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PREVALENCE AND AMINOGLYCOSIDE RESISTANCE PATTERN OF *ACINETOBACTER BAUMANNII* ISOLATED FROM HOSPITALIZED PATIENTS IN LAHORE, PAKISTAN

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ABSTRACT

Acinetobacter baumannii is a gram-negative bacterium which poses a major public health threat responsible for severe and invasive infection (mostly nosocomial) associated with high mortality rate. The multidrug resistant (MDR) poses a main clinical issue specifically due to its increasingly resistance to antibiotics such as aminoglycosides. The study was aimed to isolate *A. baumannii* from different source of clinical specimen and evaluate their antimicrobial resistance pattern against aminoglycosides. The study was conducted from November 2023 to May 2024 and 85 clinical samples were processed including blood (in culture bottles), wound swab, urine (in catheters). Samples were analyzed on Blood agar, CLED agar, L-agar and then incubated at 34^{0}C for 24hours. Gram staining was to differentiate bacteria and gram-negative isolates were biochemically analyzed. *A. baumannii* was confirmed by citrate positive, catalase positive and oxidase, indole negativity. Kirby Bauer disk diffusion method was used to asses antimicrobial susceptibility testing on Muller Hinton agar and results were analyzed according to CLSI guidelines. The frequency of *A. baumannii* was 36.47% with men having slightly higher rates (55%) than females (45%). The most common source of isolates was urine (54%) followed by wound swab (25%) and blood (19%). The resistance pattern for aminoglycosides was streptomycin (70.96%), amikacin (51.66%) neomycin (45.16%), gentamycin (35.48%) and tobramycin (32.25%). The findings highlight the critical necessity for ongoing antimicrobial resistance monitoring and implementation of appropriate antibiotic stewardship policies in healthcare settings to minimize the development of *A baumannii.*

Keywords: Antibiotic Resistance, MDR, Antibiotic susceptibility testing

INTRODUCTION

Bacteria are the most diverse forms of life on Earth, habituating an incredible variety of ecological niches. Most of the species are non-pathogenic, some act as opportunistic or obligate pathogens, main cause factor for infectious diseases. These are classified on the basis of shape, metabolism, and cell wall composition, with Gram staining for differentiating between gram-positive and gram-negative organism. *A baumannii*, a gram-negative bacterium, has evolved as one of the most significant therapeutically pathogen due to is incredible flexibility and tendency for multidrug resistance mechanism **(Mukhopadhyay *et al*., 2024)**.

A. baumannii is aerobic, catalase positive, oxidase negative, immobile coccobacillus pathogen. It is commonly observed as an opportunistic disease that affects mostly immunocompromised patients, particularly those having long term hospitalization or invasive medical interventions like catherization, ventilation, or surgery. It can habitat everywhere in nature, including soil, water, and human skin and mucous membrane. Its ability to survive on both dry and damp hospital surfaces provides a survival advantage that aids in nosocomial transmission, making it a dangerous healthcare associated pathogen **(Scoffone *et al*., 2025)**.

A. baumannii taxonomy has traditionally been complicated. Before being grouped into the genus Acinetobacter, early isolates were frequently mislabeled as Mima polymorpha and Herellea vaginicola, among others. More than 50 species are now known, although just a handful, notably *A. baumannii*, *A. calcoaceticus,* and *A. lwoffii*, are reliably linked to human disease. *A. baumannii* is thought to be the most virulent of the group, according to both clinical and animal model research. Because of its pathogenic potential and resistance features, the World Health Organization has designated it as a priority pathogen on its list of serious threats to global health **(Li *et al*., 2025)**.

Clinically, *A. baumannii* causes a wide range of illnesses. Nosocomial pneumonia especially ventilator associated pneumonia is a common clinical presentation of with 30% to 70% mortality rates in cases involving multidrug resistance bacteria. These bacteria can also cause urinary tract infection, bloodstream infections, meningitis and less commonly endocarditis. Community-acquired pneumonia caused by *A. baumannii* has also been observed, mainly in Southeast Asia and Australia, where it frequently progresses to septic shock and respiratory failure. Invasive infections are linked to poor clinical outcomes, especially in critically ill or elderly patients, and therapy is confounded by the pathogen's resistance to numerous antibiotic classes **(Ajoseh *et al*., 2025)**.

Because of their high bactericidal action, aminoglycosides such as gentamicin, amikacin, and tobramycin have long been used to treat *A. baumannii* infections. These antibiotics work by attaching to the bacterial 30S ribosomal subunit, limiting protein synthesis and causing misreading of mRNA. However, the advent of aminoglycoside resistance has severely limited their therapeutic utility. Resistance mechanisms include enzymatic aminoglycoside modification, changes in ribosomal target locations, active efflux pumps, and biofilm formation. Genes for aminoglycoside-modifying enzymes (AMEs), including as acetyltransferases, nucleotidyltransferases, and phosphotransferases, are very common and frequently carried on mobile genetic elements, allowing for rapid dissemination **(Kishk *et al*., 2021)**.

The molecular basis of aminoglycoside resistance has been the subject of extensive research. Molecular characterization techniques such as polymerase chain reaction (PCR), ribotyping, and whole-genome sequencing have provided vital insights into the genetic causes of resistance. Such approaches allow for the identification of resistance genes, transmission mechanisms, and associations with certain *A. baumannii* clonal lineages **(Yamada *et al*., 2025)**.

Patients infected with resistant strains of *A. baumannii* typically have longer hospital admissions, higher healthcare costs, and higher mortality rates than those infected with susceptible strains. The global proliferation of resistant clones has further reduced treatment choices, leaving only last-resort drugs like colistin and tigecycline as viable therapy. Even these, however, are under increasing threat from evolving resistance. Therefore, sound management strategies must be included not only the restricted use of current antibiotics, but also exploring the combination therapies, infection control measure and establish alternative therapeutic approaches such as novel antimicrobial peptides and phage therapy **(Shi *et al*., 2025)**.

The increasing prevalence of *A. baumannii* aminoglycoside resistance signify the importance of continued surveillance and research. The resistance pattern from clinical isolates can be used to enhance empirical therapy, develop antimicrobial stewardship initiatives, and shape public health policies. In addition, to prevent the spread of multidrug resistant bacteria, understanding of genetic and molecular pathways that cause resistance could speed up the establishment of new techniques.

(Ji *et al*., 2025) This study was conducted to analyzed and identify the prevalence of A baumannii among different hospital settings in Lahore and to enhance infection control practices by endowing to antimicrobial oversight efforts.

MATERIAL AND METHODS

Study Design

A cross sectional study was planned at The University of Lahore, Institute of Medical Laboratory Technology. The study aims to identify and map out trends of aminoglycoside resistant Acinetobacter baumannii from different hospital settings in Lahore Pakistan.

Study Setting

The study was conducted in different hospital settings in Lahore, (the University of Lahore Teaching Hospital, Mayo Hospital, Jinnah hospital, and Services hospital,) on hospitalized patients.

Duration

This study was conducted in a time duration of 7 months from November 2023 to May 2024.

Sampling

Samples were collected from various hospital settings from particularly infected patients and collected samples were transferred to the laboratory under aseptic conditions.

Sample Size

A total number of 85 samples were collected from patients from different wards of hospitals.

Sample Selection

The samples selected for the study were wound infection swabs, Urine samples, and Blood samples.

Ethical Approval

The ethical approval was obtained from the department of University Institute of Medical Laboratory Technology, Faculty of Allied Health Sciences, The University of Lahore.

Inclusion Criteria

•The samples selected for this experimental study were from patients admitted in ICU, Medical Unit, and Surgical Ward.
•The age criteria that were selected was between 5 years to 63 years.

Exclusion Criteria

•The samples from OPD were not included in the study.
•People under 5 years and above 63 years of age were also not included in this experiment.
•Moreover, the hospital staff, workers, and housekeeping were also excluded from this criterion.

Sample collection

Samples were collected from different wards, particularly from the ICU, Medical Unit, and Surgical unit. Sampling was conducted by swabs for wound infections while some of them were collected from direct blood veins and urine (catheter). The wound infection was conducted through the swabbing technique. Sterile swabs were used, and after swabbing, swabs were immediately preserved in a sterile normal saline solution. Samples were taken to the laboratory within an hour. For Blood samples, after drawing blood, culture bottles (liquid broth) were used for bacterial growth. After positive results, inoculation was performed on Blood and MacConkey agar. After which further isolation and identification steps were followed.
Urine samples from patients were taken into sterile containers that were processed within an hour and inoculation was performed on CLED agar culture plates.

Isolation of bacteria

For the isolation of bacteria selective media and enriched media were used. The basic nutrient agar is used for the isolation of most bacteria easily, is mostly used for refreshing bacterial colonies, and has basic nutritional needs for bacteria. Using the swabs, the samples were inoculated on L- Agar which is an enriched media. The plates were incubated at 37C for 24 hours. After the completion of incubation, the plates were observed, and results were recorded for the presence of bacteria that forms complex streak. Blood agars give colorless nonhemolytic shiny mucoid colonies of *Acinetobacter baumannii.*

Identification and characterization

Identification of isolates was performed through colony morphology, gram stanning, biochemical testing, and antibiotic susceptibility testing was performed for identification of aminoglycoside-resistant bacteria

Morphological Characterization

To identify the bacteria through morphological characteristics gram staining was performed. A small amount of bacterial culture was taken using an inoculation loop and spread onto a clean microscopic slide. In the staining procedure crystal violet stain followed by iodine, ethanol and then counter stain of safranin were used. The results were recorded under a microscope by using 40 X and 100 x magnification power. Gram-positive bacteria appear purple or blue while Gram-negative bacteria give pink or red. Under a microscope, *Acinetobacter baumannii* appears to be a short round rod-shaped bacterium halfway between a rod and a ball.

Biochemical characterization

Catalase test

Catalase is a common enzyme found in nearly all living organisms exposed to oxygen which catalyzes the decomposition of hydrogen peroxide to water and oxygen. It is a very important enzyme in protecting the cell from oxidative damage by reactive oxygen species. *Acinetobacter baumannii* is a confirmed catalase positive organism. To perform this test coagulase reagent which is hydrogen peroxide (H2O2) was used. For this purpose, a drop of coagulase reagent is placed on a glass clean slide. Taking a bacterial colony and mixing it with coagulase reagent produces bubbles. Measure the results within 30 seconds, the oxygen bubbles are produced by decomposition of hydrogen peroxide by catalase enzyme.

Citrate utilization test

A. baumannii is positive for citrate utilization. This means that it can utilize citrate as a carbon source for growth. The enzyme citrate lyase helps break down citrate into pyruvate and carbon dioxide, which can then be used by the bacterium. For this purpose, Simmons citrate agar is prepared in a glass tube. Streak the slant back and forth with a light inoculum picked from the center of a well-isolated colony. Incubate aerobically 37C for 24 hours. Observe a color change from green to blue along the slant. Blue color represents a positive test. Citrate utilization test is important as it helps in determining the ability of an organism to utilize citrate as a sole source of energy, differentiating members of Enterobacteriaceae, and identifying gram-negative pathogens and environmental isolates.

Coagulase test

Acinetobacter baumannii are confirmed coagulase negative, which determines if a bacteria can produce bound coagulase. This exoenzyme converts fibrinogen in blood plasma into fibrin which forms a clot. Blood plasma is used to check the activity of bacteria. For this purpose, a suspension of saline and plasma is prepared and poured into glass tubes. Several bacterial colonies are inoculated in it and are placed in incubation at 37C for 4-6 hours. Examine after every half an hour for clotting in it.

Analytical Profile Index (API 20)

API20 was used, and the suspension was inoculated into the cups for individual tests and was incubated for 24 hours at 37C. After the incubation, a few reagents were added in some wells and the results were observed according to the specific codes for positive and negative results and organisms were identified according to their identification number.

Antimicrobial susceptibility testing

Antimicrobial susceptibility testing was performed using the Kirby Bauer Disk diffusion method on Mueller Hinton agar according to the Clinical and Laboratory Standards Institute (CLSI) guidelines (2023 edition). Antibiotics tested were: amikacin, tobramycin, gentamycin, neomycin, and streptomycin. The agar plates were inoculated with standard suspension of bacteria. The antibiotic discs were punched on the agar plates followed by incubation for 24 hours at 37C. The results were recorded for the susceptibility pattern by measuring the diameter of the zone of inhibition and reported as sensitive, resistant, or intermediate according to the CLSI.

Statistical Analysis

Statistical analysis was performed using SPSS version 26.0. Descriptive statistics were calculated as frequencies and percentages for categorical variables.

RESULTS

During the study period, a total of 85 samples collected from hospitalized patients, 31 (36.47%) were detected as *Acinetobacter baumannii* isolates (Table 1). These isolates were further tested for antimicrobial resistance patterns, hospital ward distribution, source of infection and demographic characteristics. Among the confirmed cases, the distribution of isolates according to gender showed a slightly higher frequency among male patients as compared to female patients (Figure 1).

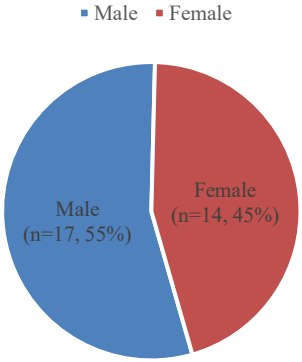

Figure 1 Age-wise distribution of Acinetobacter baumannii isolates demonstrating the number of positive cases across different age groups of hospitalized patients

The age wise distribution demonstrated that highest concentration of isolates was obtained from patients aged 16-30 years, while the minimum frequency was observed among patients older than 60. (Table 2 Table 3, Figure 2).

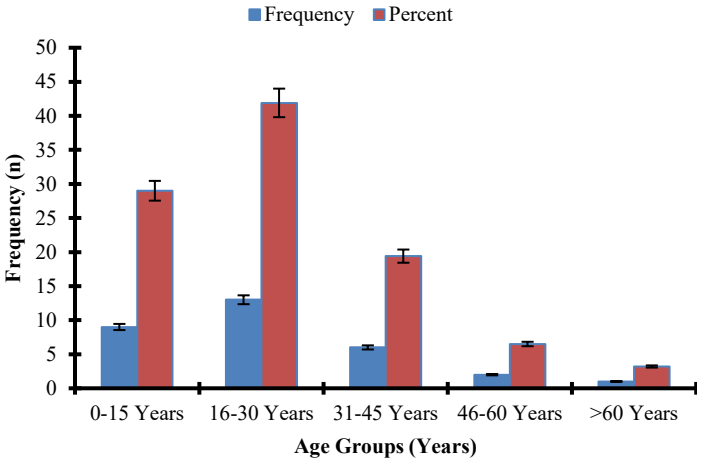

Figure 2 Comprehensive demographic distribution of ARAB isolates across specific patient age categories

The most common source of *A. baumannii* was urine samples, followed by wound and blood samples (Figure 3).

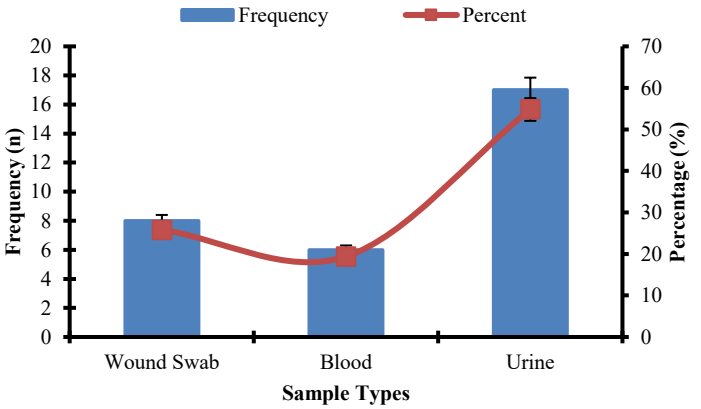

Figure 3 Distribution of ARAB isolates according to sample type (urine, wound swab, and blood), illustrating the relative frequency of isolates from each clinical specimen

The distribution analysis among hospital wards indicated that the surgical units contributed the highest volume of *A. baumannii* isolates, followed by medical wards and intensive care units (Figure 4).

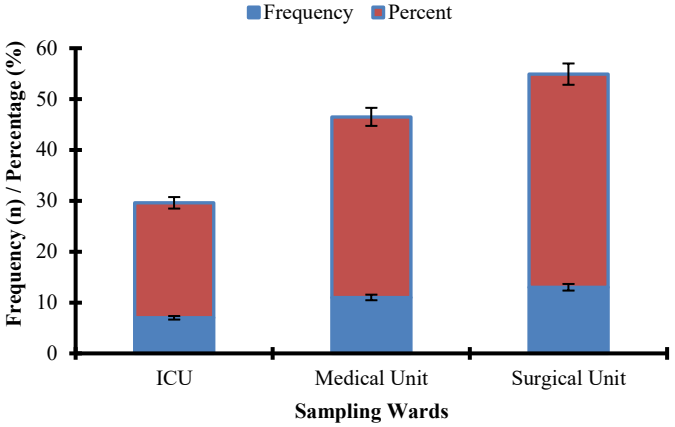

Figure 4 Ward-wise distribution of ARAB isolates, showing the number of cases identified in surgical units, medical wards, and intensive care units

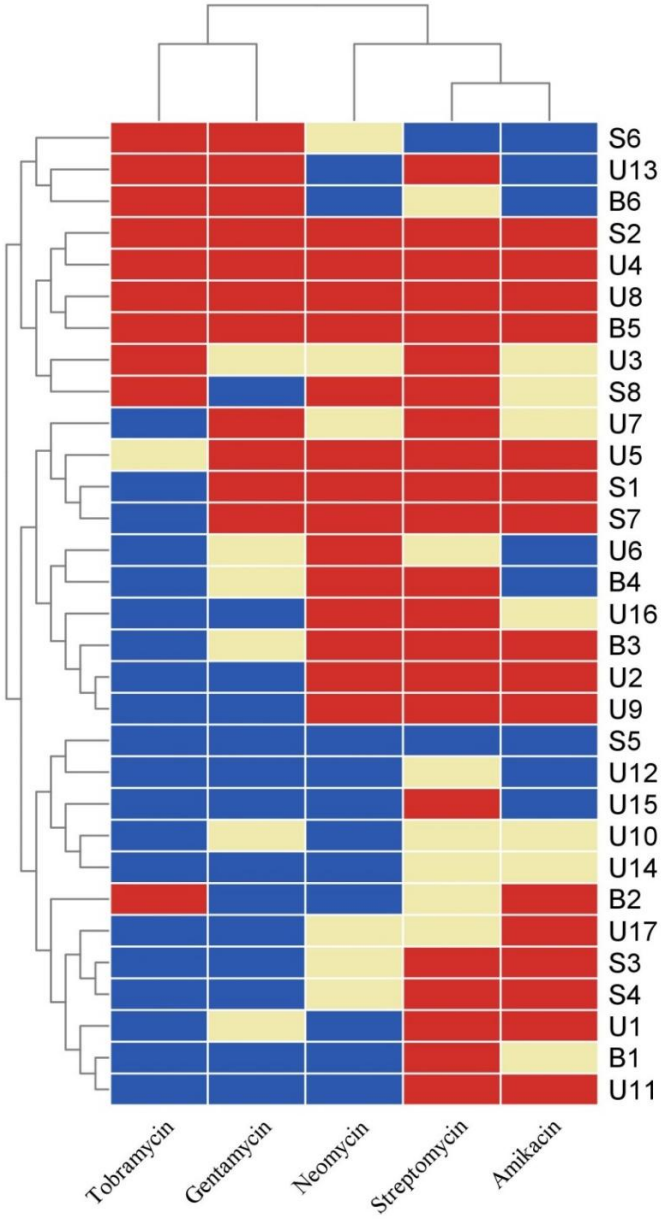

Figure 5 Clustering analysis of antibiotic resistance profiles of ARAB strains in the sample population. Blue color indicates sensitivity, yellow intermediate, and red resistance

The antimicrobial susceptibility analysis demonstrated variable resistance pattern across the evaluated aminoglycosides. Streptomycin showed highest resistance frequency followed by amikacin and neomycin while gentamicin and tobramycin exhibited lower resistance and high sensitivity against *A. baumannii* (Table 4). Correlation analysis using point-biserial correlation revealed weak associations between antibiotic-resistance and variables such as age, gender, sample type, and hospital ward (Table 5). The heat map demonstrated distinct clusters of ARAB strains, highlighting shared resistance traits particularly among aminoglycosides and providing the identification of multidrug-resistant lineages within the clinical setting (Figure 5).

Table 1 Distribution of positive sample types by sampling wards

Sample Types	Sampling Wards			
	ICU	Medical Unit	Surgical Unit	Total
Wound Swab	3 (9.7)	4 (12.9)	1 (3.2)	8 (25.8)
Blood	2 (6.5)	1 (3.2)	3 (9.7)	6 (19.4)
Urine	2 (6.5)	6 (19.4)	9 (29.0)	17 (54.8)

Table 2 Distribution of sample types by gender and age groups in the sample population

Sample Types	Age Groups (Years)					Gender	
	0-15	16-30	31-45	46-60	>60	Male	Female
Wound Swab	2 (6.5)	4 (12.9)	1 (3.2)	0 (0.0)	1 (3.2)	4 (12.9)	4 (12.9)
Blood	3 (9.7)	1 (3.2)	2 (6.5)	0 (0.0)	0 (0.0)	4 (12.9)	2 (6.5)
Urine	4 (12.9)	8 (25.8)	3 (9.7)	2 (6.5)	0 (0.0)	9 (29.0)	8 (25.8)

Table 3 Antibiotic resistance pattern in ARAB by patient gender. n (%)

Antibiotics	Gender		
	Male	Female	p-value
Neomycin	8 (25.8)	6 (19.4)	0.950
Streptomycin	11 (35.5)	11 (35.5)	0.393
Tobramycin	6 (19.4)	4 (12.9)	0.516
Gentamycin	4 (12.9)	7 (22.6)	0.209
Amikacin	8 (25.8)	8 (25.8)	0.837

Table 4 Variations in antibiotic resistance patterns of ARAB by sample types and sampling wards. n (%)

Antibiotics	Wound Swab	Blood	Urine	p-value	ICU	Medical Unit	Surgical Unit	p-value
Neomycin	4 (12.9)	3 (9.7)	7(22.6)	0.357	4 (12.9)	3 (9.7)	7 (22.6)	0.406
Streptomycin	6 (19.4)	4 (12.9)	12(38.7)	0.083	5 (16.1)	8 (25.8)	9 (29.0)	0.676
Tobramycin	3 (9.7)	3 (9.7)	4(12.9)	0.703	2 (6.5)	0 (0.0)	8 (25.8)	0.007
Gentamycin	4 (12.9)	2 (6.5)	5(16.1)	0.524	4 (12.9)	2 (6.5)	5 (16.1)	0.483
Amikacin	5 (16.1)	3 (9.7)	8(25.8)	0.874	4 (12.9)	6 (19.4)	6 (19.4)	0.925

Table 5 Point Biserial correlation of various parameters with antibiotic resistance

Parameters	Antibiotics									
	Neomycin		Streptomycin		Tobramycin		Gentamycin		Amikacin	
	ρ	p-value	ρ	p-value	ρ	p-value	ρ	p-value	ρ	p-value
Age	0.040	0.830	-0.170	0.361	0.016	0.933	0.153	0.411	-0.143	0.443
Gender	-0.026	0.891	0.213	0.250	-0.034	0.857	0.316	0.083	0.107	0.567
Sample Types	-0.165	0.376	0.139	0.455	-0.126	0.498	-0.091	0.628	-0.060	0.750
Sampling Wards	-0.027	0.886	0.078	0.676	0.309	0.091	-0.066	0.725	-0.027	0.885

DISCUSSION

The prevalence of *A. baumannii* clinical isolates in a hospital setting was 36.47%, which is comparable to reports from Central America. Latin America and the Middle East and Eastern Asia, where prevalence was 100%, 100% and 64.6% in tertiary care centers. These rates reflect the persistence of *A. baumannii* as a significant nosocomial pathogen, particularly in environments with high antimicrobial pressure **(Lim *et al*., 2019)**. The average age of the affected patients was 24 (range: 5-63 years). While *A. baumannii* infections are typically linked with the elderly or immunocompromised people, **(Kim *et al*., 2012)** our data show that younger populations are also profoundly affected. In trauma and burn wards, younger adults account for a significant number of admissions **(Song *et al.*, 2016)**. The age distribution in our analysis implies that *A. baumannii* is not limited to traditional high-risk populations, but rather poses a concern across a broad demographic spectrum. Males had a somewhat higher prevalence (55%) than females (45%), according to gender analysis. Gender differences were not statistically significant ($p > 0.05$), suggesting that both sexes are equally susceptible to infection from hospital-associated risk factors. Other regional investigations have shown similar gender patterns, with male predominance being ascribed more to healthcare exposure than to innate biological sensitivity **(Liu *et al*., 2025)**. Urine had the greatest percentage of isolates (54%), followed by wound swabs (25%), and blood cultures (19%).

These results contrast with worldwide patterns, where the clinical profile of *A. baumannii* is dominated by bloodstream infections and ventilator-associated pneumonia **(De Benedetto *et al*., 2022)**. The prevalence of urine isolates in our environment may be a reflection of regional healthcare customs, such as the extensive use of urinary catheterization, and emphasizes the necessity of more stringent infection control protocols related to devices. Alarming resistance levels to aminoglycosides were found by antimicrobial susceptibility testing for streptomycin (70.96%), amikacin (51.61%), neomycin (45.16%), gentamicin (35.48%), and tobramycin (32.25%). Significant variations in resistance rates among the investigated aminoglycosides were confirmed by statistical comparisons ($p < 0.05$).

Compared to gentamicin and tobramycin, streptomycin resistance was noticeably higher, indicating that streptomycin is no longer clinically useful in treating *A. baumannii* infections. resistance to amikacin (more than 50%) is particularly alarming, as it is often regarded as the most effective aminoglycoside against multidrug-resistant gram-negative bacteria. Similar resistance levels (74.4%) were recorded in Iran **(Keikha *et al.*, 2023)**. Neomycin resistance (45.16%) is consistent with regional data, indicating its poor effectiveness. Although resistance to gentamicin and tobramycin is decreasing, their reduced efficacy remains a therapeutic problem, particularly in resource-poor areas where treatment options are limited.

The significant aminoglycoside resistance rates reported in this investigation are consistent with global surveillance data. Resistance mechanisms are most likely mediated by aminoglycoside-modifying enzymes, efflux pumps, and biofilm formation, all of which cause treatment failure. Although antibiotics such as streptomycin and neomycin are not routinely used for the treatment of *A. baumannii* infections, they were included in this study to evaluate resistance patterns and enable comparison with regional and historical data. Clinically, these resistance patterns restrict the efficacy of aminoglycosides in monotherapy and lower their value in combination therapies. As a result, dependence shifts to last-resort treatments like colistin and tigecycline, however rising resistance to these drugs has also been reported **(Zhang *et al.*, 2023)**.

CONCLUSION

This study presented a major incidence of aminoglycosides resistant *A. baumannii* in those patients who are hospitalized (36.47%) with urine being the most prevalent source of isolation. The antimicrobial resistance pattern across age group revealed higher resistance for streptomycin, and amikacin whereas lower resistance was seen for neomycin, tobramycin and gentamycin. Males presented higher resistance to neomycin and tobramycin while higher resistance to streptomycin, gentamycin and amikacin was seen in females. The surgical units had peak number of *A. baumannii* cases and tobramycin resistance diverse significantly by ward, highlighting the importance of clinical context on research trends. The limited effectiveness of aminoglycosides against *A. baumannii* signify the utility of ongoing surveillance, ward specific antibiograms and strong antimicrobial stewardship endeavors. Future multicenter research involving molecular analysis are encouraged to better characterize the resistance patterns and provide more effective cure and mitigation efforts.

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