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RESEARCH ARTICLE
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# CIMTris METHOD FOR DETECTING CARBAPENEMASE IN CLINICALLY ISOLATED NLFs AMIDST RISING DRUG RESISTANCE

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**Abstract:**
**Background*:*** Carbapenemase-producing non-lactose fermenting gram-negative bacilli (NLFGNB), especially *Acinetobacter baumannii* and *Pseudomonas aeruginosa*, pose a major threat in healthcare settings due to rising antimicrobial resistance and limited treatment options.
**Objectives**: This study aimed to investigate the prevalence of carbapenemase production in NLF bacteria isolated from clinical specimens.
**Methods*:*** A total of 207 clinical NLFGNB isolates were tested using the CIMTris method, which involves incubating isolates in Tris-HCl buffer with meropenem discs, followed by zone-of-inhibition analysis on *E. coli*-inoculated agar plates.
**Results:** Of 207 isolates, 28.0% were carbapenemase-positive, 10.6% indeterminate, and 60.9% negative. Highest positivity was seen in *A. baumannii* (48.1%), followed by *P. aeruginosa* (15.7%) and *A. lwoffii* (16%). The CIMTris method showed 97.6% sensitivity and 92.6% specificity.
**Conclusion:** CIMTris is a rapid, cost-effective, and sensitive phenotypic method for detecting carbapenemase in NLFGNBs, especially effective for *A. baumannii*. However, molecular testing is recommended for indeterminate results to ensure early detection and containment of emerging resistance in high-risk hospital settings.

**Keywords:** Carbapenemase producing organism, CIMTris Method, Non-lactose fermenting Gram-negative bacilli, Multidrug-resistance, Carbapenemase, Phenotypic detection.

## INTRODUCTION

The rise of antimicrobial resistance (AMR) poses a serious global health problem. The World Bank highlights the severe economic impacts of AMR, predicting major global GDP losses by 2050.(1) Carbapenemase-producing organisms (CPOs) play a large role in this issue by making many life-saving antibiotics ineffective. This leads to longer hospital stays, higher mortality rates, and significant economic costs. Among CPOs, non-lactose fermenting gram-negative bacilli (NLFGNB) such as *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, and *Stenotrophomonas maltophilia* are particularly concerning because they can make carbapenem antibiotics ineffective.(2) The World Health Organization (WHO) prioritizes carbapenem-resistant *Acinetobacter baumannii* (CRAB) as a critical threat in its BPPL-2024 report due to the mortality associated with antibiotic-resistant infections. It is estimated to be the leading pathogen in South Asia and other low- and middle-income countries, especially compared to high-income countries.(3)

The increasing worry about NLFGNB comes from their widespread presence, ability to form biofilms, and natural resistance to many antibiotics.(4, 5) Hospitals provide ideal conditions for NLFGNB biofilm growth due to factors such as medical devices, catheters, and frequent use of antibiotics, which promote resistant strains. These biofilms are tough against antibiotics for several reasons, including the interaction with exopolymers, enzyme release, and the presence of efflux pumps.(6, 7, 8) Biofilms also increase bacterial mutability and horizontal gene transfer. This encourages the rise of multidrug resistance, which often goes unnoticed by common susceptibility tests.(9)

The identification of a carbapenemase-producing *P. aeruginosa* strain in 1988 marked the beginning of the CPO problem, likely related to the excessive use of carbapenems.(10) Quick and accurate detection of carbapenemase production is crucial for choosing the right antibiotic treatment and for implementing infection control measures.

Current phenotypic tests like mCIM (modified Carbapenem Inactivation Method) and Carba NP are effective for detecting carbapenemases in *P. aeruginosa*, but they are not suitable for *A. baumannii.(11)* This is because *A. baumannii* has a low-permeability membrane that limits these tests.(12) Additionally, the most common carbapenemases it produces (OXA-type) are weak, resulting only in reduced susceptibility to carbapenems. The high resistance observed is primarily due to other mechanisms, such as porin mutations, rather than the carbapenemase itself.(13)

In this study, we used the CIMTris method to detect carbapenemase activity in NLFGNBs, including *A. baumannii*.(14) This method involves a straightforward protocol that extracts bacteria using a 0.5 M Tris-HCl buffer solution, incubates them with meropenem discs, and looks for hydrolysis zones on indicator plates. Overall, CIMTris provided a sensitivity of 97.6% and a specificity of 92.6% for carbapenemase detection in NLFGNBs.(14) These results suggest that CIMTris is a promising alternative to current methods, particularly for *A. baumannii*, where established tests like mCIM have limitations.

## MATERIAL AND METHODS

NLFGNB's were identified using a combination of classical phenotypic methods, including colony morphology, Gram stain, motility, enzyme detection, and carbohydrate/amino acid metabolism tests with comparing control strains.

Modified Kirby-Bauer disc diffusion technique with commercially available Antibiotic discs was performed on Mueller-Hinton agar plates according to CLSI guidelines to assess antibiotic sensitivity.(15)

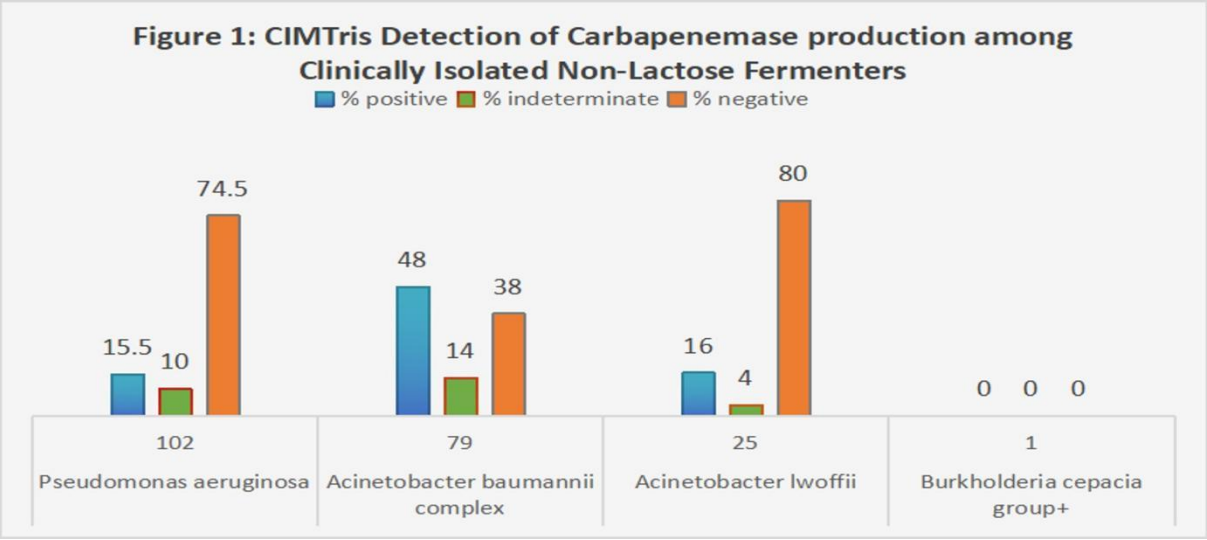
CIMTris method was employed for phenotypic detection of Carbapenemase producing NLFGNBs.(16, 17, 18) In the CIMTris method, a 10-µl loopful of bacteria from each isolate was

emulsified in 400 µl of 0.5 M Tris-HCl buffer (pH 7.6) and vortexed for 10 to 15 s.(11) A 10µg meropenem disk was added to each of three tubes. The tubes were incubated at 35°C for 2 h. Following incubation, the disk was extracted from the suspension using an inoculation loop and positioned on MHA plate that had been inoculated with a susceptible *E. coli* indicator strain (ATCC 29522). The agar plate was then incubated at $35^0C \pm 2^0C$. Inoculation of the MHA plate with the indicator strain involved streaking a suspension (inoculum turbidity with that of standard 0.5 McFarland) in three directions using a sterile cotton swab. The plates were incubated for 18 h at 35°C, and the diameter (mm) of each inhibition zone was measured. Carbapenemase activity was regarded as positive if the inhibition zone measured 6 to 15 mm or measured 16 to 18 mm with satellite colonies. Carbapenemase activity was regarded as negative if the inhibition zone measured ≥19 mm and as indeterminate if the inhibition zone measured 16 to 18 mm without satellite colonies.(16, 17, 18)

*E. coli* ATCC 25922 was used as the negative control (non-CPO) and an in-house Carbapenemase producer strain of *A. baumannii* was used as the positive control and we assess the consistency of CIMTris results by conduct the test multiple times on the same isolates by different technicians to ensure consistent and reliable results.

**RESULT :**

A total of 207 NLFGNB were analyzed using the CIMTris phenotypic method for carbapenemase detection as shown in Figure 1. The species distribution included 102 isolates of *P. aeruginosa*, 79 isolates from the *A. baumannii* complex, 25 isolates of *Acinetobacter lwoffii*, and one isolate of the *Burkholderia cepacia* group.




Overall, 28.0% (58/207) of NLFGNB isolates were positive for carbapenemase production, 10.6% (22/207) yielded indeterminate results, and 60.9% (126/207) tested negative.

Out of 102 *P. aeruginosa* isolates, 15.5% (16/102) demonstrated carbapenemase activity, while 10.0% (10/102) were categorized as indeterminate (zones of 16–18 mm without satellite colonies), and 74.5% (76/102) were negative. Carbapenemase-positive *P. aeruginosa* strains were predominantly recovered from wound swabs (75%), followed by urine samples (19%) and blood cultures (6%). Among the 79 isolates of *A. baumannii complex*, 48.1% (38/79) were carbapenemase-positive, 13.9% (11/79) were indeterminate, and 38.0% (30/79) tested negative. The highest positivity was observed in respiratory specimens (62%), especially from sputum and endotracheal tube tips. Wound swabs and urine samples accounted for 17% and 12% of positives, respectively. Invasive sources such as central venous catheter tips and pleural fluid contributed to over 60% of carbapenemase-positive *A. baumannii* isolates. Of the 25 *A. lwoffii* isolates, only 16%

(4/25) were positive for carbapenemase, while 4% (1/25) were indeterminate, and 80% (20/25) were negative. Only a single isolate from the *Burkholderia cepacia* group was tested and found negative. Due to the limited sample size, no conclusions can be drawn for this organism, however it is reported for completeness.

**DISCUSSION:**

In the current research, carbapenemase production was screened for in 207 NLFGNB's using CIMTris method. Among the ceabapenemase producer *A. baumannii* complex and *P. aeruginosa* are the most prevalent pathogens among the NLFGNBs and the result agrees with the critical pathogen list established by World Health Organisation.(3) The occurrence of carbapenem resistance among *A. baumannii* and *P. aeruginosa* has been reported in different geographic regions, although the results showing high heterogeneity but still *A. baumannii* complex and *P. aeruginosa* are the key carbapenemase producer.(19, 20, 21)

Interestingly, carbapenemase activity was highest in the *A. baumannii* complex at 48.1% (38/79) positive, 13.9% indeterminate, and 38% negative. This concurs with recent literature reporting *A. baumannii* as a frequent carrier of carbapenemase genes, particularly in hospitals, and underlines its clinical significance in multidrug resistance.(22) In this study *Acinetobacter spp*. was found to present high carbapenemase positivity in a wide range of samples. The highest proportion (62%) was in respiratory samples (namely sputum, tracheal aspirate, Tip of ET tube etc), consistent with its well-documented role in ventilator-associated pneumonia (VAP). Wound swabs (17%) and urine (12%) also had positive isolates, further underlining the organism's role in other healthcare-associated infections. This was also observed in a study in Iran where *A. baumannii* was recovered from ventilator-associated pneumonia and burn wound colonization.(23) A study in an apex trauma center in North India emphasized the clinical significance of multidrug-resistant (MDR) pathogens recovered in respiratory samples in VAP.(24) Although the actual rate of carbapenemase positivity in *Acinetobacter spp*. is not mentioned, the study makes a point regarding the problem posed by MDR organisms in VAP treatment.(24) A study in an intensive care unit (ICU) in Bangladesh also isolated *Acinetobacter spp*. as a leading VAP pathogen, emphasizing the importance of identifying local patterns of resistance in empirical antibiotic selection.(25) This is in line with the global concern that increasing resistance demands custom-fit treatment regimens.(26) A study in Punjab, India, emphasized that healthcare-associated infections due to invasive medical devices in ICUs are of high risk, increasing stays and costs.(27) The high carbapenemase positivity in *Acinetobacter spp*. from respiratory specimens is in line with its critical role in VAP in Indian and other Asian studies.(25, 28, 29) Of particular concern is the emergence of multidrug-resistant strains, which requires ongoing surveillance, early detection strategies, and tailored antibiotic regimens.(24, 30) Indeterminate *Acinetobacter spp*. results were also encountered in different types of specimens, including wound swab, blood culture, sputum, and ET tube tips. Detection of indeterminate isolates from invasive sites like blood and ET tubes is a red flag that requires urgent intervention. The likelihood of phenotypic conversion from indeterminate to fully resistant strains in these settings is high, especially in ICUs where there is high antibiotic pressure and invasive device utilization.

Of 102 *P. aeruginosa* isolates, 15.7% (16/102) were carbapenemase activity positive, 10% indeterminate, and 74.5% negative. Among the *P. aeruginosa* isolates, carbapenemase production was maximum among wound swabs (75%), followed by urine samples (19%) and blood culture (6%). A study conducted in Haryana, India, reported the prevalence of *P. aeruginosa* during post-operative wound infections (Ranjan et al., 2010) it indicates the high prevalence of *P. aeruginosa* among wound infections in Indian hospitals.(31) Two study in burn wound infections similarly reported *P. aeruginosa* as a leading causative agent.(32, 33) CIMTris-based indeterminate results in *P. aeruginosa* were mostly observed in bile samples (80%), sometimes in wound swabs (10%) and blood culture (10%). These borderline results—defined by inhibition zones of 16–18 mm in the absence of satellite colonies—are the most concerning. With the plasticity of the genome of *P. aeruginosa* and its capability to form strong biofilms, these indeterminate strains can evolve into full carbapenemase producers under selective antibiotic pressure. Biliary systems with stents and

chronic instrumentation are recognised reservoirs for biofilm formation, where horizontal gene transfer can be utilised for the spread of resistance.(6, 8) Several studies have documented *P. aeruginosa* isolates from various clinical samples, viz., urine, blood, and wound swabs, reflecting the opportunistic nature of the bacterium. A study in Nepal established the prevalence and antimicrobial resistance pattern of *P. aeruginosa* isolated from various clinical samples, result of which is in accordance with our present study.(32) These studies emphasize the importance of surveillance of antibiotic resistance in various sites of infection.

*A. lwoffii* was identified with a much lower proportion of positivity (16% or 4/25), one being indeterminate and the rest (80%) negative, indicating its relatively lesser role in carbapenem resistance dissemination. No result was yielded for the sole isolate of *B. cepacia* group, which may have been a technical exclusion from the CIMTris test due to technical limitations or low sample volume. These findings indicate a species-specific difference in carbapenamase production and highlight the requirement for periodic phenotypic screening to identify and restrict carbapenem-resistant pathogens in healthcare settings.

The rate of indeterminate results across all species, but particularly in *A. baumannii* (14%), suggests further molecular testing to improve diagnostic sensitivity. Taken together, these findings attest to the utility of species-specific resistance profiling to guide effective infection control and antimicrobial stewardship interventions.

## LIMITATIONS

This investigation was aimed at phenotypic detection of carbapenemase production only, but molecular typing using techniques like PCR was not performed, which would have provided more conclusive information about the different carbapenemases present. The investigation was also limited by small population and diversity of non-lactose fermenting Gram-negative bacilli (NLFGNB) isolates and hence might not be representative. High population and diversity of isolates and genotypic confirmation in future investigations are recommended.

## Conclusion:

Together, this research warrants the application of CIMTris for the early detection of carbapenemase activity in clinically relevant NLFs. Yet, it also points towards the limitation of phenotypic testing in detecting emerging or borderline resistance. Therefore, an integrated approach is necessary: (1) application of molecular tests on high-risk or indeterminate isolates, (2) enhanced surveillance of clinical specimens with invasive devices and biofilm, and (3) coordinated infection control efforts through HICC, particularly in high-burden wards such as ICUs, hepatobiliary surgery wards, and wound care units. In the absence of early interventions, indeterminate isolates would be an asymptomatic reservoir for spreading resistance through biofilms, with resulting limited treatment options and enhanced morbidity and mortality.

Therefore, the Hospital Infection Control Committee (HICC) must remain on high alert in high-risk areas, most importantly the hepatobiliary and critical care wards. Indeterminate isolates must be molecularly tested for latent carbapenemase genes so that timely therapeutic and preventive interventions can be initiated.

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