

Original Article

Molecular characterization & risk factors associated with carbapenemase-producing enterobacterales acquisition in hospitalised patients in a tertiary care setting

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Background & objectives: Carbapenem-resistant Enterobacterales (CREs) have emerged as a major global public health threat, leading to increasing morbidity, mortality, and healthcare costs. This study aimed to characterise the molecular profile of CRE isolates and identify clinical and epidemiological risk factors associated with the acquisition of carbapenemase-producing Enterobacterales (CPE).

Methods: This cross-sectional study was conducted from October 2023 to July 2024. Clinical isolates from hospitalized patients were identified and speciated using standard microbiological procedures and confirmed by the VITEK-2 compact. Confirmed CRE isolates were subjected to multiplex PCR for carbapenemase gene detection. Statistical analysis included bivariate and multivariate logistic regression to determine independent predictors of CPE acquisition.

Results: Among 104 Enterobacterales isolates, 58 (55.8%) were Carbapenemase producing (CP)-CRE, and 46 (44.2%) were non-CP-CRE. *K. pneumoniae* (40/58, 69.0%) and *E. coli* (13/58, 22.4%) were the predominant CPE species. Molecular analysis revealed that 34 (58.6%) of CPE isolates harboured only *bla*_{NDM}, 4 (6.9%) carried *bla*_{OXA-48}, and 20 (34.5%) exhibited coproduction of multiple carbapenemases. Significant risk factors for CPE acquisition included prolonged hospital stay ≥ 5 days; odds ratio (OR)=103.81, $P=0.009$], presence of surgical site infections (OR=76.78, $P=0.047$), prior carbapenem use (OR=48.61, $P=0.001$), and invasive procedures or indwelling devices (OR=34.37, $P=0.014$). CP-CRE isolates exhibited high resistance (70-90%) to fluoroquinolones, aztreonam, aminoglycosides, and tetracyclines, moderate resistance to tigecycline, and the lowest to colistin (2/58, 3.5%).

Interpretation & conclusions: This study revealed a high prevalence of CP-CRE, predominantly *bla*_{NDM}-producing *K. pneumoniae*. CP-CRE infections were associated with multidrug resistance, increased mortality, and risk factors like prolonged hospitalisation, surgical procedures, and previous carbapenem exposure.

Key words Carbapenemase - carbapenemase - resistant enterobacterales - hospital - acquired infections - NDM - risk factors

World Health Organization (WHO) has classified carbapenem-resistant Enterobacterales (CRE) as the highest priority due to their gene transfer capabilities, severity of associated infections, and significant global

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impact, particularly in lower-middle-income countries, necessitating urgent attention¹. There are significant differences in the CRE epidemiology and treatment efficacy among various resistance mechanisms in CRE². Carbapenemase production is recognized as the primary resistance mechanism in CRE, which is particularly challenging to treat because of limited therapeutic options, leading to increased difficulty and cost in patient management³. CRE strains that do not produce carbapenemases may exhibit resistance due to other β -lactamases such as extended-spectrum β -lactamases (ESBLs), AmpC β -lactamases, or certain OXA-type β -lactamases, in combination with porin loss or efflux pump overexpression, for which relatively more effective and affordable treatment options are available⁴. Carbapenemase-producing CRE (CP-CRE) is a significant threat among CRE infections in healthcare, as carbapenemase genes are predominantly located on mobile genetic elements and plasmids, greatly enhancing their ability to transfer horizontally across the same/different species. In contrast, non-CP-CRE demonstrate reduced fitness, significantly limiting their potential to spread within healthcare settings⁵. Therefore, it is crucial to understand the characteristics that distinguish CP-CRE from non-CP-CRE to develop and implement an effective strategy for CP-CRE management.

The molecular classification of β -lactamases categorizes carbapenemases into class A (mostly $bla_{KPC-type}$), B (metallo- β -lactamases such as bla_{NDM} , bla_{IMP} , and bla_{VIM} types), and D ($bla_{OXA48-like}$)³. The types of carbapenemases produced by CRE strains vary geographically across different countries and regions. As each carbapenemase enzyme exhibits distinct hydrolytic activity and resistance profiles, treatment regimens should be tailored according to the specific carbapenemase variant⁶. They are rarely detected and might not be treated appropriately due to inadequate health system capacity in India⁷. Identifying the associated molecular determinants is central to the treatment and control of CP-CRE⁸.

Understanding the regional variations in carbapenemase types produced by CRE and the clinical and epidemiological risk factors associated with CP-CRE acquisition is essential for promptly administering appropriate empirical antibiotic therapy and implementing robust infection control practices to prevent their spread. To date, no studies have been conducted on this issue in this part of India; therefore, we have planned this study to assess the molecular, clinical, and epidemiological characteristics of CP-CRE isolates.

Materials & Methods

This comparative cross-sectional study was conducted at the department of Microbiology, Indira Gandhi Medical College, a 822-bed tertiary care hospital in Nagpur, Maharashtra, India, from October 2023 to July 2024 after obtaining the ethical approval from the Institutional Ethics Committee.

Study design and selection of patients: All types of clinical samples from hospitalized patients were processed using standard microbiological procedures. For identification and speciation, bacterial isolates were subjected to a conventional biochemical panel and confirmed by the VITEK-2 compact (BioMérieux, France).

The study included only the non-duplicate Enterobacterales isolates and excluded non-Enterobacterales gram-negative bacteria, commensal Enterobacterales species for the respective site of infection, and all gram-positive bacteria. The study also excluded Enterobacterales isolates from outpatients.

Antimicrobial susceptibility testing (AST) and Quality control: The confirmed Enterobacterales species were subjected to routine AST using the Kirby-Bauer disk diffusion method on Muller-Hinton agar using commercially available antimicrobial disks (Hi-Media Mumbai, India). Isolates resistant to carbapenems (meropenem, imipenem, or ertapenem) were subjected to the modified carbapenemase inactivation method (mCIM) for the identification of phenotypic carbapenemase production. MBL producers were identified phenotypically by a combined disk test using meropenem (10 μ g) and meropenem-EDTA (10/750 μ g) disk. If the inhibition zone of the carbapenem-EDTA combination disk is ≥ 5 mm compared to that of the carbapenem disk, it is considered an MBL producer (Fig. 1). Colistin minimum inhibitory concentration (MIC) testing was performed using a colistin broth dilution. *E. coli* ATCC 25922 (Hi-Media) served as the quality control strain for both disk diffusion, mCIM, and MIC testing, with each set of tests performed on *Enterobacterales* species. The sizes of the inhibition zones and the MIC results were interpreted according to the CLSI 2023 guidelines⁹.

Detection of carbapenemase genes: The Enterobacterales species that were resistant to meropenem (10 μ g), imipenem (10 μ g), or ertapenem (10 μ g) were subjected to Multiplex-PCR (Eppendorf Master cycler EPS thermos-module, Hamburg,

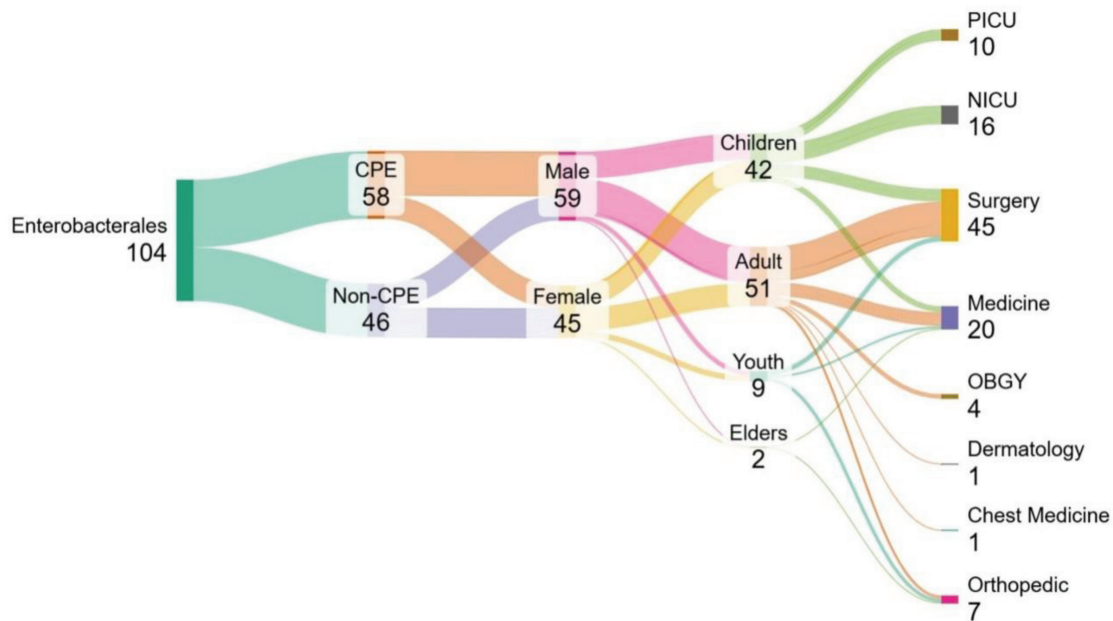


Fig. 1. Sankey diagram illustrating the demographic characteristics of the study population. (Source: SakeyMATIC.com).

Germany) for detection of the carbapenemase gene (*bla_{NDM}*, *bla_{KPC1}*, *bla_{KPC2}*, *bla_{IMP}*, *bla_{VIM}*, and *bla_{OXA-48}*) using the gene-specific primers (Supplementary Table). Amplicons from PCR were subjected to agarose gel electrophoresis for analysis, and a 100 bp DNA ladder was used for size comparison. DNA extracted from *K. pneumoniae* ATCC BAA-1705 and *K. pneumoniae* ATCC BAA-2146 was used as a positive control for the detection of KPC-2 and NDM. For IMP, VIM, KPC-1, and OXA-48 in-house isolates previously identified as positive by Sanger Sequencing for the presence of the mentioned genes were used as positive control.

Data collection: The clinical and demographic information of the patients included in the study was obtained from the medical records of the patients in the respective wards or intensive care units. The data analysed included demographic details (age, gender, and ward/unit of admission). Age groups were categorised as children (0-15 yr), youth (16-24 yr), adults (25-64 yr), and elders (≥ 65 yr); day of signs/symptoms of infection from date of admission to classify the infection into hospital-associated (HAI) if positive culture after two calendar days of hospitalisation, and community-acquired (CAI) if positive culture within two calendar days of hospitalization; comorbidities and underlying conditions, including central nervous system/neurologic disease, cardiovascular disease, cerebrovascular disease, respiratory disease,

intra-abdominal disease (gastrointestinal disease, hepatobiliary disease), genitourinary disease, diabetes mellitus, malignancy, haemodialysis, and presence of chronic wounds/nonhealing ulcers; history of previous hospitalisation within the past three months of index admission; history of broad-spectrum antibiotics use within three months of acquisition and their corresponding duration of therapy, including carbapenems, vancomycin, third and fourth-generation cephalosporins, and fluoroquinolones; invasive procedures (surgery, colonoscopy, and endoscopy) during the past three months of index admission; the invasive devices include urinary catheterization, mechanical ventilation, and central venous catheterization, and their insertion duration ranges from index admission day to acquisition of infection; and the length of hospital stay (LOS) before acquisition during the same index admission.

Statistical analysis: CRE-infected patients were grouped into CPE-infected cases and non-CP-CRE-infected controls to identify independent risk factors associated with CP-CRE acquisition in hospitalized patients in our institute.

Descriptive statistics for categorical variables are presented as frequency (percentage) and continuous variables as median (interquartile range, IQR). Using bivariate analysis, variables are compared between CP-CRE-infected and non-CP-CRE-infected to assess statistical significance by applying the chi-square test.

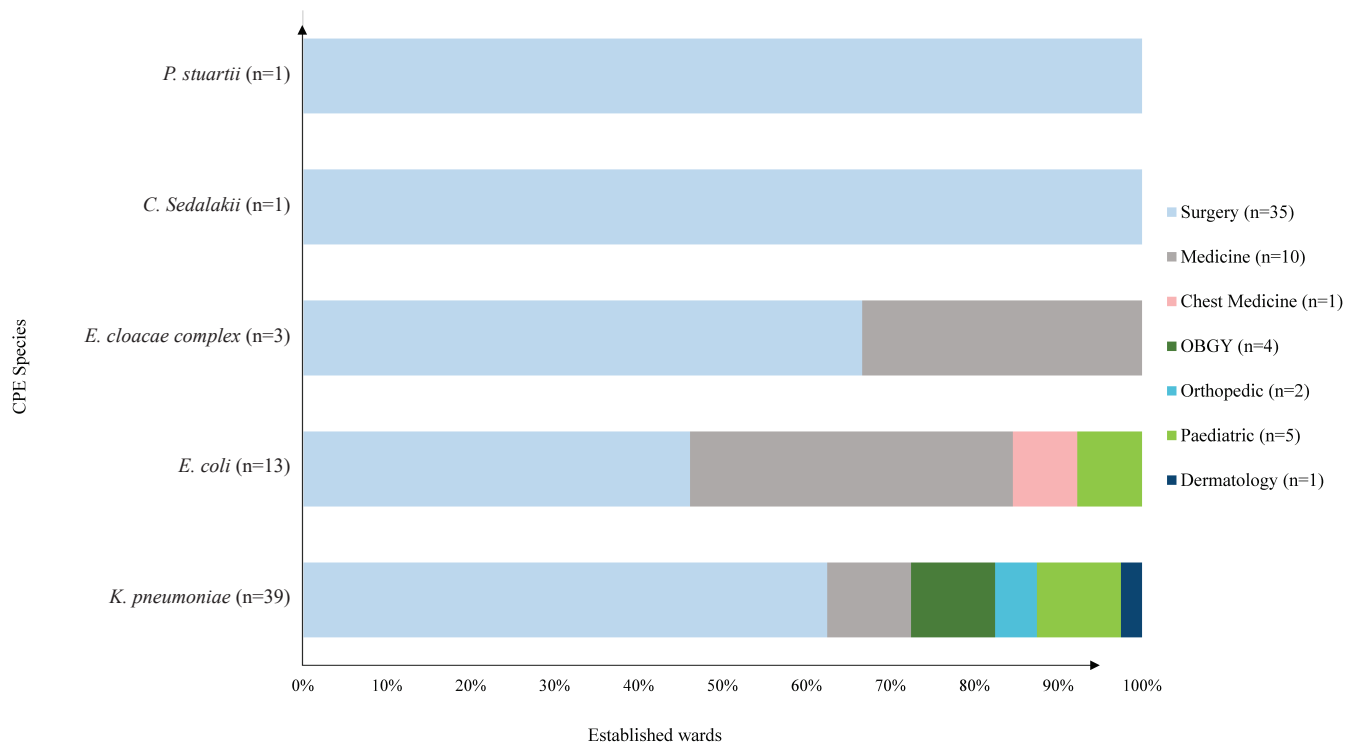


Fig. 2. Bar diagram showing the percentage of Carbapenemase-producing Enterobacteriales (CPE) species distribution across hospital established-wards.

P values < 0.05 in bivariate analysis were considered statistically significant and forwarded into multiple logistic regression analysis. Prior to conducting multivariate logistic regression, a correlation matrix was created to evaluate multicollinearity; all correlation coefficients were < 0.7 , indicating no significant multicollinearity among the variables. Adjusted odds ratio (OR), 95 per cent confidence interval (CI), and P value were calculated to predict CP-CRE acquisition based on various independent risk factors. The P value < 0.05 was considered statistically significant. STATA version 14.0 software (College Station, TX, US) was used for statistical analysis.

Results

Of 104 Enterobacteriales isolates included in the study, 58 (55.8%) were CP-CRE isolates and 46 (44.2%) were non-CP-CRE.

Demographic characteristics: Among 58 CP-CRE-infected patients, 65.5 per cent (38/58) were males, with the maximum (73.7%, 28/38) belonging to the adult age group, followed by children (13.2%, 5/38) and youth (10.5%, 4/38). Only one male elder of 82 yr. However, 34.5 per cent (20/58) were females, of whom most were adults (85%, 17/20), while only two

were children and one was an elder. CP-CRE strains were isolated from patients hospitalized in different established wards (Fig. 1).

In the non-CP-CRE infected group, there was a slight female preponderance (54.4%, 25/46), with most patients being children (68%, 17/25), followed by five youths (10.9%) and three adults (6.5%). However, among male patients (45.7%, 21/46), 18 were children, and three were adults. Most of the patients in this group were from paediatric wards (21/46, 45.7%), followed by other wards (Fig. 1).

Distribution of Enterobacteriales species: The obtained CP-CRE species are depicted in figure 2. Among non-CP-CRE isolates, the maximum isolates were *E. coli* (n=29), followed by *K. pneumoniae* (n=11), *Proteus mirabilis* (n=2), *Proteus vulgaris* (n=2), *E. cloacae complex* (n=1), and *Serratia marcescens* (n=1).

Molecular characterization of CP-CRE isolates: Of the CP-CRE isolates, 58.6 per cent (34/58) were only MBL-producers (bla_{NDM}), 6.9 per cent (4/58) were serine-carbapenemase producers (bla_{OXA-48}), and 34.5 per cent (20/58) were coproducers of MBL and serine-carbapenemases (including 17 $bla_{NDM+OXA-48}$, 1 $bla_{NDM+KPC-2}$, 1 $bla_{OXA-48+IMP}$, and 1 $bla_{NDM+KPC2+IMP}$).

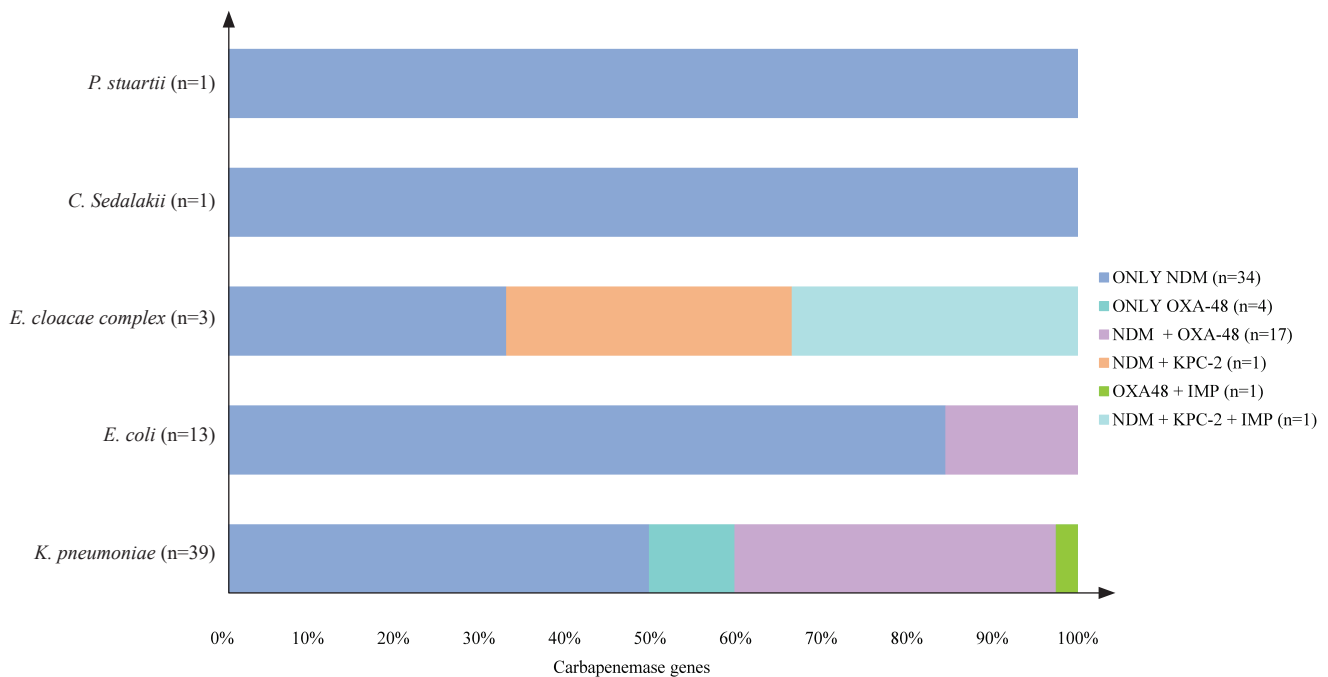


Fig. 3. Bar diagram showing the percentage of carbapenemase genes distribution across each Carbapenemase-producing Enterobacteriales species.

The distribution of carbapenemase genes among the various *Enterobacteriales species* and across different hospital-established wards is depicted in figures 3 and 4, respectively.

Comparison of clinico-epidemiological characteristics and risk factors between CP-CRE and non-CP-CRE is shown in table I. Independent risk factors identified in table I were subjected to multivariate logistic regression analysis to predict CPE acquisition.

The risk of CP-CRE acquisition was 104 times higher in patients with ≥ 5 days LOS in hospital; 35 times higher in CRE isolated from pus samples; 34 times higher in patients underwent invasive procedures or with indwelling devices; 77 times higher in surgical site infection (SSI), 21 times higher from chronic ulcer/wound; and 49 times higher in patients with history of carbapenem consumption (Table II).

Comparison of antimicrobial susceptibility of CP-CRE and non-CP-CRE isolates: Of 58 CP-CRE isolates, 55 (94.8%) were multidrug-resistant (MDR), 50 (86.2%) were extensively drug-resistant (XDR), and 16 (27.6%) were pan-drug-resistant (PDR). Almost all the isolates were resistant to routinely used cephalosporins, and BL/BL-inhibitors (except three isolates were sensitive to ceftazidime-avibactam). Monobactam resistance was observed in 91.4 per cent (53/58) isolates. However,

high resistance (70-95%) was observed against fluoroquinolones, aminoglycosides, tetracyclines, and sulfonamides classes of antimicrobials, with moderate resistance for tigecycline (63.8%) and the lowest against colistin (3.5%). None of the urinary isolates showed resistance to nitrofurantoin and fosfomycin (Table III).

In the non-CP-CRE group, 9 (19.6%) were MDR, and none of the isolates were XDR or PDR. Significant resistance was observed against amoxicillin/clavulanic acid, cefuroxime, piperacillin-tazobactam, and doxycycline. Low to moderate resistance (4-50%) was observed for the rest of the cephalosporin, aminoglycoside, and fluoroquinolone classes of drugs. The lowest resistance rates were observed in the non-CP-CRE group against ceftazidime, ceftriaxone, and tigecycline. None of the isolates showed resistance to ceftazidime-avibactam, aztreonam, colistin, nitrofurantoin, and fosfomycin (Table III).

Discussion

This study highlights a high prevalence of CP-CRE, with *K. pneumoniae* being the most commonly identified isolate. This finding is consistent with studies conducted in India and other regions¹⁰⁻¹⁴. International studies also indicate a high prevalence of CP-CRE, although colonization rates vary significantly, ranging from 17.5 per cent to 77.3 per cent^{15,16}. While

Table I. Bivariate analysis of clinical and epidemiological characteristics and risk factors associated with Carbapenemase-producing Enterobacterales				
Characteristics/Risk factors	CP-CRE (n=58), N (%)	Non-CP-CRE (n=46), N (%)	Odds ratio (95% Confidence Interval)	P value
	Cases	Controls		
Age group (yr)				
<25	11 (19.0)	39 (84.8)	-	< 0.001
≥ 25	47 (81.0)	7 (15.2)	23.80 (7.64-78.08)	
Length of stay in the hospital before acquisition during the index admission				
< 5 days	20 (34.5)	40 (87)	-	< 0.001
≥5days	38 (65.5)	6 (13)	12.66 (4.25-41.76)	
Clinical site of positive culture				
Pus/wound swab	36 (62.1)	15 (32.6)	3.38 (1.39-8.29)	0.003
Urine	10 (17.2)	25 (54.4)	0.17 (0.06-0.46)	<0.001
Drain fluid	2 (3.5)	1 (2.2)	1.60 (0.08-96.88)	0.7
Blood	4 (6.9)	2 (4.4)	1.62 (0.22-18.71)	0.58
Pleural fluid	2 (3.5)	1 (2.2)	1.60 (0.08-96.88)	0.7
Sputum/BAL	4 (6.9)	3 (6.5)	1.06 (0.17-7.63)	0.94
Ascitic fluid	1 (1.7)	0 (0)	NA	0.37
Site of acquisition of organism				
Community acquired	21 (36.2)	39 (84.8)	-	<0.001
Hospital acquired	37 (63.8)	7 (15.2)	9.81 (3.45-30.04)	
Specific infections				
Catheter associated UTI	7 (12.1)	2 (4.4)	3.01 (0.53-30.96)	0.16
Duration of catheterization (days) Median (IQR)	5 (3-25)	3.5 (3-4)		
Ventilator-associated Pneumonia	2 (3.5)	1 (2.2)	1.60 (0.08-96.88)	0.7
Duration of ventilation (days) Median (IQR)	9 (8-10)	4 (3-6)		
Central line-associated bloodstream infection	1 (1.7)	0 (0.0)	NA	0.37
Duration of central line (days) Median (IQR)	5	NA		
Catheter-related blood stream infection	2 (3.5)	0	NA	0.2035
Duration of intravenous access (days) Median (IQR)	5 (3-15)	NA		
Surgical site infection	25 (43.1)	4 (8.7)	7.95 (2.36-33.90)	<0.001
Duration of surgery (days) Median (IQR)	10 (6-30)	7 (6-10)		
Underlying disease				
Cardiovascular system disease	9 (15.5)	6 (13.0)	1.22 (0.35-4.54)	0.72
Respiratory disease	5 (8.6)	7 (15.2)	0.52 (0.12-2.10)	0.29
Central nervous system disease	1 (1.7)	0 (0.0)	NA	0.37
Intra-abdominal disease	14 (24.1)	8 (17.4)	1.51 (0.52-4.62)	0.40
Genito-urinary system disease	8 (13.8)	5 (10.9)	1.31 (0.34-5.49)	0.65
Chronic ulcer/wound	16 (27.6)	4 (8.7)	4.0 (1.14-17.60)	0.01

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Orthopedic disease	4 (6.9)	9 (19.6)	0.30 (0.06-1.20)	0.52
Other	1 (1.7)	7 (15.2)	0.9 (0.02-0.82)	0.01
Invasive procedures/indwelling devices	37 (63.8)	7 (15.2)	9.81 (3.45-30.04)	<0.001
History of the previous hospitalization during the past 3 months	14 (24.1)	8 (17.4)	1.51 (0.52-4.62)	0.40
Diabetes status				
Diabetic	9 (15.5)	7 (15.2)	1.02 (0.30-3.54)	0.97
Non-diabetic	49 (84.5)	39 (84.8)	-	
Cumulative broad-spectrum antibiotic use within 3 months before acquisition				
Penicillin	32 (55.2)	38 (82.61)	0.26 (0.08-0.70)	0.003
Duration of penicillin use (days) Median (IQR)	8 (6.5-14)	5 (3-7)		
Cephalosporins*	46 (79.3)	31 (67.4)	1.85 (0.69-4.96)	0.17
Duration of cephalosporins use (days) Median (IQR)	7 (5-10)	5 (3.5-7)		
Carbapenem (meropenem)	53 (91.4)	6 (13.0)	70.66 (17.75-304.43)	<0.001
Duration of carbapenem (meropenem) use (days) Median (IQR)	7 (5-15)	4 (3-7)		
Vancomycin	8 (13.8)	14 (30.4)	0.36 (0.12-1.06)	0.039
Duration of vancomycin use (days) Median (IQR)	7 (5-14)			
Quinolones	13 (22.4)	12 (26.09)	0.82 (0.30-2.24)	0.66
Duration of quinolones use (days) Median (IQR)	8 (5.5-10)	5 (4-8)		
Mortality	7 (12.1)	2 (4.4)	3.01 (0.53-30.96)	0.16
IQR, interquartile range				

Table II. Multiple logistic regression analysis for predicting CPE acquisition based on various independent risk factors (N=58)

Variables	Correlation (r)	Adjusted Odds ratio	95% Confidence interval	P value
Length of stay in the hospital	0.5	103.8	3.16 – 3408.94	0.009
Pus samples	0.4	35.3	1.78 – 697.12	0.019
Invasive procedures or devices	0.4	34.4	2.05 – 575.37	0.014
Surgical site infections	0.4	76.8	1.05 – 6387.99	0.047
Chronic ulcer/wound	0.3	21.2	1.02 – 435.10	0.048
Carbapenem use	0.5	48.6	4.51 – 523.63	0.001

CPE, carbapenemase-producing Enterobacterales

pneumoniae, *E. cloacae*, and *E. coli* are the dominant species within CP-CRE groups, the distribution of species differs among non-CP-CRE isolates¹²⁻²⁰.

Geographical variations in carbapenemase genes have been reported, with the Indian subcontinent recognized as a reservoir for *bla*_{NDM}-producing CRE^{14,21-23}. In our study, the majority of CP-CRE strains carried the *bla*_{NDM} gene and demonstrated multidrug resistance, leaving only a few limited treatment options, such as aztreonam, tigecycline, or colistin^{13,24}.

The predominance of isolates from surgical units and pus samples, particularly those related to surgical site infections (SSIs), underscores the risks associated with prolonged hospital stays, wound exposure, and lapses in infection control. This underscores the need to address these wards as potential hotspots for the spread of these infections.

Limited molecular diagnostic capabilities in India hinder the routine identification of resistance genes. As a result, clinicians often rely on empirical treatment

Table III. Comparison of antimicrobial susceptibility of CP-CRE and non-CP-CRE isolates

Antimicrobial class	Antimicrobial name	CP-CRE (n=58)	non-CP-CRE (n=46)	P value
		Resistance, N (%)	Resistance, N (%)	
Cephalosporins	Cefuroxime	58 (100)	30 (65.2)	<< 0.0001
	Cefpodoxime	58 (100)	12 (26.1)	<0.0001
	Ceftazidime	58 (100)	2 (4.4)	<0.0001
	Ceftriaxone	58 (100)	5 (10.9)	<<0.0001
	Cefepime	58 (100)	12 (26.1)	<0.0001
	Cefixime	58 (100)	23 (50)	<<0.0001
	Cefoxitin	58 (100)	12 (26.1)	<0.0001
β-lactam/β-lactamase inhibitor	Ceftazidime/Avibactam	57 (98.3)	0	<0.0001
	Piperacillin/Tazobactam	58 (100)	30 (65.2)	<<0.0001
	Amoxicillin/Clavulanic acid	58 (100)	44 (95.7)	0.051
Monobactam	Aztreonam	53 (91.4)	0	<0.0001
Aminoglycosides	Amikacin	44 (75.9)	23 (50)	0.006
	Gentamicin	42 (72.4)	23 (50)	0.019
Fluoroquinolones	Levofloxacin	55 (94.8)	16 (34.8)	<<0.0001
	Ciprofloxacin	54 (93.1)	14 (30.4)	<<0.0001
Tetracyclines	Doxycycline	58 (100)	30 (65.2)	<< 0.0001
	Tigecycline	37 (63.8)	4 (8.7)	<<0.0001
Folate-pathway inhibitors	Trimethoprim/Sulfamethoxazole	45 (77.6)	20 (43.5)	0.0001
Lipopeptides	Colistin	2 (3.5)	0	0.2035
Nitrofurans	*Nitrofurantoin	0 (0.0)	0	NA
Fosfomycins	*Fosfomicin	0 (0.0)	0	NA

*For urinary isolates only; CP-CRE, carbapenemase-producing carbapenem-resistant enterobacterales; non-CP-CRE, non-Carbapenemase-producing Carbapenem-resistant Enterobacterales

regimens, such as piperacillin-tazobactam, ceftriaxone, cefotaxime, meropenem, vancomycin, ciprofloxacin, doxycycline, and azithromycin, which are frequently ineffective against CP-CRE. The significantly higher mortality rates (Table I) observed in CP-CRE patients in this study support previous findings indicating worse outcomes of CP-CRE bacteraemia compared to those with non-CP-CRE infections^{12,13,24-26}. Therefore, categorizing isolates by their resistance mechanisms is crucial for optimal clinical management.

We identified six independent risk factors for CP-CRE acquisition: a hospital stay of ≥ 5 days, prior surgery, exposure to carbapenems within three months, presence of pus isolates, invasive procedures, and chronic ulcers/wounds. These findings align with previous research, reinforcing the significance of these risk factors in the context of CP-CRE transmission^{12,14,23,25-27}. Our susceptibility analysis demonstrated notable differences between CP-CRE

and non-CP-CRE strains, emphasizing the necessity for tailored empirical treatment strategies. Given the high prevalence of *bla*_{NDM} and *bla*_{NDM+OXA-48} genotypes in our isolates, this study recommends the use of aztreonam/avibactam for treating patients at high risk of CP-CRE infections. For patients deemed lower risk, ceftazidime/avibactam may be an adequate therapeutic option^{6,28}. This targeted approach will not only improve patient outcomes but also help in managing the rising threat of carbapenem-resistant infections.

The study has several limitations. First, it is a single arm study that included only inpatient isolates, which may not represent the epidemiology of CRE in the community or across other healthcare settings in central India. Second, unequal sample sizes of the CP-CRE and non-CP-CRE groups, reflecting the actual isolate distribution, which may have affected statistical power. Third, the PCR assay did not include rarer variants (e.g., bla_{NDM-5}, bla_{OXA-181}, bla_{GES}) or other reported

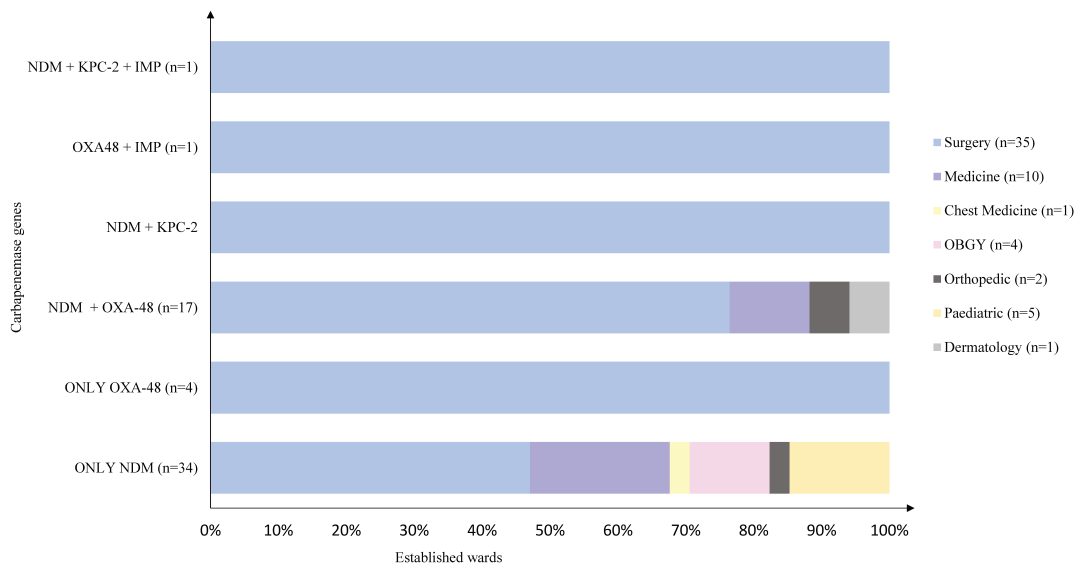


Fig. 4. Bar plots showing percentages of carbapenemase genes circulated across hospital established wards.

determinants, which might have led to under-detection of certain carbapenemase producers. Finally, the study did not investigate alternative resistance mechanisms in non-CP-CRE isolates, such as AmpC production, porin loss, or efflux pump overexpression, which may also contribute to carbapenem resistance.

The findings of this study underscore the need for robust infection prevention practices and the implementation of diagnostic and antimicrobial stewardship to limit the use of inappropriate and over-the-counter antibiotics. While most studies recommend screening for CRE-carriers and cohorting CRE-infected or colonized patients to prevent the spread of CP-CRE, these measures can be challenging to implement in both low-middle and high-income countries. Therefore, priority should be given to more cost-effective prevention strategies, such as identifying individual risk factors, reinforcing compliance with hand hygiene, environmental cleaning, and promoting antibiotic stewardship²⁹. Future prospective matched case-control studies are needed to better understand risk factors and infection outcomes.

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