



DETECTION OF CARBAPENEM RESISTANCE AND METALLO-B-LACTAMASE PRODUCTION AMONG GRAM-NEGATIVE BACTERIAL ISOLATES IN A TERTIARY CARE CENTRE.

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ABSTRACT

Background:

Carbapenem-resistant Gram-negative bacteria have emerged as a major global health threat due to their association with limited treatment options and increased morbidity and mortality. Among the various resistance mechanisms, metallo- β -lactamase (MBL) production plays a significant role in mediating resistance to carbapenem antibiotics. Continuous monitoring of resistance patterns is therefore essential to guide appropriate therapeutic decisions and infection control strategies.

Objectives: To determine the prevalence of carbapenem resistance and metallo- β -lactamase production among Gram-negative bacterial isolates and to evaluate the distribution of carbapenemase types in a tertiary care hospital setting.

Materials and Methods: This cross-sectional study included **334 Gram-negative bacterial isolates** obtained from clinical specimens. Carbapenem resistance was screened using the modified carbapenem inactivation method (mCIM). Detection of MBL production was carried out using the Double Disc Synergy Test (DDST) and confirmed by the E-test. Carbapenemase production was further evaluated using the CARBA NP test. Statistical analysis was performed to assess associations between resistance patterns and bacterial species.

Results: Out of 334 isolates, **143 (42.8%) were found to be carbapenem-resistant**, while 191 (57.2%) were carbapenem-sensitive. Among the carbapenem-resistant isolates, **67 (44.6%) were identified as MBL producers**. No significant difference was observed between the DDST and E-test methods for detecting MBL production ($p = 1.000$). The predominant carbapenemase detected was **NDM among *Escherichia coli***, whereas **OXA-48 was more commonly detected among *Klebsiella pneumoniae* and *Pseudomonas aeruginosa***. A statistically significant association was observed between bacterial species and carbapenemase production ($p < 0.01$).

Conclusion: The study demonstrates a high prevalence of carbapenem-resistant Gram-negative bacteria, with a considerable proportion of isolates producing metallo- β -lactamase enzymes. Early detection of resistant organisms using reliable phenotypic methods, along with strengthened antimicrobial stewardship and infection control practices, is essential to limit the spread of multidrug-resistant pathogens in healthcare settings.

Keywords: GNB, MBL, NDM, mCIM, OXA-48 carbapenemase, antimicrobial resistance

INTRODUCTION

Carbapenems are regarded as highly effective broad-spectrum antibiotics and are widely used as **last-resort therapeutic agents** for the treatment of severe infections caused by multidrug-resistant Gram-negative bacteria, particularly in critically ill and hospitalized patients. These antibiotics play a crucial role in managing infections caused by organisms resistant to extended-spectrum β -lactam antibiotics. However, the **increasing emergence of carbapenem-resistant Gram-negative organisms** has become a major global health concern, significantly limiting therapeutic options and leading to increased morbidity, mortality, and healthcare costs [1,2].

Among the various mechanisms responsible for carbapenem resistance, the production of **metallo- β -lactamase (MBL) enzymes** is considered one of the most important and clinically significant mechanisms. These enzymes possess the ability to hydrolyze a wide range of β -lactam antibiotics, including carbapenems, thereby rendering them ineffective. Furthermore, organisms producing MBL enzymes frequently exhibit resistance to multiple classes of antibiotics, resulting in the emergence of multidrug-resistant strains that are difficult to treat [3,4].

Early and accurate detection of **MBL-producing organisms** is therefore essential for guiding appropriate antimicrobial therapy, implementing effective infection control measures, and preventing the spread of resistant pathogens within healthcare settings. Routine surveillance of carbapenem resistance and screening for MBL production are strongly recommended as part of hospital infection control policies and antibiotic stewardship programs [5,6].

In view of the rising prevalence of carbapenem-resistant pathogens and their clinical significance, the **present study was undertaken to evaluate carbapenem resistance and the prevalence of metallo- β -lactamase (MBL) production among Gram-negative bacterial isolates** obtained from hospitalized patients.

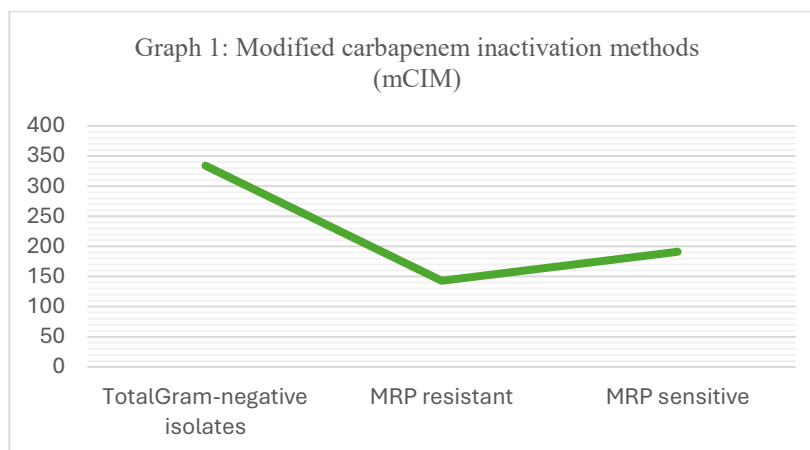
MATERIALS AND METHODS

This cross-sectional study was conducted in the Department of Microbiology at *Index Medical College & Hospital, Malwanchal University, Indore, Madhya Pradesh*.

A total of 334 Gram-negative bacterial isolates obtained from clinical samples were included in the study. Carbapenem resistance was screened using the modified carbapenem inactivation method (mCIM). Detection of metallo- β -lactamase (MBL) production was performed using the double disc synergy test (DDST), followed by confirmation with the E-test method. In addition, carbapenemase production was assessed using the CARBA NP test. All procedures were carried out in accordance with standard microbiological protocols and recommended guidelines.

RESULTS

Out of 334 Gram-negative isolates, 143 (42.8%) were found to be **carbapenem-resistant** by the **modified carbapenem inactivation method (mCIM)**, while 191 (57.2%) were sensitive. The difference between resistant and sensitive isolates was **statistically significant** ($\chi^2 = 6.90$, $p = 0.0086$). These findings indicate a considerable prevalence of carbapenem resistance, highlighting the need for **routine phenotypic screening** to support early detection and appropriate antimicrobial management (**Graph 1**).



Among the **carbapenem-resistant isolates**, **67 isolates (44.6%)** were identified as **metallo- β -lactamase (MBL) producers**, while **76 isolates (53.1%)** were found to be **MBL-negative**. These findings indicate that a **substantial proportion of carbapenem resistance** among Gram-negative isolates may be attributed to **MBL production**, highlighting the clinical importance of detecting MBL-producing strains for effective antimicrobial therapy and infection control.

Statistical analysis showed **no significant difference** between DDST and E-test methods ($p = 1.000$), indicating **identical detection rates** for MBL production. This suggests that **DDST is comparable to the E-test** for phenotypic detection of MBL-producing isolates. (Table 1)

Table 1: Comparison of screening and confirmatory tests in the phenotypic detection of MBL Production.						
Total no. of GNB isolates	CRE	MBL by the double disc synergy test		MBL by E-test		p-value =1.000
334	143	Positive	Negative	Positive	Negative	
		67(46.9%)	76(53.1%)	67(46.9%)	76(53.1%)	

The distribution of **carbapenemase types** among carbapenem-resistant isolates revealed that **New Delhi metallo- β -lactamase (NDM)** was the **predominant enzyme detected in *Escherichia coli*** isolates. In contrast, **OXA-48-type carbapenemase** was more frequently identified among ***Klebsiella pneumoniae* and *Pseudomonas aeruginosa*** isolates. Other carbapenemase types, including **Verona integron-encoded metallo- β -lactamase (VIM)** and **imipenemase (IMP)**, were detected **less frequently** among the isolates. These findings indicate a variable distribution of carbapenemase enzymes across different bacterial species, with **NDM and OXA-48 emerging as the major contributors to carbapenem resistance** in the present study.

Statistical analysis showed a **significant association** between bacterial species and carbapenemase production detected by the **CARBA NP test** ($\chi^2 = 20.80$, $df = 8$, $p = 0.0077$). (Table 2)

Table 2: Detection of carbapenemase-producing genes by the CARBA NP test				
CARBA NP test	<i>E. coli</i> (n=17)	<i>Klebsiella spp.</i> (n=13)	<i>Pseudomonas spp.</i> (n=7)	Chi-square =20.80 p-value < 0.01
NDM	9	4	0	
OXA-48	1	5	6	
VIM	4	1	0	
IPM	3	1	1	
Kpc(K)	0	2	0	

DISCUSSION

The emergence and rapid spread of carbapenem-resistant Gram-negative bacteria has become a major therapeutic and epidemiological challenge worldwide. In the present study, **143 (42.8%) of 334**

Gram-negative isolates were identified as carbapenem-resistant, indicating a considerable burden of resistance among clinical isolates. The prevalence observed in this study is comparable with findings reported by **Mohanty et al.**, who documented carbapenem resistance rates ranging from **35% to 45%** among Gram-negative isolates in tertiary care settings [7]. Likewise, **Sisay et al.** reported a substantial proportion of carbapenem-resistant organisms among hospitalized patients, highlighting the increasing difficulty in managing infections caused by multidrug-resistant bacteria in developing regions [8]. The statistically significant difference observed between carbapenem-resistant and sensitive isolates in the present study further emphasizes the need for routine resistance monitoring and antimicrobial stewardship programs.

In the current study, **67 (44.6%) of carbapenem-resistant isolates were found to produce metallo- β -lactamase (MBL)**, suggesting that MBL production plays a critical role in mediating carbapenem resistance. Similar proportions of MBL production have been reported in previous investigations. **Walsh et al.** highlighted the increasing emergence of MBL-producing Gram-negative organisms as a key factor contributing to multidrug resistance worldwide [3]. Additionally, **Nordmann et al.** observed that nearly **40–50% of carbapenem-resistant isolates** in hospital environments were associated with MBL production, which is consistent with the findings of the present study [1].

However, the identification of a considerable proportion of **MBL-negative carbapenem-resistant isolates** in the present study suggests the presence of alternative resistance mechanisms. These may include alterations in outer membrane permeability, increased activity of efflux pumps, and production of other classes of carbapenemases such as OXA or KPC enzymes. Similar mechanisms have been described by **Queenan and Bush**, who emphasized the diversity of carbapenem resistance pathways among Gram-negative organisms [2]. This highlights the importance of adopting multiple diagnostic approaches to accurately identify resistance patterns.

The evaluation of phenotypic detection methods in this study revealed **no statistically significant difference between the Double Disc Synergy Test (DDST) and the E-test method ($p = 1.000$)** for detecting MBL-producing isolates. These findings indicate that DDST may serve as a practical and economical alternative to more sophisticated testing methods, especially in laboratories with limited resources. Similar conclusions were drawn by **Yong et al.**, who reported comparable reliability between phenotypic screening techniques and confirmatory methods in identifying MBL-producing isolates [4]. The use of accessible and cost-effective detection techniques is particularly important in resource-constrained healthcare systems.

The distribution of carbapenemase types observed in this study revealed that **New Delhi metallo- β -lactamase (NDM)** was predominantly detected among *Escherichia coli*, while **OXA-48-type carbapenemase** was more frequently identified among *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*. This pattern is consistent with previously reported regional trends. **Nordmann et al.** and **Kumarasamy et al.** reported that NDM and OXA-48 enzymes are among the most encountered carbapenemases in Asian countries, particularly in India, where their dissemination has been widely documented [1,9]. The predominance of NDM-producing organisms in the Indian subcontinent has been recognized as a major contributor to the spread of carbapenem resistance and poses significant challenges for antimicrobial therapy.

Furthermore, the present study demonstrated a **significant association between bacterial species and carbapenemase production**, indicating variability in resistance patterns among different Gram-negative organisms. Similar observations were reported by **Pitout and Laupland**, who emphasized that carbapenemase distribution often varies depending on bacterial species and geographic location [6]. The detection of multiple carbapenemase types in the present study supports the concept that resistance mechanisms are becoming increasingly diverse, thereby complicating treatment decisions and infection control strategies.

Overall, the findings of this study indicate a substantial prevalence of carbapenem-resistant Gram-negative bacteria, with a significant proportion of isolates demonstrating MBL production and multiple carbapenemase types. These results agree with previously published studies conducted in tertiary care hospitals and reinforce the growing concern regarding antimicrobial resistance. The study underscores the urgent need for continuous surveillance, early detection of resistant isolates,

strict infection control practices, and judicious use of antibiotics to limit the spread of resistant pathogens within healthcare settings.

CONCLUSION

The present study revealed a **substantial prevalence of carbapenem-resistant Gram-negative bacteria**, indicating a growing challenge in the management of bacterial infections in healthcare settings. A significant proportion of carbapenem-resistant isolates were identified as **metallo- β -lactamase producers**, confirming the important role of MBL enzymes in mediating resistance to last-resort antibiotics. The predominance of **NDM and OXA-48 carbapenemases** among major Gram-negative pathogens further highlights the evolving complexity of antimicrobial resistance patterns.

The findings also demonstrated that the **Double Disc Synergy Test (DDST) showed comparable performance to the E-test**, suggesting that DDST can be used as a reliable and cost-effective screening method in routine diagnostic laboratories, particularly in resource-limited settings.

Overall, the increasing prevalence of carbapenem resistance observed in this study emphasizes the urgent need for **routine surveillance, early detection of resistant isolates, strict infection control practices, and rational antibiotic usage**. Implementation of effective antimicrobial stewardship programs is essential to prevent the further spread of multidrug-resistant organisms and to preserve the effectiveness of currently available antibiotics.

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