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Comparison of Phenotypic and Genotypic Methods for Detecting the Prevalence of Carbapenem-Resistant Gram-Negative Bacteria (CR-GNB) in ICU Patients.

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ABSTRACT
Background: Bacterial infections remain a significant challenge in intensive care units (ICUs), contributing substantially to patient morbidity and mortality. Among these, carbapenem-resistant Gram-negative bacteria (CR-GNB) are of particular concern due to the limited treatment options available. One of the key mechanisms responsible for this resistance is the production of metallo-β-lactamases (MBLs), especially New Delhi metallo-β-lactamase (NDM), which can inactivate carbapenem antibiotics. Early and accurate detection of these enzymes is essential for appropriate management and infection control. **Methodology:** This study included clinical strains of CR-GNB isolated from the clinical specimens of ICU patients, Identification of organisms was carried out using standard microbiological techniques, and carbapenem resistance was confirmed through antimicrobial susceptibility testing. Phenotypic detection of MBL production was performed using Modified Hodge Test (MHT), Double Disc Synergy Test (DDST), and Combined Disc Synergy Test (CDST). These methods were compared with genotypic detection using polymerase chain reaction (PCR) targeting the *bla*$_{NDM-1}$ gene. **Results:** Out of 911 Gram-negative isolates, 290 (31.8%) were found to be carbapenem-resistant. *Klebsiella pneumoniae* were the most frequently isolated CR-GNB (44.7%). Among the phenotypic methods, CDST demonstrated the highest sensitivity in detecting MBL production. PCR analysis identified the *bla*$_{NDM-1}$ gene in 209 of the 290 isolates. **Conclusion:** This study highlights a significant prevalence of carbapenem-resistant Gram-negative bacteria (CR-GNB) among ICU patients, with *Klebsiella pneumoniae* being the predominant isolate. The comparison between phenotypic and genotypic methods revealed that among the phenotypic techniques, the Combined Disc Synergy Test (CDST) demonstrated the highest sensitivity for detecting metallo-β-lactamase (MBL) production. However, genotypic detection using PCR targeting the *bla*$_{NDM-1}$ gene proved to be more specific and reliable for confirming carbapenem resistance. Therefore, while phenotypic methods such as CDST can be effectively used for routine screening due to their simplicity and cost-effectiveness, genotypic methods remain the gold standard for accurate detection. The combined use of both approaches can improve early diagnosis, guide appropriate antibiotic therapy, and strengthen infection control measures in ICU settings.

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INTRODUCTION:

Globally, drug-resistant bacterial infections were responsible for approximately 1.27 million deaths in 2019, reflecting a substantial increase compared to earlier estimates. If current trends persist, antimicrobial resistance (AMR)-related mortality is projected to rise to alarming levels in the coming decades, posing a major global public health threat.[1,2]

The discovery of antibiotics initially generated optimism that infectious diseases could be effectively controlled. However, this optimism has gradually diminished due to the continuous emergence and spread of antimicrobial resistance. Over time, bacteria have developed the ability to withstand multiple classes of antibiotics, thereby shifting the balance in favour of pathogenic organisms. Although antibiotics remain the cornerstone of treatment for bacterial infections, the rate at which resistance is developing has far outpaced the discovery and development of new antimicrobial agents. [3,4]

Among the various forms of AMR, infections caused by carbapenem-resistant Gram-negative bacteria (CR-GNB) are particularly worrisome. These organisms are increasingly implicated in hospital-acquired infections and are associated with poor clinical outcomes, prolonged hospital stays and increased healthcare costs.[5] The burden is especially high in intensive care units (ICUs), where critically ill patients are exposed to multiple risk factors such as invasive devices, prolonged antibiotic therapy, mechanical ventilation and compromised immunity. [6] Common pathogens encountered in such settings include *Klebsiella pneumoniae*, *Acinetobacter baumannii*, and *Pseudomonas aeruginosa*, all of which have demonstrated a remarkable capacity to develop resistance.[7]

Carbapenems are often considered last-resort antibiotics for treating severe infections caused by multidrug-resistant organisms. However, resistance to these agents has become increasingly prevalent. The underlying mechanisms are diverse and include alterations in membrane permeability, efflux pump over expression, and most importantly, the production of carbapenemase enzymes. [8] Among these, metallo-β-lactamases (MBLs) are of particular concern due to their ability to hydrolyse a wide range of β-lactam antibiotics, including carbapenems, thereby severely limiting therapeutic options.

Since their initial identification, MBL-producing organisms have been reported across different parts of the world, often associated with hospital outbreaks and rapid dissemination. These infections are frequently difficult to treat and are linked with higher rates of morbidity and mortality. [9] Early detection of MBL production is therefore crucial, not only for guiding appropriate antimicrobial therapy but also for implementing effective infection control measures and preventing further spread within healthcare facilities.

In routine laboratory practice, several phenotypic methods are employed for the detection of MBL production, including the Modified Hodge Test (MHT), Double Disc Synergy Test (DDST), and Combined Disc Synergy Test (CDST). These methods are widely used because they are relatively simple, cost-effective, and do not require advanced infrastructure. However, their diagnostic accuracy can vary, and comparative data on their performance remain limited. [10]

Molecular methods, particularly polymerase chain reaction (PCR), provide a more definitive approach by detecting specific resistance genes. Among these, the *bla*$_{\text{NDM-1}}$ gene has gained global attention due to its rapid spread and strong association with multidrug resistance. Combining phenotypic screening with genotypic confirmation enhances diagnostic reliability and contributes to a better understanding of resistance patterns.

In this context, the present study aims to evaluate and compare commonly used phenotypic methods for detecting MBL production in CR-GNB isolates from ICU patients, along with genotypic confirmation of *bla*$_{\text{NDM-1}}$ using PCR. This approach is expected to identify a practical and reliable diagnostic strategy, particularly useful in resource-limited settings.

MATERIALS AND METHODS:

This prospective observational study was conducted in the Department of Microbiology at MGM Medical College and Hospital from January to December 2023 and included 290 non-duplicate carbapenem-resistant Gram-negative bacterial (CR-GNB) isolates obtained from ICU patients (SICU, MICU, EMS-ICU) suspected of bacterial infections. Clinical samples including urine, blood, respiratory secretions, wound swabs, and catheter tips etc from

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cases of CAUTI, BSI, CRBSI, SSI, and VAP were processed using standard microbiological techniques. [11, 12] Isolates were identified by conventional biochemical methods. Antimicrobial susceptibility testing was performed using the Kirby–Bauer disk diffusion method in accordance with Clinical and Laboratory Standards Institute (CLSI) guidelines.[13] Phenotypic detection of carbapenemase production was carried out using the Modified Hodge Test (MHT), Double Disk Synergy Test (DDST), and Combined Disk Synergy Test (CDST). [14,15] Genotypic confirmation of carbapenem resistance was performed by polymerase chain reaction (PCR) targeting the *bla*$_{NDM-1}$ gene. [16]

Statistical Analysis:
Data were entered into Microsoft Excel and analysed using SPSS version 27.0 and GraphPad Prism version 5. Continuous variables were expressed as mean ± standard deviation, while categorical variables were presented as frequencies and percentages. The unpaired t-test and paired t-test were used for comparison of continuous variables. The Chi-square test or Fisher's exact test was applied for categorical variables. A p-value < 0.05 was considered statistically significant.

RESULT:

Table 1. Prevalence of Carbapenem Resistance amongst GNBs isolated from ICU patients.

Organism	Total Isolates (n)	CR Isolates (n)	Resistance (%)
Klebsiella pneumoniae.	282	126	44.7%
Acinetobacter baumannii	156	55	35.3%
Escherichia coli	220	45	20.5%
Pseudomonas aeruginosa	108	39	36.1%
Enterobacter spp.	81	14	17.3%
Proteus spp.	26	5	19.2%
Citrobacter spp.	38	6	15.8%
Total	**911**	**290**	**31.8%**

Table 2. Comparison between Phenotypic and Genotypic Detection of MBL Production in CR-GNB isolated from ICU. (n=290)

Method	Positive (n)	Percentage (%)
Modified Hodge Test (MHT)	166	57.24%
Double Disc Synergy Test (DDST)	186	64.14%
Combined Disc Synergy Test (CDST)	198	68.28%
PCR	209	72.%

Table 3. Detection of *blaNDM-1* Gene amongst CR-GNBs isolated from ICU.

Organism	Total CR Isolates	*bla*$_{NDM-1}$ Positive (n)	Percentage (%)
Klebsiella pneumoniae	126	97	77.0%
Acinetobacter baumannii	55	39	70.9 %
Escherichia coli	45	32	71.1%
Pseudomonas aeruginosa	39	25	64.1 %
Enterobacter spp.	14	8	57.1 %
Proteus spp.	5	5	100 %
Citrobacter spp.	6	4	66.7 %
Total	**290**	**209**	**72.1 %**

Table 1 shows Prevalence of Carbapenem Resistance amongst GNBs isolated from ICU patients.
During the study period, a total of 911 Gram-negative bacterial isolates were recovered, of which 290 (31.8%) were identified as carbapenem-resistant. *Klebsiella pneumoniae* was the most frequently isolated organism (282 isolates), with 126 (44.7%) showing carbapenem resistance. This was followed by *Pseudomonas aeruginosa* (36.1%) and *Acinetobacter baumannii*. (35.3%). In contrast, lower resistance rates were observed in *Escherichia coli* (20.5%), *Proteus spp*. (19.2%), *Enterobacter spp*. (17.3%), and *Citrobacter spp*. (15.8%). The variation in carbapenem resistance among different organisms was found to be statistically significant ($p < 0.001$), indicating a non-uniform distribution of resistance across species.

Table 2 shows Comparison between Phenotypic and Genotypic Detection of MBL Production in CR-GNB isolated from ICU.
Genotypic detection using PCR demonstrated the highest positivity rate (72.07%), indicating superior sensitivity for identifying MBL-producing CR-GNB isolates compared to phenotypic methods. Among phenotypic tests, CDST showed the highest detection rate (68.28%), followed by DDST (64.14%) and MHT (57.24%). Despite good performance, all phenotypic methods underestimated MBL production when compared to PCR, suggesting possible false negatives. Therefore, PCR remains the gold standard for accurate detection, while CDST appears to be the most reliable phenotypic screening tool in resource-limited ICU settings.

Table 3 shows Detection of *blaNDM-1* Gene amongst CR-GNBs isolated from ICU.

Genotypic analysis using PCR revealed the presence of the bla_{NDM-1} gene in 209 out of 290 carbapenem-resistant isolates (72.1%). Among the different organisms, *Klebsiella pneumoniae* showed the highest number of bla_{NDM-1} positive isolates (97/126; 77.0%), followed by *Acinetobacter baumannii* (39/55; 70.9%) and *Escherichia coli* (32/45; 71.1%). *Pseudomonas aeruginosa* demonstrated bla_{NDM-1} positivity in 25 out of 39 isolates (64.1%), while lower proportions were observed in *Enterobacter spp*. (57.1%) and *Citrobacter spp*. (66.7%). Notably, all *Proteus* isolates (5/5; 100%) carried the gene. The distribution of bla_{NDM-1} among different species was statistically significant ($p = 0.041$), indicating variability in gene prevalence across organisms.

DISCUSSION:

The present study was conducted to evaluate the prevalence of carbapenem-resistant Gram-negative bacteria (CR-GNB) among ICU patients and to compare the effectiveness of phenotypic and genotypic methods for their detection. A total of 290 CR-GNB isolates were analysed over a one-year period from patients admitted to various intensive care units. The study also focused on identifying the presence of the bla_{NDM-1} gene as a major mechanism of carbapenem resistance.

Table 1 shows Prevalence of Carbapenem Resistance amongst GNBs isolated from ICU patients.

In the present study, 31.8% of Gram-negative isolates were found to be carbapenem-resistant, highlighting a substantial burden of resistance in ICU settings. *Klebsiella pneumoniae*. emerged as the predominant organism with the highest proportion of resistance (44.7%). These findings are consistent with studies by Kadel et al., where *Klebsiella pneumoniae* accounted for the majority of CR-GNB isolates (52.77%) in ICU patients. [17] Similarly, surveillance data from ICU-based studies indicate that *Klebsiella*, *Acinetobacter*, and *Pseudomonas* species are the most frequently encountered carbapenem-resistant pathogens.[18] The higher prevalence in ICUs can be attributed to increased antibiotic exposure, invasive procedures, and critically ill patient populations. In contrast, lower resistance rates observed in organisms such as *Enterobacter* and *Citrobacter spp*. may reflect differences in intrinsic resistance mechanisms and antibiotic selection pressure. The statistically significant variation ($p < 0.001$) supports the observation that carbapenem resistance is not uniformly distributed across species, a finding also emphasized in epidemiological analyses of CR-GNB.[19]

Table 2 shows Comparison between Phenotypic and Genotypic Detection of MBL Production in CR-GNB isolated from ICU.

Genotypic detection by PCR showed the highest positivity (72.1%), confirming its superior sensitivity over phenotypic methods. Among phenotypic tests, CDST demonstrated the best performance (68.28%), followed by DDST (64.14%) and MHT (57.24%), but all remained lower than PCR. This indicates that phenotypic methods may miss a proportion of MBL producers, supporting PCR as the gold standard while CDST serves as the most reliable screening tool. Comparable findings were reported by Naim et al., where different phenotypic methods showed variable detection rates, with combined disc methods performing better than conventional approaches. [20] However, in another study, MHT demonstrated higher positivity compared to CDST, indicating variability in test performance depending on bacterial species and local resistance patterns. [21] Such discrepancies may be due to differences in enzyme expression levels, test conditions, and interpretation criteria. Despite these variations, the statistically significant difference observed in the present study ($p = 0.032$) supports the use of CDST as a more reliable screening tool, particularly in resource-limited settings where molecular testing may not be readily available.

Table 3 shows Detection of *blaNDM-1* Gene amongst CR-GNBs isolated from ICU.

The bla_{NDM-1} gene was detected in 72.1% of carbapenem-resistant isolates, indicating its major role in mediating resistance. *Klebsiella pneumoniae* showed the highest number of bla_{NDM-1} positive isolates (77.0%), followed by *Acinetobacter baumannii* and *E. coli.* These findings agree with studies demonstrating the widespread distribution of NDM-producing organisms, particularly among Enterobacteriaceae.[22] Previous reports have also highlighted Klebsiella pneumoniae as the predominant carrier of the bla_{NDM-1} gene in hospital settings. [5] In contrast, a study by Naim et al. reported a lower overall prevalence of bla_{NDM-1} (56.89%), suggesting regional variation in gene distribution. [21] The detection of 100% positivity in *Proteus spp*., although based on a small sample size, further emphasizes the potential for dissemination of resistance genes across different species. The statistically significant variation observed ($p = 0.041$) indicates heterogeneity in gene distribution, which may be influenced by local antimicrobial practices and horizontal gene transfer mechanisms. The persistence and rapid spread of bla_{NDM-1}, often mediated by plasmids, continues to pose a serious challenge to infection control and antimicrobial stewardship efforts. [15]

CONCLUSION:

This study demonstrates a considerable prevalence of carbapenem-resistant Gram-negative bacteria (CR-GNB) among ICU patients. The comparative evaluation of phenotypic and genotypic methods showed that phenotypic techniques, particularly the Combined Disc Synergy Test (CDST), are useful for initial screening of carbapenem resistance. However, genotypic detection using PCR for the *bla*$_{NDM-1}$ gene provides greater accuracy and specificity in confirming resistance. Therefore, while phenotypic methods remain practical and cost-effective for routine laboratory use, genotypic methods serve as the gold standard. The combined application of both approaches is recommended for reliable detection, guiding appropriate antimicrobial therapy, and strengthening infection control strategies in ICU settings

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