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Microbiological Profile of *Klebsiella pneumoniae* in Intensive Care Units: A Cross-Sectional Study

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ABSTRACT

Background: Patients in intensive care units (ICUs) are highly susceptible to severe infections due to critical illness and invasive interventions. *Klebsiella pneumoniae* has emerged as a major nosocomial pathogen with increasing antimicrobial resistance, contributing to substantial morbidity and mortality.
Aim: To determine the distribution of *Klebsiella pneumoniae* in ICUs and assess carbapenemase production and colistin susceptibility among the isolates.
Methods: This hospital-based retrospective observational study was conducted over 6 months, from August 2025 to January 2026, in the ICUs of a tertiary care hospital. A total of 225 *K. pneumoniae* isolates recovered from clinical samples were included. Identification was performed using standard microbiological methods. Antimicrobial susceptibility testing was performed using the modified Kirby–Bauer disk diffusion and broth microdilution methods, in accordance with the guidelines of the Clinical and Laboratory Standards Institute (CLSI). Carbapenemase production was detected using phenotypic methods, and colistin susceptibility was determined by broth microdilution. Isolates were classified as multidrug-resistant (MDR), extensively drug-resistant (XDR), or pan-drug-resistant (PDR).
Results: The majority of isolates were recovered from the MICU (46.7%) and NICU (36.0%). Blood (50.22%) and respiratory samples (28.89%) were the most common sources. High resistance rates were observed to cephalosporins (>93%), ciprofloxacin (91.56%), aminoglycosides (75–82%), and carbapenems (73–75%). Resistance to colistin was minimal (1.33%). Carbapenemase production was detected in 37.5% of the isolates, with metallo-β-lactamase being the predominant type. XDR isolates were the most common (60.45%), followed by MDR isolates (29.33%), while PDR isolates were relatively few (1.33%).
Conclusion: A high burden of XDR *K. pneumoniae*, along with substantial carbapenem resistance, was observed in the ICUs. Colistin remained highly active against the isolates. Strengthening antimicrobial stewardship, infection prevention and control measures, and surveillance is essential to curb the emergence and spread of antimicrobial resistance.

Keywords: Klebsiella pneumoniae; Intensive Care Unit (ICU); Antimicrobial resistance (AMR); Carbapenemase production; Colistin susceptibility.

INTRODUCTION

Patients admitted to intensive care units (ICUs) are critically ill and frequently subjected to invasive procedures, making them highly susceptible to severe infections caused by virulent and opportunistic pathogens. The burden of infections in ICUs is further compounded by the increasing prevalence of antimicrobial resistance, which significantly complicates treatment, prolongs hospital stay, increases healthcare costs, and contributes to higher morbidity and mortality rates.[1]

Among the important nosocomial pathogens, *Klebsiella pneumoniae* has emerged as a major clinical concern in recent years due to its increasing resistance to multiple antibiotics and its association with serious clinical outcomes.[2] It is a Gram-negative, non-motile, capsulated, aerobic, and non-spore-forming bacillus belonging to the Enterobacterales group. *K. pneumoniae* is implicated in a wide range of infections, including hospital-acquired pneumonia, urinary tract infections, bloodstream infections, gastrointestinal infections, and soft tissue infections, accounting for a significant proportion of nosocomial infections and associated morbidity and mortality.[3]

The organism is particularly concerning due to its ability to acquire and disseminate multiple antibiotic resistance genes, leading to the emergence of multidrug-resistant (MDR) strains. This poses a growing public health threat, especially in developing countries like India.[4]

Globally, drug-resistant Enterobacterales, particularly *Klebsiella* species, have become a major public health concern, especially in ICU settings where antibiotic pressure is high.[5] The rising trend of antimicrobial resistance necessitates continuous surveillance to monitor susceptibility patterns and guide appropriate antibiotic therapy. Surveillance is essential because antibiotic usage in both hospital and community settings directly influences resistance trends and therapeutic effectiveness.[6]

Given its clinical importance, increasing resistance, and impact on patient outcomes, studying the distribution and antimicrobial susceptibility patterns of *K. pneumoniae* in ICU settings is essential for guiding infection control strategies and optimizing treatment outcomes.

Aims And Objective

The study aimed to determine the distribution of *Klebsiella pneumoniae* in various intensive care units of a hospital and to detect carbapenemase production along with assessing colistin susceptibility among these isolates.

MATERIALS & METHODS

This hospital-based retrospective observational study with microbiological analysis was conducted in the Intensive Care Units (ICUs) of a tertiary care hospital over a period of 6 months from August 2025 to January 2026.

Inclusion and Exclusion Criteria

Inclusion criteria:

- Clinical samples (blood, urine, respiratory secretions, pus, etc.) positive for *Klebsiella pneumoniae* from ICU patients.
- *Klebsiella pneumoniae* isolated from different types of clinical specimens obtained from the same individual.

Exclusion criteria:

- Duplicate isolates from the same sample source within a short time frame.
- Contaminated or improperly labeled samples.

A total of 225 *Klebsiella pneumoniae* isolates obtained from various clinical samples of ICU patients were included. In cases where multiple samples from the same patient yielded *K. pneumoniae*, each isolate was analyzed microbiologically. Demographic

and clinical data, including age, sex, ICU type, prior antibiotic use, invasive device utilization, and surgical history, were collected from medical records.

Clinical samples were processed using standard microbiological techniques, and *Klebsiella pneumoniae* was identified based on both conventional methods and automated system (MALDI-TOF MS by bioMérieux). Antimicrobial susceptibility testing was performed using the modified Kirby–Bauer disk diffusion method using AST panel of our institute and interpreted according to the Clinical and Laboratory Standards Institute guidelines M100 of the respective year.

Carbapenemase production was tested for isolates which were resistant to any of the carbapenems tested (Imipenem, meropenem, ertapenem) and was detected using phenotypic methods in accordance with CLSI M100 methodology. These included the Modified Carbapenem Inactivation Method (mCIM) and EDTA-modified CIM (eCIM) for differentiation of metallo-β-lactamases and serine carbapenemase. A 1 µL loopful of overnight bacterial culture was emulsified in 2.0 mL of tryptic soy broth (TSB) for mCIM, while a second tube containing 2.0 mL of TSB supplemented with 5 mM EDTA for eCIM. A 10 µg meropenem disk (Himedia) was immersed in each tube and incubated ambiently at 35°C ± 2°C for 4 hours±15 minutes. Following incubation, disks were transferred onto Mueller-Hinton agar plates lawn cultured with a 0.5 McFarland lawn of *Escherichia coli* ATCC 25922 indicator strain. Plates were incubated at 35°C ± 2°C for 18–24 hours. Inhibition zones were measured to the nearest millimeter. An mCIM zone of 6–15 mm or presence of pinpoint colonies within a 16-18 mm zone indicated carbapenemase production, while an eCIM zone increase of ≥ 5 mm relative to mCIM confirmed a metallo-β-lactamase phenotype. *Klebsiella pneumoniae* strains ATCC BAA-1705, BAA-2146, and BAA-1706 served as quality controls.

Colistin Minimum Inhibitory Concentration (MIC) were determined using microbroth dilution method in accordance with NCDC Standard Operating procedure for Antimicrobial Resistance Surveillance (NARS-Net). Reference-grade colistin sulfate powder was utilized to prepare serial two-fold dilutions in Cation adjusted Mueller-Hinton broth. For broth microdilution, sterile untreated plain polystyrene 96-well microtiter plates without surfactants were used. Isolates were inoculated to a final concentration of 5×10^4 CFU/mL and incubated at 35°C ± 2°C for 16–24 hours in an ambient air incubator. MIC of colistin was interpreted as the lowest concentration of colistin that completely inhibits the growth in the microdilution wells as detected by the unaided eye. Quality control was carried out using *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853.

Isolates were classified as multidrug-resistant (MDR), extensively drug-resistant (XDR), and pan drug-resistant (PDR) based on standard international definitions.

Classification of Resistance Phenotypes

Isolates were classified as **MDR, XDR, and PDR** based on standard international definitions proposed by the joint ECDC/CDC expert group led by Magiorakos AP [7]:

- **MDR:** Non-susceptibility to ≥1 agent in ≥3 antimicrobial classes.
- **XDR:** Susceptible to ≤2 antimicrobial classes.
- **PDR:** Non-susceptible to all agents in all antimicrobial classes.

Ethical approval was obtained from the Institutional Ethics Committee (No. Pharmac/IEC-GMCA/Approval/267/2026 by Institutional Ethics Committee (IEC-GMCA) Govt. Medical College Aurangabad), and the patient confidentiality was strictly maintained.

Statistical Analysis

Data were analyzed using standard statistical tests, with results expressed as frequencies, percentages, mean ± standard deviation and median. Variables were compared by

Fisher's exact test or Kruskal Wallis test, wherever appropriate. *P* <0.05 was considered as statistically significant.

RESULT

A total of 225 *Klebsiella pneumoniae* isolates obtained from 210 patients were included, with the highest proportion from the Medical Intensive Care Unit (MICU, n=105), followed by Neonatal ICU (NICU, n=81), Trauma ICU (n=25), Pediatric ICU (PICU, n=8), and ICCU (n=6). Male predominance was observed across all units (139/210, 66.19%). The mean age varied by unit, with NICU patients being neonates (6.6 ± 7.45 days), while adult ICUs showed mean ages ranging from 34 to 45.7 years. Prior antibiotic use was common, particularly in NICU (86.84%) followed by MICU and Trauma ICU (both 80%). Central catheterization was relatively high (143/210, 68.09%), mainly in MICU. Surgical intervention was noted in 38 patients (18.09%), most frequently in Trauma ICU (n=18) and MICU (n=17). (Table 1)

Table 1: Patient Demographic and Clinical Characteristics (N=210)

Variable	Cardiac Intensive Care Unit (ICCU)	Medical Intensive Care Unit	Neonatal Intensive Care Unit	Pediatric Intensive Care Unit	Trauma Intensive Unit
Total patients	**06**	**95**	**76**	**08**	**25**
Sex (Male)	05	62	48	04	20
Age Mean ± SD	45.67 ± 17.17 Yrs	37.93 ± 13.84 Yrs	6.60 ± 7.45 Days	3.90 ± 2.25 Yrs	34.00 ± 15.07 Yrs
Prior antibiotic therapy	02	76	66	01	20
Central catheter	00	82	58	00	03
Surgery performed	01	17	02	00	18

Among the total 225 samples analyzed in the study, the majority were blood samples, accounting for 113 samples (50.22%). This was followed by tracheal secretions, which constituted 65 samples (28.89%). Urine samples accounted for 30 samples (13.33%), while pus samples contributed 11 samples (4.89%). A smaller proportion of samples included fluid and sputum, with 2 samples each (0.89%). Only one sample each was obtained from a central catheter and surgical wound, accounting for 0.44% each. (Fig 1)

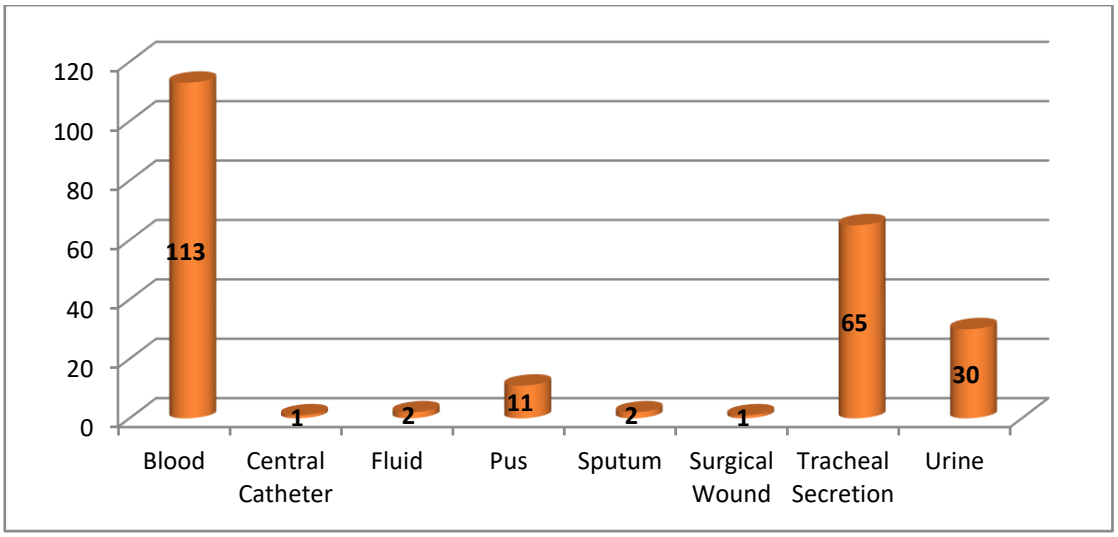

Fig 1: Specimen-wise Distribution of Samples Received of *Klebsiella pneumoniae* Isolates (N=225).

The number of isolates increased from 25 in August 2025 to 30 in September, after which it declined to 28 in October, a minor reduction from the previous month. A notable spike (73 isolates) was identified in November 2025, indicating enhanced transmission during this time period. Subsequently, the isolates decreased significantly to 25 in December 2025, then had a slight increase to 44 in January 2026. Overall, the pattern shows a transient peak in November, followed by fluctuating but somewhat lower values in subsequent months.

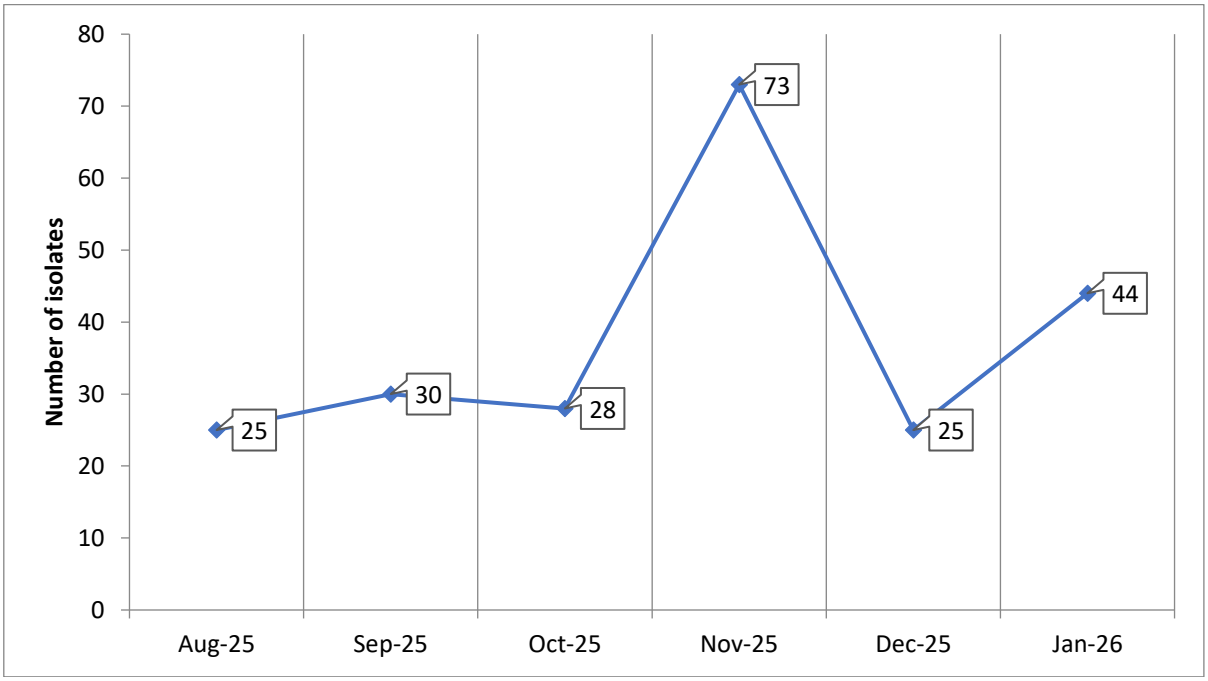

Fig 2: Time series showing number of *Klebsiella pneumoniae* isolates during the study period.

Figure 2 shows that there was a relation between time and the number of *Klebsiella* isolates, indicating considerable fluctuations during the study period.

High resistance was observed among *Klebsiella pneumoniae* isolates to most antibiotics. Cephalosporin resistance exceeded 93% (cefotaxime 94.67%, ceftriaxone 94.67%, cefuroxime 94.22%, cefoxitin 93.33%), with lower resistance to cefepime (80.44%). Ciprofloxacin resistance was 91.56%. Among aminoglycosides, resistance was 82.67% to amikacin and 75.11% to gentamicin. Carbapenem resistance ranged from 73.78% to 75.56%. Piperacillin–tazobactam showed 76.89% resistance, while cotrimoxazole had 72.89% resistance. Nitrofurantoin showed 83.33% resistance (N=30, Urinary isolates). Doxycycline (N=14, Reserved for Pus, wound swab and other sterile body fluids) demonstrated comparatively better activity (35.71% resistant). Colistin remained highly effective, with only 1.33% resistance. (Fig 2)

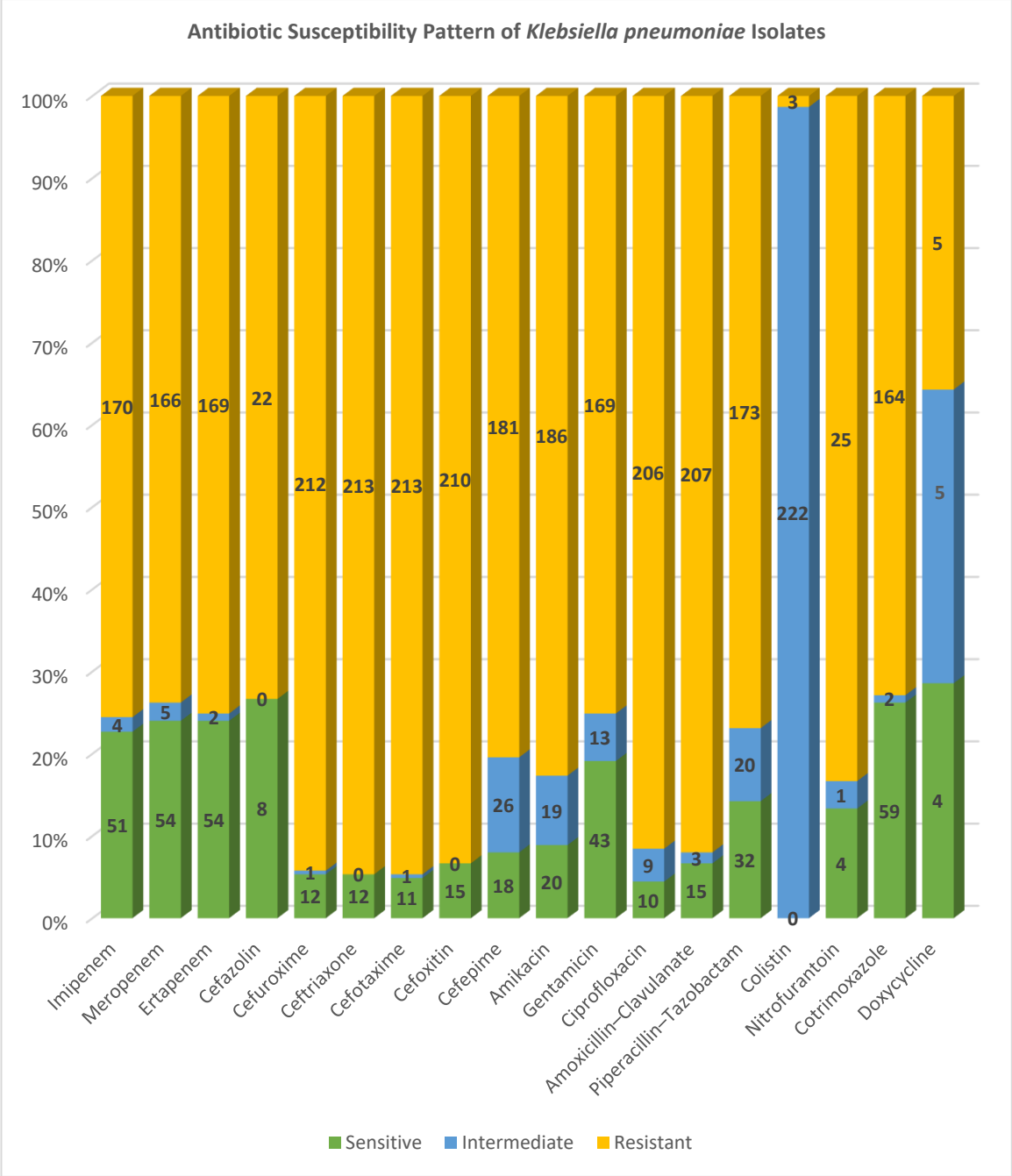

Fig 3: Distribution of Klebsiella pneumoniae Isolates According to Antibiotics Susceptibility Pattern (N=225).

Table 2 shows that the majority of *Klebsiella pneumoniae* isolates were extensively drug-resistant (XDR) (136/225, 60.45%), followed by multidrug-resistant (MDR) isolates (66/225, 29.33%). A small proportion were non-MDR (20/225, 8.89%), while pan drug-resistant (PDR) isolates were rare (3/225, 1.33%).

Table 2: Distribution of Klebsiella pneumoniae Isolates according to Antimicrobial resistance Phenotype Classification.

Phenotype Classification.	Number (n)	Percentage (%)
MDR	66	29.33%
XDR	136	60.45%
Not MDR	20	08.89%
PDR	03	01.33%
Total	**225**	**100%**

Among 170 isolates showing resistance to any of the carbapenems tested (Imipenem, meropenem, ertapenem), carbapenemase was not detected in 106 isolates (62.50%). Metallo-β-lactamase production was identified in 58 isolates (33.93%), while serine carbapenemase production was detected in 6 isolates (3.57%). (Table 3)

Table 3: Distribution of Carbapenemase Production among Carbapenem-Resistant *Klebsiella pneumoniae* Isolates.

Carbapenemase Type	Number (n)	Percentage (%)
Not detected	106	62.50%
Metallo-β-lactamase	58	33.93%
Serine carbapenemase	06	03.57%
Total	**170**	**100%**

The clinical outcomes of MICU patients were assessed during regular ICU rounds. The mean duration of hospital stay (LOS) and Median (1st and 3rd quartile) were determined across different categories of drug resistance. Statistical analysis showed that the length of hospital stay did not differ significantly across the resistance phenotype groups (Kruskal Wallis test, p-value = 0.4084).

Table 4: Length of hospital stay among patients with *Klebsiella pneumoniae* infection in MICU (N= 95)

Length of hospital stay (LOS)	Non MDR (n = 07)	MDR (n = 19)	XDR (n = 68)	PDR (n = 01)	p-value Kruskal Wallis test
Mean ± SD in Days	7 ± 5.86	6.74 ± 4.45	7.78 ± 7.71	34	0.4084
Median (Min, Max)	5 (2, 17)	6 (3, 17)	6 (2, 37)		

A total of 95 isolates were studied, of which 73 patients were discharged and 22 died. Among the Non-MDR group, 6 patients were discharged and 1 died. In the MDR group, 13 patients were discharged while 6 deaths were recorded. The majority of isolates belonged to the XDR category, where 54 patients were discharged and 14 died. The PDR group comprised a single patient, who died during the study period. Statistical analysis revealed no statistically significant association between the resistance phenotype of the isolates and patient outcome (discharge or death) (Fisher's exact test, p-value = 0.2423).

Table 5: Clinical outcome of patients with *Klebsiella pneumoniae* infection in MICU (N=95)

Type of Drug resistant found in isolates	Discharge	Death	Total	p-value Fisher's exact test
Non MDR	06	01	07	0.2423
MDR	13	06	19	
XDR	54	14	68	
PDR	00	01	01	
Total	**73**	**22**	**95**	

Only one patient admitted in the MICU was identified as having PDR strain, based on their blood and tracheal samples, which was attributed to secondary bacteraemia.

DISCUSSION

The present study demonstrates a high burden of *Klebsiella pneumoniae* infections in ICU settings, with a predominance of isolates from MICU and NICU and a clear male preponderance (66.19%). Similar male predominance has been reported by Moustafa NM et al.,[1] who observed 66.8% male patients, suggesting a consistent gender distribution pattern in ICU-associated infections. In contrast, studies by Ahmed Hasan et al. [8] and Maity SN et al. [3] reported higher prevalence among females, indicating that demographic variations may depend on regional and hospital-based factors.

In the present study, blood (50.22%) and respiratory samples (28.89%) were the most common sources of *K. pneumoniae*, which is comparable to findings by Moustafa NM et

al.,[1] who reported isolation from endotracheal aspirates, sputum, blood, and urine. However, Sonia SJ et al. [9] observed a higher prevalence from wound swabs and sputum, highlighting variability in infection sources across different healthcare settings.

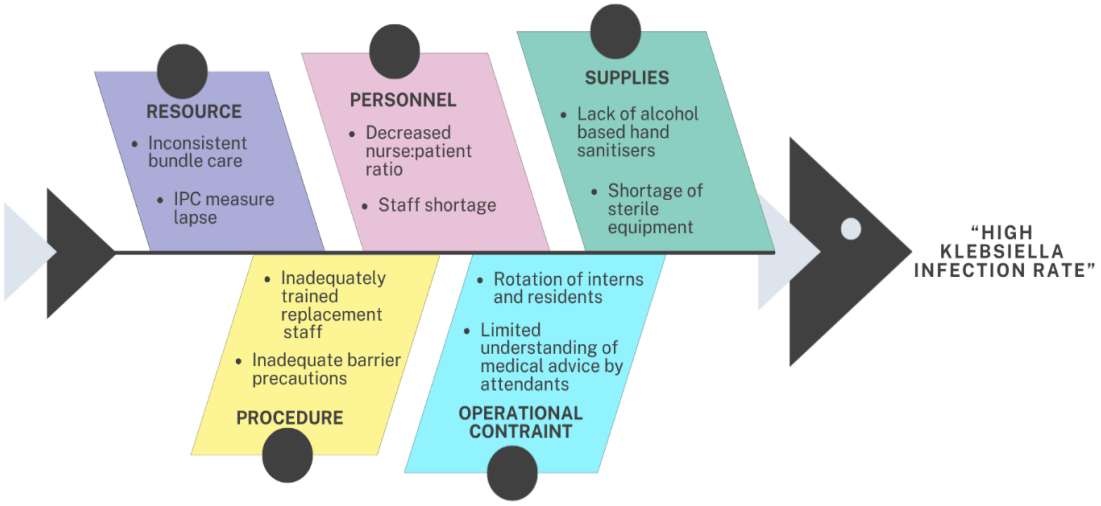

Fig 4: Fishbone analysis of the high Klebsiella infection rate identified multiple contributing factors across key domains.

The number of Klebsiella pneumoniae isolates was compared during the given time period and found to be substantially greater (N=73) in November 2025. We worked together to uncover the probable determinants of this using a fishbone diagram approach (Fig. 4).

Resource-related issues included inconsistent bundle care and lapses in infection prevention and control (IPC) measures. Personnel constraints were evident in the form of a decreased nurse-to-patient ratio and overall staff shortages largely attributed to local festival season, which resulted in inadequately trained replacement staff and insufficient adherence to barrier precautions. Supply-side limitations comprised inconsistent availability of alcohol-based hand sanitizers and a shortage of sterile equipment. Additionally, operational constraints such as frequent rotation of interns and residents, along with limited understanding of medical advice among bedside attendants, further contributed to the increased infection rates. Together, these factors highlight systemic deficiencies in clinical practices, workforce capacity, and resource allocation. Corrective and preventive actions were implemented as warranted. In response to this, regular hand hygiene audits, scrub the hub, and bundle care were reinforced, and the provision of hand sanitizer was subsequently reinstated. In the months that followed, the infection rate decreased.

We found out in the present study a high resistance to cephalosporins (>93%), fluoroquinolones (91.56%), aminoglycosides (75–82%), and carbapenems (73–75%). These findings are consistent with studies by Sonia SJ et al. [9] and Maity SN et al.,[3] who also reported high resistance to cephalosporins and β-lactam antibiotics. Similarly, Ilyas R et al. [10] documented significant resistance to multiple antibiotic classes, including carbapenems, indicating the widespread emergence of resistant strains. In contrast, Ahmed Hasan et al. [8] reported relatively low resistance to imipenem and amikacin, which differs significantly from the high carbapenem resistance observed in the present study, suggesting a rising trend of resistance over time.

The prevalence of multidrug resistance in the present study was notably high, with XDR isolates accounting for 60.45% and MDR isolates 29.33%. The predominance of XDR

isolates in the present study reflects a more alarming resistance scenario. A similar finding was reported by Sharma et al.,[11] in whose study the prevalence of XDR was 62.5%. In the same study, a comparative analysis between 2018 and 2022 revealed a concerning rise in resistance, with the proportion of XDR isolates increasing markedly from 62.5% to 93% over a period of 5 years. This study observed the frequency of PDR Klebsiella pneumoniae to be 1.33%, which is significant and corresponds to the 2.6% reported by Xiao S et al. [12] in Shanghai, China.

Carbapenem resistance in *K. pneumoniae* strains in the present study ranged from 73–76%, with carbapenemase production detected in 37.5% of isolates, predominantly metallo-β-lactamase producers. These findings are comparable to those reported by Porwal et al. [13] (44%) and Jaggi et al. [14] (53.9%). Similarly, Hussein K et al. [15] reported that most carbapenem-resistant *K. pneumoniae* isolates exhibited resistance to multiple antibiotics, indicating limited therapeutic options.

Importantly, colistin retained high efficacy in the present study, with only 1.33% resistance. This finding is comparable to those reported by Aminul P et al. [16] (0.7%) and Hussein K et al. [15] (4.5%). Similar trends have been observed in Indian studies, where Sodhi K et al. [17] reported 1.28% resistance, Khursheed N et al. [18] documented 1.7%, and Azam M et al. [19] found 3.2% resistance among *Klebsiella* isolates. These findings collectively support the continued effectiveness of colistin as a last-resort antibiotic. However, emerging resistance highlighted by Ilyas R et al. [11] remains a concern, emphasizing the need for cautious use and ongoing surveillance.

The present study demonstrated no significant association between the presence of highly resistant strains and prolonged hospital stay (p = 0.4084). Contrary to our findings Moustafa NM et al., [1] observed an increased prevalence of resistant organisms among patients with extended LOS. Similarly, patient outcomes did not differ significantly across the resistance phenotype groups (p = 0.2423). This association may be explained by several contributing elements, including prior exposure to antibiotics and presence of underlying comorbidities which predispose patients to recurrent or persistent infections, necessitating prolonged treatment. The use of invasive devices further increases the risk by providing potential entry points for pathogens.

Overall, the findings of the present study are in concordance with global literature demonstrating increasing antimicrobial resistance in *K. pneumoniae*, particularly in ICU settings. The higher proportion of XDR isolates and significant carbapenem resistance observed highlight a more severe resistance scenario compared to some earlier studies.

Limitations

This study being a single-center, hospital-based retrospective study, the findings may not be generalizable to other settings. The sample size, though adequate, was limited to ICU patients and may not reflect the overall hospital burden. Molecular characterization of carbapenemase genes was not performed, restricting precise identification of resistance mechanisms. Clinical outcomes and risk factor analysis for potential confounding factors such as underlying illness severity, comorbidities, previous hospitalisation, insufficient antibiotic dose, cross-transmission, biofilm development on invasive devices, and so on were not thoroughly evaluated.

CONCLUSION

The study demonstrates a high circulation of *Klebsiella pneumoniae* across various intensive care units, particularly in high-dependency settings such as MICU and NICU. A substantial proportion of isolates exhibited carbapenem resistance, indicating widespread carbapenemase production and limited therapeutic options. Most isolates were classified as XDR, reflecting a critical burden of antimicrobial resistance. Despite this, colistin retained high efficacy, with

minimal resistance observed. These findings highlight the urgent need for stringent infection control practices, antimicrobial stewardship, and continuous surveillance to limit the spread of resistant *K. pneumoniae* in ICUs.

Declaration by Authors

Ethical Approval: Approved (No. Pharmac/IEC-GMCA/Approval/267/2026 by Institutional Ethics Committee (IEC-GMCA).

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Conflict of Interest: The authors declare no conflict of interest.

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