

A Retrospective Study of Antibiotics Resistant Pattern and Gene Detection of Carbapenem Resistant- Klebsiella Pneumoniae

Running Title: K. pneumoniae Antibiotic Resistance: Retrospective Study

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ABSTRACT

Introduction: Carbapenemases have increasingly been reported in enterobacteriaceae worldwide. Most carbapenemases are plasmid encoded hence resistance can easily spread. Carbapenem-resistant enterobacteriaceae are reported to cause mortality in up to 50% of patients who acquire bloodstream infections. We set out to determine the burden of carbapenem resistance as well as establish genes encoding for carbapenemases in enterobacteriaceae clinical isolates obtained from Meitra Hospital, Tertiary care hospital, Kozhikode, India

Methods: In this study a total of 4384 clinical isolates previously collected from pus swabs, urine, blood, sputum, tracheal aspirates, cervical swabs, endometrial aspirates, rectal swabs, Vaginal swabs, ear swabs. All the samples were performed as microbiological examination and to find out the significant bacterial growth. In addition, all the isolates were subjected to PCR assay to confirm presence of carbapenemase encoding genes.

Results: In a retrospective study, the carbapenem resistant Klebsiella pneumonia (CRKP) incidence and distributions were analysed from January 2023 to June 2023. Clinical samples of 4384 patients. From the total 4384 patient's samples, 699 Gram Negative bacilli growth were isolated. Out of the 699 growths, 115 were Carbapenem Resistant Organism (CRO). Out of the 115 bacterial isolates

73 of them are Carbapenem Resistant -Klebsiella Pneumoniae (CR-KP).

Conclusion: This study highlights the high prevalence of carbapenem-resistant Klebsiella pneumoniae (CR-KP) in a tertiary care center in Kozhikode, Kerala. The findings stress the urgent need for robust infection control and continuous surveillance to address antibiotic resistance. Enhanced research and molecular diagnostics are essential for understanding resistance patterns and developing effective treatment strategies.

Key words: Klebsiella pneumoniae, Enterobacteriaceae family, Antibiotics Resistant, Gene detection.

I. INTRODUCTION

Klebsiella pneumoniae is an opportunistic bacterium belonging to Enterobacteriaceae family responsible for many nosocomial and community acquired infections. ^[1] Antibiotics such as carbapenems are widely used to treat infections, however the use or misuse of such antibiotics has contributed to the appearance of isolates resistant to carbapenem. ^[2] Carbapenem-resistant Klebsiella pneumoniae (CR-KP) are reported to cause mortality in up to 50% of patients who acquire infections. ^[3] The mechanism of antibiotic resistance in K.pneumoniae involve the expression of beta-lactamases, efflux mechanism, outer protein membrane or porin reduction. ^[4] Carbapenem is a

beta-lactam antibiotic which inhibits transpeptidases and prevents peptidoglycan synthesis, leading to cell death. *Klebsiella* species resistance to carbapenems is generally based on carbapenemase production or the combination of structural mutation with the production of other beta lactamases, such as AmpC cephalosporinase and ESBL. The 3 major classes of carbapenemases are Ambler Class A *Klebsiella pneumoniae* carbapenemases (KPC); Class B Metallo-beta-lactamases (MBLs) such as New Delhi MBL (NDM), Verona integrin-encoded MBL (VIM) and Imipenem resistant pseudomonas (IMP); Class D Oxacillinases (OXA)-type enzymes such as oxa-48 like carbapenemase.^[5]

The aim of the study was to investigate the characteristics of antibiotic resistant pattern and resistant gene of Carbapenem Resistant *Klebsiella pneumoniae* isolated from hospitalized patients.

II. MATERIALS AND METHODS

Study Design

This retrospective study was conducted at Meitra Hospital, a tertiary health care hospital at Kozhikode, Kerala. The study period spanned six months, from January 2023 to June 2023. The research focused on patient samples collected during this time, specifically including samples from all age groups and all human specimens. However, the study excluded samples from all bacterial species other than *Klebsiella pneumoniae* and did not consider carbapenem-sensitive strains of *Klebsiella pneumoniae*.

A total of 4384 different samples from patients visited into the Hospital were collected based on inclusion criteria and 4132 were excluded for the reasons. Consequently, 252 Patients samples with confirmed *K.pneumoniae* infections were finally enrolled in the study.

Microbiological Examination

Microbiological specimens received from different departments of the Meitra Hospital were processed at the Microbiology Laboratory. Specimens were microscopically observed after staining with Gram stain and cultured by plated in suitable culture media and incubated at 37°C. The isolated bacterial species identification and Antibiotic sensitivity test by the standard microbiological methods identified using conventional methods and the VITEK2 Compact® (bioMerieux, Marcy l'Etoile, France) automated system. Randomly Selected CR-KP isolates were

sent for PCR assay to confirm the presence of carbapenemase encoding genes.

Data collection

The infection control committee carried out daily surveillance visits of the hospital. Data from the Hospital Infectious surveillance and control unit and microbiology department were used to hospital information system and lab registry.

Statistical Analysis

The data management and analysis process will involve the utilization of Microsoft Excel and Statistical Package for the Social science (SPSS version 22.0; IBM Corp. USA). Continuous and categorical variables are presented as mean± standard deviation (SD) and number (n) with percentage (%), respectively.

III. RESULT

In a retrospective study, the carbapenem resistant *Klebsiella pneumoniae* (CRKP) incidence and distributions were analysed from January 2023 to June 2023. Clinical samples of 4384 patients shown in **Table 1**, sent for microbiological analysis, were included.

The table provides a comprehensive overview of the various sample types collected, their frequencies, and their proportions relative to the total of 4,384 samples analyzed. Urine samples constitute the largest proportion, making up 39.25% of the total, reflecting their widespread use in diagnostic tests and analyses. Blood samples follow closely, representing 37.84% of the dataset, underscoring their essential role in diagnosing and monitoring health conditions. Swab samples, at 5.95%, are also significant, highlighting their frequent use in microbiological studies. Sputum samples account for 4.99%, indicating their importance in respiratory diagnostics. Bronchial wash samples, comprising 2.25%, are utilized for examining lung cells and microorganisms. E T secretion samples, at 2.23%, are involved in specific diagnostic tests related to bodily secretions. Tissue samples make up 2.03%, crucial for histopathological evaluations and cancer detection. Ascitic fluid, pleural fluid, cerebrospinal fluid (CSF), synovial fluid, and tip samples each contribute a small but important portion of the total: 0.86%, 0.82%, 0.70%, 0.54%, and 0.52% respectively. Lastly, vaginal swab samples account for 0.34%, used in gynecological assessments. Overall, the data demonstrates a diverse range of

sample types with a predominant focus on urine and blood, reflecting their vital roles in medical diagnostics and monitoring.

From the total 4384 patient's samples, 699 Gram Negative bacilli growth were isolated shown in **Table: 2**

This table provides a detailed summary of bacterial growth frequencies and their corresponding percentages, based on a total of 699 growth instances, highlighting the prevalence of different bacterial species in the analyzed samples. *Klebsiella pneumoniae* is the most frequently encountered bacterium, representing 36.05% of the total growths, indicating its significant role as a common pathogen in the studied context. *Escherichia coli* (*E. coli*) follows as the second most common, accounting for 24.3% of the growths, and is associated with various infections, including urinary tract infections and gastroenteritis. *Pseudomonas aeruginosa* represents 20.88% of the total, reflecting its role as a significant opportunistic pathogen, particularly in immunocompromised individuals and hospital settings. The *Acinetobacter baumannii* complex is observed in 6.7% of cases and is notable for its antibiotic resistance and role in healthcare-associated infections. *Proteus* species account for 4.2% of the growths, commonly linked to urinary tract and wound infections. The *Enterobacter cloacae* complex, representing 3.71% of the growths, is associated with nosocomial infections, underscoring its importance in clinical microbiology. *Citrobacter koseri*, at 3.4%, is less common but significant in infections among neonates and immunocompromised individuals, while *Providencia* species, contributing 0.57%, are the least frequent but notable for their involvement in urinary tract and other healthcare-associated infections. Overall, the dataset reveals a diverse bacterial profile, with *Klebsiella pneumoniae* and *E. coli* being the most prevalent, highlighting their prominent roles in the infection landscape, while the presence of other species such as *Pseudomonas aeruginosa* and *Acinetobacter baumannii* complex underscores the complexity and variety of microbial infections encountered.

From the 699 growths, 115 were Carbapenem Resistant Organism (CRO) shown in **Graph: 1**

From the 115 bacterial isolates 73 of them are Carbapenem Resistant -*Klebsiella Pneumoniae* (CR-KP), The antibiotics resistant pattern on *Klebsiella pneumoniae* are noted in the **Graph 2:**

From this Carbapenem Resistant *Klebsiella Pneumoniae*, randomly selected Species are sent for

the detection of the resistant gene, results of them are shown in **graph 3:**

IV. DISCUSSION

This study provides an in-depth examination of carbapenem-resistant *Klebsiella pneumoniae* (CR-KP) within a tertiary care setting in Kozhikode, Kerala, spanning January 2023 to June 2023. The results underscore the growing challenge of antibiotic resistance in nosocomial infections and highlight critical insights into the epidemiology and resistance mechanisms of CR-KP.

Prevalence and Distribution

Out of the 4384 clinical samples analyzed, 252 were identified as *Klebsiella pneumoniae* infections, with 73 cases demonstrating resistance to carbapenems. This translates to approximately 29% of *Klebsiella pneumoniae* isolates being carbapenem-resistant. This finding reflects a significant burden of CR-KP in the hospital setting, emphasizing a need for effective infection control measures.

The distribution of bacterial species isolated from the samples revealed that *Klebsiella pneumoniae* was the most prevalent pathogen, comprising 36.05% of the total isolates. Other notable pathogens included *E. coli* (24.3%), *Pseudomonas aeruginosa* (20.88%), and *Acinetobacter baumannii* complex (6.7%). These data highlight the diverse bacterial landscape in hospital-acquired infections, where multidrug-resistant organisms frequently complicate treatment protocols.

Resistance Patterns and Mechanisms

The study's focus on CR-KP isolates revealed a complex array of resistance mechanisms. The observed resistance genes included VIM, OXA-48, and NDM, with VIM being the most prevalent. These findings are consistent with global patterns of carbapenemase production among *Klebsiella pneumoniae* strains. VIM and OXA-48 are class B metallo-beta-lactamases and class D oxacillinases, respectively, known for their ability to hydrolyze carbapenems, thus contributing to resistance.

The presence of NDM (New Delhi metallo-beta-lactamase) is particularly concerning as it is associated with a high level of carbapenem resistance and has been reported in various regions worldwide. The detection of these genes underscores the importance of molecular

surveillance to monitor and control the spread of these resistant strains.

Comparison with Other Studies

The prevalence of CR-KP in this study aligns with findings from other regional and international studies. For instance, a study from China reported a similarly high rate of carbapenem resistance among *Klebsiella pneumoniae* isolates, with VIM being the predominant resistance gene [6]. Additionally, Indian studies have also highlighted the widespread presence of NDM and OXA-48 genes among *Klebsiella pneumoniae* strains [7].

These comparative results reinforce the notion that carbapenem resistance is a widespread issue that necessitates coordinated global and local efforts to combat antibiotic resistance. The high prevalence of OXA-48 and NDM-1 in our study further emphasizes the need for surveillance and rapid identification of resistant strains to inform treatment strategies.

Clinical and Public Health Implications

The high incidence of CR-KP poses significant challenges for clinical management. Carbapenems are often reserved for treating severe infections caused by multidrug-resistant bacteria, and their reduced efficacy can lead to limited treatment options and increased morbidity and mortality. The mortality rate associated with CR-KP infections can reach up to 50%, making it crucial for hospitals to implement rigorous infection control practices, including regular screening and isolation measures.

Furthermore, the study highlights the need for ongoing education and training for healthcare professionals regarding the prudent use of antibiotics. Efforts should be made to promote antibiotic stewardship programs that minimize the misuse and overuse of carbapenems and other critical antibiotics.

Limitations and Future Directions

While this study provides valuable insights, it is not without limitations. The retrospective design restricts the ability to establish causative relationships between antibiotic resistance patterns and patient outcomes. Additionally, the study focuses solely on *Klebsiella pneumoniae*, potentially overlooking other significant contributors to antibiotic resistance.

Future research should consider longitudinal studies to track changes in resistance

patterns over time and explore the clinical outcomes associated with CR-KP infections. Further investigation into the molecular mechanisms of resistance, including the identification of novel resistance genes, will also be essential for developing effective therapeutic strategies.

V. CONCLUSION

This study provides a comprehensive analysis of the prevalence and characteristics of carbapenem-resistant *Klebsiella pneumoniae* (CR-KP) in a tertiary care setting in Kozhikode, Kerala, over a six-month period. The findings underscore the significant issue of antibiotic resistance in nosocomial infections.

The high incidence of CR-KP emphasizes the need for stringent infection control measures and ongoing surveillance to manage and mitigate the impact of antibiotic-resistant infections. Continued research and molecular diagnostics are crucial to understanding resistance patterns and guiding appropriate therapeutic strategies. This study contributes valuable insights into the epidemiology of CR-KP and serves as a basis for developing targeted interventions to combat antibiotic resistance in hospital settings.

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TABLES

Table 1: Frequency and percentage of the samples

Sample Type	Frequency	Percentage
E T SECRETION	98	2.23
URINE	1721	39.25
BLOOD	1659	37.84
SPUTUM	222	4.99
BRONCHIAL WASH	99	2.25
SWAB	324	5.95
TIP	23	0.52
TISSUE	89	2.03
ASCITIC FLUID	38	0.86
CSF	31	0.70
PLEURAL FLUID	36	0.82
SYNOVIAL FLUID	28	0.54
VAGINAL SWAB	15	0.34
TOTAL	4384	100

Table 2: 699 isolated Gram-negative Bacilli were shown.

BACTERIA	Number of Growth	percentage
AcinetoBaumanii complex	47	6.7
Citrobacter Koseri	24	3.4
Enterobacter cloacae complex	26	3.71
E coli	170	24.3
Klebsiella pneumoniae	252	36.05
Proteus species	30	4.2
Providencia species	4	0.57
Pseudomonas aeruginosa	146	20.88
Total	699	100

FIGURES

Figure 1: 115 Carbapenem Resistant Organism (CRO) were shown.

Distribution of Carbapenem Resistant Organism

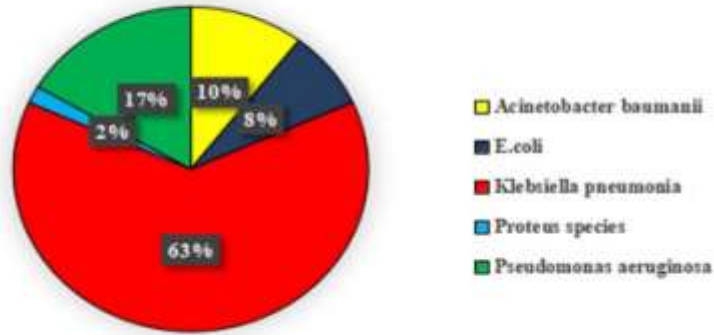


Figure 2: The antibiotics resistant pattern on Klebsiella pneumonia.

Antibiotic Resistant Pattern of Klebsiella Pneumonia

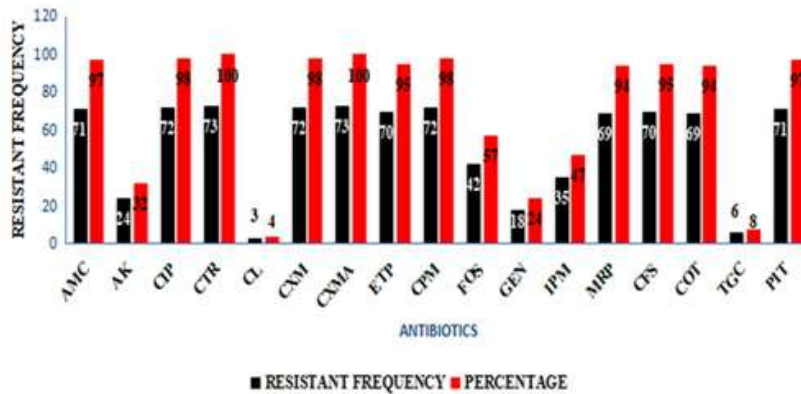


Figure3: Results of detection of the resistant gene in Carbapenem Resistant Klebsiella Pneumoniae.

FREQUENCY OF GENE DETECTION IN CRO

