RESEARCH PAPER

Prevalence and Phenotypic Detection of Carbapenemase-Producing Enterobacterales Among Hospitalized Patients at a Tertiary Care Center in Central India

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ABSTRACT

Background

Carbapenemase-producing Enterobacterales are important multidrug-resistant pathogens that cause serious hospital-associated infections. Early detection is essential for appropriate treatment and infection control. This study assessed the prevalence of carbapenemase-producing Enterobacterales using phenotypic detection methods among hospitalized patients at a tertiary care center in Central India.

Objective

To determine the prevalence of carbapenemase-producing Enterobacterales and evaluate phenotypic methods for their detection among hospitalized patients at a tertiary care center in Central India.

Materials and Methods

A hospital-based observational and cross-sectional laboratory-based study design was conducted at a tertiary care hospital in central India from January 2024 to December 2025. A total of 220 Clinical specimens were collected and processed using standard microbiological methods. Enterobacterales were identified by conventional techniques, and antimicrobial susceptibility testing was performed by the Kirby–Bauer disk diffusion method according to applicable CLSI guidelines. Carbapenemase production was detected using phenotypic methods, including Modified Hodge Test, Carba NP, and inhibitor-based tests.

Results

Among 220 Enterobacterales isolates, carbapenemase production was detected in 120 (54.5%) isolates by the MHT, 115 (52.3%) by mCIM and 116 (52.7%) by the Carba NP test. Concordant positivity by all three phenotypic methods was observed in 110 (50.0%) isolates. AST showed a high level of resistance to carbapenems and extended-spectrum β-lactam antibiotics. Carbapenemase-positive isolates demonstrated significantly higher antimicrobial resistance than carbapenemase-negative isolates, particularly to imipenem, meropenem, and ertapenem ($p < 0.001$).

Conclusion

Carbapenemase production was common among the Enterobacterales isolates, with substantial antimicrobial resistance and multidrug-resistant patterns. Integrating phenotypic carbapenemase detection with antimicrobial susceptibility testing may facilitate early identification of resistant isolates and guide appropriate antimicrobial therapy.

Keywords: Enterobacterales; Carbapenemase; Carbapenem resistance; Antimicrobial susceptibility testing; Multidrug resistance; Phenotypic detection.

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INTRODUCTION:

Hospital-acquired infections (HAIs) are infections that develop 48 hours or more after hospital admission and were not present or incubating at the time of admission. Common types of HAIs include central line-associated bloodstream infections, ventilator-associated pneumonia, catheter-associated urinary tract infections, and surgical site infections. Several factors increase the risk of HAIs, including prolonged hospitalization, invasive procedures, mechanical ventilation, immunosuppression, advanced age, diabetes mellitus, intensive care unit admission, use of indwelling devices, and repeated hospital visits. HAIs remain a significant healthcare concern because they contribute substantially to patient morbidity and mortality.[1,2]

Carbapenem drugs are the most valuable drugs for treating multi-drug resistant Gram-negative bacteria (MDR-GNB) infections and have been used for the past 10 years. However, there has been a significant growth of carbapenem-resistant organisms that cause severe damage to public health. [3,4] These antibiotics are used as a last line of defense for treating infections caused by MDR-GNBs as well as organisms that produce extended-spectrum β-lactamases, which are used to screen carbapenem resistance in laboratories, and develop resistance either through gene transfer or mutations. [5,6]

Carbapenem resistance is mainly owing to the expression of a carbapenemase enzyme, efflux pump, or porin loss. Among these, the most important and difficult mechanism is the production of the carbapenemase enzyme, because it is present on mobile genetic elements, which are easily transferable from one bacterium to another bacterium such as *Pseudomonas spp.*, *Acinetobacter spp.*, *Escherichia coli* (*E. coli*), and *Klebsiella spp.*, which the World Health Organization (WHO) has designated as high priority organisms in 2017. [7-10]

Conventional methods for detecting carbapenem resistance primarily include antimicrobial susceptibility testing by disc diffusion and determination of the minimum inhibitory concentration (MIC). These methods are widely used in routine clinical microbiology laboratories because they are relatively simple, economical, and do not require sophisticated equipment. Phenotypic methods for detecting carbapenemase production, such as the Modified Hodge Test, Carba NP test, combined disc test with EDTA, and carbapenem inactivation methods, can provide useful evidence of carbapenemase activity among Gram-negative bacterial isolates. However, differences in test performance and the time required to obtain results may affect the timely recognition of resistant organisms.

Early identification of carbapenem-resistant Gram-negative bacilli (CRGNB) is important for appropriate antimicrobial therapy and implementation of infection-control measures. Determining the prevalence and resistance patterns of CRGNB within a hospital can provide valuable information for developing local antimicrobial stewardship and infection-control strategies and may help reduce the transmission of resistant organisms and the associated healthcare burden.[11]

Against this background, the present study was undertaken to detect carbapenem resistance and carbapenemase production among Gram-negative bacterial isolates associated with hospital-acquired infections using conventional antimicrobial susceptibility testing and phenotypic carbapenemase detection methods.

DEFINING CARBAPENEM-RESISTANT GRAM-NEGATIVE ORGANISMS

In 2010, the Clinical and Laboratory Standards Institute (CLSI) revised and lowered the carbapenem susceptibility breakpoints for Enterobacteriaceae. According to the revised criteria, isolates are considered susceptible to meropenem and imipenem at MIC values ≤1 μg/mL and to ertapenem at an MIC ≤0.5 μg/mL.

Following these revised breakpoints, routine testing for carbapenemase production is no longer required solely for the purpose of reporting antimicrobial susceptibility results in isolates with elevated carbapenem MICs. When the updated CLSI breakpoints are applied, antimicrobial susceptibility results should generally be reported according to the observed MIC or zone of inhibition, irrespective of the underlying resistance mechanism. However, laboratories may continue to perform carbapenemase detection for epidemiological surveillance, infection-control purposes, and antimicrobial stewardship activities.[12,13]

Over the years, several phenotypic methods have been developed to detect carbapenemase-producing Gram-negative organisms. These methods provide useful approaches for identifying carbapenemase activity and supporting infection-control and antimicrobial-management strategies.

OVERVIEW OF PHENOTYPIC ASSAYS

Phenotypic assays are widely used for detecting carbapenemase production in cultured bacterial isolates. These methods can be broadly classified into **growth-based tests**, such as the Modified Hodge Test (MHT) and modified carbapenem inactivation method (mCIM), and **hydrolysis-based tests**, such as the Carba NP test. Inhibitor-based methods, including EDTA-based tests and the MBL E-test, can help differentiate metallo-β-lactamase activity from other carbapenemases. The selection of a phenotypic test depends on factors such as test accuracy, cost, ease of performance, turnaround time, available resources, and the local prevalence of carbapenemases.

Commonly used phenotypic methods include MHT, Carba NP, mCIM, eCIM, and inhibitor-based tests.[14,15]

MATERIAL AND METHODS:

Study design and setting

This study will employ a observational **cross-sectional laboratory-based study design** to determine the phenotypic characteristics of carbapenemase-producing Enterobacterials isolated from clinical specimens. This study conducted in the Department of Microbiology, Index Medical College Hospital and Research Centre, Indore, Madhya Pradesh, India, from January 2024 to December 2025.

Sample size

The minimum calculated sample size was approximately 216 Enterobacterial isolates. A total of **220 clinical Enterobacterials** were included in final analysis**.**

Inclusion Criteria

a) Patients of all age groups and genders admitted in the hospital

b) All clinical isolates of Gram-Negative bacteria isolated from different clinical samples will be included in this study.

c) Isolates showing reduced susceptibility or resistance to carbapenems group of antibiotics (e.g., imipenem, meropenem, ertapenem) on initial antimicrobial susceptibility testing.

Exclusion Criteria

a) Samples collected from OPD (Outpatient department) patients.

b) Isolates identified as Gram-positive bacteria, fungi, or anaerobes will be excluded.

c) Isolates showing Sensitive to carbapenems group of antibiotics (e.g., imipenem, meropenem, ertapenem) on initial antimicrobial susceptibility testing.

Study Workflow

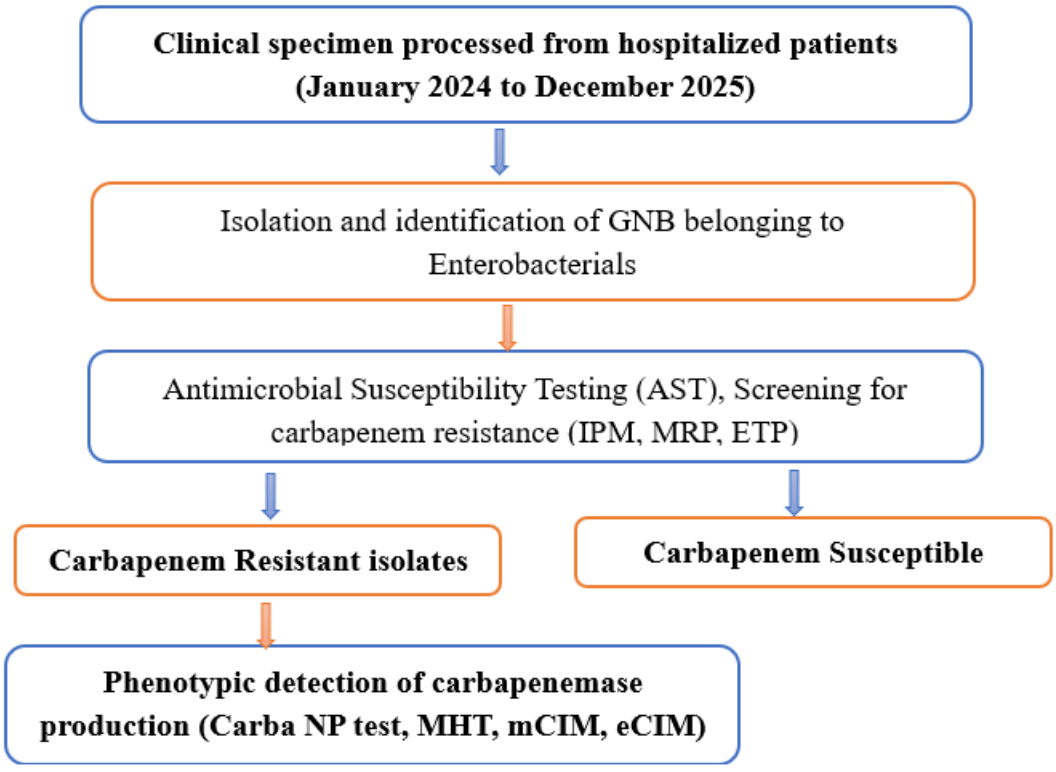


Figure 1. Study methodology and analytical workflow.

Isolation and Identification of Enterobacterales

Clinical specimens collected from hospitalized patients were processed using standard microbiological procedures. Specimens were inoculated onto appropriate culture media, including blood agar and MacConkey agar, and incubated aerobically at 37°C for 18–24 h. Urine specimens were additionally cultured on CLED agar where appropriate. Bacterial growth was assessed based on colony morphology, pigmentation, hemolysis, and lactose fermentation characteristics.

Suspected Gram-negative isolates were subjected to Gram staining and preliminary identification using oxidase and

other appropriate biochemical tests. Enterobacterales were identified and differentiated using conventional biochemical reactions, including indole, methyl red, Voges–Proskauer, citrate utilization, urease, triple sugar iron agar, and motility tests, as applicable. Identification was based on the characteristic biochemical profile of each isolate. Pure cultures were maintained on appropriate media for further antimicrobial susceptibility testing and phenotypic detection of carbapenemase production. Conventional culture and biochemical identification procedures are consistent with approaches used in clinical microbiology studies for Enterobacterales.

Antimicrobial Susceptibility Testing

Antimicrobial susceptibility testing of Enterobacterales isolates was performed by the Kirby–Bauer disk diffusion method on Mueller–Hinton agar. The antimicrobial panel included piperacillin–tazobactam, cefotaxime, ceftazidime, ceftriaxone, ciprofloxacin, amikacin, gentamicin, trimethoprim–sulfamethoxazole, imipenem, meropenem, and ertapenem. A 0.5 McFarland suspension of each test isolate was prepared and inoculated uniformly onto Mueller–Hinton agar. Antibiotic discs were applied to the inoculated plates, followed by incubation at 35–37°C for 16–18 hours. Zone diameters were measured and interpreted according to the applicable CLSI M100 criteria.

Carbapenem Screening

Enterobacterales isolates were screened for reduced susceptibility to carbapenems using ertapenem, imipenem, and meropenem. Isolates showing reduced susceptibility to one or more of these carbapenems were subjected to further phenotypic testing for carbapenemase production using the selected conventional methods. Carbapenem susceptibility testing was interpreted according to the CLSI criteria applicable to the study period.

For Enterobacterales, carbapenem susceptibility should be assessed using ertapenem, imipenem, and meropenem by disk diffusion and/or MIC testing. The CLSI breakpoints commonly cited for Enterobacterales were:

Table:1 Disk-diffusion breakpoints — CLSI 2024[16]

Carbapenem	Disk	Susceptible (S)	Intermediate (I)	Resistant (R)
Ertapenem	10 µg	≥22 mm	19–21 mm	≤18 mm
Imipenem	10 µg	≥23 mm	20–22 mm	≤19 mm
Meropenem	10 µg	≥23 mm	20–22 mm	≤19 mm

Table:2 The carbapenem MIC breakpoints for Enterobacterales: CLSI 2024[16]

Carbapenem	Susceptible (S)	Intermediate (I)	Resistant (R)
Ertapenem	≤0.5 µg/mL	1 µg/mL	≥2 µg/mL
Imipenem	≤1 µg/mL	2 µg/mL	≥4 µg/mL
Meropenem	≤1 µg/mL	2 µg/mL	≥4 µg/mL

Phenotypic Detection of Carbapenemase Production

Carbapenemase production was evaluated among Enterobacterales isolates exhibiting reduced susceptibility to carbapenems using phenotypic assays, including the Modified Hodge Test (MHT), Carba NP test, modified Carbapenem Inactivation Method (mCIM), EDTA-modified CIM (eCIM), and MBL E-test.

Modified Hodge Test (MHT):

A standardized suspension of *E. coli* ATCC 25922 was inoculated onto Mueller–Hinton agar, followed by placement of a meropenem disc (10 µg) at the center. The test isolate was streaked from the disc toward the plate periphery and incubated at 35 ± 2°C for 16–20 h. Development of a characteristic **cloverleaf-like growth pattern** toward the meropenem disc was considered positive for carbapenemase production.

Carba NP Test:

A fresh bacterial culture was suspended in the test reagent containing phenol red and imipenem. Carbapenem hydrolysis results in acid production and a corresponding color change from red/orange to yellow. A clear color change compared with the negative control was interpreted as a positive carbapenemase reaction.

Modified Carbapenem Inactivation Method (mCIM):

Test colonies were suspended in tryptic soy broth containing a meropenem disc and incubated. The disc was then transferred to Mueller–Hinton agar inoculated with *E. coli* ATCC 25922. A reduced or absent inhibition zone around the disc indicated carbapenem hydrolysis and was interpreted as mCIM positive, whereas a well-defined larger zone indicated a negative result.

EDTA-modified CIM (eCIM):

The eCIM was performed alongside mCIM using a meropenem disc in the presence of EDTA. Following incubation and transfer of the disc to an *E. coli* ATCC 25922 lawn, an increase in the inhibition zone compared with mCIM indicated inhibition of metallo-β-lactamase activity by EDTA and therefore suggested MBL production.

MBL E-test:

A 0.5 McFarland suspension of the isolate was inoculated onto Mueller–Hinton agar, and a meropenem/meropenem-EDTA E-test strip was applied. After incubation, MIC values were determined from the inhibition ellipse. A significant reduction in the meropenem MIC in the presence of EDTA, according to the manufacturer's criteria, was interpreted as evidence of MBL production.

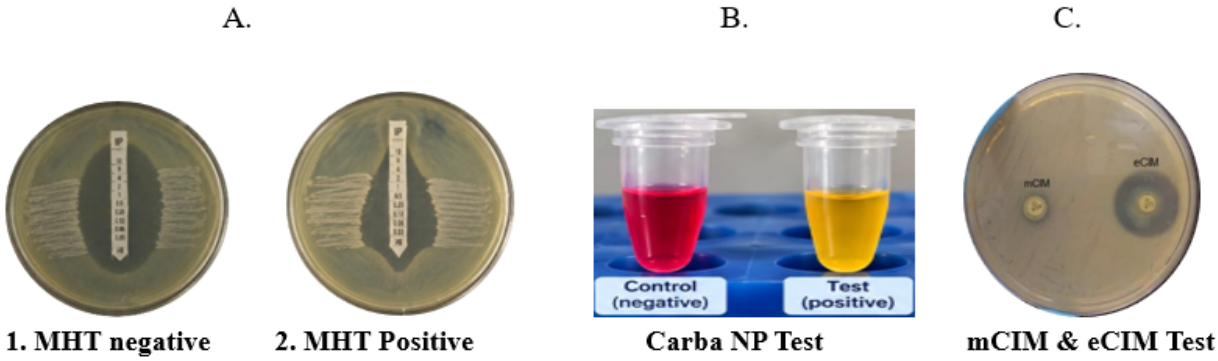


Figure:2 *(A.) Modified Hodge test (1.) Negative (2.) Positive. (B) CLSI Carba NP Negative red tube, no imipenem added , yellow positive result, imipenem added (C.) Modified carbapenem inactivation method (mCIM) positive result and eCIM results that are positive for a metallo-beta-lactamase producer, as there is inhibition of carbapenemase activity in the presence of EDTA.*

RESULT

A total of 220 non-duplicate clinical isolates of Enterobacterales were included in the study. Among these, 119 (54.1%) were identified as *Escherichia coli*, 70 (31.8%) as *Klebsiella pneumoniae*, 27 (12.3%) as *Klebsiella oxytoca*, and 4 (1.8%) as other Enterobacterales. Phenotypic carbapenemase production was detected in 120 (54.5%) isolates by the Modified Hodge Test, 115 (52.3%) by mCIM, and 116 (52.7%) by the Carba NP test.

GENDER-WISE DISTRIBUTION OF PHENOTYPIC CARBAPENEMASE DETECTION:

Among the 220 isolates, 141 (64.1%) were obtained from male patients and 79 (35.9%) from female patients. MHT positivity was 53.2% among males and 57.0% among females, while mCIM positivity was 50.4% and 55.7%, respectively. Carba NP positivity was 50.4% in males and 57.0% in females. None of these differences was statistically significant (P > 0.05).

Table:1 Gender-wise distribution of phenotypic carbapenemase detection

Gender	Total	MHT positive n (%)	mCIM positive n (%)	Carba NP positive n (%)
Male	141	75 (53.2)	71 (50.4)	71 (50.4)
Female	79	45 (57.0)	44 (55.7)	45 (57.0)
Total	**220**	**120 (54.5)**	**115 (52.3)**	**116 (52.7)**
P value		**0.691**	**0.535**	**0.423**

SPECIES-WISE DISTRIBUTION OF PHENOTYPIC CARBAPENEMASE DETECTION

Phenotypic carbapenemase positivity varied among the different bacterial species. MHT positivity ranged from 46.2% in *E. coli* to 85.2% in *K. oxytoca*. Similarly, mCIM positivity ranged from 43.7% to 81.5%, while Carba NP positivity ranged from 43.7% to 81.5%. A statistically significant association was observed between bacterial species and MHT (P = 0.003), mCIM (P = 0.004), and Carba NP (P = 0.004).

Table:2 Species-wise distribution of phenotypic carbapenemase detection

Organism	Total	MHT positive n (%)	mCIM positive n (%)	Carba NP positive n (%)
Escherichia coli	119	55 (46.2)	52 (43.7)	52 (43.7)
Klebsiella pneumoniae	70	40 (57.1)	39 (55.7)	40 (57.1)
Klebsiella oxytoca	27	23 (85.2)	22 (81.5)	22 (81.5)
Proteus vulgaris	2	2 (100)	2 (100)	2 (100)
Enterobacter spp.	1	0	0	0
Enterobacter aerogenes	1	0	0	0
Total	**220**	**120 (54.5)**	**115 (52.3)**	**116 (52.7)**
P value		**0.004**	**0.006**	**0.002**

CLINICAL SAMPLE-WISE DISTRIBUTION

Urine was the predominant clinical specimen, accounting for 119 (54.1%) isolates, followed by sputum 29 (13.2%) and pus 27 (12.3%). Carbapenemase positivity was highest among pus isolates, with 81.5% positive by MHT and 77.8% by both mCIM and Carba NP. The association between specimen type and phenotypic positivity was statistically significant for MHT (**P = 0.009**), mCIM (**P = 0.016**), and Carba NP (**P = 0.047**).

Table: 3 Clinical Sample-Wise Distribution

Clinical sample	Total	MHT positive n (%)	mCIM positive n (%)	Carba NP positive n (%)
Urine	119	65 (54.6)	62 (52.1)	62 (52.1)
Sputum	29	11 (37.9)	11 (37.9)	11 (37.9)
Pus	27	22 (81.5)	21 (77.8)	21 (77.8)
Blood	18	4 (22.2)	4 (22.2)	5 (27.8)
TA	6	2 (33.3)	2 (33.3)	2 (33.3)
ET	4	2 (50.0)	2 (50.0)	2 (50.0)
BAL	2	0	0	0
Bronchial washing	2	2 (100)	2 (100)	2 (100)
Fluid	2	2 (100)	2 (100)	2 (100)
TT	2	2 (100)	2 (100)	1 (50.0)
Tracheostomy	2	2 (100)	2 (100)	2 (100)
HVS	1	1 (100)	1 (100)	1 (100)
ET-Tip	1	1 (100)	0	1 (100)

Drain	1	1 (100)	1 (100)	1 (100)
C. tip	1	1 (100)	1 (100)	1 (100)
ETA	1	1 (100)	1 (100)	1 (100)
PICC line tube	1	0	0	0
Pleural fluid	1	1 (100)	1 (100)	1 (100)
Total	**220**	**120 (54.5)**	**115 (52.3)**	**116 (52.7)**
P value		**0.0002**	**0.0016**	**0.006**

ANTIMICROBIAL SUSCEPTIBILITY PROFILE

The isolates demonstrated substantial resistance to several antimicrobial agents. Resistance to imipenem, meropenem, and ertapenem was observed in 58.2%, 57.3%, and 55.9% of isolates, respectively. High resistance was also observed for cefazolin (85.9%), ampicillin (84.1%), cefuroxime (83.6%), ciprofloxacin (82.7%), and levofloxacin (82.7%). Minocycline showed the highest proportion of susceptible isolates (75.5%), followed by amikacin (43.6%).

Table:4 Antimicrobial Susceptibility Profile

Antibiotic	Susceptible n (%)	Intermediate n (%)	Resistant n (%)
Imipenem	92 (41.8)	0	128 (58.2)
Meropenem	92 (41.8)	2 (0.9)	126 (57.3)
Ertapenem	85 (38.6)	12 (5.5)	123 (55.9)
Ampicillin	32 (14.5)	3 (1.4)	185 (84.1)
Amoxicillin-clavulanate	71 (32.3)	6 (2.7)	143 (65.0)
Piperacillin-tazobactam	79 (35.9)	5 (2.3)	136 (61.8)
Cefazolin	29 (13.2)	2 (0.9)	189 (85.9)
Cefuroxime	33 (15.0)	3 (1.4)	184 (83.6)
Cefoxitin	45 (20.5)	4 (1.8)	171 (77.7)
Cefotaxime	43 (19.5)	10 (4.5)	167 (75.9)
Ceftazidime	54 (24.5)	1 (0.5)	165 (75.0)
Cefoperazone	58 (26.4)	0	162 (73.6)
Ceftazidime-avibactam	92 (41.8)	0	128 (58.2)
Ciprofloxacin	32 (14.5)	6 (2.7)	182 (82.7)
Levofloxacin	38 (17.3)	0	182 (82.7)
Amikacin	96 (43.6)	12 (5.5)	112 (50.9)
Gentamicin	83 (37.7)	11 (5.0)	126 (57.3)
Minocycline	166 (75.5)	23 (10.5)	31 (14.1)
Polymyxin B	3 (1.4)	166 (75.5)	51 (23.2)

Colistin	3 (1.4)	165 (75.0)	52 (23.6)
Nitrofurantoin	87 (39.5)	5 (2.3)	39 (17.7)
Nalidixic acid	23 (10.5)	6 (2.7)	102
TMP-SMX	60 (27.3)	0	156 (70.9)

OVERALL PHENOTYPIC DETECTION OF CARBAPENEMASE PRODUCTION

Among the 220 Enterobacterales isolates, carbapenemase production was detected in 120 (54.5%) isolates by MHT, 115 (52.3%) by mCIM, and 116 (52.7%) by Carba NP. The positivity rates were therefore broadly comparable across the three phenotypic methods.

Table:5 Phenotypic Detection of Carbapenemase Production

Phenotypic method	Positive, n (%)	Negative, n (%)
Modified Hodge Test (MHT)	120 (54.5)	100 (45.5)
Modified Carbapenem Inactivation Method (mCIM)	115 (52.3)	105 (47.7)
Carba NP test	116 (52.7)	104 (47.3)

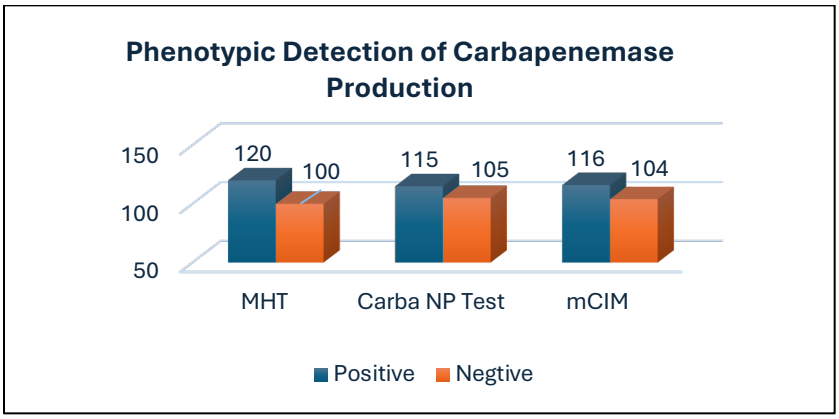


Figure:3 Phenotypic Detection of Carbapenemase Production

COMPARISON OF ALL THREE PHENOTYPIC CARBAPENEMASE DETECTION METHODS

The three phenotypic methods demonstrated high agreement. Concordance was 97.7% between MHT and mCIM, 97.3% between MHT and Carba NP, and 95.0% between mCIM and Carba NP. None of the pairwise differences was statistically significant (**P > 0.05**). Complete agreement among all three methods was observed in 209 (95.0%) isolates, including 110 (50.0%) isolates that were positive by all three methods and 99 (45.0%) that were negative by all three methods.

Table:6 Comparison of All Three Phenotypic Carbapenemase Detection Methods

Comparison	Concordant	Discordant	Concordance (%)	Exact McNemar P
MHT vs. mCIM	215/220	5/220	97.7	0.063
MHT vs. Carba NP	214/220	6/220	97.3	0.219
mCIM vs. Carba NP	209/220	11/220	95.0	1.000

DISCUSSION

Carbapenemase-producing Enterobacterales (CPE) have emerged as an important cause of antimicrobial-resistant healthcare-associated infections. Their detection is clinically relevant because carbapenem resistance may be associated with resistance to several other antimicrobial classes, thereby limiting therapeutic options and creating challenges for infection prevention and control. In the present study, phenotypic carbapenemase production was evaluated among 220 Enterobacterales isolates obtained from hospitalized patients using the Modified Hodge Test (MHT), modified Carbapenem Inactivation Method (mCIM), and Carba NP test.

In the present study, the Modified Hodge Test detected carbapenemase production in 120 (54.5%) isolates. This finding indicates a substantial occurrence of phenotypically detectable carbapenemase activity among the isolates investigated. An earlier Indian study by **Pallavi et al.** evaluated 1,072 Enterobacteriaceae isolates and detected carbapenemase activity by MHT in 35.9% of isolates. The higher proportion observed in the present study may be related to differences in study population, hospital setting, inclusion criteria, bacterial species distribution, and the proportion of isolates selected because of reduced carbapenem susceptibility. Direct comparison of these percentages should be interpreted with caution due to differences in study population and methodology.

The relatively high MHT positivity observed in the present study should also be interpreted in light of the recognized limitations of this method. MHT is based on demonstration of enhanced growth of the indicator organism around the carbapenem-containing disk and is subject to visual interpretation. Studies have reported variable performance of MHT depending on the type of carbapenemase present. In particular, false-positive and false-negative results have been described, limiting its usefulness as a stand-alone confirmatory test. A systematic review and meta-analysis involving 67 studies reported pooled sensitivity and specificity of approximately 92% and 93%, respectively, for MHT, whereas Carba NP and mCIM demonstrated higher overall diagnostic accuracy.[17]

The mCIM was positive in 115 (52.3%) isolates in the this study. The positivity rate was close to that obtained with MHT, although five fewer isolates were identified as positive. The mCIM assesses carbapenemase activity by determining whether the test organism can inactivate a carbapenem, thereby providing a functional measure of enzyme activity. The results of the present study are consistent with previous investigations demonstrating the usefulness of mCIM for detecting carbapenemase-producing Enterobacterales. In a study involving 207 Enterobacteriaceae isolates, mCIM demonstrated 100% sensitivity and specificity and performed better than several conventional screening approaches. [18]

The findings are also supported by a systematic review published subsequently, which reported pooled sensitivity and specificity of approximately 97% for mCIM for detection of carbapenemase production in Enterobacterales. The same analysis found somewhat lower pooled sensitivity and specificity for Carba NP. These findings indicate that differences between phenotypic tests may occur even when the assays are applied to the same group of organisms.[19]

In the this study, the Carba NP test detected carbapenemase production in 116 (52.7%) isolates. The proportion was comparable to that observed with mCIM and MHT. Carba NP detects carbapenem hydrolysis through a colorimetric change resulting from acidification of the reaction mixture. Its principal advantage is the relatively rapid detection of carbapenemase activity, which can facilitate early laboratory recognition of CPE. Previous comparative studies have demonstrated good performance of Carba NP, although its sensitivity can vary according to the carbapenemase type and bacterial characteristics. [20]

Yamada et al. compared MHT, Carba NP, and CIM and reported that MHT and Carba NP could produce false-negative results, particularly with some OXA-48-like and mucoid NDM-producing isolates, whereas CIM detected these isolates more consistently.**20** This observation is relevant to the present study because the small differences in positivity between MHT, mCIM, and Carba NP may reflect differences in the biochemical principles and detection capabilities of the individual methods.

An important finding of the present study was that 110 (50.0%) isolates demonstrated concordant positivity by all three phenotypic methods. This concordance provides evidence of consistent detection of carbapenemase activity among a substantial proportion of the isolates. At the same time, 99 (45.0%) isolates were negative by all three methods, while the remaining isolates showed variable combinations of positive and negative results. Such discordance has also been documented in comparative studies of carbapenemase detection methods. Differences may result from variation in enzyme expression, carbapenemase type, bacterial inoculum, growth characteristics, or the analytical principle of the test. Consequently, discrepant phenotypic results should be interpreted cautiously rather than assuming that one method is invariably capable of detecting every carbapenemase.

The present findings are particularly relevant in the context of Indian clinical microbiology, where different carbapenemase families may circulate simultaneously. An Indian study evaluating Carba NP highlighted the importance of rapid phenotypic detection in a setting where NDM-producing organisms are an important concern, while also emphasizing limitations of MHT, including the possibility of false-positive results and

failure to detect certain metallo-β-lactamases.[21] Thus, the performance of phenotypic assays may depend not only on the method itself but also on the local epidemiology of carbapenemase genes.

The antimicrobial susceptibility findings in the present study further demonstrated resistance to carbapenems and multiple other antimicrobial agents among the isolates, with multidrug-resistant patterns observed in a substantial proportion. This observation is clinically important because carbapenemase-producing organisms frequently occur within multidrug-resistant populations. Nevertheless, phenotypic carbapenem resistance should not automatically be interpreted as evidence of a specific carbapenemase mechanism. Reduced carbapenem susceptibility may also result from combinations of other resistance mechanisms, including porin alterations and other β-lactamases. Therefore, phenotypic carbapenemase testing and antimicrobial susceptibility testing provide complementary information rather than interchangeable results.

The differences observed among the three phenotypic assays also emphasize the importance of selecting appropriate laboratory methods according to the purpose of testing. The systematic review by Zhong et al. reported substantially greater overall diagnostic accuracy for Carba NP and mCIM than for MHT, supporting the use of more reliable contemporary phenotypic approaches where resources and laboratory expertise permit.[17] More recent evidence likewise indicates that mCIM generally demonstrates high diagnostic accuracy, although performance can vary according to the carbapenemase repertoire represented in the tested population. [22]

The antimicrobial susceptibility testing performed in the present study was interpreted according to CLSI M100, 34th edition (2024), which provides standardized antimicrobial susceptibility testing methods, interpretive criteria, and quality-control recommendations.[23] Use of standardized criteria is essential for meaningful interpretation and comparison of antimicrobial susceptibility results across clinical laboratories.

Taken together, the findings of the present study demonstrate a substantial proportion of phenotypically detectable carbapenemase production among the Enterobacterales isolates studied. The comparable positivity rates obtained by MHT, mCIM, and Carba NP, along with concordant positivity in 50.0% of isolates, indicate that the three methods identified a considerable common subset of carbapenemase-producing isolates. However, the observed discordance confirms that individual phenotypic assays may not detect all carbapenemase-producing isolates with identical efficiency. These findings support the use of standardized phenotypic testing as part of routine laboratory investigation, with molecular or other confirmatory approaches being particularly useful for resolving discrepant results and identifying the specific carbapenemase mechanism.

The findings should be interpreted in consideration of the study design and laboratory setting. The study was conducted among hospitalized patients at a tertiary care center and therefore reflects the bacterial population encountered in that clinical setting rather than the prevalence of carbapenemase production in the general community. In addition, phenotypic tests demonstrate carbapenemase activity but do not by themselves establish the underlying resistance gene. Consequently, the phenotypic results provide important microbiological evidence of carbapenemase production, while molecular characterization can provide further information regarding the specific genetic mechanisms involved.

LIMITATION

This study was limited by its single-centre design, which may restrict the generalizability of the findings. Phenotypic carbapenemase detection may vary according to the carbapenemase type, enzyme expression level, and laboratory conditions. Differences in test principles and subjective interpretation may also contribute to discordant results among phenotypic methods. Therefore, the findings should be interpreted primarily as institution-specific phenotypic surveillance data.

CONCLUSION

The study identified a substantial proportion of Enterobacterales isolates with phenotypically detectable carbapenemase activity among hospitalized patients. The MHT, mCIM, and Carba NP assays demonstrated comparable detection patterns, with considerable agreement among the methods. Variability in test results highlights the importance of careful interpretation and appropriate selection of phenotypic assays. Routine phenotypic detection, together with antimicrobial susceptibility testing, can support early recognition and surveillance of carbapenemase-producing isolates in hospital settings.

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