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Phenotypic Characterization of Carbapenem Resistant *Klebsiella pneumoniae* Isolated from Critical Care Areas in a Tertiary Care Teaching Hospital in Eastern Gujarat

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ABSTRACT

Carbapenem Resistant Klebsiella pneumoniae (CRKP) has emerged as a critical public health threat, particularly in intensive care settings. Carbapenems represent the last-resort treatment for multidrug-resistant Gram-negative infections, and resistance to these agents severely limits therapeutic options.

Objectives: To isolate and phenotypically characterise CRKP from clinical specimens received from critical care areas, and to determine the prevalence of metallo-β-lactamase (MBL) and Klebsiella pneumoniae carbapenemase (KPC) using phenotypic detection methods.

Methods: 200 Klebsiella pneumoniae clinical isolates from critical care areas of Zydus Medical College and Hospital, Dahod were processed using conventional phenotypic identification methods. Antimicrobial susceptibility testing was performed by the Kirby Bauer Disk Diffusion method and interpreted per CLSI guidelines 2024/2025. Carbapenem resistance was confirmed and carbapenemase genes were detected using Epsilometer (E-test) strips with EDTA (MBL) and Boronic Acid (KPC).

Results: Of 200 isolates, 113 (56.5%) were carbapenem resistant. The highest prevalence was found in urine (26.6%), pus (24.78%), and sputum (23.9%) samples. The Medical ICU contributed the majority (55.7%) of CRKP isolates. Most patients (71.7%) were below 60 years of age, and 67.26% had a history of frequent or recent hospitalization. MBL genes were detected in 12 isolates (11%) and KPC genes in 2 isolates (1.77%).

Conclusion: The high prevalence of CRKP in critical care units underscores the urgent need for robust antimicrobial stewardship programs, strict infection control practices, and hospital-specific antibiotic policies. Phenotypic methods remain practical and cost-effective tools for CRKP detection in resource-limited settings.

Keywords: *Carbapenem resistance, Klebsiella pneumoniae, MBL (Metallo β Lactamase), KPC (Klebsiella pneumoniae Carbapenemase), Phenotypic characterization, ICU (Intensive Care Unit), Antimicrobial Resistance, Eastern Gujarat.*

INTRODUCTION

Antibiotic resistance represents one of the most pressing global public health crises of the 21st century. Among the most formidable contributors to this challenge is *Klebsiella pneumoniae*, a Gram-negative encapsulated bacterium belonging to the Enterobacteriaceae family. Although it normally colonizes the human gut as a commensal organism without causing harm, it manifests as a dangerous opportunistic pathogen in hospitalized patients—particularly those in intensive care units (ICUs)—causing a spectrum of infections including pneumonia, urinary tract infections (UTIs), bloodstream infections, and wound infections.

The clinical significance of *Klebsiella pneumoniae* has escalated dramatically in recent decades due to the emergence of strains resistant to carbapenem—a class of broad-spectrum β-lactam antibiotics traditionally reserved as last-resort therapy for severe infections. Carbapenem Resistant *Klebsiella pneumoniae* (CRKP) arises primarily through the production of carbapenemase enzymes such as New Delhi Metallo-β-lactamase (NDM), *Klebsiella pneumoniae* Carbapenemase (KPC), Verona Integron-encoded Metallo-β-lactamase (VIM), and Oxacillinase-48 (OXA-48), in addition to alterations in outer membrane permeability and active drug efflux mechanisms. The horizontal transmission of resistance genes via mobile genetic elements such as plasmids and transposons further amplifies the spread of resistance within hospital environments. India carries a disproportionately high burden of CRKP infections, with critical care areas serving as hotspots for transmission. In regions like Eastern Gujarat, where tertiary care hospitals serve large rural and tribal populations, the emergence of drug-resistant pathogens in ICUs poses a particularly serious threat. Phenotypic detection methods—including the Modified Hodge Test (MHT), Carbapenem Inactivation Method (CIM), Disk Diffusion, and the Epsilometer Test (E-test)—remain essential for routine clinical microbiology laboratories due to their cost-effectiveness and accessibility, compared to expensive molecular methods such as PCR and whole-genome sequencing.

This study was undertaken to determine the prevalence and phenotypic characteristics of CRKP isolated from critical care areas of a tertiary care teaching hospital in Eastern Gujarat, and to evaluate carbapenemase gene detection using E-test strips. The findings aim to inform local antibiotic policies and infection control strategies.

MATERIALS AND METHODS

1. Study Design and Setting

This was a cross-sectional observational study conducted at the Central Laboratory, Department of Microbiology, Zydus Medical College and Hospital, Dahod, Gujarat, India, from October 2023 to November 2025. The study included 200 *Klebsiella pneumoniae* isolates from clinical specimens received from various critical care areas.

2. Specimen Collection and Culture

Clinical specimens including blood, urine, sputum, pus, endotracheal aspirate (ET), tracheostomy tube aspirate (TT), pleural fluid, and pericardial fluid were processed by standard microbiological techniques. Specimens were subjected to direct Gram staining and inoculated on MacConkey Agar (HiMedia M082), Blood Agar, and Nutrient Agar (HiMedia M001). Plates were incubated at 37°C for 18–24 hours and colonial morphology was recorded.

3. Identification of *Klebsiella pneumoniae*

Identification was based on colonial morphology, Gram stain characteristics, and biochemical reactions. *Klebsiella pneumoniae* was identified by the following profile: Gram-negative, encapsulated, non-motile thick stout bacilli; lactose-fermenting mucoid colonies on MacConkey agar; negative Indole and Oxidase; positive Citrate utilization, Urease, Lysine Decarboxylase, and Arginine Dihydrolase; TSI showing Acid/Acid with gas production and no H_2S. Quality control was performed using *Klebsiella aerogenes* ATCC 15048.

4. Antimicrobial Susceptibility Testing

Antimicrobial susceptibility testing was performed by the Kirby Bauer Disk Diffusion method on Mueller Hinton Agar (MHA, HiMedia M173) using a panel of 22 antibiotics across multiple drug classes (cephalosporins, fluoroquinolones, aminoglycosides, carbapenems, tetracyclines, and others). Results were interpreted according to CLSI guidelines 2024 and 2025. Isolates showing resistance to Meropenem (10 µg), Imipenem (10 µg), and/or Ertapenem (10 µg) were classified as CRKP.

5. Confirmation by Epsilometer Test (E-test)

All CRKP isolates were further confirmed using Ezy MIC E-test strips on MHA incubated at 37°C for 18–24 hours. The minimum inhibitory concentration (MIC) was read at the intersection of the elliptical inhibition zone with the strip:

- MBL detection: Meropenem+EDTA/Meropenem E-test strips (EM092) and Imipenem+EDTA/Imipenem E-test strips (EM078). A ≥8-fold reduction in MIC in the presence of EDTA was considered positive.
- KPC detection: Ertapenem/Ertapenem+Boronic Acid E-test strips (EM14). A ≥8-fold reduction in MIC in the presence of Boronic Acid was considered positive.

RESULTS

Overall Prevalence

Of 200 *Klebsiella pneumoniae* isolates processed from critical care areas, 113 (56.5%) were identified as Carbapenem Resistant *Klebsiella pneumoniae* (CRKP) and 87 (43.5%) were carbapenem sensitive.

Table 1: Prevalence of CRKP among *Klebsiella pneumoniae* isolates (n=200)

Category	No. of Isolates	Percentage (%)
Carbapenem Sensitive *Klebsiella pneumoniae*	87	43.5%
Carbapenem Resistant *Klebsiella pneumoniae* (CRKP)	113	56.5%
Total	200	100%

Sample-wise Distribution of CRKP

Among the 113 CRKP isolates, urine samples yielded the highest proportion (30; 26.6%), followed by pus (28; 24.78%), sputum (27; 23.9%), blood (14; 12.37%), endotracheal aspirate (7; 6.19%), tracheostomy tube aspirate (5; 4.4%), and pleural and pericardial fluid (1 each; 0.88%).

Table 2: Sample-wise distribution of CRKP isolates (n=113)

Sample Type	No. of CRKP	Percentage (%)
Blood	14	12.37%
Urine	30	26.6%
Sputum	27	23.9%
Pus	28	24.78%
Endotracheal Aspirate	7	6.19%
Tracheostomy Tube Aspirate	5	4.4%
Pleural Fluid	1	0.88%
Pericardial Fluid	1	0.88%
Total	113	100%

Age Distribution

The majority of CRKP isolates were recovered from patients in the 18–59 year age group (71; 62.83%), followed by those ≥60 years (32; 28.31%), 1 month–18 years (8; 7.07%), and neonates 0–30 days (2; 1.8%). Overall, 81 (71.7%) patients were below 60 years of age.

Table 3: Age-wise distribution of CRKP isolates (n=113)

Age Group	No. of Isolates	Percentage (%)
0–30 days (Neonates)	2	1.8%
1 month – 18 years	8	7.07%
18–59 years	71	62.83%
60 years and above	32	28.31%

Critical Area-wise Distribution

The Medical ICU (MICU) accounted for the largest share of CRKP isolates (63; 55.7%), followed by the Surgical ICU (SICU) (26; 23%), OT Recovery Room (11; 9.74%), Obstetric ICU (6; 5.3%), Paediatric ICU (3; 2.66%), ICCU (2; 1.8%), and Neonatal ICU (2; 1.8%).

Table 4: Critical area-wise distribution of CRKP isolates (n=113)

Critical Care Area	No. of Isolates	Percentage (%)
Medical ICU (MICU)	63	55.7%
Surgical ICU (SICU)	26	23%
OT Recovery Room	11	9.74%
Obstetric ICU	6	5.3%
Paediatric ICU (PICU)	3	2.66%
Intensive Critical Care Unit (ICCU)	2	1.8%
Neonatal ICU (NICU)	2	1.8%

Hospitalization History

76 (67.26%) CRKP-positive patients had a history of frequent or recent hospitalization due to co-morbidities or inadequate follow-up care. Only 37 (32.74%) had no prior hospitalization history.

Carbapenemase Gene Detection

Among the 113 CRKP isolates, MBL genes were detected in 12 (11%) and KPC genes in 2 (1.77%) isolates using phenotypic E-test methods.

Table 5: Carbapenemase gene detection by E-test in CRKP isolates (n=113)

Sample Type	KPC Detected	MBL Detected	Neither
Blood	1	2	11
Urine	0	4	26
Sputum	0	3	24
Pus	0	1	27
Endotracheal Aspirate	1	0	6
Tracheostomy Tube Aspirate	0	1	4
Pericardial Fluid	0	1	0
Total	2 (1.8%)	12 (11%)	99

DISCUSSION

The present study recorded a CRKP prevalence of 56.5% among 200 *Klebsiella pneumoniae* isolates from critical care areas—a figure considerably higher than reports from Mumbai (26%, Prabhala et al., 2023) and China (33%, Wang et al., 2018). This elevated prevalence is likely reflective of the high antibiotic selection pressure within ICU settings, the referral pattern of critically ill patients from rural and tribal districts of Eastern Gujarat, and the prolonged empirical antibiotic use in complex cases.

Urine (26.6%) and Pus (24.78%) were the most common sources, consistent with findings from Patil et al. (2022) from Indore and Cölkesen et al. (2023) from Turkey. This pattern is attributable to prolonged catheterization, biofilm formation by CRKP on medical devices, and cross-contamination from respiratory or gastrointestinal colonization sites. Sputum contributed 23.9% of isolates, reflecting the high prevalence of ventilator-associated pneumonia in MICU patients.

The predominance of CRKP in the MICU (55.7%) aligns with the complex clinical profiles of these patients, including mechanical ventilation, central venous catheterization, and broad-spectrum antibiotic exposure. Ibik et al. (2023) from Turkey reported a higher SICU proportion (35%), likely due to differing patient demographics and case mix. Age-wise,

71.7% of affected patients were below 60 years, comparable to Wang et al. (2018) (65.7%), suggesting that younger working-age adults requiring prolonged ICU care for trauma, sepsis, or post-operative complications are a key at-risk group.
The high rate of recent or repeated hospitalization (67.26%) among CRKP patients, compared to 40.6% reported by Qureshi et al. (2012) in the USA, highlights how prior healthcare exposure drives acquisition of multidrug-resistant organisms. Repeated antibiotic courses and prolonged device use compound the risk.

Kirby Bauer disk diffusion identified 56.5% of isolates as CRKP, results concordant with E-test confirmation. This is higher than Gupte et al. (2016) at 14% from Punjab, but comparable to Elmanakhly et al. (2022) at 46% from Egypt and lower than Patil et al. (2022) at 71% from Indore—variability attributable to differences in patient populations, regional antibiotic practices, and the volume of critical care referrals.

MBL gene positivity by phenotypic E-test was observed in 11% of CRKP isolates. This is markedly lower than Girlich et al. (2013) from France (47%) and Esra C. et al. (2019) from Turkey (43%), which may reflect a true epidemiological difference or the inherent limitations of phenotypic methods for MBL detection compared to molecular assays. KPC detection (1.77%) was comparable to Wang et al. (2018) at 1.37%, suggesting KPC remains less prevalent in this region compared to MBL-type resistance. The remaining CRKP isolates lacking detected carbapenemase genes may harbor resistance through porin loss, efflux pump overexpression, or non-phenotypically detectable carbapenemases—reinforcing the need for molecular characterization as a future direction.

Treatment of CRKP infections is severely constrained. Available options include colistin, tigecycline, aztreonam, and newer combination agents such as ceftazidime-avibactam and meropenem-vaborbactam. Combination regimens have demonstrated superior outcomes in several international studies and are recommended for serious CRKP infections where individual agents are insufficient.

CONCLUSIONS

This study demonstrates a high prevalence of Carbapenem Resistant *Klebsiella pneumoniae* (56.5%) in critical care areas of a tertiary care teaching hospital in Eastern Gujarat. The Medical ICU represents the most affected area, with urine and pus as the most common sample sources. Younger and middle-aged adults with frequent hospitalization history are at highest risk. While MBL gene positivity (11%) exceeds KPC (1.8%), a large proportion of CRKP isolates may carry undetected resistance mechanisms, highlighting the need for molecular studies.

Phenotypic methods including the Kirby Bauer disk diffusion and E-test remain valuable, accessible tools for CRKP detection in resource-limited settings. Comprehensive infection control strategies—encompassing hand hygiene, contact precautions, environmental decontamination, and antimicrobial stewardship—are indispensable for containing CRKP dissemination. Establishment of hospital-specific antibiotic policies informed by local surveillance data is essential to preserving the efficacy of existing therapeutic agents.

DECLARATIONS

Ethical approval

Ethical clearance was granted by the Institutional Ethics Committee of Zydus Medical College and Hospital (ZMCH/IEC/01/06(04)-2025).

Conflict of interest

None declared.

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Authors' contributions

1: Data collection, Clinical Correlation.
2: Conceptualisation, Manuscript writing.
3: Data Analysis.
4: Manuscript review and editing.

All authors approved the final manuscript.

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