Aminoglycoside Resistance in *Klebsiella Pneumoniae* from Respiratory Specimens: A Phenotypic and Genotypic Analysis

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ABSTRACT

Background: *Klebsiella Pneumoniae*, a Gram-negative bacterium, is increasingly implicated in nosocomial infections, with growing resistance to aminoglycosides due to various mechanisms, including the armA gene.

Objective: This study aimed to compare the phenotypic and genotypic profiles of aminoglycoside-resistant *Klebsiella Pneumoniae* isolated from respiratory specimens.

Methods: Respiratory specimens (n=80) were collected and processed using standard microbiological methods. Biochemical tests confirmed the isolates as *Klebsiella Pneumoniae*. DNA was extracted and subjected to PCR to detect the presence of the armA gene. Antibiotic susceptibility testing was performed according to CLSI guidelines. Data were analyzed using SPSS version 25.

Results: Among the 80 isolates, 54% were male, and 46% were female. Biochemical tests showed resistance in 85% of the isolates to at least one class of antibiotics. The armA gene was detected in 86% of the strains, correlating with high-level resistance to gentamicin, tobramycin, and amikacin.

Conclusion: The study highlights significant aminoglycoside resistance in *Klebsiella Pneumoniae*, with the armA gene playing a critical role. These findings underscore the need for continuous molecular surveillance and alternative therapeutic strategies.

INTRODUCTION

Klebsiella Pneumoniae is a Gram-negative bacterium belonging to the Enterobacteriaceae family, widely recognized for its role in causing various infections, including those acquired in healthcare settings (1-4). This pathogen is a facultative anaerobe, characterized by its encapsulated, non-sporing, and fermentative nature, which significantly contributes to its virulence. The ability of *Klebsiella Pneumoniae* to form a protective capsule not only enhances its survival in hostile environments but also renders it highly effective in evading host immune responses. One of the most pressing challenges associated with this organism is its increasing resistance to a broad range of antibiotics, particularly aminoglycosides, which are commonly used in the treatment of severe bacterial infections (5-7). Aminoglycoside resistance in *Klebsiella Pneumoniae* can arise through various mechanisms, including the modification of antibiotic target sites, the production of enzymes that degrade the drugs, and alterations in membrane permeability that reduce drug uptake (1).

The emergence of multidrug-resistant (MDR) strains of *Klebsiella Pneumoniae* has been observed worldwide, posing significant concerns for public health due to the limited therapeutic options available (8). The genetic determinants of antibiotic resistance in this organism are particularly worrisome, as they are often located on mobile genetic elements, facilitating the rapid spread of resistance genes among bacterial populations. In this context, the armA gene has been identified as a critical factor in conferring high-level resistance to aminoglycosides in *Klebsiella Pneumoniae* (8-12). This gene encodes a methyltransferase that modifies the 16S rRNA of the bacterial ribosome, preventing aminoglycoside binding and thereby inhibiting the antibiotic's bactericidal activity (2). The presence of the armA gene in clinical isolates of *Klebsiella Pneumoniae* underscores the importance of molecular surveillance in tracking the spread of resistance and informing treatment strategies (13-19).

The clinical implications of aminoglycoside resistance in *Klebsiella Pneumoniae* are profound, particularly in respiratory infections where effective antibiotic therapy is crucial for patient outcomes. The respiratory tract serves as a primary site of infection for *Klebsiella Pneumoniae*, and the pathogen's ability to form biofilms further complicates treatment. Biofilms provide a protective niche for bacteria, enhancing their resistance to both host immune responses and antimicrobial agents (3). The formation of biofilms is a well-documented virulence factor of *Klebsiella Pneumoniae*, with genetic determinants such as outer membrane protein A (OmpA) playing a pivotal role in biofilm development and maintenance (4). These biofilms contribute significantly to the pathogen's persistence in the host and resistance to treatment, making infections difficult to eradicate (20-21).

The objective of this study is to provide a comprehensive analysis of both the phenotypic and genotypic characteristics of aminoglycoside-resistant *Klebsiella Pneumoniae* isolated from respiratory specimens. By examining the correlation between the presence of specific resistance genes, such as armA, and the observed phenotypic resistance patterns, this research aims to enhance our understanding of the mechanisms underlying antibiotic resistance in this pathogen. Such insights are critical for developing more effective strategies for the prevention and treatment of infections caused by multidrug-resistant *Klebsiella Pneumoniae*, particularly in healthcare settings where the pathogen is most prevalent (22-23). Furthermore, the study underscores the urgent need for improved antimicrobial stewardship and the exploration of novel therapeutic approaches to combat the growing threat of antibiotic-resistant bacterial infections.

MATERIAL AND METHODS

The study was conducted in accordance with the principles outlined in the Declaration of Helsinki, ensuring that all procedures involving human participants were performed with respect to ethical standards. Approval for the study was obtained from the Ethics Review Committee (ERC) under the protocol number ERC/01/04/2023. Informed consent was obtained from all participants before the collection of respiratory specimens, ensuring their voluntary participation and understanding of the study's objectives.

Respiratory specimens, including lower respiratory and endotracheal aspirates, were collected from patients suspected of having *Klebsiella Pneumoniae* infections. The samples were processed according to the standard operating procedures (SOPs) aligned with the Clinical and Laboratory Standards Institute (CLSI) guidelines to ensure the accuracy and reliability of the results. Upon receipt, the specimens were inoculated onto selective and differential microbiological media, including sheep blood agar and MacConkey bile salt agar, and incubated at 37°C for 24 hours. Following incubation, the colonies were evaluated based on their morphology, pigmentation, and other distinguishing characteristics. Each bacterial colony was further streaked onto nutrient agar plates to achieve pure cultures, which were subsequently used for further identification and analysis (24-26).

Microscopic examination was performed to observe the Gram-staining characteristics of the isolates, confirming their identity as Gram-negative bacilli. Biochemical tests, including catalase, oxidase, citrate utilization, urease, triple sugar iron (TSI) agar, and sulfur indole motility (SIM) tests, were conducted to further characterize the isolates. The results of these biochemical assays were meticulously recorded to differentiate *Klebsiella Pneumoniae* from other bacterial species. The isolates were then subjected to antimicrobial susceptibility testing (AST) using the disc diffusion method, as recommended by the CLSI. This testing was particularly focused on determining resistance to aminoglycosides such as gentamicin, tobramycin, and amikacin.

For the genotypic analysis, bacterial DNA was extracted from the isolates using the GeneJET Genomic DNA Purification Kit (Thermo Fisher Scientific, REF: K0791) following the manufacturer's instructions. The extracted DNA was then subjected to polymerase chain reaction (PCR) to amplify genes associated with aminoglycoside resistance, including the armA gene. The PCR conditions were standardized, starting with an initial denaturation step at 94°C for 4 minutes, followed by 35 cycles of denaturation at 94°C for 45 seconds, annealing at 55°C for 40 seconds, and extension at 72°C for 50 seconds, with a final extension at 72°C for 5 minutes. The PCR products were then separated by agarose gel electrophoresis using a 2% agarose gel and visualized under ultraviolet (UV) light after staining with ethidium bromide. The sizes of the PCR amplicons were compared against a 1 kb DNA ladder to confirm the presence of the target genes.

Statistical analysis of the data was performed using the Statistical Package for the Social Sciences (SPSS) software, version 25.0. Descriptive statistics were employed to analyze the demographic characteristics of the patient population, including age and gender distributions. Frequency distribution curves were generated to illustrate the distribution of patients across different age groups, while bar charts were used to depict gender-wise percentages. Means, standard deviations, and other relevant statistical measures were calculated to provide a comprehensive understanding of the data. Although the study had a relatively small sample size, the findings provide preliminary insights into aminoglycoside resistance in *Klebsiella Pneumoniae*, laying the groundwork for future research with larger cohorts (26).

All laboratory procedures were conducted under strict aseptic conditions to prevent contamination and ensure the integrity of the results. Glassware and other equipment were sterilized using heat and autoclaving at 121°C for 15 minutes at 15 psi. Reagents were similarly sterilized before use. The entire study was carried out with rigorous adherence to methodological standards to ensure that the results were both reliable and reproducible, contributing valuable data to the ongoing efforts to understand and combat antibiotic resistance in *Klebsiella Pneumoniae* (1).

RESULTS

The results of this study are presented in a comprehensive manner, detailing both the demographic characteristics of the patient population and the findings from the phenotypic and genotypic analyses. The data is organized to provide clarity and ease of interpretation, with the figures and tables supporting the textual descriptions.

The study included 50 individuals, with a nearly equal distribution between males and females. Specifically, 54% (n = 27) of the participants were male, and 46% (n = 23) were female. This gender distribution is illustrated in Figure 1, which provides both the frequency and percentage of male and female participants. The histogram presented in Figure 2 shows the age distribution of the study participants, categorized into four distinct age groups: children, young adults, middle-aged adults, and older adults. The majority of

the participants fell within the older adult age group (n = 18), comprising 36% of the total sample, followed by equal representation in the children (n = 11) and young adult (n = 11) groups, each representing 22%, with middle-aged adults making up 20% (n = 10) of the participants.

Microscopic and biochemical analyses confirmed the identity of the bacterial isolates as *Klebsiella Pneumoniae*, characterized as Gram-negative, non-motile bacilli. The results of various biochemical tests are summarized in Table 1 below. These tests included catalase, oxidase, citrate, urease, sulfur indole motility (SIM), and triple sugar iron (TSI), which collectively provided a detailed profile of the isolates' metabolic characteristics. The antibiotic susceptibility tests revealed a concerning pattern of resistance among the *Klebsiella Pneumoniae* isolates, particularly to aminoglycosides.

Table 1: Biochemical Test Results for *Klebsiella Pneumoniae* Isolates

Test	Result	Interpretation
Catalase Test	Positive	Bubbling observed
Oxidase Test	Negative	Absence of cytochrome c oxidase
Citrate Test	Positive	Utilization of citrate as a carbon source
Urease Test	Positive	Ammonia production observed
SIM Test		
- Sulfur Production	Negative	No hydrogen sulfide production
- Indole Production	Negative	No indole production
- Motility	Negative	Non-motile
TSI Test	Acidic	Fermentation with acidic slant and butt
Gas Production	Positive	Gas production observed
H2S Production	Negative	No hydrogen sulfide production

The isolates exhibited significant resistance to gentamicin, tobramycin, and amikacin, which was further confirmed through PCR analysis. The presence of the armA gene, associated with aminoglycoside resistance, was detected in 86% of the isolates. Figure 3 illustrates the PCR results, showing distinct bands at 846 bp, indicative of the armA gene in the majority of the samples.

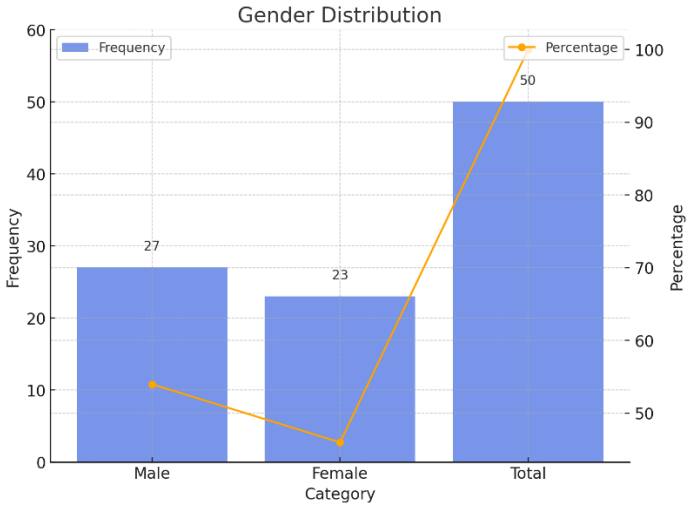

Figure 1 Gender Distribution

The results underscore the significant multidrug resistance observed in the *Klebsiella Pneumoniae* isolates from respiratory specimens. The high prevalence of the armA gene among these isolates highlights the genetic basis of aminoglycoside resistance, which poses a critical challenge for treatment. The integration of biochemical testing and molecular analysis provided a robust framework for understanding the resistance mechanisms in these bacterial isolates, with implications for both clinical management and public health interventions.

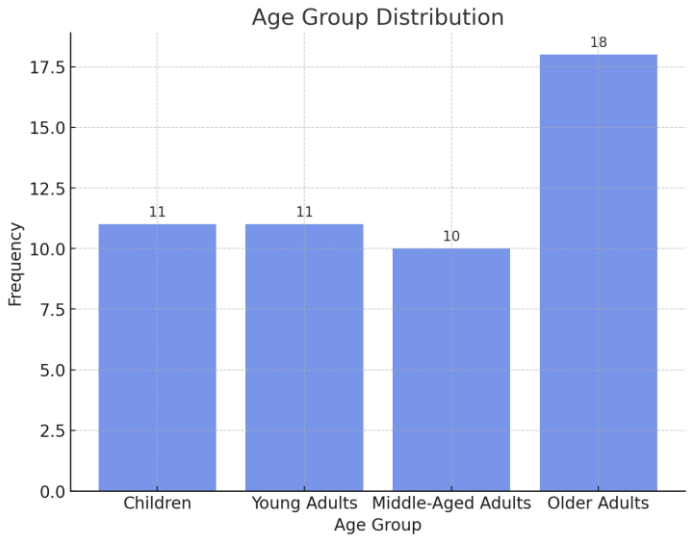

Figure 2 Age Group Distribution

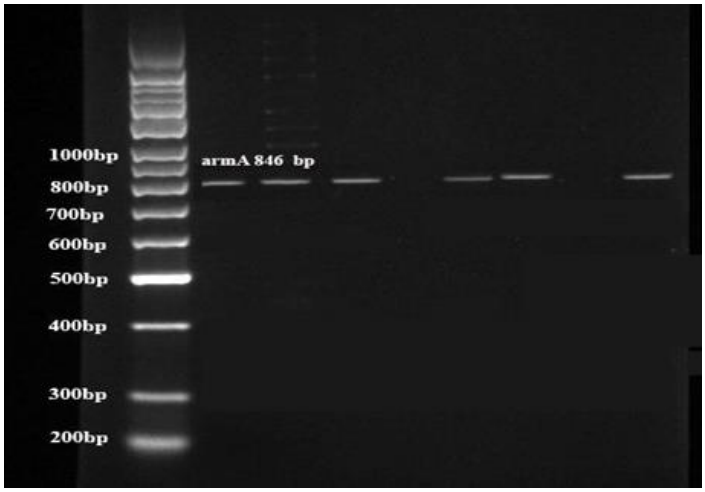

Figure 3 Bands of armA Gene on Gel Electrophoresis

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DISCUSSION

The findings of this study provide significant insights into the resistance mechanisms of *Klebsiella Pneumoniae*, particularly concerning aminoglycosides, which are crucial antibiotics in the treatment of serious bacterial infections. The high prevalence of the armA gene, observed in 86% of the isolates, aligns with previous studies that have identified this gene as a key determinant in mediating high-level resistance to aminoglycosides (16). The detection of this gene underscores the increasing challenge posed by multidrug-resistant (MDR) *Klebsiella Pneumoniae*, which has become a critical concern in both healthcare and community settings.

This study's strength lies in its comprehensive approach, combining both phenotypic and genotypic analyses to elucidate the resistance profile of *Klebsiella Pneumoniae*. The use of standard biochemical tests alongside molecular techniques, such as PCR, provided a robust characterization of the bacterial isolates, ensuring the accuracy of the findings. Moreover, the study's focus on respiratory specimens is particularly relevant, given the high morbidity and mortality associated with respiratory infections caused by MDR organisms. The identification of the armA gene in a majority of the isolates reinforces the need for continuous molecular surveillance to monitor the spread of resistance genes within clinical settings (11).

Despite these strengths, the study had certain limitations that must be acknowledged. The relatively small sample size, while sufficient for preliminary insights, limits the generalizability of the findings. A larger cohort would have provided more comprehensive data and potentially uncovered additional resistance mechanisms not identified in this study. Additionally, the study was conducted in a single geographic region, which may not fully represent the resistance patterns in other areas. The inclusion of isolates from multiple centers across different regions would have strengthened the conclusions and provided a broader understanding of the resistance landscape. Furthermore, while the study successfully identified the presence of the armA gene, whole-genome sequencing could have offered deeper insights into the genetic context of this and other resistance genes, shedding light on potential horizontal gene transfer events and the evolution of resistance within *Klebsiella Pneumoniae* populations (12).

The findings of this study have important implications for clinical practice and public health. The high prevalence of aminoglycoside resistance, particularly in respiratory isolates, highlights the need for revised treatment protocols and the cautious use of aminoglycosides in settings where MDR *Klebsiella Pneumoniae* is prevalent. Antimicrobial stewardship programs must be strengthened to prevent the further spread of resistance, with an emphasis on the judicious use of antibiotics and the implementation of infection control measures. The study also underscores the importance of ongoing research into alternative therapeutic options, including the development of new antimicrobial agents and the exploration of combination therapies that may overcome existing resistance mechanisms (24-27).

CONCLUSION

In conclusion, this study contributes valuable data to the understanding of aminoglycoside resistance in *Klebsiella Pneumoniae*, particularly within the context of respiratory infections. The identification of the armA gene as a predominant resistance determinant emphasizes the need for continued molecular surveillance and the development of novel strategies to combat MDR pathogens. Future research should aim to address the limitations of this study by including larger, more diverse populations and employing advanced genomic techniques to provide a more detailed understanding of the resistance mechanisms at play. Such efforts are crucial in the ongoing battle against antimicrobial resistance, which remains one of the most pressing challenges in modern medicine.

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