membranes and solubilize enzymes. Incubation: A 10 µgmeropenem susceptibility disk was immersed in the suspension. The tube was incubated at 35°C for 2 hours to allow potential carbapenemases to hydrolyze the meropenem. Indicator Application: Following incubation, the disk was removed using a sterile loop and placed onto an MHA plate previously inoculated with a lawn of a carbapenem-susceptible Escherichia coli indicator strain (ATCC 25922).

The lawn was prepared by streaking a 0.5 McFarland standard suspension of the indicator strain. Final Incubation and Readout: Plates were incubated at 35°C ± 2°C for 18 hours. Interpretation Criteria: Positive: An inhibition zone of 6–15 mm, or a zone of 16–18 mm containing satellite colonies (indicating hydrolysis of meropenem allowing the indicator strain to grow). Negative: An inhibition zone of ≥19 mm (indicating the meropenem disk remained potent). Indeterminate: An inhibition zone of 16–18 mm without the presence of satellite colonies. Quality Control: Validity of the test results was ensured by running concurrent controls: *E. coli* ATCC 25922 served as the negative control (non-CPO), and a verified in-house carbapenemase-producing *A. baumannii* strain served as the positive control.

Consistency was verified by having different technicians replicate the test on selected isolates.

Results

Demographic and Microbiological Profile: A total of 351 NLFGNB clinical isolates were analyzed. The species distribution was as follows: *Pseudomonas aeruginosa* (n=172, 49.0%), *Acinetobacterbaumannii* complex (n=134, 38.2%), *Acinetobacterlwoffii* (n=42, 11.9%), *Burkholderiacepacia* group (n=2, 0.6%), and *Stenotrophomonasmaltophilia* (n=1, 0.3%). (Figure 1).

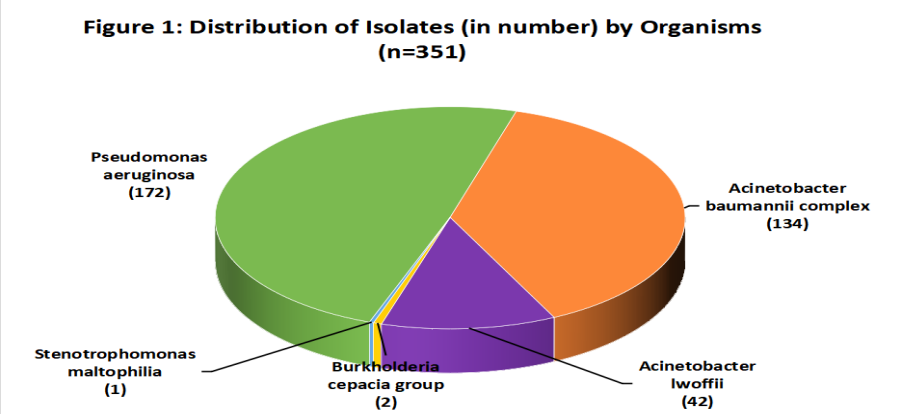

Figure 1: Distribution of isolates (in number) by organism (n=351)

Prevalence of Carbapenemase Activity: Using the CIMTris method, 29.3% (103/351) of all NLFGNB isolates demonstrated confirmed carbapenemase activity. A significant proportion, 11.7% (41/351), yielded indeterminate results, while 59.0% (207/351) were non-producers. (Table 1)

Table 1: Proportion of Positive, Indeterminate and Negative Result by Organism for Carbapenemase Production

Organism (Sample Size)	% Positive (Carbapenemase)	% Indeterminate	% Negative
Acinetobacterbaumannii complex (n=134)	49.30%	14.20%	36.50%
Pseudomonas aeruginosa (n=172)	16.90%	11.60%	71.50%
Acinetobacterlwoffii (n=42)	16.70%	4.80%	78.50%
Stenotrophomonasmaltophilia (n=1)	100% #	0%	0%
Burkholderiacepacia group (n=2)	0%	0%	100%

#- Intrinsic Carbapenemase producer.

Species-Specific Analysis: *Acinetobacterbaumannii* complexexhibited the highest rate of carbapenemase production. Out of 134 isolates, 49.3% (66/134) were positive. Indeterminate results were observed in 14.2% (19/134) of cases. Resistance was most profound in respiratory specimens. Approximately 62% of isolates from sputum and endotracheal tube tips were positive, correlating strongly with ventilator-associated pneumonia (VAP). Invasive sources such as central venous catheter tips and pleural fluid contributed to over 60% of the positive burden.

Among 172 *Pseudomonas aeruginosa*isolates, 16.9% (29/172) were positive for carbapenemase production, with 11.6% (20/172) classified as indeterminate. Carbapenemase-positive strains were predominantly recovered from wound swabs (76%), followed by urine samples (18%) and blood cultures (6%). Notably, 80% of the indeterminate results in this species were derived from bile samples. *Acinetobacterlwoffii*showed lower resistance potential, with 16.7% (7/42) positivity and 4.8% (2/42) indeterminate results. The single isolate of *Stenotrophomonasmaltophilia* detected in this study was positive for carbapenemase production, consistent with intrinsic resistance mechanisms. *Burkholderiacepacia* both isolates tested were negative.

Discussion

Our study reports a significant burden of carbapenemase production (29.3%) among clinical isolates, utilizing the CIMTris method. The distribution of pathogens aligns with global surveillance data, reaffirming *A. baumannii* and *P. aeruginosa* as the primary drivers of Gram-negative resistance. Additionally, the detection of a carbapenemase-positive *Stenotrophomonasmaltophilia* isolate underscores the diversity of resistance threats in the hospital environment.[5, 17, 18]

Our finding that 49.3% of *A. baumannii* complex isolates are carbapenemase producers highlights a

concerning trend in critical care medicine. This rate concurs with recent literature identifying *A. baumannii* as a pervasive nosocomial pathogen equipped with potent resistance mechanisms. The high positivity rate in respiratory samples (62%) is particularly alarmist, corroborating the organism's established role in Ventilator-Associated Pneumonia (VAP).(19, 20) Similar patterns have been documented in Iran and Bangladesh, where *Acinetobacter* spp. dominate ICU infections.[21, 22] The association with invasive devices (ET tubes, central lines) underscores the role of biofilm formation in facilitating persistence and resistance. The detection of indeterminate results in invasive samples is critical; these isolates may represent strains with low-level enzyme expression or hetero-resistance, which can rapidly evolve into high-level resistance under selective pressure.

While the overall positivity rate for *P. aeruginosa* (16.9%) was lower than that of *Acinetobacter*, the site-specific prevalence is noteworthy. The dominance of carbapenemase-positive strains in wound swabs (76%) reflects the organism's predilection for chronic wounds and burns, consistent with studies from Haryana and Nepal.[23, 24] A unique finding of this study is the clustering of indeterminate *P. aeruginosa* results in bile samples (80%). The hepatobiliary system, often subjected to stenting and chronic instrumentation, acts as a niche for biofilm formation. Bacteria within biofilms often exhibit altered metabolic states that may affect phenotypic test readouts.[25] These "borderline" strains are potential reservoirs for future outbreaks, as horizontal gene transfer within biliary biofilms can disseminate resistance traits to other gut commensals.[26]The CIMTris method demonstrated utility in detecting resistance in organisms where standard tests often fail. By utilizing Tris-HCl for extraction, the assay likely overcomes the permeability barrier of the *Acinetobacter* cell wall, allowing for more reliable detection of intracellular carbapenemases. The positive detection of *Stenotrophomonasmaltophilia*, a known producer of the L1 metallo-β-lactamase, further validates the assay's capability to detect diverse carbapenemases typical of NLF bacteria.[27] However, the 11.7% indeterminate rate across all species suggests that phenotypic testing alone has limits. These results may be attributable to weak carbapenemases (like certain OXA variants) or alternative resistance mechanisms such as porin loss or efflux pump overexpression, which CIMTris detects only partially.[12]

Limitations

This study was limited to phenotypic detection. Molecular characterization (PCR/Sequencing) was not performed, precluding the identification of specific carbapenemase genotypes (e.g., NDM, VIM, OXA, L1). Additionally, the sample size for non-major pathogens like *B. cepacia* and *S. maltophilia* was small, limiting generalizability for those species.

Conclusion

The present study validates the CIMTris method as a feasible, sensitive screening tool for detecting carbapenemase activity in clinical NLFGNB isolates, particularly in resource-constrained settings where molecular testing is not routine. The high prevalence of carbapenemase-producing *A. baumannii* in respiratory units and *P. aeruginosa* in surgical wards, alongside the presence of intrinsically resistant *Stenotrophomonasmaltophilia*, necessitates aggressive infection control protocols.

We propose a tiered diagnostic stewardship model:

1. Routine Screening**:** Implementation of CIMTris for all NLF isolates from critical care units.
2. Molecular Reflex Testing**:** Mandatory genotypic verification for all "indeterminate" isolates to rule out latent resistance genes.
3. Targeted Surveillance**:** Focused monitoring of high-risk reservoirs, specifically hepatobiliary and burn units, to mitigate the spread of biofilm-associated resistance.

   Early detection via robust phenotypic methods, followed by stringent isolation precautions, remains the cornerstone of combating the silent epidemic of multidrug-resistant NLFGNBs.

Reference

1. WorldBank. Drug-Resistant Infections A Threat to Our Economic Future. Washington, DC; 2017.
2. Walsh TR. Emerging carbapenemases: a global perspective. Int J Antimicrob Agents. 2010; 36S3:S8-S14.
3. Nordmann P, Dortet L, Poirel L. Carbapenem resistance in Enterobacteriaceae: here is the storm! Trends Mol Med. 2012;18(5):263-72.
4. WHO. ″Global antimicrobial resistance and use surveillance system (GLASS) report 2022″. Geneva; 2022.
5. WHO. Bacterial Priority Pathogens List, 2024. Bacterial pathogens of public health importance to guide research, development and strategies to prevent and control antimicrobial resistance. Geneva: World Health Organization; 2024; 1-72.
6. Hsu L-Y, Apisarnthanarak A, Khan E, Suwantarat N, Ghafur A, Tambyah PA. Carbapenem-Resistant *Acinetobacterbaumannii* and *Enterobacteriaceae* in South and Southeast Asia. ClinMicrobiol Rev. 2017;30(1):1-22.

7. Cornaglia G, Akova M, Amicosante G, Cantónd R, Cauda R, Docquier J-D, et al. Metallo-beta-lactamases as emerging resistance determinants in Gram-negative pathogens: open issues. Int J Antimicrob Agents. 2007;29(4):380-8.
8. Thummeepak R, Kongthai P, Leungtongkam U, Sitthisak S. Distribution of virulence genes involved in biofilm formation in multi-drug resistant *Acinetobacterbaumannii* clinical isolates. IntMicrobiol. 2016;19(2):121-9.
9. Karen D Allen HTG. Hospital outbreak of multi-resistant *Acinetobacter*: an air borne mode of spread. J Hosp Infect. 1987; 9(2):110-l 19.
10. Simner PJ, Johnson JK, Brasso WB, Anderson K, Lonsway DR, Pierce VM, et al. Multicenter Evaluation of the Modified Carbapenem Inactivation Method and the Carba NP for Detection of Carbapenemase-Producing *Pseudomonas aeruginosa* and *Acinetobacterbaumannii*. J ClinMicrobiol 2018;56(1):1-10.
11. Uechi K, Tada T, Shimad K, Kuwahara-Arai K, Arakaki M, Tome T, et al. A Modified Carbapenem Inactivation Method, CIMTris, for Carbapenemase Production in Acinetobacter and *Pseudomonas* Species. J ClinMicrobiol. 2017; 55:3405–10.
12. Potron A, Poirel L, Nordmann P. Emerging broad-spectrum resistance in *Pseudomonas aeruginosa* and Acinetobacterbaumannii: Mechanisms and epidemiology. Int J Antimicrob Agents. 2015;45(6):568-85.
13. CLSI. Performance Standards for Antimicrobial Susceptibility Testing. 33rd ed: Clinical and Laboratory Standards Institute, USA; 2023.
14. Tamma PD, Simner PJ. Phenotypic Detection of Carbapenemase-Producing Organisms from Clinical Isolates. J ClinMicrobiol. 2018;56(11):e01140-18.
15. Kuchibiro T, Komatsu M, Yamasaki K, Tatsuya Nakamura, Hisaaki Nishio, Kimura K, et al. Comparison of the performance of three carbapenem inactivation methods for the detection of carbapenemase-producing gram-negative bacilli. J Infect Chemother. 2021; 27:1634–8.
16. Kuchibiro T, Komatsu M, Yamasaki K, Nakamura T, Hisaaki Nishio, Nishi I, et al. Evaluation of the modified carbapenem inactivation method for the detection of carbapenemase-producing Enterobacteriaceae. J Infect Chemother. 2017; xxx:1-5.
17. Gniadek TJ, Carroll KC, Simner PJ. Carbapenem-Resistant Non-Glucose-Fermenting Gram-Negative Bacilli: the Missing Piece to the Puzzle. J ClinMicrobiol. 2016;54(7):1700-10.
18. Bohra R, Wadhwa R, Bala K. Isolation and characterization of lactose and non-lactose fermenting bacteria from tertiary care hospital and their antimicrobial susceptibility test. Asian J Pharm Clin Res. 2017;10(2):201-5.
19. Gupta V, Chhina D, Chhina RS, Lyall KDS, Suri P. Ventilator-associated pneumonia in intensive care units of a tertiary care hospital in Punjab. Journal of Patient Safety and Infection Control. 2015; 3:139–41.
20. Khurana S, Mathur P, Kumar S, Soni KD, Aggrawal R, Batra P, et al. Incidence of Ventilator-associated Pneumonia and Impact of Multidrug-Resistant Infections on Patient's Outcome: Experience at an Apex Trauma Centre in North India. Indian J Med Microbiol. 2017; 35:504-10.
21. Sarda C, Fazal F, Rello J. Management of ventilator-associated pneumonia (VAP) caused by resistant gram-negative bacteria: which is the best strategy to treat? Expert Rev Respir Med. 2019;13(8):787–98.
22. Ahsan AA, Barai L, Faruq MO, Fatema K, Ahmed F, Saha DK, et al. Antibiotic Resistance Pattern among Bacteria causing Ventilator Associated Pneumonia in An Intensive Care Unit of Bangladesh. Bangladesh Crit Care J 2016;4(2):69-73.
23. Ranjan KP, Ranjan N, Bansal SK, Arora DR. Prevalence of *Pseudomonas aeruginosa* in Post-operative Wound Infection in a Referral Hospital in Haryana, India. J Lab Physicians. 2010;2(2):74-7.
24. Maharjan R, Shrestha B, Shrestha S, Angbuhang KB, Lekhak B, Nepal K. Detection of Metallo-β-Lactamases and Carbapenemase Producing *Pseudomonas aeruginosa* Isolates from Burn Wound Infection. Tribhuvan University journal of microbiology. 2020;7(1):67-74.
25. Kievit TRD, Parkins MD, Gillis RJ, Srikumar R, Ceri H, Poole K, et al. Multidrug efflux pumps: expression patterns and contribution to antibiotic resistance in *Pseudomonas aeruginosa* biofilms. Antimicrob Agents Chemother. 2001;45(6):1761-70.
26. Cho HH, Kwon KC, Kim S, Park Y, Koo SH. Association between Biofilm Formation and Antimicrobial Resistance in Carbapenem-Resistant *Pseudomonas Aeruginosa*. Ann Clin Lab Sci. 2018;48(3):363-8.
27. Terrier CL, Masseron A, Uwaezuoke NS, Edwin CP, Ekuma AE, Olugbeminiyi F, et al. Wide spread of carbapenemase-producing bacterial isolates in a Nigerian environment. J Glob Antimicrob Resist. 2020;21:321-3.