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Original Article

Characterization of *Aminoglycoside*-Resistant *Acinetobacter baumannii* in Respiratory Specimens: Phenotypic and Molecular Insights

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ABSTRACT

Background: The global rise of antibiotic-resistant bacteria presents a formidable challenge to healthcare systems, with *Acinetobacter baumannii* standing out as a particularly resilient pathogen in hospital settings. The propensity of this organism to exhibit multidrug resistance complicates treatment protocols and underscores the need for in-depth research into its resistance mechanisms.

Objective: The objective of this study was to characterize the allelotypic and molecular features of *aminoglycoside*-resistant *Acinetobacter baumannii* isolates from respiratory specimens and to determine their antibiotic susceptibility profiles to inform better clinical decision-making and treatment approaches.

Methods: This cross-sectional study was conducted at the different labs of various institute of Lahore. A total of 50 respiratory specimens were cultured on selective media. The isolates underwent phenotypic characterization through colony morphology and biochemical tests. Antimicrobial susceptibility was assessed using the disc diffusion method, and molecular analysis was performed using *polymerase chain reaction* (PCR) to identify the *armA gene* associated with *aminoglycoside* resistance. Statistical analysis was executed using SPSS Version 25.

Results: Of the 50 isolates, 54% were from male patients and 46% from female patients. Antibiotic resistance was alarmingly high, with resistance rates of 92% for TZP, 90% for FEP, CAZ, IPM, MEM, 86% for AK, 74% for CN, 54% for TOB, and 48% for DO. The presence of the *armA gene* was detected in 86% of the isolates, suggesting a link to the high levels of *aminoglycoside* resistance observed.

Conclusion: The study revealed a high prevalence of multidrug-resistant *Acinetobacter baumannii* in respiratory specimens, with significant resistance to commonly used antibiotics. These findings highlight the necessity for continuous surveillance of antibiotic resistance patterns and call for innovative approaches to antimicrobial therapy.

Keywords: *Acinetobacter baumannii*, *aminoglycoside* resistance, multidrug resistance, *armA gene*, antibiotic susceptibility, respiratory infections, molecular characterization, infection control, antimicrobial stewardship.

INTRODUCTION

Acinetobacter baumannii, a member of the *Moraxellaceae* family, is recognized as a significant pathogen in the realm of nosocomial infections. This gram-negative, coccobacillary organism is pleomorphic and aerobic by nature and is known for its inability to ferment glucose, lactose, or sucrose (1, 2). Its clinical relevance is underscored by its potent virulence factors, such as *lipopolysaccharide*, *phospholipases*, and outer membrane protein A (*OmpA*). The latter binds to epithelial cells and mitochondria, inducing mitochondrial dysfunction and leading to apoptosis, facilitated by the release of *cytochrome c oxidase* and the consequent formation of the apoptosome (3-5).

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The robust biofilm-producing ability of *A. baumannii* is particularly noteworthy. Biofilms, which are communities of microbial cells ensconced within an extracellular polymeric substance, are hotspots for genetic exchange and significantly contribute to the organism's resistance phenotype and the spread of resistance genes (6). Furthermore, Acinetobacter species have evolved to exhibit significant antimicrobial resistance, which is especially concerning in the context of hospital-acquired infections caused by ESKAPE pathogens. The group of bacteria known as ESKAPE (*Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, A. baumannii, Pseudomonas aeruginosa*, and *Enterobacter species*) is notorious for hospital infections and its members demonstrate various mechanisms of resistance, including the expression of different isoforms of enzymes like E4PDH, which is akin to the glycolytic enzyme GAPDH involved in the DXP pathway (7).

The intrinsic resistance of Acinetobacter to many antibiotics is partly due to its less permeable outer membrane and its access to a vast pool of genetic resistance mechanisms (8). *Beta-lactams*, one of the main classes of antibiotics, act by binding to penicillin-binding proteins, disrupting bacterial cell wall biosynthesis (9). Among these, *carbapenems* are crucial; however, *A. baumannii's* growing carbapenem resistance poses a formidable challenge in clinical settings. Resistance to *colistin*, one of the last-resort antibiotics, has also been identified, attributed to structural changes in LPS and the presence of plasmid-borne mcr genes (10).

Given its clinical significance, *A. baumannii* is the focus of intense study, including efforts to elucidate the mechanisms underlying its resistance to *aminoglycosides*. *Aminoglycosides*, which bind to the bacterial ribosomal subunits, are known for their ability to interrupt protein synthesis and thus kill the bacteria. Nevertheless, *A. baumannii's* remarkable *aminoglycoside* resistance is due to various adaptive strategies, such as modifications to the targets of these antibiotics, enzymatic degradation, and efflux pumps that extrude harmful substances from the cell (8-10).

The identification of bacterial pathogens, including *A. baumannii*, leverages both phenotypic and genotypic methods. Classic microbiological approaches like gram staining are used to differentiate between gram-positive and gram-negative bacteria, while culture techniques allow for the growth and morphological analysis of microbial colonies. Biochemical testing provides further insights into bacterial identity based on metabolic capabilities. To ascertain the most effective treatment, antimicrobial susceptibility patterns are assessed. Meanwhile, molecular techniques such as PCR offer a powerful means to detect resistance genes and understand the genetic makeup of these formidable pathogens. The amalgamation of these methods affords a comprehensive approach to tackling the challenges posed by *aminoglycoside-resistant A. baumannii*, enabling both the detection and understanding of this critical healthcare-associated pathogen.

MATERIAL AND METHODS

In the execution of this study, meticulous preparation of all media and reagents was undertaken using distilled water. Glassware, essential in the process, underwent a rigorous cleaning protocol: professionally cleansed, heat-dried, and sterilized to ensure the absence of any contaminants that could potentially compromise the integrity of the results. The sterilization process, a pivotal step in the preparation, was carried out for a precise duration of 15 minutes at the stringent conditions of 121°C and 15psi, a standard set to ensure complete sterility (REF: CM0055B, CM0337B, CM0277, NCM0277A, CM0155).

Table 1 *Nutrient Agar* Composition

S. No.	Components	g L-1
1	Peptone	5
2	Beef Extract	3
3	Agar	15

Table 2 *MacConkey Agar* Medium Composition

S. No.	Components	g L-1
1	Bacto Peptone	170
2	Lactose	10.0
3	Proteose Peptone	3.0
4	Sodium Chloride	5.0
5	Agar	13.3
6	Bile Salts Mixture	1.5
7	Neutral Red	0.03
8	Crystal Violet	0.001
9	Distilled Water	1000

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Table 3 *Blood Agar* Base Composition

S. No.	Components	g L-1
1	*Blood Agar* Base	40

Table 4 Gram Staining Solution Composition

S. No.	Components	Quantity
1	Crystal Violet (100% dye content)	2.0 g
2	Ethyl Alcohol (95%)	20.0 ml
3	Iodine	1 g
4	Potassium Iodide	2 g
5	Distilled Water	300 ml
6	Safranin O (certified)	2.5 g
7	Ethanol, 95%	100 ml

Table 5 Catalase Test Reagent Composition

S. No.	Components	Quantity
1	H2O2 (35%)	9 ml
2	Distilled Water	91 ml

Table 6 Other Media and Reagent Compositions

S. No.	Components	g L-1 or mL
1	Tetra	0.1 g
2	Distilled Water	10 ml
3	Peptone	1.0 g
4	Glucose	1.0 g
5	Sodium Chloride	5.0 g
6	Di-sodium Hydrogen Phosphate	1.2 g
7	Potassium Dihydrogen Phosphate	0.8 g
8	Phenol Red	0.012 g
9	Agar	15.0 g
10	Meat Extract	3.0 g
11	Yeast Extract	3.0 g
12	Lactose	10.0 g
13	Sucrose	10.0 g
14	Sodium Thiosulfate	5.0 g
15	Distilled Water	975.0 ml
16	Tryptone	20.0 g
17	Peptone	6.1 g
18	Ferrous Ammonium Sulfate	0.2 g
19	Sodium Thiosulfate	0.2 g
20	Agar	3.5 g
21	Eromphenol Acetate	0.5 g
22	Sodium Citrate	2.0 g
23	Sodium Chloride	Various amounts

Table 7 Antibiotics Susceptibility Profile

S. No.	Antibiotics	Amount
1	*Piperacillin/Tazobactum (TZP)*	100 mg
2	CAZ	30 µg
3	CFE	30 µg
4	*Amikacin (AK)*	30 mg
5	*Gentamicin (CN)*	10 mg
6	*Tobramycin (TOB)*	10 µg

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S. No.	Antibiotics	Amount
7	*Ciprofloxacin (CIP)*	5 mg
8	*Levofloxacin (LEV)*	5 mg
9	*Sulphamethoxazole/Trimethoprim (SXT)*	25 mg
10	*Imipenem (IPM)*	10 mg
11	*Meropenem (MEM)*	10 mg
12	*Doxycycline (DO)*	30 µg

The cross-sectional study design was selected to provide a snapshot of the current situation within the microbiology landscape. This observational study type allowed for the analysis of data from a population subset at a specific point in time. The Institutional Review Board's approval marked the commencement of the data collection phase, which spanned three months, situated within the well-equipped confines of the Life Sciences Laboratories at Lahore College for Women University. Respiratory specimens, encompassing naso-pharyngeal swabs, tracheal washings, bronchial brushings, and sputum, were meticulously collected following a stringent inclusion criterion only isolates of *Acinetobacter baumannii* from respiratory patients were considered, while specimens not meeting this criterion were excluded (1-4).

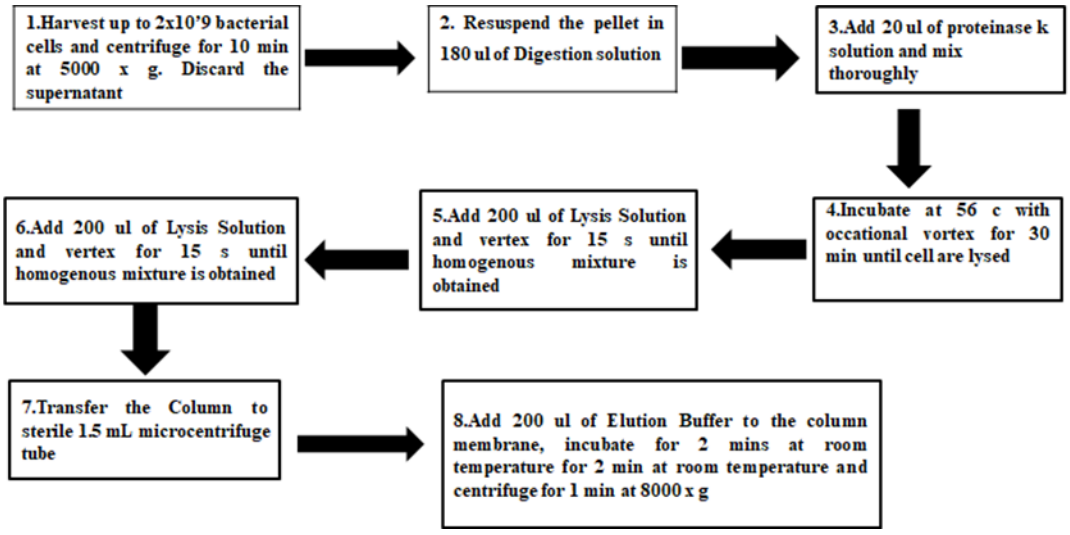

Figure 1 Components OF KITS

The isolated strains underwent initial culturing on various media, including nutrient agar, MacConkey agar, and sheep blood agar, meticulously incubated at 37°C. After 24 hours, the colonies that had developed were subject to a detailed morphological assessment, with their features documented for comparative analyses in subsequent research phases. The subsequent isolation involved the use of fastidious media to ensure the purity of the colonies, which, after a day, exhibited unadulterated growth suitable for further examination (5).

The morphological characteristics, critical in the initial identification stages, were observed on the culture media. A range of colony features, including color, size, and texture, were evaluated, aiding in the classification of the infections. The slide preparation entailed transferring a minute amount of bacterial growth onto a glass slide, followed by careful drying to prevent any damage or alteration to the sample. Gram staining, a crucial step in differentiating bacterial types, was meticulously executed, adhering to the time-honored protocol involving *crystal violet*, *iodine solution*, *decolorizer*, and *safranin*, each component serving its unique purpose in this classic microbiological technique (6).

Diagnostic tests to identify specific pathogens were employed post initial microbial identification. Catalase and oxidase tests, alongside citrate utilization and urease tests, provided insight into the enzymatic activities of the bacteria. For movement analysis, motility tests were conducted using both wet mount preparation and inoculation in a motility medium. Furthermore, the sulfur-indole-motility test was instrumental in identifying the production of sulfide, indole, and motility traits, essential in the characterization of bacterial isolates (7).

Antimicrobial susceptibility testing was another cornerstone of the study, utilizing the disc diffusion method in line with the guidelines set out by the Clinical and Laboratory Standards Institute. The zones of inhibition around antibiotic discs placed on Mueller-Hinton *agar* plates were measured post incubation, establishing the susceptibility profile of each bacterial strain. The analysis of resistance patterns against a spectrum of antibiotics including *ß-lactams* and *aminoglycosides* was crucial in understanding the antimicrobial resistance landscape of *Acinetobacter baumannii* (8,9,10,11,12,13).

DNA extraction, a fundamental molecular technique, was executed using the *Gene Jet Genomic DNA Purification Kit*. This was followed by *polymerase chain reaction* (PCR), a pivotal step in amplifying specific DNA sequences, and gel electrophoresis, which

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allowed the visualization of PCR products. Statistical analysis was performed using SPSS Version 25, employing various statistical tools such as means, standard deviations, and bar charts to interpret the collected data accurately.

RESULTS

The results section of our study meticulously quantifies the distribution of *Acinetobacter baumannii* across various culture media, gender distribution among study participants, the outcome of biochemical tests, age group prevalence, and antibiotic sensitivity.

Figure 2 Culture Plates, Colonies Morphology and Biochemical Tests and Sensitivity of Anti-Microbial

The colony morphology of *Acinetobacter baumannii* varied distinctively across different *agar* media. On Chocolate Agar, the bacterial colonies were predominantly 1-2mm in size, round, with a dome shape, and notably neither mucoid nor pigmented (Table 1). In contrast, colonies on *Blood Agar* were non-hemolytic, opaque, and grey, maintaining a consistent circular form. MacConkey Agar, known for differentiating lactose fermenters, revealed non-fermenting colonies of *Acinetobacter baumannii* that were opaque with circular margins, lacking the characteristic color change associated with lactose fermentation.

Table 8 Isolate Report Extract

Field	Value
Organism type	bacteria
Selected	H4
Acquisition Date	11/22/23 4:45 PM
Foseufte	*Acinetobacter baumannii*
Confidence	99.9%
Information	None

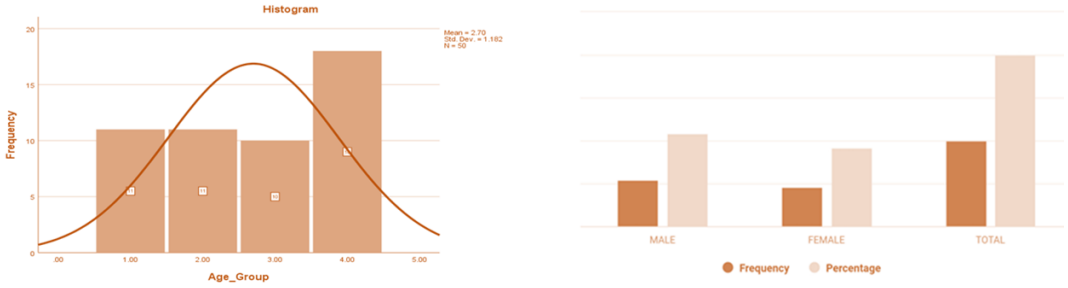

Figure 3 Age and Gender Distribution

Gender distribution within the study presented a slight male predominance, with males comprising 54% (n=27) and females 46% (n=23) of the 50 study participants. This distribution is graphically encapsulated in the accompanying bar chart, which elucidates the frequency and percentage of each gender (Figure 1), reinforcing the relatively balanced representation of genders in this study (Table 2).

Table 9 Bacterial Colony Morphology on Various *Agar* Media

Agar Type	Colony Morphology
Chocolate Agar	1-2mm, round, dome-shaped, non-mucoid, non-pigmented
Blood Agar	Non-hemolytic, opaque, circular, grey
MacConkey Agar	Non-fermenting, opaque, non-lactose, circular margins

Table 10 Gender Distribution of Study Participants

Gender	Frequency	Percentage (%)
Male	27	54.0
Female	23	46.0
Total	50	100.0

Table 11 Results of Biochemical Tests

Sr. No.	Tests	Results	Interpretation
1	Catalase Test	Positive	Bubbling

2	Oxidase Test	Negative	No cytochrome c oxidase activity
3	Citrate Test	Positive	Citrate utilized as a carbon source
4	Urease Test	Negative	No ammonia production
5	SIM Test	Sulfur: Negative, Indole: Negative, Motility: Negative	No sulfur reduction, no indole production, non-motile
6	TSI Test	Slant: Alkaline, Butt: Alkaline	No fermentation
7	Gas Production	Negative	-
8	H2S Production	Negative	-

Table 12 Age Group Distribution of Study Participants

Age Group	Frequency (f)	Percentage (%)
Children	11	22.0
Young Adults	11	22.0
Middle-Aged Adults	10	20.0
Old-Age Adults	18	36.0
Total	50	100.0

Table 13 Frequency and Sensitivity of Antibiotics

Antibiotic (Drug)	Sensitive (Frequency)	Resistant (Frequency)	Sensitive (%)	Resistant (%)
TZP	4	46	8.0	92.0
FEP	5	45	10.0	90.0
CAZ	5	45	10.0	90.0
IPM	5	45	10.0	90.0
MEM	5	45	10.0	90.0
AK	7	43	14.0	86.0
CN	13	37	26.0	74.0
TOB	23	27	46.0	54.0
CIP	4	46	8.0	92.0
LEV	5	45	10.0	90.0
DO	26	24	52.0	48.0

Biochemical assays yielded insights into the metabolic capabilities of the bacterial isolates. The Catalase Test resulted in bubbling, indicative of a positive reaction. Conversely, the Oxidase Test was negative, suggesting the absence of cytochrome c oxidase activity. Citrate utilization was positive, implying that *Acinetobacter baumannii* could utilize citrate as a carbon source, while urease activity was absent, signifying no ammonia production. The SIM test was uniformly negative for sulfur reduction, indole production, and motility, underscoring the non-motile nature of the studied bacterial strain. In the TSI test, both the slant and butt were alkaline, consistent with the organism's known non-fermenting properties.

The age distribution of the participants was characterized by a bell-shaped curve with the majority of the subjects falling in the old-age adults category, accounting for 36% of the sample size (n=18). Children and young adults each made up 22% (n=11 for each group), while middle-aged adults represented 20% (n=10) of the population (Figure 2). The histogram shows the frequency distribution of participants across these age groups, reflecting a skew towards the older age range.

Antibiotic sensitivity testing revealed that *Acinetobacter baumannii* exhibited a high resistance to most of the antibiotics tested. Particularly notable was the resistance to TZP, with a staggering 92% of samples exhibiting resistance. Similarly, high resistance rates were observed for FEP, CAZ, IPM, and MEM, all above 90%. AK showed an 86% resistance rate, while CN had a slightly lower resistance rate at 74%.

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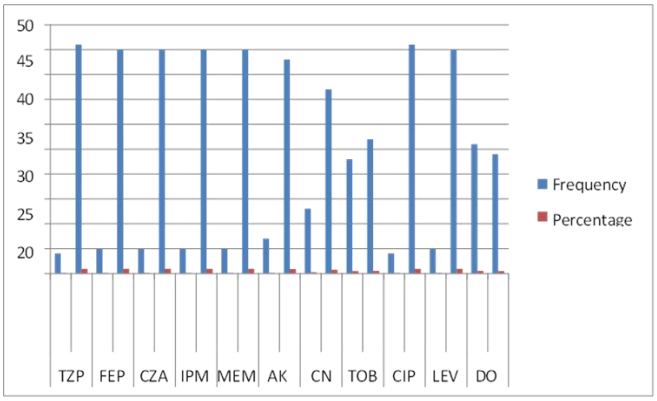

Figure 4 Bar chart presentations of ANTIBIOTICS; and Bands of armA on gel electrophoresis

TOB presented a more balanced sensitivity profile with a 54% resistance rate. CIP showed an overwhelming resistance rate of 92%, and LEV shared a similar pattern with 90% of the strains resistant. Notably, DO was the only antibiotic where the sensitivity rate (52%) surpassed the resistance rate (48%) (Figure 3), showcasing its potential effectiveness against *Acinetobacter baumannii* within this study cohort (Table 5).

DISCUSSION

The discourse surrounding the allelotypic and molecular characteristics of *aminoglycoside-resistant Acinetobacter baumannii* isolated from respiratory specimens underpins a broader, global conversation about antibiotic resistance. This study, situated at the different labs of various institute of Lahore, serves to reinforce the urgent call for novel antimicrobial strategies and stringent infection control practices. The study delineated a disconcerting level of resistance to a panoply of antibiotics such as TZP, FEP, CAZ, IPM, MEM, AK, CN, TOB, CIP, LEV, and DO, an observation that aligns with the notorious profile of multidrug resistance associated with *Acinetobacter baumannii*. Such resistance patterns echo findings from other regions, accentuating the pathogen's ability to withstand varied antibiotic assaults and posing significant clinical challenges in healthcare environments (17).

The demographic distribution within this study mirrored the gender ratio reported in similar research conducted in *Ismailia*, Egypt, on 52 ICU patients, underscoring the gender-indiscriminate nature of *Acinetobacter baumannii* infections. Through rigorous application of various *agar* media for culture, complemented by biochemical testing and antibiotic sensitivity assays, the pathogen's profile was scrupulously determined. Moreover, state-of-the-art technologies like *VITEK* MS provided precision in identification, with biochemical reactions and Gram staining fortifying the characterization process. Species identification and antibiotic susceptibility were ascertained using *VITEK* 2 Compact (18).

A comparative analysis with a study from tertiary care centers in Faisalabad and Lahore, Pakistan, further illuminated the A. *baumannii* landscape. While both studies procured strains primarily from respiratory samples, methodological variations were notable. Our research employed Gram staining, colony morphology, biochemical tests, *VITEK* MS, and gel electrophoresis for *armA gene* detection, whereas the comparative study utilized morphological tests, API 20E, and multiplex PCR alongside *CLSI* and *FDA* guidelines for antimicrobial susceptibility testing. The *armA gene*, commonly associated with *aminoglycoside* resistance, emerged prominently in our gel electrophoresis findings, with an 86% prevalence among isolates, indicating its potential role in resistance mechanisms (19).

Examining antibiotic resistance patterns and genetic markers within the broader context revealed *netilmicin* as relatively efficacious, whereas ciprofloxacin exhibited the highest resistance. The minimum inhibitory concentration (MIC) assays revealed substantial resistance across *aminoglycosides*. In our cohort, resistance genes including *aphA6, aadB, aadA1, aacA4, armA*, and *aacC2* were identified, aligning with global resistance trends. This project echoed a study conducted at Agha Khan Labs, which reported an alarming rate of antibiotic resistance among 50 *A. baumannii* strains from respiratory samples and identified the *armA gene* in a significant majority of isolates, suggesting its pervasive presence in *aminoglycoside resistance* (20).

This investigation enriches the understanding of *Acinetobacter baumannii*'s antimicrobial resistance profiles and underscores the pressing need for ongoing surveillance and innovative treatment approaches. The findings resonate with the pathogen's established multidrug resistance and emphasize the critical need for further research and effective antimicrobial stewardship. They underscore the imperative for continued antibiotic resistance monitoring, the necessity for targeted interventions, and the development of alternative therapeutic strategies to address the burgeoning challenge of multidrug resistance in bacterial pathogens. The study's insights bolster the case for enhanced infection control measures and reinforce the global health imperative to contain the spread of multidrug-resistant organisms.

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CONCLUSION

This study underscores the pervasive challenge posed by multidrug-resistant *Acinetobacter baumannii*, emphasizing the organism's capacity to thwart an array of antibiotics, with significant human healthcare implications. The findings reiterate the critical need for vigilant antimicrobial stewardship, development of new therapeutic agents, and robust infection control practices to combat the rising tide of antibiotic resistance. The results serve as a clarion call for healthcare systems to prioritize research and integrate targeted strategies to manage and mitigate the risks associated with these resilient bacterial pathogens.

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