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Original Research Article

NDM and OXA-48 Dominance among Carbapenem-Resistant Enterobacterales in an Indian Tertiary Care Hospital

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Abstract: Carbapenem-resistant Enterobacterales (CRE) infections, which are associated with high morbidity and mortality, are increasingly reported worldwide, particularly in healthcare settings. This observational study investigated patients with CRE infections at an Indian tertiary care hospital. The infections predominantly affected elderly individuals: the 61–80 age group accounted for 59.4% of cases, while the 71–80 subgroup alone comprised 38.6% of patients. Regarding hospital stay duration, nearly half of the patients (48.3%) were hospitalized for 0–10 days, whereas 31.0% remained hospitalized for 31 days or more. The most frequently utilized indwelling devices were Foley catheters, followed by nebulizers, nasogastric (NG) tubes, and ventilators. Microbiologically, *Klebsiella pneumoniae* was the primary isolate (58.6%), followed by *Escherichia coli* (27.3%), *Pseudomonas aeruginosa* (6.1%), *Acinetobacter baumannii* (2.0%), non-lactose fermenters (2.0%), and *Providencia rettgeri*, *Enterobacter cloacae*, and *Aspergillus* spp. (2.0% each). This study highlights the clinical and microbiological characteristics of CRE isolated from hospitalized patients. Genetic analysis revealed that blaNDM and blaOXA-48 were the most prevalent carbapenemase genes, while blaKPC and blaIMP occurred less frequently. Notably, several isolates co-harbored multiple resistance genes (such as blaNDM and blaOXA-48, suggesting the emergence of multidrug-resistant variants with complex resistance determinants.

Keywords: Carbapenem-Resistant Enterobacterales (CRE), BlaNDM Gene, BlaOXA-48 Gene, ICU Infections.

INTRODUCTION

Carbapenem-resistant Enterobacterales (CRE) represent one of the most urgent threats in modern healthcare, given their resistance to carbapenems, long regarded as last-resort antibiotics for severe Gram-negative infections [1]. Associated with high morbidity and mortality, CRE infections are increasingly reported worldwide, particularly in hospital settings. Their capacity to spread resistance genes across bacterial species further complicates treatment and infection control, underscoring the need for accurate detection and targeted management strategies [1, 2].

Antibiotic therapy is targeted, which means treatment is designed to match the resistance and genetics of the cause of the infection. [3]. This is especially important for carbapenem-resistant Enterobacterales (CRE) that are resistant to a range of first-line antibiotics. Identifying the mechanism of resistance (KPC, NDM or OXA-48 enzyme production) will allow the clinician to choose the most effective agent and also reduce the unnecessary use of broad-spectrum antibiotics to ensure that resistance is not further developed and the clinical outcomes improve [4].

Some indwelling medical devices, such as urinary catheters, central venous catheters, ventilators and feeding tubes, are significant risk factors for CRE infection. These devices give surfaces for attachment of bacteria and biofilm formation, which can help the bacteria to survive and grow. CRE colonization or infection is more likely to occur in hospitalized patients requiring long-term use of the device, and contaminated equipment and longer hospital stays are additional factors in spread between patients in hospital settings [5].

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Molecular tests are still the most accurate way to identify the genetic cause of carbapenem resistance. Real-time PCR can detect specific carbapenemase genes such as blaKPC, blaNDM, blaOXA-48, blaVIM, blaIMP – with rapid, extremely sensitive detection and can identify resistance genes even if they are not phenotypically expressed [6].

The objective of this study was to analyze the clinical characteristics, sociodemographic factors, comorbidities, clinical picture and antibiotic treatment received by patients with carbapenem-resistant Enterobacterales (CRE) infection in a tertiary care hospital in India, focusing on the type of carbapenemase genes detected and the associated antibiotic treatment.

MATERIALS AND METHOD

An observational study was carried out on patients with carbapenem resistant Enterobacteriaceae infections in an Indian tertiary care hospital. This study examined and evaluated the clinical histories of hundred individuals with CRE infection, as recorded in hospital records. To protect patients' privacy, the data was made anonymous in accordance with the hospital ethics committee requirements. In addition, the researchers examined the patients' medical records to learn about their sociodemographic characteristics, comorbidities, clinical symptoms, the type of genes detected andantibiotic therapy.

Microbial Investigation

Clinical samples were obtained from hospitalized patients with suspected bacterial infection and handled in an aseptic manner, such as urine, blood, sputum, bronchoalveolar lavage (BAL) fluid, wound swabs, pus, endotracheal aspirates and tissue samples. The specimens were sent off immediately to the Department of Microbiology where they were processed under the standard microbiological procedures. Samples were inoculated onto the appropriate culture medium (Blood agar, MacConkey agar, and Chocolate agar) and incubated at 35-37°C for 18-24 hours under suitable atmospheric conditions. After incubation, bacterial colonies were checked for their colony morphology, Gram staining characteristics and biochemical properties. Conventional microbiological methods were used to identify the bacterial isolates and the automated VITEK® 2 Compact system was used to confirm the conventional results and generate antimicrobial susceptibility testing (AST) results.

The results of antimicrobial susceptibility testing were interpreted based on the guidelines of CLSI. Isolates with resistance or reduced susceptibility to carbapenems (imipenem, meropenem, and ertapenem) were classified as suspected carbapenem-resistant Enterobacterales (CRE).

The confirmed CRE isolates were used for the genomic DNA extraction using commercial genomic DNA extraction kit according to the manufacturer's protocol for definitive confirmation. Specific primers targeting the major carbapenemase genes, blaNDM, blaOXA-48, blaKPC, blaVIM, and blaIMP were used in molecular detection by real-time Polymerase Chain Reaction (PCR). Amplification and detection were performed through standardized PCR and the presence of one or more of the genes was used as evidence of the production of carbapenemases. Culture, automated identification, phenotypic assays and molecular testing were used together, resulting in accurate identification and characterization of CRE isolates from hospitalized patients.

Ethical Considerations

The study was conducted after obtaining approval from the Institutional Ethics Committee and the concerned hospital authorities, in accordance with institutional guidelines. Patient confidentiality was strictly maintained throughout the study. Personal identifiers such as patient names and hospital identification numbers were kept confidential and were not disclosed during data analysis or publication. As this was a retrospective observational study using anonymized hospital records and laboratory data, no direct intervention was performed on the patients.

Phenotypic Detection Methods

Automated VITEK® 2 System

VITEK® 2 Compact is an automated bacterial identification system and antimicrobial susceptibility testing system. Bacterial suspension is loaded in cards and a series of biochemical substrates and antibiotics are preloaded in a biochemical identification and AST card, also adjusted to 0.5 McFarland. The system monitors the growth and metabolic activity data of the sample to determine the identification data and MICs. Isolates that have resistance to meropenem, imipenem, and ertapenem are considered potential CRE when using the CLSI breakpoints. Its speed and reliability have led it to become a major tool used for routine clinical screening for resistant organisms.

Kirby-Bauer Disk Diffusion Method

The standard phenotypic assays inoculated a 0.5 McFarland bacterial suspension onto Mueller-Hinton (MH) agar, followed by the application of disks containing meropenem, imipenem and ertapenem. Inhibition zone diameters are measured after 16-18 hours incubation at 35-37°C and compared with the CLSI breakpoints to determine if the isolate is susceptible, intermediate, or resistant. Isolate is identified as a candidate CRE if reduced carbapenems susceptibility is reported.

Modified Carbapenem Inactivation Method (mCIM)

This is a CLSI-recommended direct assay for the detection of carbapenemase production. The meropenem disk is placed in a tryptic soy broth suspension of the test organism and incubated for four hours with the bacteria, in which carbapenemase producing bacteria will degrade and inactivate meropenem. The disk is then placed on a Mueller-Hinton plate that is pre-inoculated with carbapenem susceptible *E. coli* ATCC 25922. If there is no inhibition zone after overnight incubation, the sample is considered to have carbapenemase activity.

EDTA-Modified Carbapenem Inactivation Method (eCIM).

eCIM is a similar product to mCIM, but also chelates zinc ions needed for activity of Metallo-β-lactamases (NDM, VIM, IMP). The meropenem disk is again streaked onto an *E. coli* ATCC 25922 lawn and left to incubate overnight. A zone of inhibition ≥ 5 mm in diameter over the mCIM result confirms the presence of a Metallo-β-lactamase, a useful tool to distinguish between the different classes of carbapenemase.

Carba NP Test

This is a quick biochemical test for the presence of carbapenemase activity by hydrolysis of imipenem. The solution is then combined with imipenem and the pH indicator phenol red; the bacteria present in the solution will be capable of hydrolyzing the imipenem if the solution turns from red to yellow or orange, indicating that imipenem has been acidified by the hydrolysis and lowered the pH. If a non-producer: Leave the solution red. Carba NP is one of the fastest phenotypic tests available – results are available within 2 hours.

The Modified Hodge Test (MHT)

MHT spreads the test organism over a lawn of *E. coli* ATCC 25922 which is carbapenem susceptible. If the disk is a carbapenemase producer, it will break down the antibiotic on the disk and the susceptible strain will grow towards the disk to create a distinctive cloverleaf-shaped depression (positive). Originally, the use of MHT was common, but it has been largely replaced by mCIM because of its lack of specificity and high false positive and negative rates.

Genotypic Detection Methods

Polymerase Chain Reaction (PCR)

The gold standard test to confirm organisms as CPEs is still PCR. It amplifies gene specific sequences and identifies resistance genes directly without having to infer resistance from the phenotype, using gene-specific primers. Real-time PCR was used in reviewed studies to detect blaKPC, blaNDM, blaOXA-48 and blaVIM after DNA extraction from bacterial isolates. It is very sensitive and specific but only detects genes it is searching for, and requires specialized equipment and trained personnel.

Isothermal Amplification

Isothermal amplification eliminates the need for the thermal cycler, and amplify the DNA at a constant temperature. Loop-Mediated Isothermal Amplification (LAMP) identifies blaNDM, blaKPC, blaOXA-48, blaVIM and blaIMP, the results being calculated by using fluorescent or colour change. It is simple, fast and is a good substitution for PCR when resources are limited.

DNA Microarray

Multiple resistance genes are identified simultaneously using DNA microarrays that hybridize labelled bacterium DNA with a chip containing gene-specific probes for blaKPC, blaNDM, blaOXA-48, blaVIM and blaIMP. Positive hybridization results in fluorescent signals, marking multiple genes in a single test. The method is useful for surveillance and epidemiological studies, but logistical and resource requirements are currently prohibitive of routine clinical application.

Whole Genome Sequencing (WGS)

WGS provides the most comprehensive view, providing the complete DNA sequence of a bacterial genome, including resistance genes, virulence factors and plasmids in a single analysis. It not only detects blaKPC, blaNDM, blaOXA-48, blaVIM, and blaIMP, but also provides a genetic map of the relationships between isolates, and is therefore essential for outbreak investigation and surveillance. That power is expensive, though, and requires a sophisticated sequencing and bioinformatics infrastructure, as well as turnaround time, which prevents it from being routinely used.

RESULTS

The 100 patients with carbapenem-resistant Enterobacterales (CRE) infection were mostly seen in elderly, with 59.4% in the age group 61 – 80 years. The 71 – 80 years group alone accounted for 38.6% of patients [**Fig. 1**]. The most common clinical specimen was urine (43%), followed by wound swabs, BAL fluid, endotracheal aspirates and blood, with urinary tract infection being the predominant clinical presentation [**Fig. 2**]. The most common comorbidities were diabetes mellitus and hypertension, which frequently occurred together with chronic kidney disease, chronic lung disease, neurological disorders and cardiovascular disease.

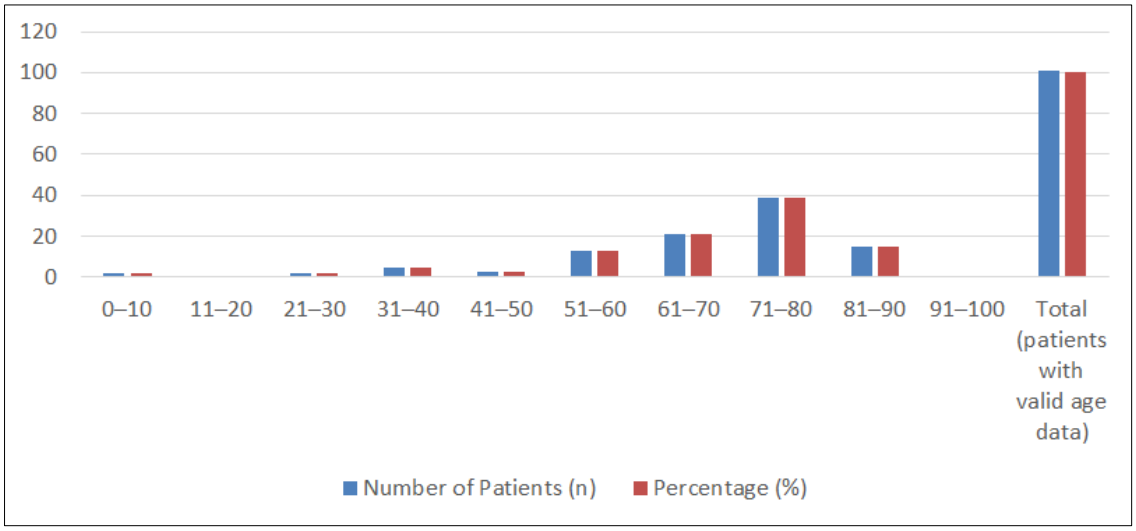

Fig. 1: Age-wise Distribution of Patients

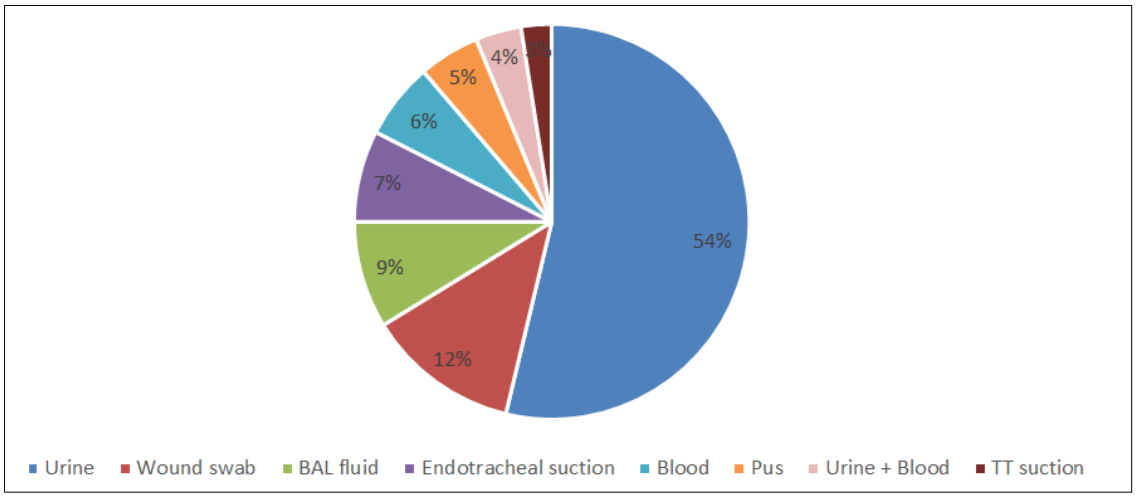

Fig. 2: Clinical Specimen Distribution

Almost half of the patients (48.3%) spent 0–10 days in hospital and 31.0% spent 31 days or more. The most frequently used indwelling device was the Foley catheter (32 patients), followed by nebulizers (29), Ryle's/NG Tubes (24) and ventilators (14) [**Fig. 3**].

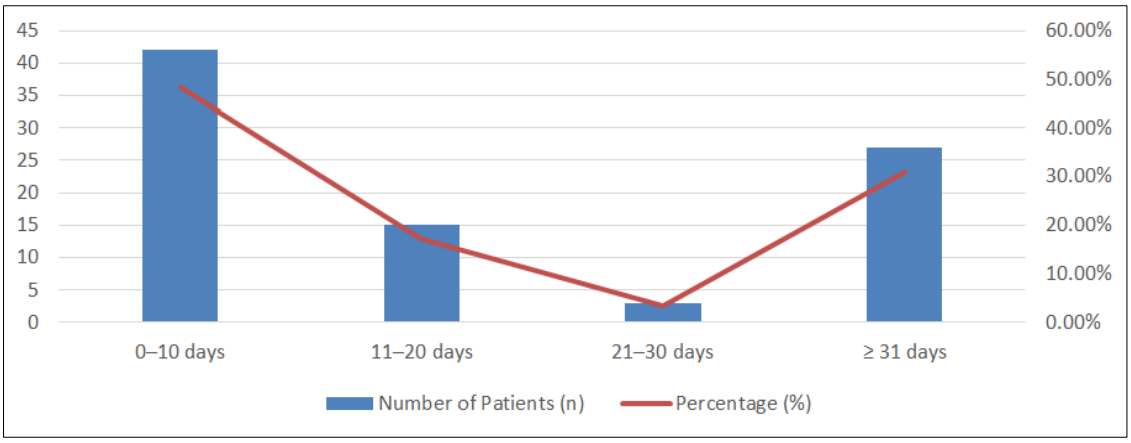

Fig. 3: Duration of Hospital Stay

The major isolate was *Klebsiella pneumoniae* (58.6%), followed by *Escherichia coli* (27.3%), *Pseudomonas aeruginosa* (6.1%), *Acinetobacter baumannii* (2.0%) and non-lactose fermenters (2.0%) and *Providencia rettgeri*, *Enterobacter cloacae* and *Aspergillus* spp. (2.0% each). (1.0% each). The most common carbapenemase gene was blaNDM (66.7%), followed by blaOXA-48 (61.6%), with a number of isolates having both genes, indicating the presence of multi-drug-resistant variants. The presence of blaKPC, blaIMP and blaVIM were found at very low levels (3.0%, 2.0% and 1.0%, respectively) [**Fig. 4**].

Individual Gene	Number of Isolates Carrying the resistance Gene	Percentage of Isolates (%)
NDM	66	66.7
OXA-48	61	61.6
KPC	3	3
IMP	2	2
VIM	1	1

Fig. 4: Carbapenemase Gene Distribution

Treatment was customized according to the gene profile: blaNDM+ entered into ceftazidime-avibactam plus aztreonam (usually with tigecycline and/or polymyxin B); blaOXA-48+ were treated with ceftazidime-avibactam alone; and dual-gene isolates were treated with ceftazidime-avibactam plus aztreonam plus another two drugs. The most commonly used overall agent was ceftazidime-avibactam.

DISCUSSION

The demographic and microbiological profile observed in this study aligns closely with findings from other Indian tertiary-care cohorts, while also highlighting some notable contrasts. The predominance of *Klebsiella pneumoniae* (58.6%) as the leading CRE isolate is consistent with a retrospective review from a tertiary referral hospital, which similarly reported *K. pneumoniae* as the most frequently isolated organism, accounting for 77% of isolates in a cohort with a mean age of 54 years, most of whom were immunosuppressed and ICU-admitted [7].

The high correlation of indwelling device and CRE acquisition observed in this data set, particularly urinary catheterization, is similar to risk factors observed throughout the literature. The Indian study focused on urinary tract infections found that surgical procedures, dialysis, use of indwelling catheter, mechanical ventilation and prolonged or ICU hospitalization are known risk factors for CRE colonization or infection [8]. This is supported by a separate tertiary-care analysis, which showed that mechanical ventilation and indwelling devices were both significantly associated with mortality on univariate analysis, along with prior hospitalization, antibiotic exposure and duration of time in the intensive care unit [9].

The gene distribution is NDM and OXA-48 dominance, with low numbers of KPC, similar to a landmark Indian ICU study where NDM-1 was the most prevalent carbapenemase in the gut of patients screened on day 1 and 4 of ICU admission [10]. Supporting this, the earlier single-Centre Indian carriage study is the first from India to show high CRE gut carriage among hospitalized patients, highlighting the need for better antimicrobial stewardship [11].

Klebsiella pneumoniae (58.6%) was the major pathogens among the bacterial isolates while Escherichia coli (27.3%) was the next most common pathogen. This study shows that *K. pneumoniae* was the most common CRE pathogen in hospitalized patients and the recent study by Halder *et al*., (2025) [13], which revealed that the most common CRE pathogen in hospitalized patients in tertiary-care hospitals in India was carbapenem-resistant *K. pneumoniae*.

The blaNDM gene was the most prevalent (66.7%) followed by blaOXA-48 family (61.6%) and blaKPC, blaIMP and blaVIM were very rarely found. Several isolates were found to carry multidrug-resistant (MDR) strains with multiple resistance determinants, blaNDM and blaOXA-48, indicating the circulation of MDR strains. The results are similar to those of Ibaideya *et al*., (2024) [14], who determined the blaNDM and blaOXA-48 genes to be the predominant carbapenemase genes found in *E. coli* and K. pneumoniae, and to those of Halder *et al*., (2025) [13], who identified *K. pneumoniae* isolates carrying blaNDM, blaOXA-48-like and blaKPC-2 genes in India.

CONCLUSION

This study highlights the clinical and microbiological characteristics of carbapenem-resistant

Enterobacterales (CRE) isolated from hospitalized patients. The overall conclusion of the present study agrees with the previous studies, but some differences were seen too. blaNDM and blaOXA-48 were the most common carbapenemase genes, while blaKPC and blaIMP were found less frequently, unlike North America and Europe, where KPC is more frequently detected. Urine was the most common clinical specimen, whereas wound swabs were the second most frequent source of isolates, a difference from some studies that found bloodstream or respiratory specimens as the major sources of isolates. Furthermore, multiple genes of resistance were observed in several of the isolates from the present study such as blaNDM and blaOXA-48, which suggest the development of multi-drug-resistant variants with multiple resistance determinants. This variation may be attributed to regional differences in carbapenemase distribution, patient population, regional antimicrobial use and infection control measures.

There are some limitations of this study. The study was single-centre and therefore results might not be generalizable to other tertiary care centres or to areas with varying resistance epidemiology. Secondly, the sample size is relatively small (100 patients), which could reduce the statistical power of the subgroups (such as the less prevalent genes blaKPC, blaVIM and blaIMP). Third, the use of retrospective hospital data can be limited by the presence of incomplete or inconsistent medical records, including data on comorbidities, prior antibiotic use, and duration of devices. Fourth, the study did not account for long-term follow-up of discharged patients, meaning that outcomes like reinfection, readmission or late mortality could not be evaluated. Lastly, although molecular testing was focused on the most clinically relevant genes for carbapenemase, other, less common, or novel resistance mechanisms that were not in the primer set may not have been captured, potentially underestimating the genetic diversity of resistance in the study population.

Conflict of Interest: The authors declare no conflict of interest related to this study.

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