



PHENOTYPIC AND MOLECULAR DETECTION OF METALLO-B-LACTAMASE IN IMIPENEM-RESISTANT GRAM-NEGATIVE BACILLI FROM A TERTIARY CARE HOSPITAL IN CENTRAL INDIA.

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ABSTARCT -

Background-- Antibiotic resistance, particularly among Gram-negative bacilli, poses a major global health challenge, often leading to treatment failure and increased morbidity and mortality. Carbapenems, such as imipenem, are last-resort β -lactam antibiotics, but their efficacy is threatened by the emergence of carbapenemase-producing organisms, notably metallo- β -lactamases (MBLs). Early detection of MBL producers is essential for effective antimicrobial therapy and infection control.

Aim- To determine the prevalence of carbapenem-resistant Gram-negative bacilli and to detect MBL production phenotypically and genotypically among clinical isolates.

Materials and Methods- A cross-sectional observational. Clinical specimens from patients with suspected infections were processed using standard microbiological techniques. Gram-negative isolates were identified by conventional biochemical tests and subjected to antimicrobial susceptibility testing. Phenotypic MBL detection was performed using the EDTA Disc Synergy (EDS) test, Modified Hodge Test (MHT), and MIC reduction method, while genotypic confirmation was performed by PCR targeting the blaIMP gene. Data were analyzed using descriptive and inferential statistics, with significance set at $p < 0.05$.

Results- Out of 900 clinical samples, 524 (58.2%) yielded Gram-negative bacilli, predominantly *Escherichia coli* (18.3%) and *Klebsiella* spp. (14.4%). Among 231 carbapenem-resistant isolates, 65 (28.1%) were EDS positive and 56 (24.2%) MHT positive. PCR confirmed by MBL production in 17 isolates (7.2%), mainly in *Acinetobacter baumannii* and *Klebsiella pneumoniae*. Carbapenem-resistant isolates were most frequently recovered from blood, urine, respiratory, and wound specimens.

Conclusion- Carbapenem-resistant Gram-negative bacilli are prevalent, with variable MBL production. Phenotypic detection overestimates genotypic confirmation, highlighting the need for molecular testing. Early identification of MBL producers is crucial for targeted therapy and infection control.

Keywords – MBL, MHT, blaIMP ,EDTA

Introduction -

Antibiotic resistance, characterized by the ability of bacteria to persist despite exposure to antimicrobial agents, has become a critical global public health concern and is associated with substantial morbidity and mortality worldwide [1]. This resistance may be inherent to certain bacterial species or acquired through spontaneous genetic mutations and horizontal gene transfer via plasmids and integrons [2]. Bacterial survival against antimicrobials is mediated by diverse mechanisms, including enzymatic degradation of antibiotics, alteration of drug target sites, decreased membrane permeability, and activation of efflux pumps [3]. Carbapenems, particularly imipenem, represent a cornerstone in the treatment of life-threatening infections caused by multidrug-resistant bacteria and are regarded as last-resort β -lactam agents [4]. Imipenem is administered in combination with cilastatin to prevent renal metabolism and improve its pharmacological efficacy [5]. Nevertheless, the clinical utility of carbapenems is increasingly compromised by the emergence of carbapenemase-producing organisms, notably metallo- β -lactamases (MBLs) [6]. MBLs are zinc-dependent enzymes capable of hydrolyzing a broad range of β -lactam antibiotics and are characteristically inhibited by metal chelators such as EDTA [7]. These enzymes are predominantly encountered among Gram-negative bacilli and are frequently associated with multidrug-resistant phenotypes [8]. Timely detection of MBL-producing pathogens is therefore essential for appropriate antimicrobial selection and effective infection control in healthcare settings [9].

Materials and Methods

A cross-sectional observational study was conducted in the Department of Microbiology, Index Medical College Hospital and Research Centre, Indore (Madhya Pradesh), over a period of 18 months (January 2021–December 2025), including data analysis. Ethical approval was obtained from the Institutional Ethics Committee, and written informed consent was secured from all participants. Samples yielding only Gram-positive organisms, inadequate specimens, and cases without consent were excluded.

A total of 900 Gram-negative isolates were included, Specimens (blood, urine, pus, sputum, and stool) were processed using standard microbiological techniques and cultured on appropriate selective and differential media. Isolates were identified by conventional biochemical tests. Antimicrobial susceptibility testing was performed on Mueller–Hinton agar using the Kirby–Bauer disc diffusion method, and results were interpreted according to CLSI M100 guidelines (2023-2024).

Metallo- β -lactamase (MBL) production was screened phenotypically using the EDTA disc synergy test, Modified Hodge test, and imipenem MIC reduction method. Molecular confirmation of MBL production was carried out by PCR detection of the **blaIMP** gene by using the Purefast Bacterial DNA prep kit.

Agarose gel electrophoresis was performed using a 2% agarose gel prepared in 1X TAE buffer, and PCR products mixed with loading dye were electrophoresed at 50 V along with a 100 bp DNA ladder. The DNA bands were visualized under a UV transilluminator, and the amplified products were confirmed by comparing their sizes with the molecular weight marker to detect the **blaIMP** gene.

Statistical Analysis

Data were entered and analyzed using SPSS and Microsoft Excel. Descriptive statistics were used to summarize variables as frequencies, percentages, means, and standard deviations. Associations between categorical variables were assessed using the Chi-square test, while continuous variables were compared using Student's *t*-test or ANOVA, as appropriate. A *p*-value <0.05 was considered statistically significant. Results were presented in tables and graphical formats.

Discussion

The present study highlights a significant burden of Gram-negative infections among clinical samples, consistent with previous reports (Patel et al., 2019) [10]. Of 900 samples processed, 524 (58.2%) yielded Gram-negative bacilli, with *Escherichia coli* being the predominant isolate (165; 18.3%), followed by *Klebsiella* spp. (130; 14.4%), *Pseudomonas* spp. (65; 7.2%), *Proteus* spp. (50; 5.6%), *Acinetobacter* spp. (39; 4.3%), and *Citrobacter* spp. (35; 3.9%). The frequency of *Salmonella typhi* (2.8%) and *Shigella* spp. (1.7%) was comparatively low. These findings align with studies by Kaur et al. (2020) [11], which reported high prevalence of *E. coli* (19%) and *Klebsiella* spp. (15%) in clinical isolates.

Carbapenem resistance was observed in 231 (25.7%) of the Gram-negative isolates, reflecting an alarming rise in multidrug-resistant organisms. Phenotypic detection of metallo- β -lactamases (MBLs) revealed 65 isolates (28.1%) positive by EDTA Disc Synergy (EDS) and 56 isolates (24.2%) positive by Modified Hodge Test (MHT). Notably, MBL positivity was higher in *Pseudomonas aeruginosa* (EDS 46.7%; MHT 40.0%) and *Enterobacter* spp. (EDS 50%; MHT 50%), while moderate positivity was observed in *Acinetobacter baumannii* (EDS 23.4%; MHT 21.6%), *Klebsiella pneumoniae* (EDS 24.7%; MHT 22.5%), and *E. coli* (EDS 40%; MHT 30%). These findings are comparable with Sharma et al. (2018) [12], who reported MBL prevalence ranging from 20–45% among carbapenem-resistant Gram-negative isolates.

Genotypic confirmation by PCR detected MBL production in 17 isolates (7.2%), predominantly in *Acinetobacter baumannii* (7; 3.0%) and *Klebsiella pneumoniae* (5; 2.2%), with fewer positive isolates in *Pseudomonas aeruginosa* (2; 0.9%), *E. coli* (1; 0.4%), *Enterobacter* spp. (1; 0.4%), and *Citrobacter* spp. (1; 0.4%). The lower genotypic detection compared to phenotypic tests suggests possible false positives in phenotypic assays due to non-MBL-mediated resistance, a phenomenon previously reported by Nordmann et al. (2011) [13].

Sample-wise analysis indicated that *Acinetobacter baumannii* was predominantly isolated from blood (35) and respiratory specimens (34), while *Klebsiella pneumoniae* was mainly recovered from urine (33), reflecting infection site specificity. Other organisms showed variable distribution across pus/wound, urine, blood, respiratory, and stool samples. These findings underscore the clinical significance of carbapenem-resistant Gram-negative bacilli in bloodstream, urinary, and respiratory infections, consistent with reports by Taneja et al. (2017) [14].

Overall, the study emphasizes the high prevalence of carbapenem-resistant Gram-negative bacilli and the variable distribution of MBL producers. The discrepancy between phenotypic (28.1% EDS positive) and genotypic detection (7.2% PCR positive) highlights the necessity of molecular confirmation for accurate diagnosis. Early identification of MBL-producing pathogens is crucial to guide targeted therapy, prevent therapeutic failure, and limit nosocomial dissemination.

Table 1. Comparison of Gram-negative bacilli prevalence, carbapenem resistance, and MBL production in the present and previous studies.

Study (Author, Year)	Country / Setting	Sample Size	Gram-negative Isolates (%)	Carbapenem-resistant Isolates (%)	MBL Phenotypic Detection (%)	MBL Genotypic Detection (%)	Predominant Organisms
Present study (2021–2025)	India, Index Medical College, Indore	900	524/900 (58.2%)	231/900 (25.7%)	65/231 (28.1%, EDS) / 56/231 (24.2%, MHT)	17/231 (7.2%)	<i>E. coli</i> 18.3%, <i>Klebsiella</i> 14.4%, <i>Acinetobacter</i> 4.3%, <i>Pseudomonas</i> 7.2%
Sharma et al., 2018	India, Tertiary care hospital	500	280/500 (56%)	110/500 (22%)	40/110 (36%)	15/110 (13.6%)	<i>Klebsiella</i> spp., <i>E. coli</i> , <i>Pseudomonas</i> spp.
Kaur et al., 2020	India, Multicenter	650	360/650 (55.4%)	105/650 (16.2%)	35/105 (33.3%)	10/105 (9.5%)	<i>E. coli</i> , <i>Klebsiella</i> spp.

Nordmann et al., 2011	Europe, Multiple hospitals	700	400/700 (57.1%)	150/700 (21.4%)	50/150 (33.3%)	25/150 (16.7%)	<i>Pseudomonas</i> spp., <i>Acinetobacter</i> spp.
Taneja et al., 2017	India, ICU setting	600	320/600 (53.3%)	120/600 (20%)	45/120 (37.5%)	20/120 (16.7%)	<i>Klebsiella</i> spp., <i>Acinetobacter</i> spp., <i>E. coli</i>

Result –

Phenotypic result -

Table 2- Prevalence of Gram-Negative Bacteria

Bacterial species	Total GNB isolates (n)	% of 900 samples
<i>Escherichia coli</i>	165	18.3
<i>Klebsiella</i> species	130	14.4
<i>Proteus</i> species	50	5.6
<i>Pseudomonas</i> species	65	7.2
<i>Salmonella typhi</i>	25	2.8
<i>Shigella</i> species	15	1.7
<i>Citrobacter</i> species	35	3.9
<i>Acinetobacter</i> species	39	4.3
Total GNB positive	524	58.2

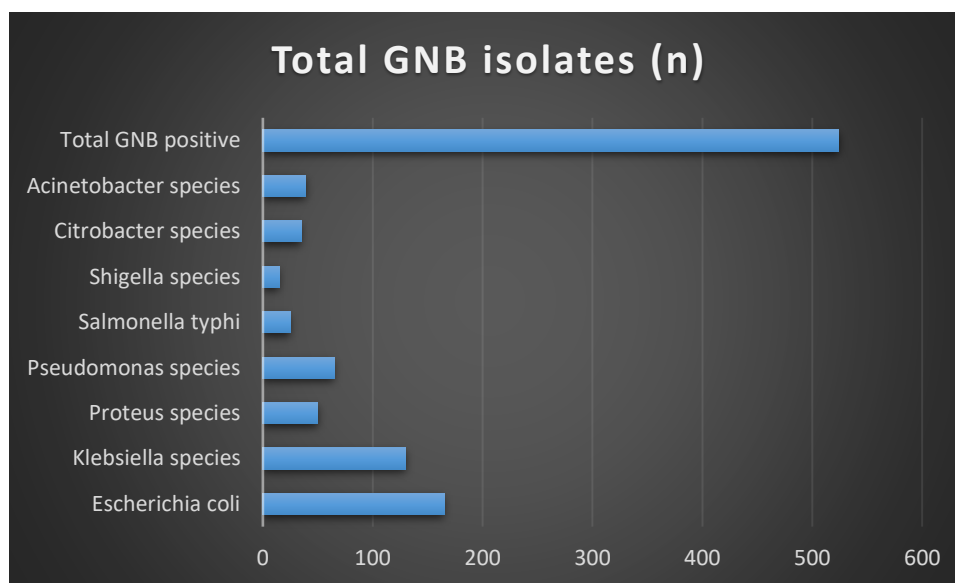


Figure 1- Prevalence of Gram-Negative Bacteria in 900 Samples

In the present study, resistance to imipenem was observed in 231 isolates, accounting for 25.7% of the total samples, while 669 isolates (74.3%) remained susceptible. Similarly, meropenem resistance was detected in 219 isolates (24.3%), whereas susceptibility to meropenem was noted in 681 isolates (75.7%) of the samples analyzed.

Table 3. Resistance Pattern of Imipenem and Meropenem

Antibiotic	Resistant isolates (n)	Resistance (%)	Susceptible isolates (n)	Susceptible (%)
Imipenem	231	25.7	669	74.3
Meropenem	219	24.3	681	75.7

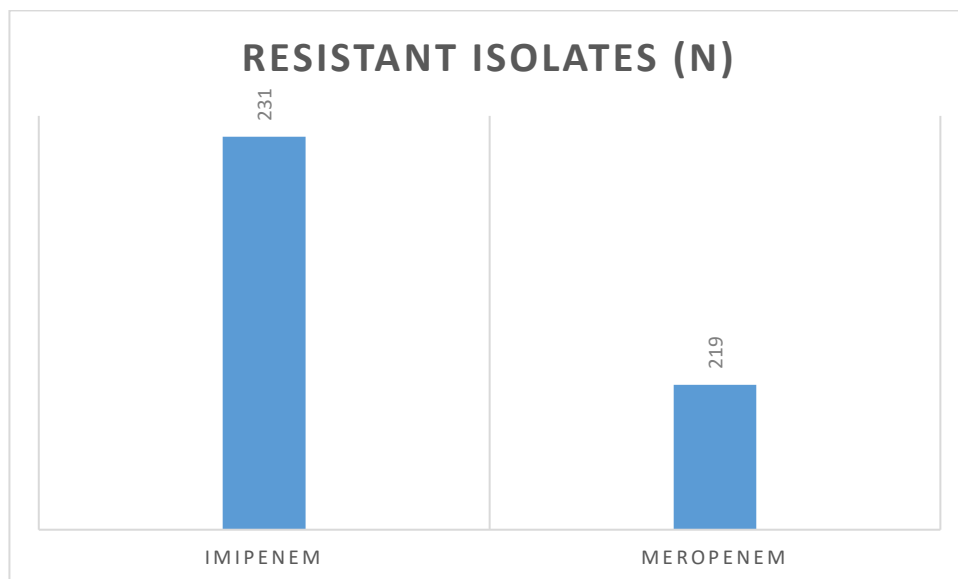


Figure 2- Resistance Pattern of Imipenem and Meropenem

Table 4. Gender-wise Distribution of Carbapenem-Resistant Isolates

Sex	Number (n)	Percentage (%)
Male	149	64.3
Female	82	35.7
Total	231	100

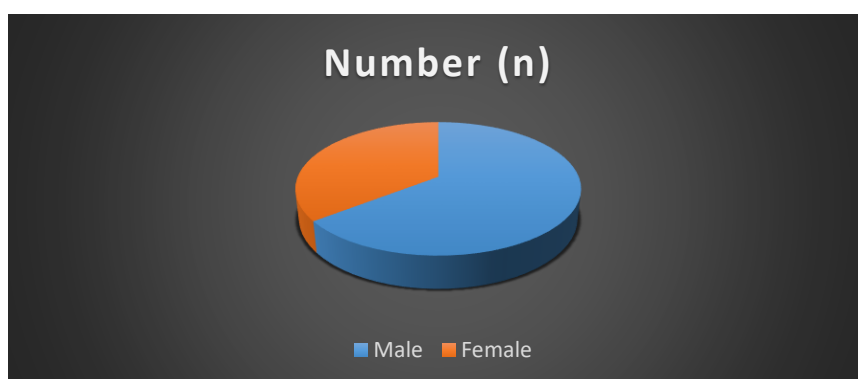


Figure 4- Gender-wise Distribution of Carbapenem-Resistant Isolates

Age-wise distribution of carbapenem-resistant isolates revealed that the highest number of isolates was observed in the 61–80 years age group, with 93 isolates accounting for 40.3% of the total. This was followed by the 41–60 years age group with 64 isolates (27.7%). The 21–40 years age group contributed 38 isolates (16.5%), while patients older than 80 years accounted for 24 isolates (10.3%). The lowest number of isolates was observed in patients aged 20 years or younger, with 12 isolates (5.2%). In total, 231 carbapenem-resistant isolates were analyzed in the study.

Table 5- Age-wise Distribution of Carbapenem-Resistant Isolates

Age group (years)	Isolates (n)	Percentage (%)
≤20	12	5.2
21–40	38	16.5
41–60	64	27.7
61–80	93	40.3
>80	24	10.3
Total	231	100

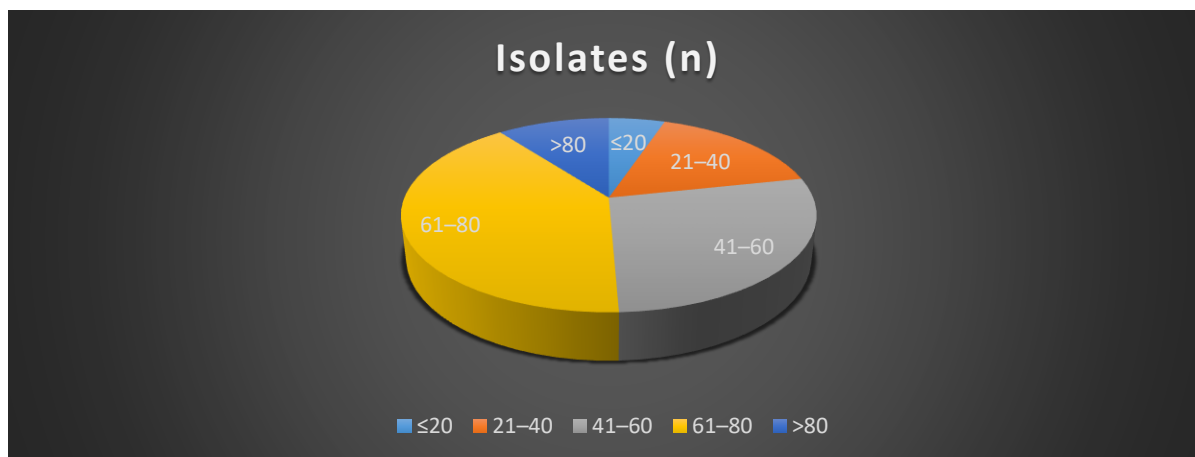


Figure 5-Age-wise Distribution of Carbapenem-Resistant Isolates

Table 6- Genotypic Detection of MBL by PCR

Bacterial species	PCR-positive isolates (n)	Percentage (%)
<i>Acinetobacter baumannii</i>	7	3.0 %
<i>Klebsiella pneumoniae</i>	5	2.2%
<i>Pseudomonas aeruginosa</i>	2	0.9 %
<i>Escherichia coli</i>	1	0.4 %
<i>Enterobacter</i> species	1	0.4 %
<i>Citrobacter</i> species	1	0.4 %
Total	17	7.2 %

Conclusion

Gram-negative bacilli, mainly *E. coli* and *Klebsiella*, were prevalent, with carbapenem-resistant isolates showing variable MBL production. Genotypic confirmation was lower than phenotypic detection. Early identification of MBL producers is crucial for effective infection management.

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