



## Prevalence and Characteristics of Colistin-Resistant Bacteria: A Retrospective Study from a Tertiary Care Hospital in Jamshedpur, Jharkhand, India

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### Abstract:

*Klebsiella pneumoniae* is an important opportunistic pathogen associated with a variety of healthcare-associated infections. The rise of colistin resistance in carbapenem-resistant *K. pneumoniae* represents a significant global health threat, as colistin is frequently used as a last-resort antibiotic that requires continuous monitoring for effective antimicrobial therapy and infection control strategies. Present studies evaluate the prevalence and in-vitro antibiotics antibiotic susceptibility of *K. pneumoniae* clinical isolates at a tertiary care hospital, along with their demographic and clinical distribution. Clinical isolates from a tertiary care hospital, along with their demographic and clinical distribution, were analyzed in this retrospective study, which included 556 Gram-negative bacterial isolates obtained from various clinical specimens between December 2023 and March 2024. The VITEK-2 automated system was used for bacterial identification and antimicrobial susceptibility testing. The distribution of *K. pneumoniae* isolates by gender, age group, clinical specimen and hospital location was evaluated. We also assessed patterns of colistin resistance and susceptibility to other available antibiotics. A Chi-square test was applied to find the association of colistin resistance with demographic variables, (where  $p < 0.05$ ). Extensive resistance to  $\beta$ -lactams, carbapenems, fluoroquinolones, and aminoglycosides was detected by antibiotic susceptibility testing. In-vitro susceptibility was minimal for fosfomycin (22.7%), ertapenem (18.2%), tigecycline (13.6%) and gentamicin (9.1%), indicating the presence of extensively drug-resistant strains. The emergence of colistin-resistant *K. pneumoniae* with extensive drug resistance and serious therapeutic limitations has become a significant public health issue, as this study highlighted. These results highlight the importance of surveillance against antimicrobial resistance, strong infection control policies and practices, and effective implementation of programs related to antimicrobial stewardship in order to limit transmission of resistant strains. Early detection and judicious use of antibiotics are the keys to ensuring colistin and other last-line antibiotics remain effective.

**Keywords:** *Klebsiella pneumoniae*, Colistin resistance, Antimicrobial resistance, Nosocomial infections, Multidrug resistance, ICU infections.

## Introduction:

Antimicrobial resistance (AMR) is widely recognized as one of the most serious threats to global public health, healthcare systems and sustainable development [1]. The urgent proliferation and spread of multidrug-resistant (MDR) and extensively drug-resistant (XDR) Gram-negative bacteria have markedly restricted available therapeutic options, resulting in increased morbidity, mortality, prolonged hospital stay and escalating healthcare costs [2]. Out of the few remaining treatment options, colistin, an old antibiotic discovered in the 1950s has witnessed resurgence a resurgence as a last-treatment option for carbapenem-resistant *Enterobacteriaceae* (CRE) [2].

Colistin works by binding to lipopolysaccharides (LPS) on the outer membrane of Gram negative Gram-negative bacteria, thereby causing cell lysis and death [3]. The major resistance mechanism to colistin is through chromosomal mutations that change the structural components of lipid A, resulting in reduced affinity for the drug [4]. Even more worrisome is the emergence of plasmid-mediated mobile colistin resistance (*mcr*) genes that allow for horizontal transfer of resistance between bacterial species. Several variants of the *mcr* gene (*mcr*-1 to *mcr*-10) have been reported around the world since its initial discovery, demonstrating that resistance determinants can evolve and spread rapidly [5].

The global dissemination of colistin-resistance genes has been linked to inappropriate use of antimicrobials, over-the-counter sales of antibiotics, empirical broad-spectrum therapy and the extensive application of polymyxins in veterinary and agricultural settings [6]. Given the high prevalence of infectious diseases, misuse of antibiotics, poor infection control practices and inadequate AMS programs, AMR is particularly high in developing countries like India. The increasing rate of polymyxin resistance observed in hospital isolates underscore the need for consistent monitoring studies [7].

Healthcare settings, specifically intensive care units (ICUs), represent key reservoirs for the emergence and dissemination of colistin-resistant pathogens [8]. Longer hospitalization, mechanical ventilation, invasive procedures, immunocompromised status and extensive antibiotic use are the important risk factors for antibiotic resistant strain selection [9]. Studies indicate that colistin-resistant isolates are commonly linked with nosocomial infections including ventilator-associated pneumonia, bloodstream infection, urinary tract infection and wound infection [10].

Within the Indian context, multiple studies have described *mcr* genes among clinical Gram-negative isolates from tertiary care setups highlighting the risk for horizontal transmission of resistance [11]. A cross-sectional study in a tertiary care hospital in India found *mcr* gene variants in various Gram-negative bacteria, underscoring the clinical significance of regular surveillance and molecular characterization of resistant isolates. Such findings reinforce the need for local epidemiologic data to inform infection control measures and empirical antibiotic therapy [12].

The co-occurrence of colistin resistance with resistance to other classes of antibiotics (e.g., carbapenems, cephalosporins and fluoroquinolones) has led to the emergence of pan-drug-resistant (PDR) pathogens [13]. Infecting these organisms greatly complicates treatment decision making, and they can result in therapeutic failure. This scenario adds more fuel to the fire, mainly in the resource limited health-care set up as they often lack access to advanced diagnostics facilities and newer classes of antimicrobials. Surveillance of patterns of antimicrobial resistance is critical to help guide the selection of appropriate antibiotics and ultimately optimize patient outcomes [14].

Although colistin resistance is a subject of increasing global concern, there are limited data from eastern Indian states (including Jharkhand) on the epidemiology, prevalence and microbiological profile of colistin-resistant isolates. This regional antimicrobial resistance data is important for this reason; resistance patterns vary dramatically by geography, the infrastructure of hospitals, infection control practices and antibiotic

prescribing trends. Hence local data can be pivotal for strengthening hospital antibiotic policies, enhancing infection prevention measures and supporting national AMR surveillance programs [15].

Due to growing global and national concern about colistin resistance and the dearth of regional data from Jharkhand, this retrospective study aimed to determine the prevalence and distribution of colistin-resistant Gram-negative bacilli (GNB) isolated from different clinical samples in a tertiary care hospital. The other objective of this study is to determine the antimicrobial susceptibility profiles of the isolates and their association with clinical settings. These findings could aid in contributing to improvements in the understanding of antimicrobial resistance trends and provide a foundation for developing evidence-based strategies for improving infection control and rational antibiotic use.

## Material and Methods

### Data Collection

A retrospective study was conducted at the TATA Main Hospital, a tertiary care hospital in Jamshedpur, Jharkhand, India, spanning from December 2023 to March 2024. Bacterial strains were isolated from various clinical specimens, including blood, urine, sputum, endotracheal aspirate, and pus, collected from suspected patients across different hospital departments such as the outpatient department (OPD), inpatient department (IPD), intensive care unit (ICU), and neonatal intensive care unit (NICU). Key clinical details such as age, gender, clinical presentations, geographic location, prior antibiotic usage, along with the date and origin of bacterial isolation, were recorded for subsequent analysis.

### Identification and Antibiotics susceptibility test

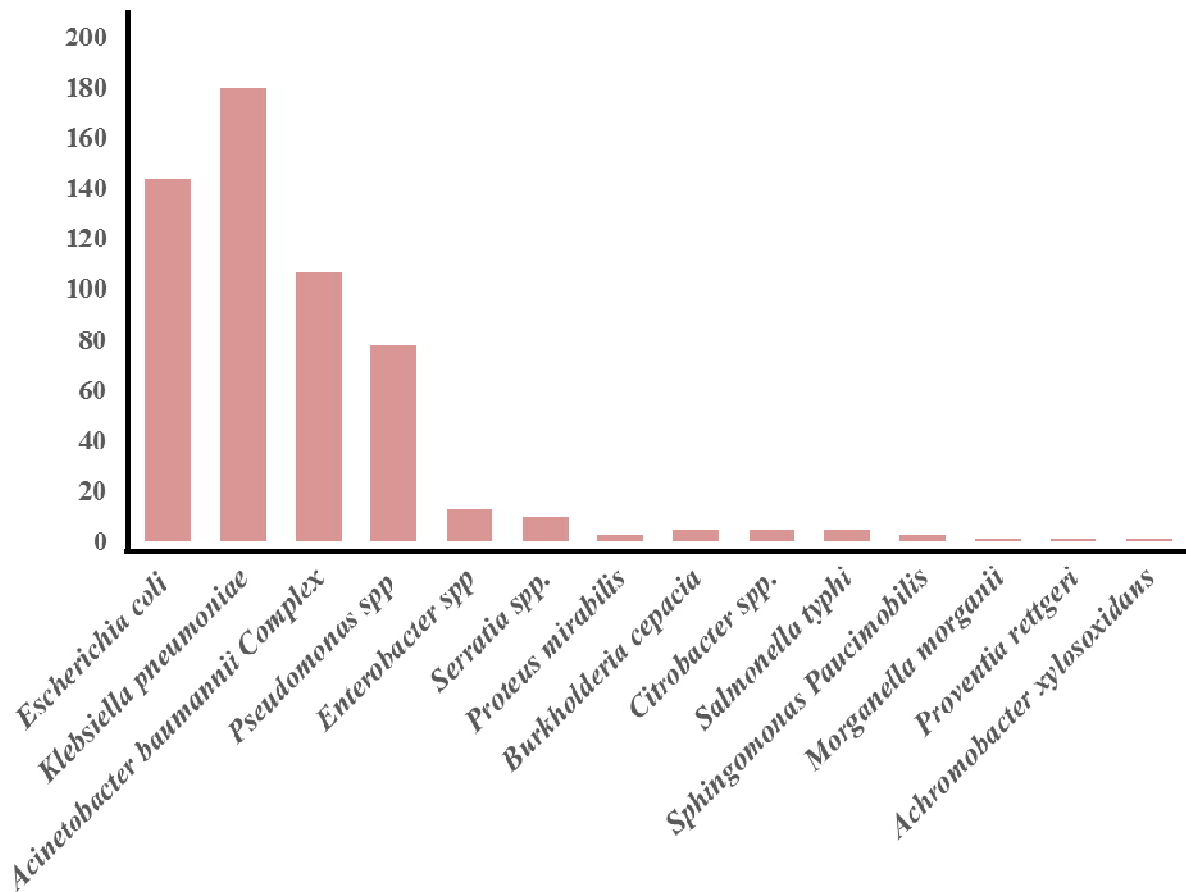
*Klebsiella* spp. from blood agar enriched media and visually identified as mucoid colonies on cysteine lactose electrolyte-deficient (CLED) agar media were identified using either the VITEK2 compact system (bioMérieux, USA) by GN card. The antibiotics susceptibility tests were performed using either the VITEK2 compact system (bioMérieux, USA). Colistin resistant was determined by broth microdilution method using 96 wells plate. Quality control strains used for MIC determination were *Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* ATCC 700603 and *Pseudomonas aeruginosa* ATCC 27853. Interpretation of results was carried out in accordance with the guidelines defined by the Clinical and Laboratory Standards Institute (CLSI) in 2021 (M100-S21) [16].

### Statistical analysis

Statistical analysis was performed using Statistical Package for Social Sciences version 17.0 (SPSS, Chicago, IL, USA). All reported P-values were two-sided, with statistical significance set at  $P < 0.05$ .

## Results

Identification of five hundred fifty six positive bacterial cultures using Vitek automatic system demonstrated fourteen different genera (**Figure 1**). As shown in **Figure 1**, the predominant isolate was *Klebsiella pneumoniae* (32.13%, n=180) and *Escherichia coli* (25.71%, n=144), collectively accounting for approximately 57.84% of the total isolates. Among non-fermenting Gram-negative bacilli, the *Acinetobacter baumannii* complex (19.10%, n=107) and *Pseudomonas aeruginosa* (13.92%, n=78) were notably prevalent, underscoring their role as important nosocomial pathogens. Other Gram-negative genera detected in smaller proportions included *Enterobacter* spp. (2.34%), *Serratia* spp. (1.80%), *Burkholderia cappacia* (0.9%), *Citrobacter* spp. (0.9%), *Salmonella typhi* (0.9%), and a few rare isolates such as *Proteus mirabilis*, *Sphingomonas paucimobilis*, *Morganella morganii*, *Providencia rettgeri*, and *Achromobacter xylosoxidans* (each below 1%) (**Figure 1**).



**Figure1: Bar chart showing the percentage distribution of bacterial genera among clinical isolates (N = 556).**

Out of 556 Gram-negative bacterial isolates, 32.37% (n=180) isolates were belong to *Klebsiella* spp. *K. pneumoniae* isolates across various clinical specimens revealed that the highest proportion was recovered from **endotracheal aspirates** (41.11%, n=74), followed by **urine samples** (31.67%, n=57). **Wound swabs, pus, and tracheostomy tube swabs** contributed to 8.89% (n=16), blood cultures accounted for 6.67% (n=12), while **sputum** specimens yielded 5.00% (n=9) and 6.67% (n=12) sample were unknown (**Table2**).

The age-wise distribution of patients from which *K. pneumoniae* were isolated revealed a markedly higher prevalence of infections among the elderly population. Individuals aged **above 61 years** accounted for the majority of cases 77.71% (n=136), indicating a significant susceptibility in this age group. In comparison, the **51–60 years** group comprised 13.71% (n=24), followed by **31–50 years** with 8.00% (n=14), and the **20–30 years** group with only 3.34% (n=6) (**Table2**). Although, Chi-square analysis showed no statistically significant association between colistin resistance and gender ( $\chi^2 = 0.207$ ,  $p = 0.649$ ), hospital location ( $\chi^2 = 1.420$ ,  $p = 0.233$ ), or age group ( $\chi^2 = 0.104$ ,  $p = 0.747$ ). Although higher resistance proportions were observed in ICU patients and elderly populations, these differences were not statistically significant ( $p > 0.05$ ).

The overall prevalence of colistin resistance among *Klebsiella pneumoniae* isolates was **12.22%**. A slightly higher resistance rate was observed among male patients (13.33%) compared to females (11.11%). Clinical source analysis showed the highest resistance among endotracheal aspirates (16.21%) and blood samples (16.67%), suggesting an association with invasive infections. Age group analysis demonstrated the highest resistance in patients aged 51–60 years (20.83%). ICU isolates showed higher resistance (16.67%) compared to non-ICU isolates, indicating the possible impact of antibiotic selection pressure and critical care exposure (**Table 2**).

**Table2: *Klebsiella* spp. isolated during study period (December 2023 to March 2024)**

Property	<i>Klebsiella pneumoniae</i> N=180 (%)	Colistin resistant <i>Klebsiella pneumoniae</i> N=22 (%)
Gender		
Male	90 (50)	12(54.54%)
Female	90 (50)	10(45.45%)
Clinical sources		
Urine	57 (31.67)	6(27.27%)
Blood	12 (6.67)	2(9.09%)
Endotracheal Aspirate	74 (41.11)	12(50.00%)
Wound Swab/Pus/TT Swab	16 (8.89)	2 (9.09%)
Sputum	9 (5.00)	0
Unknown	12 (6.67)	0
Age group		
20-30years	6 (3.33%)	1 (4.54%)
31-50years	14 (7.77%)	1 (4.54%)
51-60years	24 (13.33%)	5(22.72%)
>61years	136 (75.55%)	15(68.18%)
Hospitalsites		
ICU/CCU	54 (30.00%)	9 (40.90%)
IPD	110 (61.11%)	10 (45.45%)
OPD	13 (7.22%)	3 (13.63%)
BCU	3 (1.66%)	0

ICU-Intensive Care Unit, IPD-Inpatients Department, OPD-Out Patients Department, BCU-Burn Care Unit,

Antibiotics susceptibility pattern of these *K. pneumoniae* showed that 12.22% (n=22) isolates were colistin resistant. The majority of the colistin-resistant Gram-negative isolates (22) were susceptible to a narrow range of antibiotics. All (100%) isolates were resistant to  $\beta$ -lactam/ $\beta$ -lactam inhibitor combinations, cephalosporins, meropenem, ciprofloxacin, amikacin and trimethoprim-sulfamethoxazole. Fosfomycin (22.7%), ertapenem (18.2%) and tigecycline (13.6%) were associated with limited susceptibility. Gentamicin was sensitive in 9.1% isolates, while imipenem showed intermediate sensitivity in 9.1% isolates. Overall resistance pattern suggest the existence of extensively drug-resistant phenotypes with very few therapeutic options.

## Discussion

In the current study, *Klebsiella pneumoniae* (32.13%) and *Escherichia coli* (25.71%) were found to be the most common Gram-negative pathogens responsible for about 58% of all isolates. Recent antimicrobial surveillance studies have reported similar findings with *Enterobacteriaceae* being the most prevalent pathogens causing healthcare-associated infections due to their high genetic flexibility and potential for acquiring resistance [16]. The high percentage of non-fermenting Gram-negative bacilli in particular, *Acinetobacter baumannii* complex (19.10%) and *Pseudomonas aeruginosa* (13.92%), confirms their acknowledged role as important nosocomial pathogens. These organisms are commonly linked to ventilator-associated pneumonia, bloodstream infections, and device-associated infections in ICU settings with high antibiotic selection pressure [17].

We report that *K. pneumoniae* was frequently isolated more from endotracheal aspirates (41.11% isolates), while urine samples were 31.67%. Similar results have been shown in recent hospital-based studies, with respiratory specimens, and especially tracheal aspirates, being the most frequent source of multidrug-resistant *K. pneumoniae* [18], particularly in intubated patients. This can be attributed to long-term hospital care, invasive procedures and biofilm development in medical apparatuses, thereby promoting bacteria survival and transmission.

According to age distribution, infections were mostly in patients over 61 years (75.55%). This observation is further supported by recent epidemiological studies showing that elderly patients are at increased risk for *K. pneumoniae* infections due to immunosenescence, underlying comorbidities, repeated healthcare exposure, and frequent antibiotic use [19]. In this study, increased resistance rates were not only seen among ICU patients but also older adults; however, none reached statistical significance that could be driven by the limited sample size.

In this study, the overall prevalence of colistin resistance among *K. pneumoniae* isolates was 12.22%, which is not different from reports estimating global prevalence frequencies: recent estimates have indicated approximately 13% colistin resistance in clinical isolates [20]. This rising trend of colistin resistance is alarming, as it is one of the last remaining therapeutic options for carbapenemase-producing *Enterobacterales*. Chromosomal mutations such as alterations in the *mcrB* gene have been associated with emergence of resistance; *mcr* genes on plasmids are likewise implicated [21].

The elevated proportion of resistance were observed among invasive clinical samples, including endotracheal aspirates and blood cultures in this study points to the potential impact of invasive interventions and extended antibiotic exposure on promoting resistant pathogen development. Outbreaks of colistin-resistant *K. pneumoniae* in intensive care units have been previously described, underscoring the need for stringent measures to maintain infection control and prevent transmission [22].

Our antibiotic susceptibility testing data showed marked resistance to  $\beta$ -lactam/ $\beta$ -lactam inhibitor combinations, cephalosporins, carbapenems, fluoroquinolones and aminoglycosides in the current study suggesting that these isolates show extensive drug resistant (XDR) phenotypes [16]. The limited susceptibility seen in the present study also supports mounting evidence of both narrow therapeutic options for treating these types of infections.

These results clearly demonstrate the need of ongoing AMR surveillance, antiseptic stewardship programme and proper infection prevention measures. To prevent the emergence and spread of resistance to last-resort antibiotics, regular specificity monitoring of colistin [17] and responsible antibiotic prescribing practices are warranted. Importantly, the rise of colistin-resistant *K. pneumoniae* described herein poses a serious therapeutic dilemma and highlights the need for early detection in conjunction with antimicrobial stewardship policies and reinforced infection control measures to combat this emerging threat provided by antimicrobial resistance.

## Conclusion

The present study demonstrates that *Klebsiella pneumoniae* remains one of the most predominant Gram-negative pathogens associated with clinical infections, particularly in hospitalized and critically ill patients. The detection of 12.22% colistin resistance among *K. pneumoniae* isolates is of serious concern because colistin represents a last-resort therapeutic option for the management of infections caused by carbapenem-resistant Enterobacterales. The higher recovery of isolates from endotracheal aspirates and ICU settings indicates the significant role of invasive procedures, prolonged hospital stay, and antibiotic selection pressure in the development and dissemination of resistant strains. The antimicrobial susceptibility profile revealed extensive resistance to multiple classes of antibiotics including  $\beta$ -lactams, carbapenems, fluoroquinolones, and aminoglycosides, suggesting the circulation of extensively drug-resistant (XDR) strains with limited therapeutic alternatives. The partial susceptibility observed for Fosfomycin, tigecycline, and gentamicin indicates that combination therapy and susceptibility-guided treatment may remain the only viable clinical options in such cases. Overall, these findings emphasize the urgent need for strengthened antimicrobial stewardship programs, continuous resistance surveillance, and strict infection prevention and control measures to limit the emergence and spread of colistin-resistant pathogens in healthcare settings.

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**Conflict of interest: None**

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