Original Article

DOI: 10.7860/JCDR/2026/84251.24464

Microbiology Section

Distribution of Carbapenemase Genes among Clinical Isolates of *Escherichia coli* and *Klebsiella* spp. in a Tertiary Care Centre: A Cross-sectional Study

RADHA KUNJALWAR[1], DIPIKA SHAW[2], GARGI MUDEY[3], NISHA AKOIJAM[4], KAUSHIK SAHOO[5]

(cc) BY-NC-ND

ABSTRACT

Introduction: Carbapenemase-Producing Enterobacterales (CPE), especially *Escherichia coli* and *Klebsiella* spp., have become a serious global health concern due to their role in nosocomial infections, limited treatment options, and high mortality. Resistance is primarily mediated by carbapenemase genes, often undetected by conventional phenotypic methods. Early molecular tool detection is crucial for effective infection control and antimicrobial stewardship.

Aim: To detect carbapenemase-producing *E. coli* and *Klebsiella* spp. using both phenotypic mCIM (modified carbapenem inactivation method) and eCIM (EDTA-modified carbapenem inactivation method) and genotypic Reverse Transcriptase Polymerase Chain Reaction (RT-PCR) methods at a tertiary healthcare centre in Central India.

Materials and Methods: This cross-sectional study was conducted in the Department of Microbiology, Jawaharlal Nehru Medical College, Sawangi, Wardha, Maharashtra, India. A total of 205 non-duplicate clinical isolates were collected from June 2022 to June 2024. Antimicrobial susceptibility testing was performed by the Kirby-Bauer disk diffusion method as per Clinical and Laboratory Standards Institute (CLSI) 2022 guidelines. Carbapenemase production was confirmed phenotypically using mCIM and eCIM. Genotypic detection of carbapenemase genes (bla_{NDM}, bla_{OXA}-48, bla_{KPC}, bla_{VIM}, and bla_{IMP}) was done by real-time PCR. Data were analysed using descriptive statistics; p-value<0.05 was considered significant.

Results: Out of 205 isolates, 115 (56.1%) were confirmed as carbapenemase producers, with 94 (81.7%) identified as Metallo-β-Lactamase (MBL) producers and 21 (18.3%) as serine carbapenemase producers. RT-PCR detected carbapenemase genes in 60 of 73 phenotypically positive isolates (82.19%). bla_{NDM} gene was most prevalent 41 (56.16%), followed by bla_{OXA-48} 35 (47.94%), bla_{KPC} 11 (15.06%), bla_{VIM} 9 (12.32%), and bla_{IMP} 1 (1.36%). Co-occurrence of these genes was also common, with 13 isolates showing both NDM and OXA-48.

Conclusion: The study highlights a high prevalence of NDM and OXA-48 carbapenemase genes among *E. coli* and *Klebsiella* spp. in Central India. The predominance of MBLs emphasises the urgent need for routine molecular diagnostics, stringent infection control, and antibiotic stewardship to curb the spread of these highly resistant organisms. The combination of phenotypic and rapid genotypic methods enables timely identification of resistance patterns, which is essential for targeted therapy, infection control, and healthcare settings.

Keywords: Antimicrobial resistance, Antimicrobial stewardship, Carbapenemase production

INTRODUCTION

Enterobacterales are a large family of gram-negative rods usually found in the colon of humans and animals. They are a significant source of nosocomial infections as well as community-acquired illnesses [1,2]. Carbapenems are beta-lactam antibiotics with the largest scope of any beta-lactam antibiotic, making them appropriate for treating infections caused by Multidrug-Resistant (MDR) gram-negative bacteria [3].

Enterobacterales, notably *Escherichia coli* and *Klebsiella* spp., can evolve into Carbapenem-Resistant Enterobacterales (CRE). CREs that creates carbapenemases, enzymes that degrade Carbapenems and similar antibiotics, rendering them ineffective, is known as CPE. As a result, CPE is a subset of all CRE [4]. Drugs used to treat CPE infections are partly based on the mechanism of resistance to carbapenem. The most common mechanism of carbapenem resistance in CPE is due to the production of carbapenem-hydrolysing enzymes called carbapenemase, which are either serine beta lactamase or MBL [5]. Ambler classes A, B, and D are the Carbapenem-hydrolysing beta-lactamases. They have been documented worldwide among the Enterobacterales. Class A - KPC, Class B - IMP, VIM and NDM-1, and Class D- OXA-48 are the most clinically significant types. Class A and D antibiotics cleave the β-lactam ring using a serine-based hydrolytic action. Class B antibiotics are metallo-beta-lactamases, which rely on a zinc ion in the active site [3,6].

CPE infections are mostly seen in patients with ventilators, urinary catheters, or intravenous catheters, and prolonged treatment with antibiotics, underlying co-morbidities, extremes of age, Intensive Care Unit (ICU) stay, immunosuppression, and prior antibiotic exposure are all considered associated with increased risk for CRE [6]. CPE was identified as a significant danger by the Centres for Disease Control and Prevention (CDC) in 2013, even more so than Methicillin-Resistant *Staphylococcus aureus,* as well as MDR Tuberculosis (MDR-TB) [2]. *K. pneumoniae* is among the top three bacteria of global concern in the World Health Organisation (WHO) report due to rising antibiotic resistance, making treatments increasingly ineffective [3]. Carbapenemase detection in Enterobacterales is crucial, as it is associated with other resistance mechanisms leading to MDR, Extensively Drug-Resistant (XDR), and Pan-Drug Resistance (PDR) [7].

Thus, in order to begin an efficient empirical and antibiotic susceptibility guided therapy, it is crucial to know the prevalence of carbapenem resistance, carbapenemase prevalence, and kind of carbapenemase produced by the bacteria [5]. As the carbapenem resistance rates are high, rapid detection using tests with a short turnaround time is essential to ensure early detection and timely

implementation of control measures in hospitals is very important for guiding appropriate antimicrobial therapy [8,9]. This study provides molecular epidemiological data on carbapenemase-producing *E. coli* and *Klebsiella* spp. from a tertiary healthcare centre in Central India using a combined phenotypic (mCIM/eCIM) and real-time PCR approach. It simultaneously detects five major carbapenemase genes and demonstrates a predominance of MBLs with frequent co-existence of bla_{NDM} and bla_{OXA-48}. This study highlights the practical utility of mCIM-eCIM for routine laboratories and provides sample-wise and species-wise gene distribution data, from this region.

In the present study, the aim was to assess the detection of carbapenemase production among clinical isolates of *Escherichia coli* and *Klebsiella* spp., by different phenotypic and genotypic methods in a tertiary rural healthcare facility.

The primary objective was to detect carbapenemase production in the isolates by Multiplex PCR for bla_{NDM}, $bla_{OXA-48,}$ $bla_{KPC,}$ $bla_{VIM,}$ and bla_{IIMP}, and the secondary objective was to determine carbapenemase production in these isolates by mCIM and eCIM.

MATERIALS AND METHODS

This cross-sectional study was conducted in the Department of Microbiology, Jawaharlal Nehru Medical College, Sawangi (Meghe), Wardha, Maharashtra, India, from June 2022 to June 2024. Institutional Ethics Committee approval was obtained- Approval Reference No.: DMIMS (DU/IEC/2022/1055). As the study was on isolates recovered from specimens received in the Department of Microbiology, written informed consent was not required.

Sample size: Sample size was calculated on the basis of Daniel's formula for cross-sectional studies. The formula used was $n=\{z^2\ p\ (1-P)\}/d^2$ [10]. This was time-bound study; all subjects available in the study were taken into consideration. The exact sample size was 205 non-duplicate specimens that were received in the Department of Microbiology.

Inclusion criteria: This study included 205 non-duplicate *E. coli* and *Klebsiella* spp. isolates from various clinical samples (urine, pus, vaginal swab, blood, sputum, and body fluids like cerebrospinal, pleural, ascitic, and peritoneal fluid) received in the microbiology laboratory.

Exclusion criteria: Stool samples and rectal swabs were excluded from the study.

Study Procedure

Specimens were processed using standard methods on blood agar, chocolate agar, and MacConkey's agar and incubated at 35±2°C aerobically for 16-24 hours [11]. Blood samples were cultured using BacT/ALERT 3D system (Biomerieux, France). Identification was performed using the conventional method and automated system (VITEK 2 compact system, Biomerieux, France). These isolates were screened for carbapenem resistance i.e., imipenem and meropenem using the Kirby-Bauer disc diffusion method on Mueller–Hinton agar, as per Clinical Laboratory Standard Institute (CLSI) M100-Ed32, 2022 guidelines. Minimum inhibitory concentration (MIC) of colistin was determined using the Colistin Broth Disk Elution (CBDE) method [12].

Phenotypic Detection

Detection of carbapenem resistance- Imipenem and meropenem-resistant *E.coli* and *Klebsiella* spp., identified by the modified Kirby-Bauer method per CLSI M100-Ed32, 2022 guidelines, were tested for carbapenemase production using phenotypic methods [12].

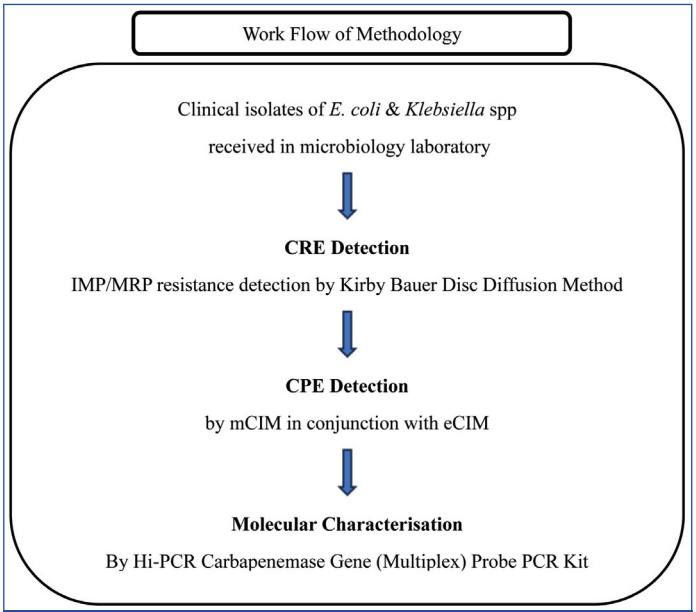
MBL and Serine carbapenemase detection-Imipenem and meropenem-resistant isolates were tested for carbapenemase production using mCIM and eCIM as per CLSI 2022 guidelines. mCIM detects carbapenemases in Enterobacterales, while eCIM differentiates MBL from serine carbapenemases. *E. coli* (ATCC® 25922) served as the meropenem-susceptible indicator strain. eCIM is interpretable only if mCIM is positive [12].

MDR organisms were defined as isolates showing non-susceptibility to at least one agent in three or more antimicrobial categories. Extensively Drug-Resistant XDR organisms were defined as isolates showing non-susceptibility to at least one agent in all but two or fewer antimicrobial categories, i.e., the isolate remains susceptible to only one or two antimicrobial categories [13].

Genotypic Detection

Overnight bacterial culture was used for DNA extraction using the HiPurA® Bacterial Genomic DNA Purification Kit (MB505) according to the manufacturer's instructions. Real-Time PCR was used for the identification of bla_{NDM}, bla_{IMP}, bla_{VIM}, bla_{KPC}, and bla_{OXA-48} genes. PCR was done by the MBPCR132 Carbapenemase Gene (multiple) Probe PCR-kit from Hi-PCR. The purpose of multiplex probe PCR kit is to identify particular gene regions that code for different carbapenemase enzymes. In both master mixes, the internal control was identified in the Cy5.5 channel. In a single-tube reaction, the kit enables the sensitive and precise identification of single and co-present carbapenemase-encoding genes. The PCR program was set and carried out in BIO-RAD Thermocycler for PCR (CFX96 Real-time system) settings as per the manufacturer's instructions.

Quality Control: Hi-PCR® Carbapenemase Gene (Multiplex) Probe PCR Kit from HiMedia was tested for nonspecific DNase activity, endonuclease contamination, and exonuclease contamination in each lot. The study isolates were processed through a structured workflow for comprehensive detection and characterisation of resistance mechanisms [Table/Fig-1].



[Table/Fig-1]: Workflow of methodology for the study isolates.

STATISTICAL ANALYSIS

Statistical analysis was performed using IBM Statistical Package for the Social Sciences (SPSS) Statistics for Windows, Version 25.0. Descriptive statistics were expressed as mean ± standard deviation for continuous variables and percentages for categorical variables. A p-value <0.05 was considered statistically significant.

RESULTS

A total of 205 *Klebsiella* spp. and *E. coli* isolates from all clinically relevant samples were included in the study. The majority of the samples were from surgery ward 52 (25.36%), followed by medicine 42 (20.48%) and medicine ICU 22 (10.73%) [Table/Fig-2].

The data revealed that the largest age group was individuals aged 41 to 60 years, comprising 73 patients (35.6% of the total). This was

Wards	n (%)
Surgery	52 (25.36)
Medicine	42 (20.48)
MICU	22 (10.73)
Paediatrics	18 (8.78)
Neuro-ICU	12 (5.85)
SICU	11 (5.36)
PICU	11 (5.36)
Orthopaedic	11 (5.36)
Urology	7 (3.41)
Gynaecology	7 (3.41)
NICU	6 (2.92)
Others	7 (3.41)
Total	6 (2.92)

[Table/Fig-2]: Ward-wise distribution of study isolates.

followed by the 21 to 40 years age group, with 44 patients (21.5%), and the above 60 years age group, with 47 patients (22.9%). The youngest age group, 0 to 20 years, included 41 patients (20%). Age distribution of patients in the study, as given in [Table/Fig-3].

Minimum age (years)	0
Maximum age (years)	86
Mean ± Std.	41.51±23.04

[Table/Fig-3]: Age descriptive statistics.

From 205 isolates, majority of the organisms were *Klebsiella* spp. 114 (55.6%) followed by *E. coli* 91 (44.4%). *E. coli* showed high resistance to several antibiotics such as ceftriaxone and cefotaxime 83 (91.2%), ciprofloxacin 81 (89.01%), amoxicillin and clavulanic acid 77 (84.61%), cefepime 73 (80.21%), ceftazidime 71 (78.02%), piperacillin-tazobactam 70 (76.92%), 114 were *Klebsiella* spp., including *K. pneumoniae* and *K. oxytoca* (1 isolate), which exhibited high resistance to cefotaxime 103 (0.35%), ceftriaxone 102 (89.47%,) ciprofloxacin 101 (88.59%), Cefepime 100 (87.72%), *E. coli* and *Klebsiella* spp. both showed high susceptibility to tigecycline 100%, followed by colistin 89 (97.4%) and 111 (98.23%), respectively. Resistance pattern is mentioned in the [Table/Fig-4].

Antibiotics	*Escherichia coli* n=91 (44.4%)	*Klebsiella spp* n=114 (55.6%)
Ceftriaxone (CTR)	83 (91.2%)	102 (89.47%)
Cefotaxime (CTX)	83 (91.2%)	103 (90.35%)
Ciprofloxacin (CIP)	81 (89.01%)	101 (88.59%)
Amoxyclav (AMC)	77 (84.61%)	95 (83.33%)
Cefepime (CPM)	73 (80.21%)	100 (87.72%)
Ceftazidime (CAZ)	71 (78.02%)	95 (83.33%)
Piperacillin-tazobactam (PIT)	70 (76.92%)	90 (78.95%)
Co-trimoxazole (COT)	65 (71.42%)	80 (70.17%)
Ampicillin (AMP)	63 (69.23%)	NA
Gentamycin (GEN)	51 (56.04%)	70 (61.40%)

[Table/Fig-4]: Antibiotic resistance profile of *E. coli* and *Klebsiella* spp.
NA*- *Klebsiella* spp is intrinsically resistant to AMP.

The resistance profile of the clinical isolates shows that 104 (50.7%) were XDR, 44 (21.5%) were MDR, and 57 (27.8%) were non-applicable to these categories.

Detection of Carbapenemase Production by Phenotypic Methods

Among 205 total isolates, 115 (56.1%) were found to be carbapenemase-producing; 94 (81.74%) MBL producers and 21 (18.3%) serine carbapenemase producers.

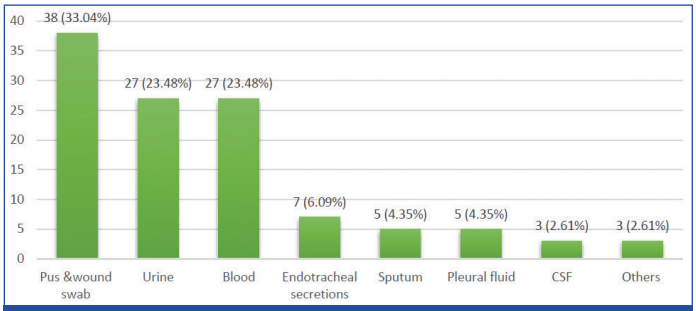
Quality Control Strains used for mCIM- *K. pneumoniae ATCC® BAA -1705™* as a positive-control, *K. pneumoniae* ATCC® BAA -1706™ as a negative control, and for eCIM- *K. pneumoniae* ATCC® BAA -2146™ as a positive-control, *K. pneumoniae* ATCC®BAA -1705™ as a negative control. [Table/Fig-5,6] depicts the prevalence of MBL and Serine carbapenemase producers in the study isolates. Total carbapenemase production was 57.01% (n=65) in *Klebsiella* spp. and 54.94% (n=50) in *E. coli*.

[Table/Fig-5]: mCIM in conjunction with eCIM.

Organisms	MBL Producers (n=94)	Serine Carbapenemase (n=21)
Klebsiella spp. (n=65)	52 (80%)	13 (20%)
E. coli (n=50)	42 (84%)	8 (16%)
Total (n=115)	94 (81.7%)	21 (18.3%)

[Table/Fig-6]: MBL and Serine carbapenemase producers.

The majority of the organisms were isolated from pus and wound swabs, 38 (33.04%), followed by urine and blood, 27 (23.48%) each which is shown in [Table/Fig-7].



[Table/Fig-7]: Sample-wise distribution in CPE.

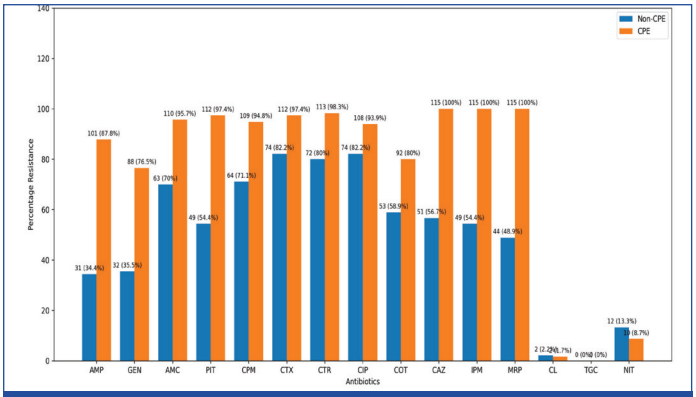
Antimicrobial Resistance Pattern of CPE and Non-CPE

Similar antimicrobial resistance patterns of carbapenemase-producing study isolates were noted among both *E. coli* and *K. pneumoniae*. All isolates of both species (100%) were resistant to imipenem, meropenem, and ceftazidime as detailed in [Table/Fig-8].

Out of 73 carbapenemase-producing isolates, carbapenemase genes were detected in 60 (82.19%) isolates using real-time PCR. [Table/Fig-9] shows prevalence of genes responsible for carbapenem resistance in CPE isolates. NDM carbapenemase gene was the predominant gene 41 (56.16%), followed by OXA-48 35 (47.94%), KPC 11 (15.06%), VIM 9 (12.32%), and IMP 1 (1.36%) as shown in the [Table/Fig-10]. Thirteen isolates out of 73 were negative for all the carbapenemase genes.

Distribution of carbapenemase genes in different Samples

The NDM was the only gene predominant in all the combinations. The predominant genes in the blood isolates were OXA-48 5 (31.25%), followed by NDM 3 (18.75%), and NDM+OXA-48, 3 (18.75%), NDM+KPC+OXA-48 2 (12.5%). In pus, 16 (46.3%) of the



[Table/Fig-8]: Antibiogram data of CPE & Non-CPE isolates (Antimicrobial resistant profile).
*Mentioned in n (%)
*AMP: ampicillin; Gen: Gentamycin; AMC: Amoxicillin–Clavulanate; PIT: Piperacillin-tazobactam; CPM: Cefepime; CTX: Cefotaxime; CTR: Ceftriaxone; CIP: Ciprofloxacin; COT: Cotrimoxazole; CAZ: Ceftazidime; IPM: Imipenem; MRP: Meropenem; CL: Colistin; TGC: Tigecycline; NIT: Nitrofurantoin; CPE: Carbapenemase producing **Enterobacterales**; *AMP- intrinsic resistance to *Klebsiella* spp.

Organisms	Distribution of Genes				
	NDM	KPC	VIM	IMP	OXA-48
Klebsiella pneumoniae (n=48)	26 (54.16%)	8 (16.66%)	7 (14.58%)	1 (2.08%)	23 (47.91%)
E. coli (n=25)	15 (60%)	3 (12%)	2 (8%)	0 (0%)	12 (48%)
Total (n=73)	41 (56.16%)	11 (15.06%)	9 (12.32%)	1 (1.36%)	35 (47.94%)

[Table/Fig-9]: Prevalence of genes responsible for Carbapenem Resistance in CPE isolates.

Genes combination	*Klebsiella pneumoniae* (n= 48)	*E. coli* (n=25)	Total (n=73)
NDM	8 (16.66)	6 (24)	14 (19.17)
KPC	2 (4.16)	1 (4)	3 (4.1)
VIM	2 (4.16)	0	2 (2.73)
OXA48	9 (18.75)	4 (16)	13 (17.8)
Two gene combinations			
NDM+KPC	1 (2.08)	1 (4)	2 (2.73)
NDM+VIM	3 (6.25)	0	3 (4.1)
NDM+OXA48	8 (16.66)	5 (20)	13 (17.8)
VIM+OXA48	1 (2.08)	0	1 (1.36)
Three gene combinations			
NDM+KPC+OXA48	5 (10.41)	1 (4)	6 (8.21)
NDM+VIM+IMP	1 (2.08)	0	1 (1.36)
NDM+VIM+OXA48	0	2 (8)	2 (2.73)
Negative for all Genes	8 (16.66)	5 (20)	13 (17.8)
Total	48	25	73

[Table/Fig-10]: Distribution of genes in CPE isolates.

most prevalent genes were OXA-48 3 (18.75%), NDM with OXA-48 3 (18.75%), followed by NDM + KPC + OXA - 48, 3 (18.75%). In the urine sample, NDM 4 (36.36%) was the predominant gene, followed by NDM+OXA-48, 3 (27.27%). Only two genes were found in CSF samples was NDM and OXA-48. [Table/Fig-11] shows the distribution of various carbapenemase genes in different samples included in the study.

[Table/Fig-12,13] show the amplification plots of various carbapenemase genes detected.

DISCUSSION

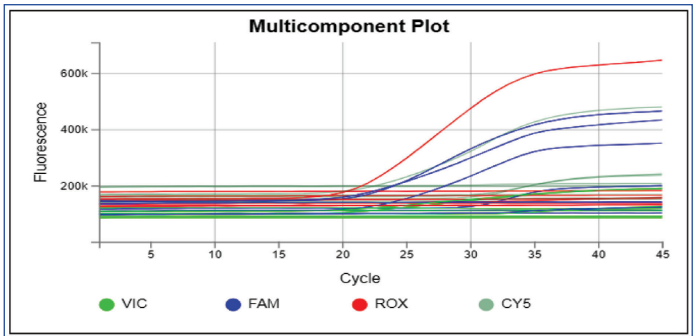
Carbapenem resistance is increasingly recognised as a critical threat, particularly in healthcare-associated infections, leading to elevated morbidity, mortality, and economic burden due to prolonged hospital stays and the need for last-resort therapies [13,14]. In this study, both phenotypic and genotypic approaches are used to investigate CPE, aiming to better understand resistance patterns and curb the transmission of these pathogens within hospitals. This was almost a decade ago, and now the prevalence of CPE has risen. In the past few years, the prevalence of CPE has peaked globally, including in India, as proven by some Indian literature [5,15]. Among the 205 isolates, 115 (56.1%) were carbapenemase producers. The prevalence among *Klebsiella* spp. and *E. coli* was 57.01% and 54.94%, respectively. This prevalence was significantly higher than earlier reports from India, which documented CPE rates ranging from 4% to 18.9% nearly a decade ago [15,16]. The majority of carbapenemase-producing isolates in the present study were recovered from pus and wound swabs (33.04%), followed by urine and blood samples (23.48% each). This contrasts with earlier findings reporting lower proportions of CPE from pus samples (42.3%, and 51%) [16,17].

However, the frequency of CP-CRE in blood samples (19-22.3%) aligns closely with this study's results [17-19]. *Klebsiella* spp. was the predominant carbapenemase-producing organism in the present cohort, consistent with other reports documenting prevalence ranging from 46% to 73.4% [5,15,20-22]. A study from Saudi Arabia reported 100% resistance to both imipenem and meropenem, while Baskaran V et al., documented 78.2% resistance to IPM and 97.6% to MRP [5,7]. The imipenem resistance observed in this study aligns with these findings, whereas the meropenem resistance was comparatively lower.

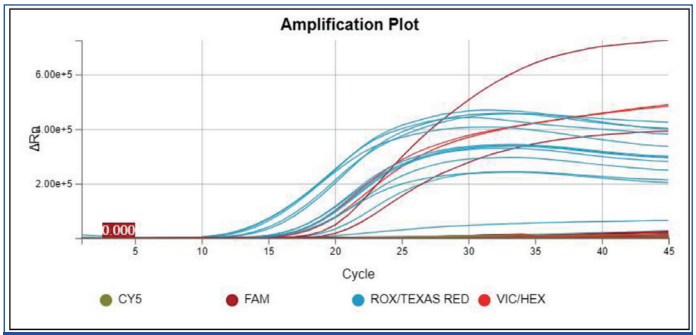
In this study, the mCIM results showed a high prevalence of carbapenemase production, with 56.1% of the isolates testing positive. The present study noted 81.74% of MBL production as they were positive for eCIM, and 18.26% Serine carbapenemase was seen. A similar study demonstrated higher MBL production by mCIM in conjunction with eCIM, and carbapenemase production was reported, as 52.8%, which was in concordance with the

Genes	ET	Blood	Sputum	CSF	Pus	Wound swab	Urine	*Others
NDM (n=14)	1	3	2	1	1	2	4	0
KPC (n=4)	0	1	0	0	1	1	1	0
VIM (n=2)	0	0	0	0	2	0	0	0
OXA48 (n=13)	0	5	1	1	3	1	0	2
NDM+KPC (n=2)	0	0	0	0	1	0	1	0
NDM+VIM (n=2)	0	1	0	0	1	0	0	0
NDM+OXA48 (n=13)	0	3	1	0	3	1	3	2
VIM+OXA48 (n=1)	0	0	0	0	0	0	0	1
NDM+KPC+OXA48 (n=6)	0	2	0	0	3	0	1	0
NDM+VIM+IMP (n=1)	0	0	0	0	0	0	1	0
NDM+VIM+OXA48 (n=2)	0	1	0	0	1	0	0	0
Total (n=60)	1	16	4	2	16	5	11	5

[Table/Fig-11]: Distribution of carbapenemase genes in different samples.



[Table/Fig-12]: Amplification plots of New Delhi metallo-β-lactamase (NDM), *Klebsiella pneumoniae* carbapenemase (KPC), and Verona integron-mediated metallo β-lactamase (VIM) are displayed; *VIC-KPC, FAM-NDM, ROX-IMP, CY5-VIM.



[Table/Fig-13]: The amplification plots of OXA-48.
*CY5- OXA-58, FAM- OXA-51, ROX- OXA-48, HEX-OXA-23

current study [19]. The present study results accurately identified carbapenemase producers and further distinguished between serine andMBL producers.

Genotypic characterisation by real-time PCR confirmed the presence of carbapenemase genes in 82.19% of the phenotypically positive isolates. The most frequently detected gene was NDM (56.16%), followed by OXA-48 (47.94%), KPC (15.06%), VIM (12.32%), and IMP (1.36%). Notably, 17.81% of phenotypically positive isolates were negative for all tested genes, suggesting the potential presence of other untested or novel carbapenemase genes or alternate resistance mechanisms. In the present study, the most commonly identified carbapenemase gene was NDM, detected in 56.16% of isolates, followed by OXA-48 (47.94%) and KPC (15.06%). Given its global spread, NDM is likely to become the most prevalent carbapenemase gene worldwide. In the present study, 82.19% (60 out of 73) of isolates were genotypically positive for carbapenemase genes, comparable to positivity rates reported in recent studies (87.2% and 90.3%) [17,23]. Among the positive isolates, co-existence of resistance genes was frequently observed. The most common combinations involved NDM with other genes: NDM + OXA-48, NDM + VIM, NDM + KPC, and VIM + OXA-48. Co-occurrence of three genes was less frequent (12.32%) but notable. Specific combinations included NDM + KPC + OXA-48 (8.21%), NDM + VIM + OXA-48 (2.73%), and NDM + VIM + IMP (1.36%). IMP gene was detected alone in a few isolates (1.36%), similar to findings by Vamsi SK et al., [17]. Additionally, VIM was found in 12.32% of isolates, consistent with Traczewski MM et al., who reported 15.45% VIM and 1.16% IMP [24].

Interestingly, this study's data showed 6.25% of isolates harbouring both VIM and NDM, which was lower than previous reports of 39.6%, 14.6%, and 17.3% co-occurrence rates [5]. Some studies have reported differing gene prevalence; for instance, a study showed KPC (51.6%) as the most common gene, followed by NDM (35.7%) and OXA-48-like (7.3%), contrasting with the present study findings [25]. Similarly, while the present study showed NDM predominance, others reported OXA-48 as the most prevalent gene, with rates of 40.7% and 83.3%, compared to NDM at 14.8% and 16.7%, respectively [26,27].

Various studies across India have reported CPE prevalence rates ranging from 14-69%. [5] Specifically, studies from northern India reported 24% prevalence [28], Western Maharashtra around 12.26% [29], and South India approximately 30% [30]. The high frequency of NDM and OXA-48 genes, both of which encode MBL, indicates that carbapenemase production is a major mechanism of resistance in our region. The variation in gene prevalence across studies is likely influenced by geographical differences, testing methods, and the bacterial species involved.

Limitation(s)

The study has focused on isolates from specific healthcare facilities, which could be different in other settings. Furthermore, the sample size may have been insufficient to capture the full diversity of carbapenemase-producing *Enterobacteriaceae* circulating in the healthcare setting, which could limit the robustness of the study findings. Additionally, five carbapenemase genes were investigated, while other carbapenemase genes were not included. Future research with larger sample sizes, along with more comprehensive detection methods, is needed to overcome these limitations and provide a more accurate assessment of carbapenem resistance.

CONCLUSION(S)

This study underscores the significant burden of CPE s in Central India, with MBLs emerging as the predominant resistance mechanism. Molecular analysis demonstrated the presence of important carbapenem resistance genes, including co-occurrence of multiple resistance determinants in certain isolates, highlighting the growing challenge of antimicrobial resistance. Collaborative efforts among healthcare facilities, public health authorities, and research institutions are essential to monitor resistance trends, strengthen surveillance, share data, and implement effective infection control and antimicrobial stewardship practices.

Funding: This study was financially supported by the Indian Council of Medical Research (ICMR Reference ID: MD22JUN-0259).

REFERENCES

[1] Dahab RA, Ibrahim AM, Altayb HN. Phenotypic and genotypic detection of carbapenemase enzymes producing gram-negative bacilli isolated from patients in Khartoum State. F1000Research. 2017;6:1656.
[2] Tsai YM, Wang S, Chiu HC, Kao CY, Wen LL. Combination of modified carbapenem inactivation method (mCIM) and EDTA-CIM (eCIM) for phenotypic detection of carbapenemase-producing Enterobacteriaceae. BMC Microbiol. 2020;20(1):315.
[3] Oduyebo O, Falayi O, Oshun P, Ettu A. Phenotypic determination of carbapenemase producing enterobacteriaceae isolates from clinical specimens at a tertiary hospital in Lagos, Nigeria. Niger Postgrad Med J. 2015;22(4):223.
[4] Centers for Disease Control and Prevention. Healthcare-associated infections: Tracking CRE [Internet]. Atlanta (GA): CDC; [cited 2026 May 18]. Available from: CDC Healthcare-associated Infections Tracking CRE.
[5] Baskaran V, Jeyamani L, Thangaraju D, Sivaraj R, Jayarajan J. Detection of carbapenemase production by modified carbapenem inactivation method among carbapenem resistant Gram-negative bacilli at a tertiary care centre in Coimbatore, Tamil Nadu, India. Natl J Lab Med [Internet]. 2023 [cited 2026 May 18];12(2):MO11–MO14.
[6] Alemayehu T, Asnake S, Tadesse B, Azerefegn E, Mitiku E, Agegnehu A, et al. Phenotypic detection of carbapenem-resistant gram-negative bacilli from a clinical specimen in Sidama, Ethiopia: A cross-sectional study. Infect Drug Resist. 2021;14:369-80.
[7] Michodigni NF, Nyachieo A, Akhwale JK, Magoma G, Kimang'a AN. Molecular identification of co-existence of carbapenemase and extended-spectrum β-Lactamase genes in *Klebsiella pneumoniae* clinical isolates, and their phylogenetic patterns in Kenya. Adv Microbiol. 2021;11(08):399-415.
[8] Begum N, Shamsuzzaman SM. Emergence of carbapenemase-producing urinary isolates at a tertiary care hospital in Dhaka, Bangladesh. Ci Ji Yi Xue Za Zhi Tzu-Chi Med J. 2016;28(3):94-98.
[9] Pragasam AK. A pilot study on carbapenemase detection: Do we see the same level of agreement as with the CLSI observations. J Clin Diagn Res [Internet]. 2016 [cited 2024 Jul 6];10(7):DC09-DC13. Available from: https://jcdr.net/article_fulltext.asp?id=8154.
[10] Ravikant, Jeya M, Felix AJW. Prevalence and risk factor analysis of faecal carriage of carbapenem resistant Enterobacteriaceae in a tertiary care hospital. J Clin Diagn Res [Internet]. 2019 [cited 2026 May 18];13(5):DC01-DC04. Available from: https://jcdr.net/article_fulltext.asp?id=12839.
[11] J. G. Collee. Mackie & McCartney practical medical microbiology [Internet]. 14th ed. Churchill Livingstone, New York, 1996;

[12] Lewis JS. II (2022). *CLSI 2022:* Performance Standards for Antimicrobial Susceptibility Testing (32nd ed.) [PDF]. Clinical and Laboratory Standards Institute. https://clsi.org/media/wi0pmpke/m100ed32_sample.pdf.
[13] Magiorakos AP, Srinivasan A, Carey RB, Carmeli Y, Falagas ME, Giske CG, et al. Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: An international expert proposal for interim standard definitions for acquired resistance. Clin Microbiol Infect. 2012;18(3):268-81.
[14] Campos-Madueno EI, Moradi M, Eddoubaji Y, Shahi F, Moradi S, Bernasconi OJ, et al. Intestinal colonization with multidrug-resistant Enterobacterales: Screening, epidemiology, clinical impact, and strategies to decolonize carriers. Eur J Clin Microbiol Infect Dis. 2023;42(3):229-54.
[15] Pawar KS, Mohite TS, Shinde VR, Patil RS, Karande SG. Carbapenem-resistant Enterobacteriaceae: Prevalence and bacteriological profile in a tertiary teaching hospital from rural western India. Indian J Microbiol Res. 2020;5(3):342-47.
[16] Datta P, Gupta V, Garg S, Chander J. Phenotypic method for differentiation of carbapenemases in Enterobacteriaceae: Study from north India. Indian J Pathol Microbiol. 2012;55(3):357.
[17] Vamsi SK, Moorthy RS, Hemiliamma MN, Chandra Reddy RB, Chanderakant DJ, Sirikonda S. Phenotypic and genotypic detection of carbapenemase production among gram negative bacteria isolated from hospital acquired infections. Saudi Med J. 2022;43(3):236-43.
[18] Modi CM, Singh SP, Pandya YG, Patel CP, Patel RM. Prevalence of carbapenem resistant Enterobacteriaceae in a tertiary care hospital of Gujarat, India. J Clin Diagn Res [Internet]. 2021 [cited 2026 May 18];15(4):DC05-DC09. Available from: https://jcdr.net/article_fulltext.asp?id=14771.
[19] Abid FB, Tsui CKM, Doi Y, Deshmukh A, McElheny CL, Bachman WC, et al. Molecular characterization of clinical carbapenem-resistant Enterobacterales from Qatar. Eur J Clin Microbiol Infect Dis. 2021;40(8):1779-85.
[20] Gaur S, Gahlot R, Sherwani N, Netam S, Dadarya S. Characterizing carbapenemase production in Enterobacterales through combined disk test and genetic profiling. Indian J Microbiol Res. 2024;11(2):97-102.
[21] Pilli R, Rani AD, Sunitha B, Venna SC. Detection of carbapenemase producing Gram-negative bacteria at a tertiary care hospital: A cross-sectional study. Natl J Lab Med [Internet]. 2024 [cited 2026 May 18];13(1):MO01-MO05.
[22] Abdeta A, Bitew A, Fentaw S, Tsige E, Assefa D, Lejisa T, et al. Phenotypic characterization of carbapenem non-susceptible gram-negative bacilli isolated from clinical specimens. PloS One. 2021;16(12):e0256556.
[23] Garg A, Garg J, Kumar S, Bhattacharya A, Agarwal S, Upadhyay GC. Molecular epidemiology & therapeutic options of carbapenem-resistant Gram-negative bacteria. Indian J Med Res. 2019;149(2):285-89.
[24] Traczewski MM, Carretto E, Canton R, Moore NM; Carba-R Study Team. Multicenter evaluation of the xpert carba-r assay for detection of carbapenemase genes in gram-negative isolates. J Clin Microbiol. 2018;56(8):e00272-18.
[25] Han R, Shi Q, Wu S, Yin D, Peng M, Dong D, et al. Dissemination of carbapenemases (KPC, NDM, OXA-48, IMP, and VIM) among carbapenem-resistant enterobacteriaceae isolated from adult and children patients in China. Front Cell Infect Microbiol. 2020;10:314.
[26] Joshi DN, Shenoy B, MV B, Adhikary R, Shamarao S, Mahalingam A. Prevalence of carbapenem-resistant Enterobacteriaceae and the genes responsible for carbapenemase production in a tertiary care hospital in South India. Eur Med J [Internet]. 2023 [cited 2026 May 18];8(1):84-92. Available from: https://www.emjreviews.com/microbiology-infectious-diseases/article/prevalence-of-carbapenem-resistant-enterobacteriaceae-and-the-genes-responsible-for-carbapenemase-production-in-a-tertiary-care-hospital-in-south-india/.
[27] Belouad EM, Benaissa E, El Mrimar N, Bssaibis F, Maleb A, Elouennass M. Predominance of OXA-48 carbapenemase-producing enterobacterales in a Moroccan Hospital. Int J Microbiol. 2023;2023:1-10.
[28] Gupta E, Mohanty S, Sood S, Dhawan B, Das BK, Kapil A. Emerging resistance to carbapenems in a tertiary care hospital in north India. Indian J Med Res. 2006;124(1):95-98.
[29] Nair PK. Prevalence of carbapenem resistant Enterobacteriaceae from a tertiary care hospital in Mumbai, India. J Microbiol Infect Dis. 2013;03(04):207-10.
[30] Abhishek S, Naik SS, Leela KV, Maheswary D. Genotypic distribution and antimicrobial susceptibilities of carbapenemase-producing enterobacteriaceae isolated in tertiary care hospital in South India. J Pure Appl Microbiol. 2022;16(4):2488-95.

PARTICULARS OF CONTRIBUTORS:
1. Senior Resident, Department of Microbiology, Jawaharlal Nehru Medical College, DMIHER, Wardha, Maharashtra, India.
2. Assistant Professor, Department of Microbiology, Jawaharlal Nehru Medical College, DMIHER, Wardha, Maharashtra, India.
3. Professor and Head, Department of Microbiology, Jawaharlal Nehru Medical College, DMIHER, Wardha, Maharashtra, India.
4. Junior Resident, Department of Microbiology, Jawaharlal Nehru Medical College, DMIHER, Wardha, Maharashtra, India.
5. Junior Resident, Department of Microbiology, Jawaharlal Nehru Medical College, DMIHER, Wardha, Maharashtra, India.

NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:
Dr. Gargi Mudey,
Professor and Head, Department of Microbiology, Jawaharlal Nehru Medical College, DMIHER, Sawangi Meghe, Wardha, Maharashtra, India.
E-mail: gargi.microbiology@dmiher.edu.in

PLAGIARISM CHECKING METHODS: [Jain H et al.]
- Plagiarism X-checker: Nov 25, 2025
- Manual Googling: Jun 13, 2026
- iThenticate Software: Jun 16, 2026 (1%)

ETYMOLOGY: Author Origin

EMENDATIONS: 8

AUTHOR DECLARATION:
- Financial or Other Competing Interests: As declared above
- Was Ethics Committee Approval obtained for this study? Yes
- Was informed consent obtained from the subjects involved in the study? NA
- For any images presented appropriate consent has been obtained from the subjects. NA

Date of Submission: **Sep 13, 2025**
Date of Peer Review: **Dec 13, 2025**
Date of Acceptance: **Jun 18, 2026**
Date of Publishing: **Oct 01, 2026**