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Evaluation of Colistin Susceptibility in ICU Isolates of Carbapenemase-Producing *Klebsiella pneumoniae*

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Abstract: ***Introduction: The emergence of colistin-resistant carbapenem-resistant Klebsiella pneumoniae poses a significant threat in intensive care units, reducing available treatment options and complicating patient management. Monitoring its occurrence and distribution is essential for guiding infection control and antimicrobial stewardship strategies. Materials and Methods: A total of 242 carbapenem-resistant Klebsiella pneumoniae isolates were collected from intensive care unit patients. Initial screening for colistin resistance was conducted using VITEK 2, followed by confirmation with the reference Broth Microdilution method. Data on patient demographics, specimen type, and ICU source were recorded and analyzed. Results: Out of 242 carbapenem-resistant Klebsiella pneumoniae isolates, 18 (7.4%) were identified as colistin-resistant by VITEK 2, and 13 (5.4%) were confirmed by Broth Microdilution. colistin resistant Klebsiella pneumonaie isolates were predominantly from male patients (9/13, 69.2%), with most patients in the 41–60 years age group. Department-wise, the majority were recovered from the Neuro intensive care unit (8/13, 61.5%). Respiratory specimens were the most common source (8/13, 61.5%), including endotracheal aspirates (5/13, 38.5%) and sputum (3/13, 23.1%). Conclusion: The detection of colistin resistant Klebsiella pneumonaie in intensive care unit settings highlights the urgent need for accurate diagnostic methods, ongoing surveillance, and robust infection control practices. Implementation of antimicrobial stewardship programs is critical to curb the spread of these highly resistant pathogens.***

Keywords: colistin resistance, *Klebsiella pneumoniae,* intensive care unit, antimicrobial stewardship, infection control

1. Introduction

Klebsiella pneumoniae, a member of the Enterobacteriaceae family, is commonly isolated from clinical samples. It causes primary pneumonia and is also implicated in extrapulmonary infections such as meningitis in infants, urinary tract infections, enteritis, and septicaemia.[1] It is a leading cause of hospital-acquired infections and often harbors resistance genes like extended-spectrum β-lactamases (ESBLs). Due to ESBL production, third- and fourth-generation cephalosporins have limited utility, making carbapenems the preferred treatment option.[2] *K. pneumoniae* has also developed resistance to carbapenems through plasmid-mediated carbapenemase production.[3] In such infections, colistin is often used as a last-resort therapeutic option.[4] Colistin is highly cationic due to the presence of L-diaminobutyric acid residues. It binds to the negatively charged phosphate groups of lipid A in lipopolysaccharides of Gram-negative bacteria, displacing stabilizing calcium and magnesium ions. This interaction disrupts the outer membrane, leading to loss of integrity, leakage of cellular contents, and ultimately bacterial cell death.[5] The rise of colistin-resistant *K. pneumoniae* is concerning due to limited treatment options and high mortality associated with these infections.[6] The main objective of this study was to evaluate the antibiotic sensitivity of of patients with colistin-resistant *K.pneumonaie* isolates obtained from all clinical samples from different Intensive Care Unit's (ICU's) of the Hospital.

2. Materials and Methods

This cross-sectional study was conducted over a period of six months in the ICU of a tertiary care hospital in Meerut, Uttar Pradesh, to evaluate colistin resistance in clinical isolates of carbapenemase producing *K. pneumoniae*. Various clinical specimens, including blood, urine, bronchoalveolar lavage (BAL), and body fluids were collected and processed in the microbiology laboratory following standard protocols.[7] Non-urine and non-blood samples were inoculated onto 5% sheep blood agar, chocolate agar, and MacConkey agar, while urine specimens were cultured on cysteine lactose electrolyte-deficient (CLED) agar. Blood cultures were performed using the BacT/ALERT 3D automated system (bioMérieux, Marcy-l'Étoile, France), and positive cultures were sub-cultured onto solid media for bacterial growth. Presumptive identification of *K. pneumoniae* was based on colony morphology and Gram staining, and species-level identification along with antibiotic susceptibility testing, including colistin, was performed using the VITEK-2 Compact automated system (bioMérieux, Marcy-l'Étoile, France) with GN identification cards and AST-N405 susceptibility cards. Colistin susceptibility was interpreted according to CLSI and EUCAST guidelines, and data on resistant isolates were analyzed to determine prevalence among ICU patients, with associated patient clinical profiles recorded to assess patterns of infection and potential risk factors.

Confirmation of colistin resistance was performed using the gold-standard broth microdilution (BMD) method following CLSI guidelines (M07-A09).[8] Cation-adjusted Mueller-

Hinton broth (CaMHB) was used, and colistin sodium methanesulfonate (HiMedia Pvt. Ltd.) was prepared in serial two-fold dilutions ranging from 0.25 to 64 µg/mL. Bacterial suspensions were added to each well to achieve a final concentration of 10^5 CFU/mL. To ensure accuracy, each assay included a drug-free growth control and a non-inoculated sterility control. Additionally, *Escherichia coli* ATCC 25922 was used as a negative control, with expected MIC values of 0.5–2 µg/mL. After overnight incubation at 37°C, the MIC for colistin was recorded for each isolate.

Figure 1: Colonies of Overnight Incubated Lactose fermenting *K. pneumoniae* on MacConkey Agar

3. Result

A total of 242 carbapenem-resistant *K.pneumoniae* (CRKP) isolates were recovered from ICU patients during the study period. Among these, 18 isolates (7.4%) exhibited growth on culture indicative of potential colistin resistance and were included in the analysis. Subsequent confirmation using the gold-standard BMD method verified colistin resistance in 13 of these isolates, highlighting a prevalence of confirmed colistin resistance of 5.4% among carbapenem-resistant ICU isolates.

The colistin-resistant *K.pneumoniae* (COLRKP) isolates were predominantly from males 9 (69.2%) compared to females 4 (30.8%) (p = 0.0197). Most males belonged to the age group of 41–60 years (23.1%), while among females too, the majority were also in the 41–60 years group (15.4%). Among males, 15.4% were in the 17–25 years age group, 15.4% in the 26–40 years group, and 7.7% were either 1–16 years or >60 years. In females, 7.7% were in the 17–25 years and >60 years age groups, with no females in the 1–16 years or 26–40 years groups. The p-value for age distribution was 0.756, with a chi-square statistic of 2.0028.

Table 1: Age and gender-wise distribution of COLRKP (n = 13)

Age group (years)	Male, n (%)	Female, n (%)
1–16	1 (7.7%)	0
17–25	2 (15.4%)	1 (7.7%)
26–40	2 (15.4%)	0
41–60	3 (23.1%)	2 (15.4%)
>60	1 (7.7%)	1 (7.7%)
Total	9 (69.2%)	4 (30.8%)

On department/unit-wise distribution, the majority of COLRKP isolates were recovered from the Neuro ICU, accounting for 8/13 (61.5%) of cases, followed by the Medical ICU (MICU) with 3/13 (23.1%) and the Respiratory ICU (RICU) with 2/13 (15.4%) with P = 0.091[Table 2]. Regarding sample-wise distribution, respiratory specimens accounted for the largest proportion of isolates, with 8/13 (61.5%) obtained from respiratory sample: ET aspirates 5/13 (38.5%) and sputum 3/13 (23.1%). Blood samples contributed 5/13 (38.5%) of isolates.

Table 2: COLRKP isolates among different hospital units (n=13)

ICU Type	Number of COLRKP Isolates	Percentage (%)
Neuro ICU	8	61.5
Medical ICU	3	23.1
Respiratory ICU	2	15.4
Total	13	100

4. Discussion

This study highlights the increasing difficulties in managing COLRKP, and emphasizes the role of the hospital environment in maintaining and spreading these pathogens. It is noteworthy for several reasons. Primarily, the study reports a hospital-wide occurrence of COLRKP ICU of a tertiary care hospital. Previous reports have also documented prolonged or repeated outbreaks of similar resistant isolates, indicating the persistent challenge of controlling such organisms in healthcare settings.[9]

In the present study, a total of 242 CRKP isolates were recovered from ICU patients, of which 18 isolates (7.4%) initially demonstrated potential colistin resistance by VITEK 2. Confirmation using the BMD method verified colistin resistance in 13 isolates, giving a prevalence of 5.4% among CRKP isolates. This finding aligns with Jian *et al.*[10] who previously reported 6.1% prevalence rates of colistin resistance among CRKP in ICUs. The discrepancy between VITEK 2 and BMD results highlights the known limitations of automated systems in detecting colistin resistance and reinforces the importance of BMD as the reference standard. In our study, the patients with colistin-resistant CRKP had a mean age of 41.2 ± 1.8 years, with a predominance of males (69.2%). These findings are in line with previous reports; for instance, an observational study by Diwane *et al.*[11] at a tertiary care centre involving 30 hospitalized patients reported a mean age of 59.1 ± 16.4 years, with 63.3% of cases being male. Similarly, another study from South India on colistin-resistant isolates noted a mean patient age of 58.3 years, also demonstrating a higher incidence among males compared to females.[12]

In our study, colistin-resistant *Klebsiella pneumoniae* (COLRKP) isolates were most frequently recovered from respiratory specimens (61.5%), including endotracheal aspirates (38.5%) and sputum (23.1%), followed by blood samples (38.5%). The predominance of respiratory isolates is consistent with *Richter et al.*[13] and Singh *et al* [14] which also documented higher rates of COLRKP from respiratory sources.

Furthermore, the majority of COLRKP isolates in our study originated from Neuro ICU patients, reflecting the high

vulnerability of critically ill patients to such infections. This observation aligns with earlier studies that reported a significant proportion of COLRKP isolates from Neuro ICU.[15]

The increased susceptibility of ICU patients is likely attributable to factors such as prolonged hospitalization, use of invasive medical devices, and compromised immune defences.

5. Conclusion

Effective management of COLRKP requires continuous surveillance, accurate diagnostic methods, and strict infection control practices in healthcare settings especially among ICU's. Proactive antimicrobial stewardship and preventive strategies are essential to limit the emergence and spread of these extensively drug-resistant pathogens.

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