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Detection of Colistin Resistance by Broth Micro dilution among Multidrug-Resistant Klebsiella Species in an Intensive Care Unit of a Tertiary Care Institute, Manipur

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Abstract

Background: The increasing prevalence of multidrug-resistant (MDR) Klebsiella species has led to the reintroduction of Colistin as a last-resort antibiotic. However, emerging resistance to Colistin is a growing concern, particularly in intensive care units (ICUs).

Materials and Methods: A total of 86 non-duplicate MDR Klebsiella isolates (Klebsiella pneumoniae, n=63; Klebsiella oxytoca, n=23) were included in this study. MDR screening was performed using the Kirby–Bauer disc diffusion method. Colistin minimum inhibitory concentration (MIC) was determined using the Broth micro dilution (BMD) method according to National AMR Containment Programme guidelines. Interpretation was done as per CLSI M100 (2025). Statistical analysis was performed using SPSS version 27.0.

Results: Out of 86 isolates, 40 (46.5%) were resistant (MIC ≥4 µg/mL) and 46 (53.5%) were intermediate (MIC ≤2 µg/mL). Significant associations were observed between Colistin resistance and ICU stay >7 days (p=0.032) and specimen type (p=0.002).

Conclusion: A high prevalence of Colistin resistance was observed among MDR Klebsiella isolates. Routine MIC testing using Broth micro dilution and implementation of strict antimicrobial stewardship are essential.

Keywords: Colistin Resistance, Broth Micro Dilution, MDR, ICU, Antimicrobial Resistance

Introduction

Antimicrobial resistance (AMR) is a major global health threat, particularly in hospital settings. Among Gram-negative pathogens, Klebsiella pneumoniae and Klebsiella oxytoca are significant causes of healthcare-associated infections, especially in intensive care units (ICUs) [1, 2].

The emergence of carbapenem-resistant Klebsiella species has necessitated the use of Colistin as a last-resort antibiotic [3, 4]. However, resistance to Colistin is increasingly being reported, further limiting treatment options. Resistance mechanisms include chromosomal mutations such as inactivation of the mgrB gene, as well as plasmid-mediated mcr genes that facilitate horizontal transmission [5,7].

In regions with high antimicrobial usage, such as tertiary care medical institute in Manipur, the burden of MDR organisms is particularly high [8,9]. Conventional susceptibility testing methods are unreliable for Colistin due to poor diffusion; therefore, Broth micro dilution (BMD) is recommended as the gold standard [10].

Objectives:

This study was conducted to determine the prevalence of Colistin resistance among MDR Klebsiella isolates in ICU patients and to analyze associated clinical factors.

Materials and Methods

Study Design and Setting

This prospective cross-sectional study was conducted from April 2024 to February 2026 in the Bacteriology Laboratory, Department of Microbiology, with different clinical specimens collected from various intensive care units (ICUs) of Jawaharlal Nehru Institute of Medical Sciences (JNIMS), Imphal, Manipur.

Ethical considerations

Ethical approval for the study was obtained from the Institutional Ethics Committee (IEC), Jawaharlal Nehru Institute of Medical Sciences (JNIMS), Imphal, Manipur, India (Approval No. 46/PGT, dated 25 April 2024). The study was conducted in accordance with the ethical standards of the institutional research committee and the principles of the Declaration of Helsinki.

Inclusion Criteria

- Critically ill ICU patients aged 18 years and above, of either sex
- Patients with microbiologically confirmed culture-positive infections

Exclusion Criteria

- Patients who did not provide consent to participate
- Patients who had received broad-spectrum antibiotics within the preceding 48 hours
- Patients aged less than 18 years
- Pregnant and lactating women, considering potential contraindications of certain antimicrobial agents
- Repeat isolates from the same patient, to avoid duplication of data

Sample Processing and Identification

Clinical samples received from ICU patients were processed as per standard microbiological procedures. Identification of Klebsiella species was performed by streaking on culture media like Nutrient agar, Blood agar, Mac Conkey agar, CLED agar for urine samples and then incubated the culture plates at 37ºC for 18-24 hrs. Aerobic Gram negative organisms were isolated and identified by standard microbiological techniques like colony morphology, pigment production, Gram staining, Hanging drop motility and standard biochemical reactions including catalase test, oxidase test, Indole test, citrate utilization test, urease test, triple

sugar iron (TSI) agar, Hugh –Leifson oxidative – fermentation test, nitrate reduction test and sugar fermentation tests –glucose, lactose, sucrose, maltose and mannitol.

Screening for Multidrug Resistance

Antimicrobial susceptibility testing was performed using the Kirby–Bauer disc diffusion method on Mueller–Hinton agar using commercially available disc(Hi-media). The different antimicrobials used were Gentamicin 10µg, Ceftazidime (30µg), Ceftriaxone (30µg), Piperacillin/ Tazobactam (100µg/10µg), Imipenem (10µg), Ciprofloxacin (5µg), Trimethoprim/ Sulfamethoxazole(1.25/23.75µg), Cefepime 30 µg, Fosfomycin 200µg(urinary isolates). The AST was interpreted following CLSI guidelines 2025. Escherichia coli ATCC 25922 was used as control strain during the study[11]. Panel of drugs used for MDR was defined as resistance to at least one agent in three or more antimicrobial classes.[12]

Colistin MIC Determination

The minimum inhibitory concentration (MIC) of Colistin was determined by the standard broth micro dilution (BMD) method in accordance with the National AMR Containment Programme guidelines for aerobic Gram-negative bacteria. Colistin sulfate powder with a potency of 737 µg/mg, as specified in the Certificate of Analysis, was used for preparation of the stock solution. The required quantity of the powder was calculated based on potency to obtain a final concentration of 1000 µg/mL (1 mg/mL) in sterile distilled water. The stock solution was aliquoted into sterile cryovials and stored at −80°C until further use.

Working solutions were prepared by diluting the stock solution in cation-adjusted Mueller–Hinton broth (Ca-MHB), ensuring that the pH of the medium was maintained between 7.2 and 7.4. Serial two-fold dilutions of Colistin were prepared in sterile 96-well micro titer plates to achieve a concentration range of 0.25–16 µg/mL.The bacterial inoculum was standardized to approximately 5×10^5 CFU/mL and inoculated into each well.

The micro titer plates were incubated at 35±2°C for 16–20 hours in ambient air. The MIC was defined as the lowest concentration of Colistin that completely inhibited visible bacterial growth.

Quality Control and Interpretation

Quality control was performed with each batch of testing using Escherichia coli ATCC 25922, and the obtained MIC values were ensured to be within the acceptable range of 0.25–2 µg/mL. Interpretation of results was carried out according to CLSI M100 (2025) guidelines.

Statistical Analysis

Data were analyzed using SPSS version 27.0. Chi-square test and Fisher's exact test were applied. A p-value <0.05 was considered statistically significant.

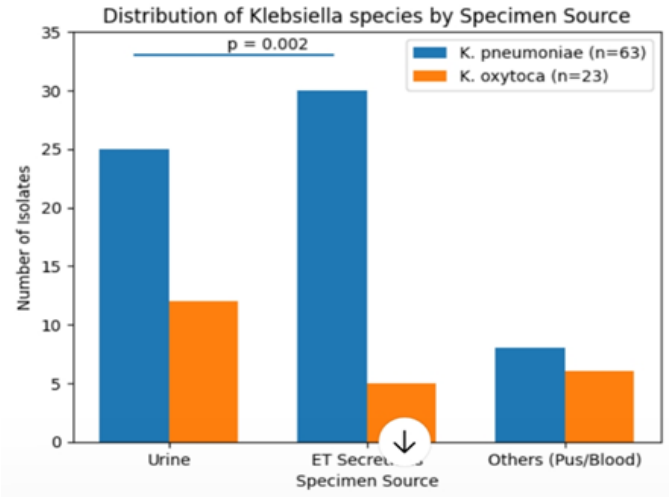
Results

A total of 86 MDR Klebsiella isolates were studied, of which Klebsiella pneumoniae (73.3%) was the predominant species, followed by Klebsiella oxytoca (26.7%). Urine (42.7%) and end tracheal secretions (40.9%) were the most common sources of isolates.

Species Distribution Across Specimens

Figure 1: Distribution of Klebsiella pneumoniae and Klebsiella oxytoca across clinical specimens. Klebsiella pneumoniae predominated across all specimen types, especially in urine and ET secretions.

Figure 1: Distribution of Klebsiella pneumoniae and Klebsiella oxytoca isolates across different specimen sources.

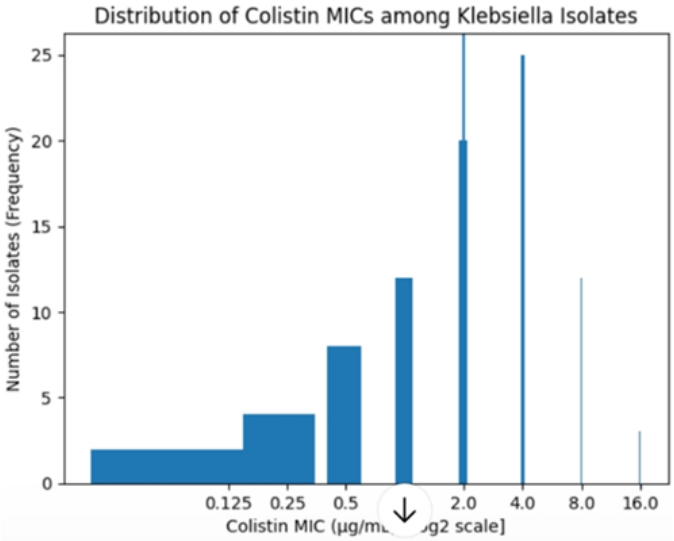


A statistically significant difference was observed between urine and endo tracheal secretion isolates (p = 0.002).

Colistin MIC Distribution

Figure 2: Frequency distribution of Colistin MIC values. Most isolates clustered around MIC values of 2 µg/mL and 4 µg/mL.

Figure 2: Distribution of Colistin MIC values among Klebsiella isolates showing a gradual shift toward higher MICs ("MIC creep"). The vertical line at 2 µg/mL indicates the susceptibility breakpoint.

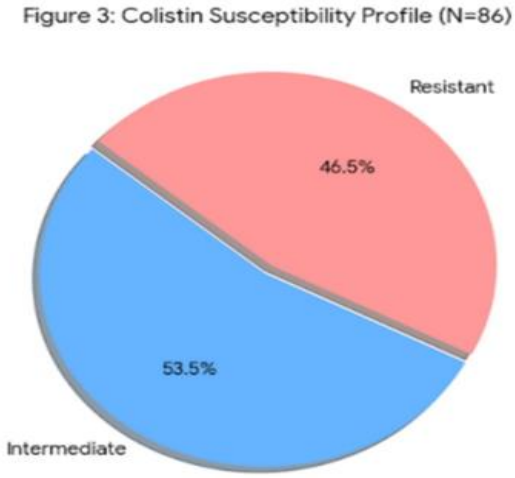


Overall Susceptibility Pattern

Figure 3: Colistin susceptibility profile.

Intermediate: 53.5%

Resistant: 46.5%



Species-wise Resistance

K. pneumoniae: 47.6% resistant

K. oxytoca: 43.5% resistant

Statistical Findings

Significant associations were observed between: ICU stay >7 days and resistance (p=0.032). Specimen type and resistance (p=0.002)

Discussion

The prevalence of Colistin resistance observed in the present study (46.5%) indicates a concerning rise in resistance within the ICU setting of Manipur. This high rate likely reflects the intense selective pressure encountered in tertiary care centers, where Colistin is frequently employed as a last-line therapeutic option for infections caused by carbapenem-resistant organisms.

Findings from other regions of India show a comparable upward trend. Umratkar[et al.13] reported a marked increase in polymyxin resistance among Gram-negative bacilli in Central India, suggesting that progression from carbapenem resistance to Colistin resistance may occur

rapidly in high antibiotic-use environments. Similarly, Singh et al. [14] documented elevated Colistin MICs among Klebsiella isolates in North Indian ICUs, particularly in patients requiring prolonged ventilatory support. This observation is consistent with the present study, where endo tracheal secretions constituted a significant proportion (40.9%) of isolates.

In South Indian settings, Poonam et al. [15] reported a Colistin resistance rate of approximately 38% among Klebsiella pneumoniae, which is slightly lower than the 47.6% observed in our study. Such differences may reflect regional variations in antimicrobial usage patterns, infection control practices, and circulating bacterial strains.

In contrast, considerably lower resistance rates have been reported from other parts of the country. Hait et al. [16] documented a resistance rate of 12.5% in East India, while Yadav et al. [17] observed a rate of 15.2% in a multicentric analysis. These discrepancies may be attributed to differences in patient populations, antibiotic stewardship policies and healthcare infrastructure. Additionally, surveillance data from North-Eastern India by Ananda et al. [18] indicated that although carbapenem resistance was prevalent, Colistin resistance had not reached the levels observed in our ICU cohort, suggesting a more localized escalation in resistance within this setting.

An important methodological strength of the present study is the inclusion and differentiation of both Klebsiella pneumoniae and Klebsiella oxytoca. While many studies focus primarily on K. pneumoniae, our findings demonstrate that K. oxytoca also contributes significantly to the resistance burden, with 43.5% of isolates showing resistance. Furthermore, the statistically significant association between specimen type and resistance ($p = 0.002$) suggests that respiratory sources, particularly endo tracheal secretions, play a key role in harboring resistant strains.

A major concern highlighted in this study is the high proportion of isolates categorized as intermediate (53.5%) according to CLSI 2025 criteria [10]. Previous studies by Panigrahi et al. [19] have emphasized that such isolates often lie at the threshold of therapeutic failure. This pattern may represent a gradual upward shift in MIC values, commonly referred to as "MIC creep," as also supported by Agarwal et al. [20]. The predominance of isolates within this range raises concern that a substantial proportion may soon transition to full resistance under continued antibiotic pressure.

Conclusion

The present study demonstrates that Colistin resistance is increasingly prevalent among MDR Klebsiella isolates in ICU settings in Manipur. With a substantial proportion of isolates already resistant and the remaining clustering within the intermediate MIC range, there is a clear indication of diminishing susceptibility to this last-resort antibiotic.

These findings underscore the urgent need for routine implementation of Broth microdilution for accurate MIC determination, along with strengthening of antimicrobial stewardship practices and infection control measures. Early identification and continuous surveillance are essential to limit further escalation and preserve the clinical utility of Colistin.

Strengths of the Study

- Use of Broth micro dilution (gold standard) for Colistin MIC determination
- Inclusion of both Klebsiella pneumoniae and Klebsiella oxytoca
- Focus on ICU population

- Statistical analysis of risk factors

Limitations of the Study

- Lack of molecular characterization
- No clinical outcome correlation

Availability: The data supporting the findings of this study are available within the article or from the corresponding author upon reasonable request.

Conflicts of Interest: The authors declare that there is no conflict of interest regarding the publication of this paper.

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