

Screening for Carbapenemase Production in Gram Negative Bacilli: A Phenotypic Study in a Tertiary Care Hospital

Jani V¹, Chaudhary A², Solanki R³

¹Senior Resident, ²Junior Resident, ³Professor,
Department of Microbiology,
Dr. M.K. Shah Medical College and Research Centre, Gujarat University, Ahmedabad, India.

Corresponding Author: Dr. Vaibhavi Jani

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ABSTRACT

Introduction: Carbapenem-resistant Gram-negative bacilli (CR-GNB) are a major global health concern due to limited therapeutic options and rapid dissemination of resistance mechanisms. Carbapenemase production is a key contributor to resistance, necessitating reliable and cost-effective detection methods.

Aim: To evaluate and compare Modified Hodge Test (MHT), Modified Carbapenem Inactivation Method (mCIM), and EDTA-modified CIM (eCIM) for detection of carbapenemase production among Gram-negative bacilli.

Materials and Methods: This prospective observational study was conducted in the Department of Microbiology of a tertiary care hospital from January to March 2026. A total of 100 non-duplicate carbapenem-resistant Gram-negative bacilli isolated from 910 clinical samples were included. Identification was performed using standard microbiological methods and VITEK 2 Compact. Antimicrobial susceptibility testing was done as per CLSI guidelines. Carbapenemase production was detected using MHT, mCIM, and eCIM. Diagnostic performance of MHT was evaluated using mCIM as the reference method.

Results: *Acinetobacter* spp. (37%) and *Klebsiella* spp. (30%) were the predominant isolates. Carbapenemase production was detected in 73% isolates by mCIM and 60% by MHT. mCIM demonstrated higher detection rates across all organisms. eCIM identified metallo- β -lactamase production in a subset of isolates. Compared to mCIM, MHT showed sensitivity of 73.97%, specificity of 77.78%, positive predictive value of 90.00%, negative predictive value of 52.50%, and diagnostic accuracy of 75.00%. Agreement between MHT and mCIM was moderate ($\kappa=0.45$). High susceptibility to colistin and tigecycline was observed.

Conclusion: mCIM is more reliable than MHT for detection of carbapenemase production, while eCIM aids in identifying metallo- β -lactamases. Early phenotypic detection is essential for appropriate therapy and infection control in resource-limited settings.

Keywords: Carbapenem-resistant Gram-negative bacilli (CR-GNB), Carbapenemase, Modified Carbapenem Inactivation Method (mCIM), Modified Hodge Test (MHT), Phenotypic detection

INTRODUCTION

Carbapenem-resistant Gram-negative bacilli (GNB) have emerged as a major global health concern due to the rapid rise of multidrug-resistant organisms and limited therapeutic options. The widespread production of β -lactamases, including extended-spectrum β -lactamases (ESBLs) and AmpC enzymes, has contributed to increasing resistance among Enterobacterales, *Acinetobacter baumannii*, and *Pseudomonas aeruginosa* [1,2]. Carbapenems, often considered last-resort antibiotics, are extensively used in the management of severe infections; however, the emergence of carbapenem resistance has significantly compromised their efficacy [3]. Carbapenemase production is one of the most important mechanisms of resistance, as these enzymes can hydrolyze a broad range of β -lactam antibiotics [5]. Major carbapenemases include KPC, NDM, VIM, IMP, and OXA types, which are frequently associated with mobile genetic elements, facilitating rapid dissemination across bacterial species [6]. This poses a serious challenge for infection control and antimicrobial stewardship. Accurate and timely detection of carbapenemase production is essential for appropriate patient management and prevention of hospital outbreaks [3,4]. While molecular methods are considered the gold standard, they are not widely available in resource-limited settings. Hence, phenotypic methods such as the Modified Hodge Test (MHT), Modified Carbapenem Inactivation Method (mCIM), and EDTA-modified CIM (eCIM), recommended by the Clinical and Laboratory Standards Institute, remain practical alternatives [7,8].

MATERIALS & METHODS

Aim:

To evaluate and compare MHT, mCIM, and eCIM for detection of carbapenemase production among Gram-negative bacilli.

Study Design and Setting

This prospective observational study was conducted in the Department of Microbiology at Dr. M. K. Shah Medical College and Research Centre, a tertiary care hospital, over a period of three months from January 2026 to March 2026. The study was designed to include 100 non-duplicate carbapenem-resistant Gram-negative bacilli isolates. To achieve this sample size, a total of 910 clinical specimens were processed during the study period.

Study Population

Non-duplicate carbapenem-resistant Gram-negative bacilli isolated from clinically significant samples were included in the study.

Inclusion Criteria

- Clinically significant non-duplicate gram-negative bacilli isolates
- Isolates demonstrating carbapenem resistant (imipenem/meropenem)

Exclusion Criteria:

- Duplicate isolates from the same patient
- Gram-positive organisms, fungi, and non-bacterial pathogens

Identification of Isolates

Bacterial identification was performed using standard microbiological techniques, including colony morphology on Nutrient Agar, Blood Agar, and MacConkey Agar, followed by conventional biochemical tests and or using an automated system (VITEK 2 Compact). Isolates belonging to Enterobacterales, *Acinetobacter spp.*, and *Pseudomonas spp.* were included.

Antimicrobial Susceptibility Testing

Antimicrobial susceptibility testing (AST) was performed by the Kirby–Bauer disk diffusion method and an automated system (VITEK 2 Compact) in accordance with Clinical and Laboratory Standards Institute (CLSI) guidelines. [9] Colistin was tested by broth microdilution (BMD), while tigecycline interpretation was based on FDA

breakpoints for Enterobacterales [10] and article based criteria for *Acinetobacter* spp. [11]

Phenotypic Detection of Carbapenemase Production

1. Modified Hodge Test (MHT)

The Modified Hodge Test was performed as per CLSI guidelines¹². A 0.5 McFarland suspension of *Escherichia coli* ATCC 25922 was inoculated onto Mueller–Hinton agar to form a lawn culture. A 10 µg

imipenem disk was placed at the center of the plate. Test organisms were streaked from the edge of the plate toward the disk. Plates were incubated at 35–37°C for 16–24 hours.

Interpretation: A cloverleaf-like indentation along the streak line, as shown in **Figure 1**, was considered positive for carbapenemase production. Absence of distortion indicated a negative result.

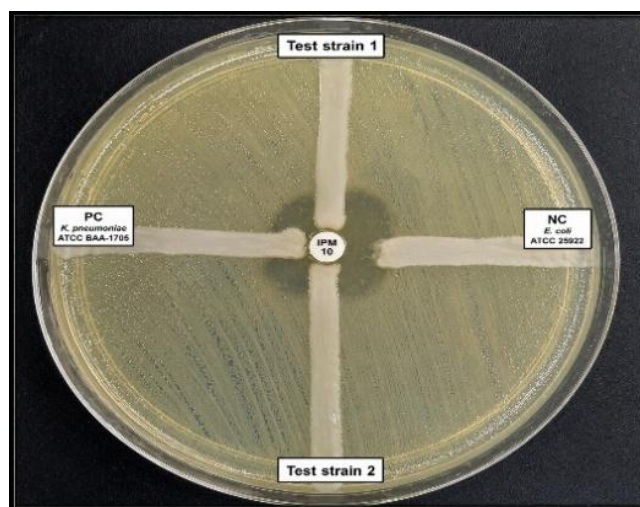


Figure 1. Modified Hodge Test (MHT) demonstrating carbapenemase production.

2. Modified Carbapenem Inactivation Method (mCIM) and EDTA-modified CIM (eCIM)

The mCIM and eCIM tests were performed as per CLSI guidelines [9]. A loopful of test organism (1 µL for Enterobacterales and 10 µL for *Pseudomonas aeruginosa*) was emulsified in 2 mL Tryptic Soy Broth (TSB). Two tubes were prepared: one without EDTA (mCIM) and one with EDTA (eCIM). A 10 µg imipenem disk was added to each tube and incubated at 35 ± 2°C for 4 hours.

Following incubation, the disks were placed on Mueller–Hinton agar plates inoculated with *E. coli* ATCC 25922 and incubated overnight.

Interpretation: A zone diameter of 6–15 mm, as shown in **Figure 2A**, was considered positive for carbapenemase production, while a zone diameter of ≥19 mm indicated a negative result. An increase in zone size in the presence of EDTA, as shown in **Figure 2B**, indicated metallo-β-lactamase production.

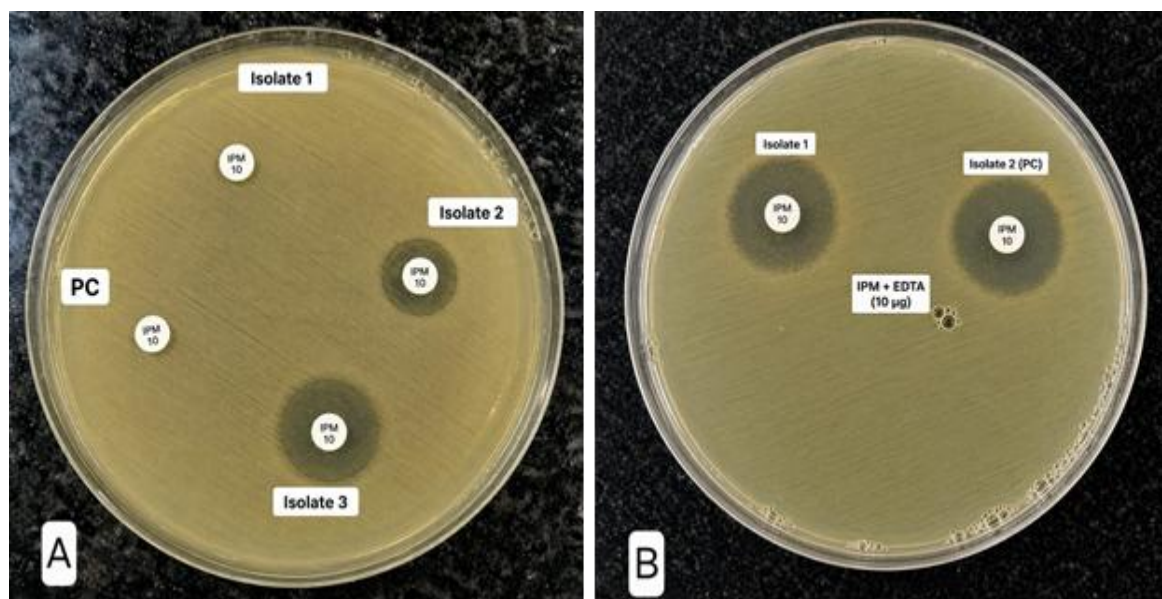


Figure 2: mCIM and eCIM demonstrating carbapenemase and metallo-β-lactamase (MBL) production

RESULT

A total of 910 clinical samples were processed, of which 100 non-duplicate carbapenem-resistant Gram-negative bacilli (CR-GNB) were identified and included in the study. Among these, *Acinetobacter spp.* were the most common isolates (37%), followed by *Klebsiella spp.* (30%),

Escherichia coli (15%), *Enterobacter spp.* (12%), and *Pseudomonas aeruginosa* (6%). Sample-wise and department-wise distribution of isolates are shown in Figure 3 and Figure 4, respectively.

A total of 43 isolates were obtained from ICU, with *Acinetobacter spp.* (44%) and *Klebsiella spp.* (34%) being the predominant organisms (Figure 4).

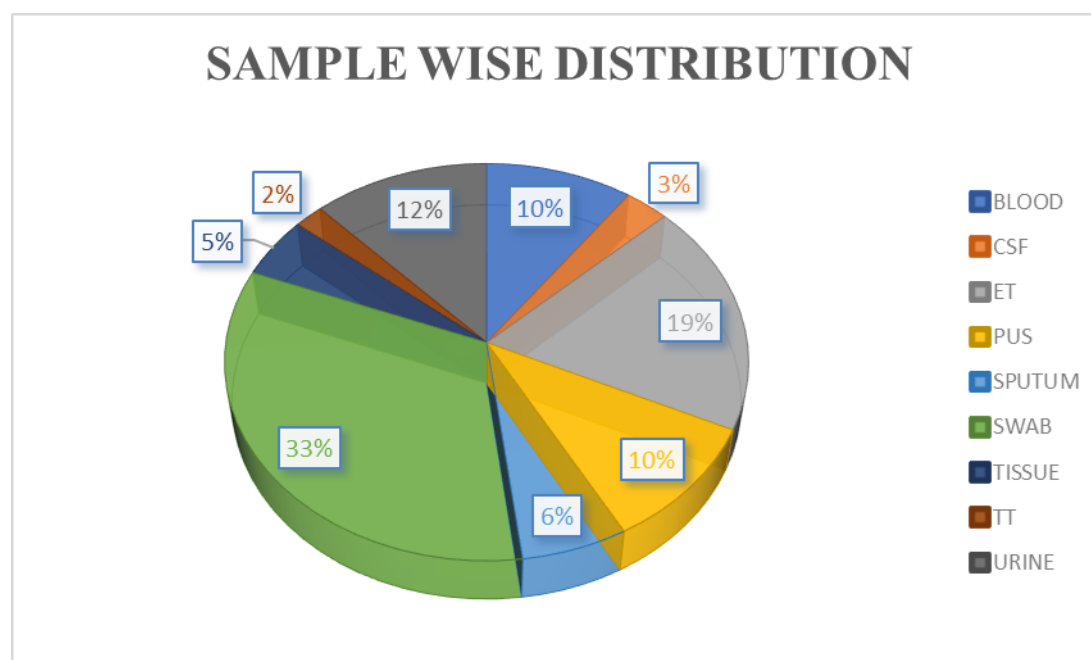


Figure 3. Sample-wise distribution of carbapenem-resistant Gram-negative bacilli isolates (n= 100).

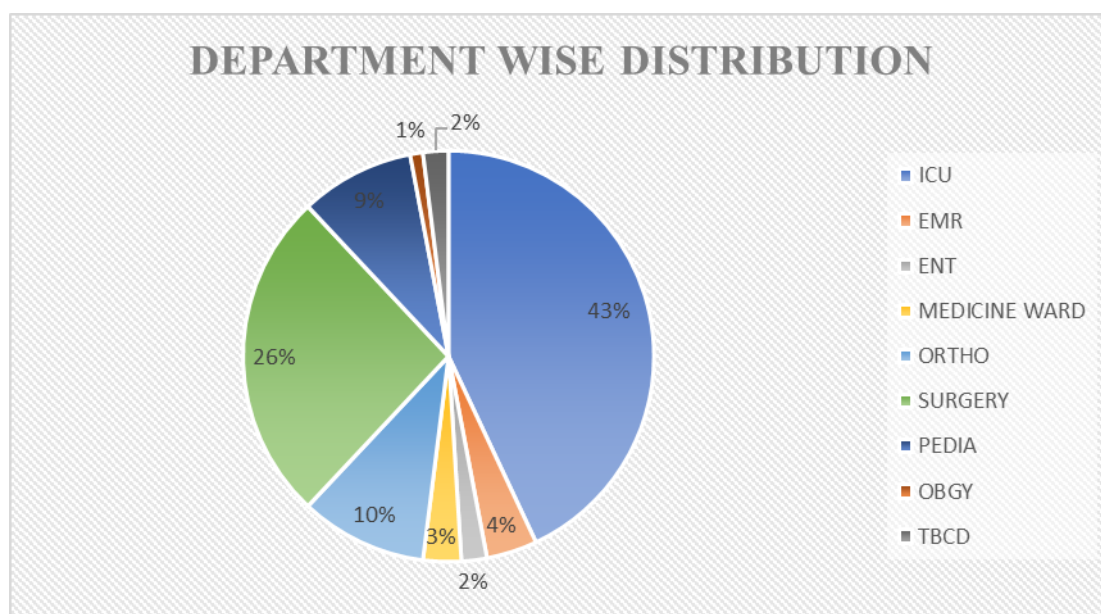


Figure 4. Department-wise distribution of carbapenem-resistant Gram-negative bacilli isolates (n = 100).

Carbapenemase production was detected in 73% of isolates by mCIM and 60% by MHT. Organism-wise comparison showed higher detection rates by mCIM across all

isolates, particularly in *Escherichia coli* (87%) and *Enterobacter spp.* (83%) (Table 1). eCIM identified metallo- β -lactamase production in a subset of isolates.

Table 1. Comparison of phenotypic test results (MHT, mCIM, and eCIM) among carbapenem-resistant Gram-negative bacilli isolates (n = 100).

S. No	Organism (n=100)	MHT Positive n (%)	mCIM Positive n (%)	eCIM Positive n (%)
1.	<i>Acinetobacter spp.</i> (37)	21 (57%)	25 (68%)	17 (46%)
2.	<i>Klebsiella spp.</i> (30)	18 (60%)	23 (77%)	11 (37%)
3.	<i>Escherichia coli.</i> (15)	13 (87%)	13 (87%)	11 (73%)
4.	<i>Enterobacter spp.</i> (12)	8 (67%)	10 (83%)	7 (58%)
5.	<i>Pseudomonas aeruginosa</i> (6)	0 (0)	2 (33%)	2 (33%)

MHT: Modified Hodge Test; mCIM: Modified Carbapenem Inactivation Method; eCIM: EDTA-modified Carbapenem Inactivation Method.

On comparison with mCIM (reference method), MHT demonstrated a sensitivity of 73.97%, specificity of 77.78%, positive predictive value of 90.00%, negative predictive value of 52.50%, and overall accuracy of 75.00%. The area under the

ROC curve was 0.76, indicating moderate diagnostic performance. Cohen's kappa coefficient for agreement between MHT and mCIM was 0.45, indicating moderate agreement between the two methods (Table 2, Figure 5).

Table 2. Comparison of Modified Hodge Test (MHT) with mCIM (reference method)

MHT Result	mCIM Positive	mCIM Negative	Total
Positive	54	6	60
Negative	19	21	40
Total	73	27	100

Receiver Operating Characteristic (ROC) - MHT vs mCIM

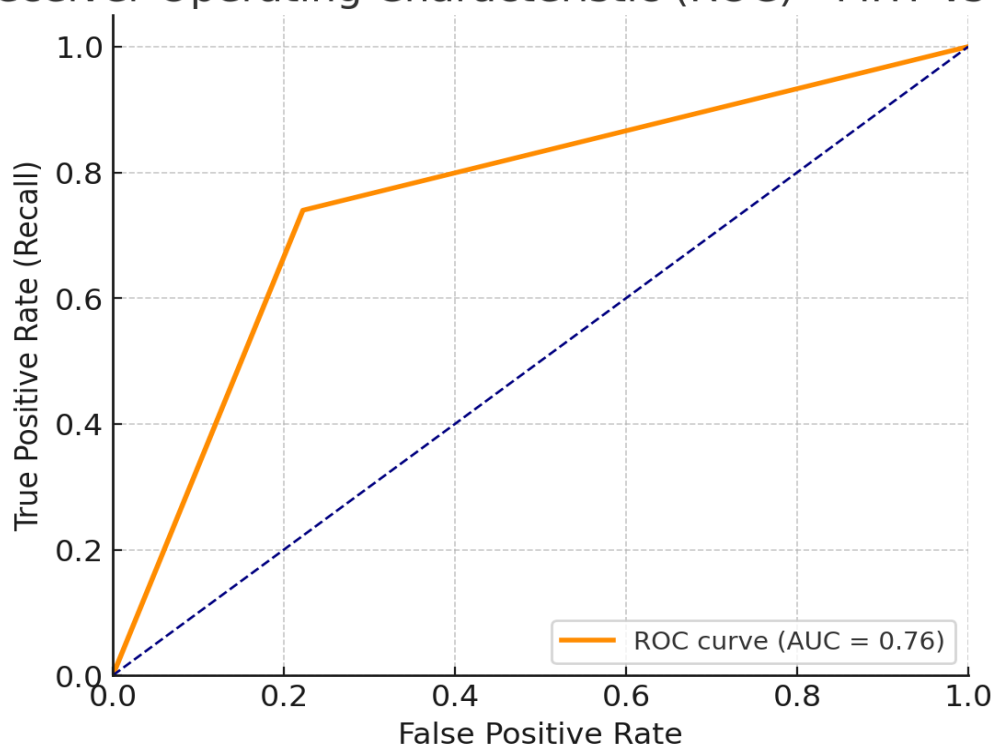


Figure 5. Receiver operating characteristic (ROC) curve showing diagnostic performance of MHT compared to mCIM. The ROC curve demonstrates moderate diagnostic accuracy of MHT, with an area under the curve (AUC) of 0.76.

Antibiotic susceptibility patterns are depicted in Figure 6, showing high susceptibility to colistin and tigecycline, with marked resistance to fluoroquinolones and other commonly used antibiotics.

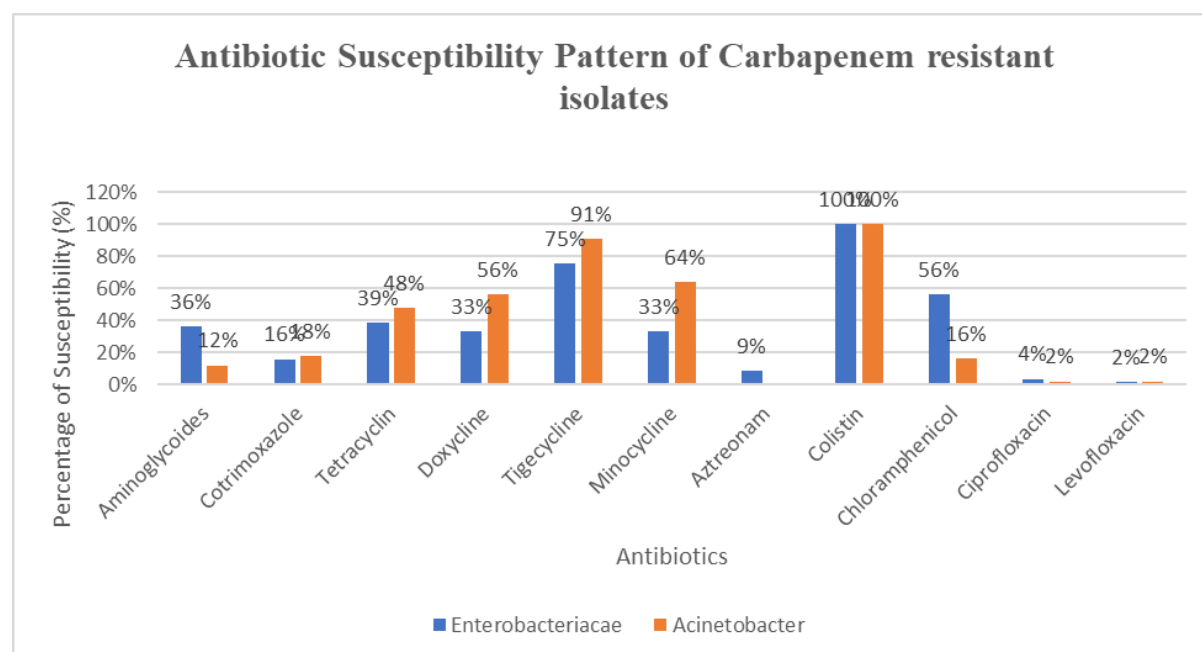


Figure 6. Antibiotic susceptibility pattern of carbapenem-resistant Gram-negative bacilli isolates (n=100).

DISCUSSION

The present study highlights the increasing prevalence of carbapenem-resistant Gram-negative bacilli (CR-GNB) in a tertiary care hospital, with *Acinetobacter spp.* and *Klebsiella spp.* emerging as the predominant isolates. These findings are consistent with previous studies by Venkataraman R et al. [13] and Garg A et al. [14], which also reported a higher prevalence of these organisms, particularly in intensive care settings.

In the present study, mCIM demonstrated higher detection rates of carbapenemase production (73%) compared to MHT (60%), indicating superior sensitivity. Similar findings have been reported by Khare et al. [1] and Cheemala et al. [2], who observed better diagnostic performance of mCIM over MHT. In the present study, mCIM demonstrated higher detection rates than MHT (73% vs 60%). The lower specificity of MHT may be explained by false-positive results, which are known to occur due to ESBL production in combination with porin loss. These findings suggest that mCIM is a more reliable phenotypic method for detection of carbapenemase production.

The moderate sensitivity (73.97%) and specificity (77.78%) of MHT in the current study further support its limited reliability as a standalone screening method.

The use of eCIM enabled identification of metallo- β -lactamase (MBL) production among carbapenemase-producing isolates. This aligns with findings by Tamma et al. [8], who emphasized the importance of combining mCIM and eCIM for accurate phenotypic differentiation of carbapenemase types, particularly in resource-limited settings where molecular methods are not feasible.

The phenotypic differentiation of carbapenemase types observed in the present study has important clinical relevance. Identification of metallo- β -lactamase (MBL) producers by eCIM can guide appropriate antimicrobial therapy, as these isolates are not effectively treated with ceftazidime–avibactam alone. In such cases, combination therapy with ceftazidime–

avibactam and aztreonam is beneficial, as aztreonam is stable to MBL hydrolysis while avibactam inhibits co-produced serine β -lactamases. In contrast, isolates producing KPC or OXA-type carbapenemases may respond to ceftazidime–avibactam, highlighting the utility of simple phenotypic methods in guiding targeted therapy, particularly in resource-limited settings.

The higher proportion of isolates from ICU settings observed in this study is in agreement with earlier reports, which attribute this to factors such as prolonged hospital stay, invasive procedures, and increased antibiotic exposure [15,16]. The predominance of *Acinetobacter spp.* and *Klebsiella spp.* in ICU further underscores their role as major nosocomial pathogens.

Antibiotic susceptibility patterns revealed high susceptibility to colistin and tigecycline, while resistance to fluoroquinolones and other commonly used antibiotics was markedly high. These findings are comparable to those reported by Nation et al. [16] and Karah et al. [17], highlighting the limited therapeutic options available for treating infections caused by carbapenem-resistant organisms.

Overall, the present study emphasizes the importance of early and accurate detection of carbapenemase production using reliable phenotypic methods. Timely identification aids in prompt implementation of infection control measures, including patient cohorting or isolation of carbapenem-resistant cases, strict adherence to hand hygiene practices, and surveillance for early detection of outbreaks. Strengthening antimicrobial stewardship programs, along with these infection control strategies, is essential to limit the transmission and prevent hospital outbreaks of multidrug-resistant pathogens.

Limitations: The study was conducted over a short duration at a single center with a limited sample size, which may affect the generalizability of the findings. Molecular methods for confirmation of carbapenemase genes were not performed. Additionally, the Modified Hodge Test (MHT) was included

for comparative and academic purposes, despite its known lower specificity and current CLSI discouragement.

Recommendations: Further multicentric studies with larger sample size and longer duration are recommended. Incorporation of molecular methods would improve diagnostic accuracy. Routine use of reliable phenotypic methods such as mCIM and eCIM, along with strengthened infection control and antimicrobial stewardship, is essential.

CONCLUSION

Carbapenemase-producing Gram-negative bacilli are increasingly prevalent, particularly in ICU settings. The study demonstrates that mCIM is a more reliable phenotypic method for detection compared to MHT, while eCIM aids in identifying metallo- β -lactamase production. Phenotypic differentiation has direct clinical relevance, as MBL producers may require ceftazidime-avibactam with aztreonam, whereas KPC and OXA-type producers may respond to ceftazidime-avibactam. Early and accurate detection, along with effective infection control and antimicrobial stewardship, is essential to limit the spread of these multidrug-resistant organisms.

Declaration by Authors

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Conflict of Interest: The authors declare no conflict of interest.

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