Original Article

Occurrence of Carbapenem Resistant Gram Negative Bacteria by Using Modified Carbapenem Inactivation Method

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Abstract

Background: The rapid emergence of carbapenem-resistant Gram-negative bacteria has become a major concern in healthcare institutions worldwide. Carbapenems are considered the drugs of choice for severe infections caused by multidrug-resistant organisms however, increasing resistance has significantly compromised their effectiveness. The aim is to determine the occurrence of carbapenemase-producing Gram-negative bacteria among clinical isolates and characterize carbapenemase types using phenotypic methods. **Material and Methods:** An observational study was conducted in the Department of Microbiology, Teerthankar Mahaveer hospital. A total of 209 Gram-negative bacterial isolates recovered from various clinical specimens were included. Carbapenemase production was detected by Carbapenem inactivation methods (CIM) including modified CIM (mCIM) and EDTA CIM (eCIM) to identify metallo-β-lactamases (MBLs) and serine carbapenemases as per Clinical laboratory standards institute (CLSI) standards, M100 2025. **Results:** Among 209 isolates, 66 (31.6%) were confirmed as carbapenemase producers. Male patients constituted 56.06% of positive cases, while females represented 43.94%. The highest frequency was observed in the 61-80 year age group. Intensive care units (ICUs) accounted for majority of carbapenemase-producing isolates particularly the Medical Intensive Care Unit (MICU). Escherichia coli was the most common organism (43.9%) followed by Klebsiella pneumoniae (31.8%) and Pseudomonas aeruginosa (24.2%). Of the 66 carbapenemase producers 42 (63.6%) were Metallo-beta-lactamase (MBL) producers and 24 (36.4%) were serine carbapenemase producers. **Conclusion:** A considerable occurrence of carbapenemase-producing Gram-negative bacteria was identified in hospitalized patients, particularly in critical care settings. Phenotypic methods such as mCIM and eCIM provide reliable and affordable tools for routine detection and classification of carbapenemases, facilitating timely infection control interventions.

Keywords: Carbapenem resistance, Carbapenemase, mCIM, eCIM and antimicrobial resistance.

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Introduction

Antimicrobial resistance has emerged as one of the greatest threats to global public health. The increasing occurrence of multidrug-resistant bacterial pathogens has complicated the management of infectious diseases and has resulted in prolonged hospitalization increased healthcare costs and higher mortality rates.[1,2] Among resistant organisms, Gram-negative bacteria have gained particular attention because of their ability to acquire and disseminate multiple resistance determinants.[3,4]

Carbapenems are broad-spectrum β-lactam antibiotics widely used for the treatment of infections caused by multidrug-resistant Gram-negative organisms. These agents are considered the last therapeutic option for severe infections caused by extended-spectrum β-lactamase-producing bacteria.[5]

Carbapenem resistance occurs through several mechanisms, including porin loss, efflux pump overexpression, alterations in penicillin-binding proteins and production of carbapenemases.[6,7] Among these mechanisms, carbapenemase production is the most clinically significant because carbapenemase genes are often plasmid-mediated and capable of rapid horizontal transfer among bacterial species.[4,6]

Carbapenemases are classified into Ambler Classes A, B, C and D. Class A enzymes include KPC. Class B enzymes comprise metallo-β-lactamases (MBLs) such as NDM, IMP and VIM. Class D carbapenemases include OXA-type enzymes.[4,5]

The increasing occurrence of carbapenemase-producing organisms has become a serious challenge in healthcare settings. Early detection is essential for appropriate antimicrobial therapy, surveillance and infection control. Molecular methods provide accurate detection but are expensive and not universally available. Therefore, phenotypic methods such as mCIM and eCIM offer cost-effective alternatives for routine diagnostic laboratories.[8,9]

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Materials and Methods

A hospital-based observational study was conducted in the Department of Microbiology, Teerthankar Mahaveer Hospital, Moradabad, Uttar Pradesh after approval by Institutional Ethical Committee TMU Moradabad with Ref no. (TMU/IEC/2024-25/PG/006).

Various clinical specimens including urine, blood, pus, sputum, endotracheal aspirates and swabs were processed according to standard microbiological procedures. Specimens were inoculated onto Blood agar and MacConkey agar and incubated at 37°C for 18-24 hours. Antimicrobial susceptibility testing was performed using the Kirby-Bauer disk diffusion method on Mueller-Hinton agar according to CLSI recommendations, M100 2025.[10]

Modified carbapenem inactivation method procedure: As per CLSI guidelines, a loopful of bacterial culture was suspended in tryptic soy broth and incubated with a meropenem disk for four hours. The disk was then placed on Mueller-Hinton agar inoculated with carbapenem-susceptible E. coli ATCC 25922. Following incubation, inhibition zones were measured and interpreted according to CLSI criteria. For eCIM, EDTA was added to the bacterial suspension before incubation with the meropenem disk. An increase in the inhibition zone diameter compared with mCIM indicated metallo-β-lactamase production, while the absence of a significant change suggested serine carbapenemase activity.[8,10]

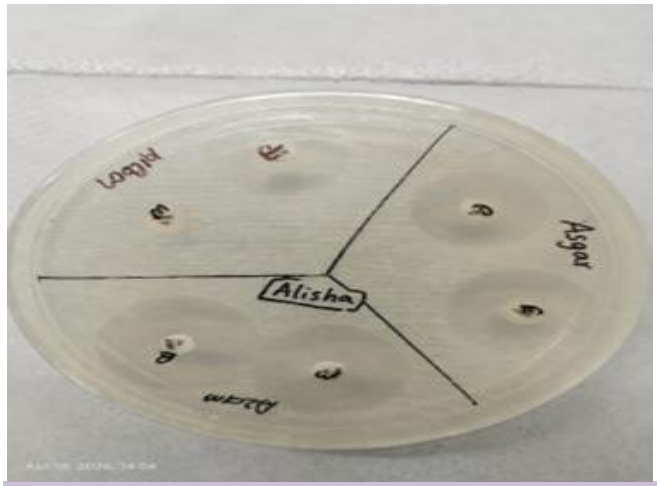

Image 1: Modified carbapenemase inactivation showing carbapenemase producer isolate

Statistical Analysis

Data were entered into Microsoft Excel and analyzed using descriptive statistical methods. Results were expressed as frequencies, percentages and proportions.

Results

Out of 209 clinical samples were processed during this study. Carbapenemase production was detected in 66 isolates using the Carbapenem Inactivation Method (CIM). Consequently, the occurrence of carbapenemase-producing organisms within the study population was calculated to be 31.37%.

Figure 1: Carbapenem resistant organism (CRO) status

Among the 66 carbapenem resistant organism positive cases, 37(56.06%) were identified in males, while 29(43.94%) were found in females. This indicates a higher occurrence of carbapenemase production in males compared females.

The distribution of CRO positive cases varied across different age groups.

Among the 66 cases the maximum number was observed in the 61-80 year age, with 21 cases, followed by the 41-60 years group, which had 19 cases. The 21-40 year and 0-20 years groups had 16 and 9 cases respectively while the lowest was seen in individuals over 80 years with only 1 case. This trend suggests carbapenem production is more common in older age groups, particularly between 41-80 years.

Escherichia coli was the predominant carbapenemase-producing organism (29 isolates), followed by Klebsiella pneumoniae (21 isolates) and Pseudomonas aeruginosa (16 isolates).

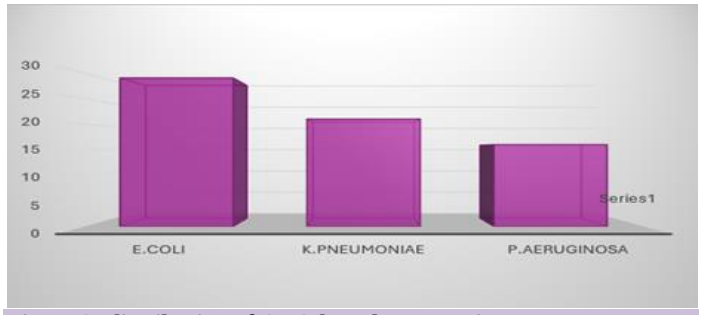

Figure 2: distribution of CRO based on organisms

Metallo-β-Lactamase Producers

Among 42 MBL-producing isolates:

Figure: 3 Distribution of MBL Producing isolates

Among 24 serine carbapenemase-producing isolates:

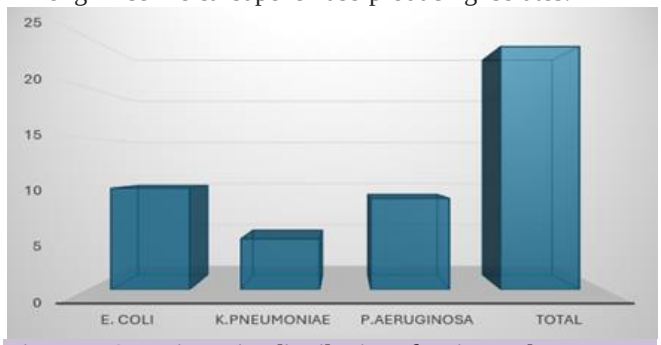

Figure 4: Organism wise distribution of serine carbapenemase producers

Discussion

Carbapenem-resistant Gram-negative bacteria have emerged as a major public health concern worldwide because they are associated with limited therapeutic options, prolonged hospitalization, increased healthcare expenditure and higher mortality rates.[1] The increasing occurrence of carbapenemase-producing organisms in healthcare settings poses a significant challenge to clinicians and microbiologists.[2] In the present study carbapenemase production was detected among clinical Gram-negative isolates using the Modified Carbapenem Inactivation Method (mCIM) and EDTA-Carbapenem Inactivation Method (eCIM) which are CLSI-recommended phenotypic methods for the detection and differentiation of carbapenemases.[6]

The overall occurrence of carbapenemase-producing organisms in the present study was 31.6% (66/209). This occurrence is comparable to the findings reported by Mohanty et al., who documented carbapenem resistance in 33.1% Gram-negative isolates and Gupta et al., who reported a prevalence of 28.4%.[11,12] However, higher prevalence rates of 41.2% and 45% have been reported by Wattal et al. and Datta et al., respectively.[13,14]

Among the carbapenemase-producing isolates, males accounted for 56.06%, whereas females represented 43.94%. Similar findings were reported by Veeraraghavan et al., who observed male predominance (58%) among carbapenem-resistant infections.[15]

Age-wise analysis revealed that the highest number of carbapenemase-producing isolates occurred in the 61-80 years age group (31.8%), followed by the 41-60 years age group (28.8%). Similar observations have been documented by Gupta et al. and Falagas et al., who demonstrated increased carbapenem resistance among elderly patients.[12,16]

Majority of carbapenemase-producing isolates were recovered from intensive care units. In the present study, the MICU accounted for 24.2% of positive cases, followed by the SICU (21.2%), RICU (18.2%) and HDU (18.2%). Similar findings were reported by Nordmann et al. and Meletis, who observed that intensive care units represent the principal reservoirs for carbapenem-resistant organisms.[4,6]

With respect to bacterial species distribution, Escherichia coli was the predominant carbapenemase-producing organism (43.9%), followed by Klebsiella pneumoniae (31.8%) and Pseudomonas aeruginosa (24.2%). Similar findings were reported by Gupta et al., who found E. coli to account for approximately 41.5% of carbapenem-resistant isolates.[12]

Among carbapenemase-positive isolates, 42 (63.6%) were identified as metallo-β-lactamase (MBL) producers, whereas 24 (36.4%) were serine carbapenemase producers. The predominance of MBLs observed in the present study is comparable to the findings of Manoharan et al., who reported MBL production in approximately 65% of carbapenem-resistant isolates.[17]

Urine was the most common specimen source for MBL-producing organisms, accounting for 33.3% of isolates, followed by blood (21.4%) and swabs (16.7%). Similar observations have been reported by Amjad et al., who found urinary isolates to be the predominant source of MBL-producing Enterobacterales. Organism-wise analysis of MBL producers demonstrated that E. coli accounted for 45.23% followed by K. pneumoniae (38.09%) and P. aeruginosa (16.66%). These findings are consistent with report from an Indian tertiary care center where Enterobacterales constitute the major reservoir of metallo-β-lactamase genes.[18]

Among serine carbapenemase producers, E. coli remained the most common organism (41.66%), followed by P. aeruginosa (37.5%) and K. pneumoniae (20.83%). Similar trends have been reported by Queenan and Bush, who highlighted the increasing contribution of Class A and Class D carbapenemases among Enterobacterales and non-fermenting Gram-negative Bacilli.[5]

Conclusion

The occurrence of carbapenemase-producing Gram-negative bacteria in the present study was 31.6%. Escherichia coli was the predominant organism, and metallo-β-lactamases represented the most common carbapenemase type. Routine screening using mCIM and eCIM is recommended for early detection and characterization of carbapenemase-producing isolates. Effective antimicrobial stewardship and strict infection control measures are essential to limit the spread of carbapenem resistance.

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Conflicts of interest

There are no conflicts of interest.

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