



THE MOLECULAR CHARACTERISTICS AND ANTIMICROBIAL RESISTANCE OF ACINETOBACTER BAUMANNII ISOLATES FROM HOSPITAL-ACQUIRED INFECTIONS IN INTENSIVE CARE UNIT

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Abstract

Background: *Acinetobacter baumannii* is a major cause of hospital-acquired infections in intensive care units (ICUs), notable for its multidrug resistance and biofilm-forming ability, which complicate treatment and infection control.

Aims: This study aimed to determine the antimicrobial resistance patterns, biofilm formation, and molecular characteristics, including carbapenemase and metallo- β -lactamase (MBL) genes, of ICU-associated *A. baumannii* isolates.

Materials and Methods: A cross-sectional observational study was conducted over 18 months including 6 months data analysis in ICU patients with infections >48 hours after admission. Clinical specimens (blood, urine, respiratory samples, pus) were processed for culture and identification. Phenotypic identification included Gram staining, biochemical tests, and PCR targeting blaOXA-51. Antibiotic susceptibility was determined by Kirby–Bauer disk diffusion and MIC testing. Molecular characterization involved real-time PCR for blaOXA-51 and MBL genes (blaNDM, blaVIM, blaIMP). Biofilm formation was assessed qualitatively (tube method) and quantitatively (microtiter plate assay).

Results: All 126 isolates (100%) were confirmed as *A. baumannii* via blaOXA-51 PCR. Biofilm production was observed in 63% of isolates, with 18.3% strong, 26.9% moderate, and 17.5% weak producers. Multidrug resistance was detected in 73% of isolates, while 38.1% were extensively drug-resistant; only 3% were pandrug-resistant. MBL genes were identified in 6.3% of isolates (blaNDM-1: 2.4%, blaVIM: 2.4%, blaIMP: 1.6%).

Conclusion: ICU-associated *A. baumannii* exhibits high multidrug drug resistance and substantial biofilm-forming capacity, with low prevalence of MBL genes. Continuous antimicrobial surveillance, molecular characterization, and biofilm-targeted strategies are crucial for infection control and optimizing patient outcomes.

Introduction

Acinetobacter baumannii has become a leading cause of hospital-acquired infections, especially in intensive care units (ICUs), due to its ability to survive on surfaces, colonize medical devices, and resist multiple antibiotics.^{1,2} Infections caused by this pathogen, including ventilator-associated pneumonia, bloodstream infections, and urinary tract infections, are associated with high morbidity and mortality.³

The rapid emergence of multidrug-resistant and carbapenem-resistant strains has significantly limited treatment options, making management of ICU infections increasingly challenging.⁴ Molecular characterization of *A. baumannii* isolates is essential to understand the mechanisms of resistance and virulence, monitor their spread, and guide effective infection control measures.⁵

Despite global attention, data on the antimicrobial resistance patterns and genetic profiles of ICU-associated *A. baumannii* in many regions remain limited. Studying these isolates can provide crucial insights for improving patient outcomes and controlling hospital outbreaks.⁵

Material and method

Study Design and Setting

A **cross sectional observational study** was conducted in the Department of Microbiology, involving ICU-admitted patients. Clinical specimens received for culture and sensitivity will be processed for isolation of ***Acinetobacter baumannii***.

Study Duration- This study was conducted in 18 months including 6 months data analysis.

Study Population-

ICU patients with suspected after **>48 hours of admission**.

Inclusion Criteria

- ICU patients with a hospital stay of more than 48 hours.
- Patients showing clinical signs or suspicion of infection.
- Positive culture confirming *Acinetobacter baumannii*.
- Informed consent obtained from patient or guardian.

Exclusion Criteria

- Patients not admitted to ICU or with ICU stay less than 48 hours.
- Pediatric or neonatal patients.

Sample Size –

- $n = Z^2 \times P \times (1 - P) / d^2$
 - Z = statistics for a level of confidence
 - P = Expected prevalence or proportions
 - p = prevalence of *Acinetobacter baumannii* infections in Central India (assumed as **18% = 0.18**, based on regional hospital surveillance data)
- $$n = (1.96)^2 \times 0.18 \times 0.82 / (0.05)^2 \approx 340$$

Ethical consideration- Ethical clearance was obtained from the Institutional Ethics committee (IEC), Ethical clearance letter has been attached.

Sample Collection and Processing

Clinical specimens (respiratory samples, catheterized urine, blood, pus) was collected aseptically from ICU patients.

- **Blood:** Collected by venepuncture, inoculated into BHI broth, incubated, and subcultured.
- **Respiratory samples:** Sputum, BAL, ET tips, TT, and endotracheal aspirates processed using dilution technique; $\geq 10^5$ CFU/mL considered significant.

- **Urine:** Catheterized urine cultured on CLED agar with calibrated loop; $\geq 10^5$ CFU/mL indicates bacteriuria.
- **Wound/pus:** Directly inoculated onto blood and MacConkey agar.

Phenotypic Identification of *Acinetobacter baumannii*

- Samples cultured on blood and MacConkey agar at 37°C for 18–24 h.
- Preliminary identification: Gram staining, colony morphology, standard biochemical tests.
- Confirmation: PCR targeting intrinsic *blaOXA-51* gene.

Antibiotic Susceptibility Testing

- Performed by Kirby–Bauer disk diffusion on Mueller–Hinton agar using 0.5 McFarland inoculum.
- Antibiotics tested: piperacillin, ceftazidime, cefepime, imipenem, meropenem, aminoglycosides, colistin, fluoroquinolones.
- Interpretation per CLSI guidelines, MDR/XDR classification applied.
- Carbapenem-resistant isolates confirmed by MIC using agar dilution.

Molecular Characterization

Real-time PCR

- Genomic DNA extracted from overnight cultures (Qiagen kit) standardized to 1.0 McFarland.
- PCR targeting *blaOXA-51* gene performed in 20 μ L reaction; 34 cycles with annealing at 55°C.
- Ct ≤ 35 indicates positive for *A. baumannii*.

MBL Gene Detection

- Multiplex real-time PCR for *blaIMP*, *blaVIM*, and *blaNDM* using Hi-PCR kit (Himedia).
- DNA extracted via commercial kit or rapid boiling-lysis; A260/A280 1.7–2.0 acceptable.
- Fluorescence detection: FAM (*blaNDM*), HEX/VIC (*blaVIM*), Cy5 (*blaIMP*), ROX internal control.
- Ct ≤ 35 indicates positive; absence of amplification indicates negative.

Biofilm Assay

- **Qualitative:** Tube method for preliminary screening.
- **Quantitative:** Microtiter plate assay using OD measurements; categorized as negative, weak, moderate, or strong biofilm producers.

Result –

All 126 isolates (100%) tested positive for the **blaOXA-51** gene, confirming their identity as *Acinetobacter baumannii*. The universal presence of this intrinsic gene highlights its reliability as a species-specific molecular marker.

Table – 1 Gene detection by PCR

Gene Detected	No. of Positive Isolates	Percentage (%)	Interpretation
blaOXA-51 positive	126	100%	Intrinsic gene confirming <i>A. baumannii</i> species
blaOXA-51 negative	0	0%	Non- detected

Among the 126 *Acinetobacter baumannii* isolates, 23 (18.3%) were classified as strong biofilm producers, 34 (27.0%) as moderate producers, and 22 (17.5%) as weak producers. The remaining 47 isolates (37.3%) did not exhibit any biofilm formation. Overall, approximately two-thirds of the isolates demonstrated the ability to form biofilms, a virulence trait that likely contributes to their persistence in ICU environments and increases their tolerance to antimicrobial agents.

Table 2- Biofilm Formation Categories among *A. baumannii* Isolates

S.N.	Biofilm Category	No. of Isolates (n=126)	Percentage (%)
1	Strong Biofilm Producers	23	18.3%
2	Moderate Biofilm Producers	34	26.9%
3	Weak Biofilm Producers	22	17.5%
4	Non-Biofilm Producers	47	37.3%
Total	—	126	100%

Among the 126 *Acinetobacter baumannii* isolates, 92 (73.0%) were identified as multidrug-resistant (MDR), exhibiting resistance to at least one agent in three or more antibiotic classes, including β -lactams, aminoglycosides, and fluoroquinolones. Forty-eight isolates (38.1%) were classified as extensively drug-resistant (XDR), showing resistance to nearly all tested antibiotics except for limited options such as polymyxins or tigecycline. In contrast, 34 isolates (27.0%) remained susceptible to most antibiotics. Notably, four isolates (3.2%) were pandrug-resistant (PDR), demonstrating resistance to all tested antimicrobials, including colistin. These findings underscore the high prevalence of MDR and XDR *A. baumannii* in ICU settings, highlighting the significant therapeutic challenges posed by these pathogens.

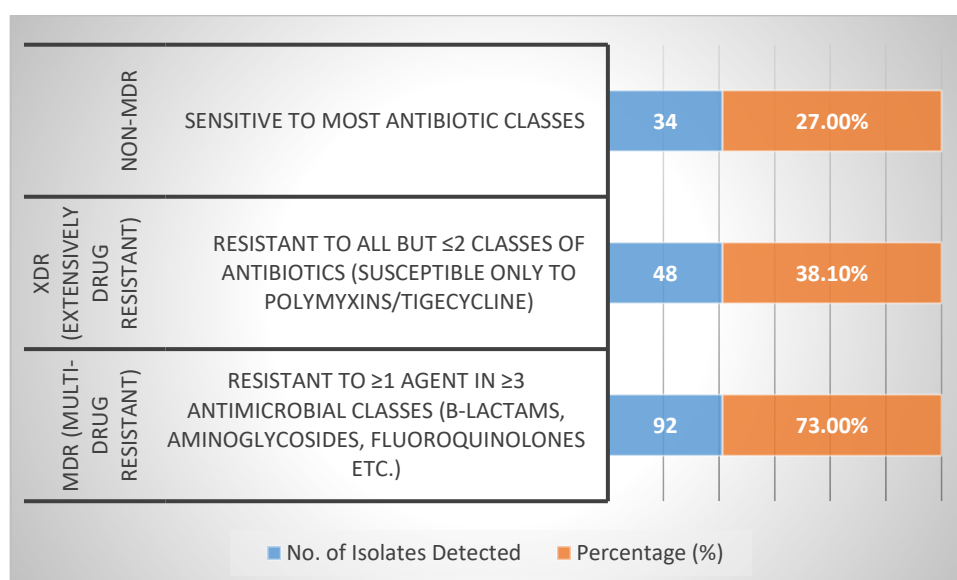


Figure 1- *Acinetobacter baumannii* isolates based on drug resistance pattern

Among the 126 *Acinetobacter baumannii* isolates, the **blaNDM-1** gene was detected in 3 isolates (2.4%), **blaVIM** in 3 isolates (2.4%), and **blaIMP** in 2 isolates (1.6%). Overall, 8 isolates (6.3%) tested positive for MBL genes, indicating a relatively low prevalence of these carbapenemase determinants in the study population.

Table 3- Detection of MBL Genes in *A. baumannii*

S.N.	MBL Gene Detected	Positive Isolates (n)	Percentage (%)
1	blaNDM-1	3	2.4%
2	blaVIM	3	2.4%
3	blaIMP	2	1.6%
—	Total MBL-positive isolates	8	6.3%

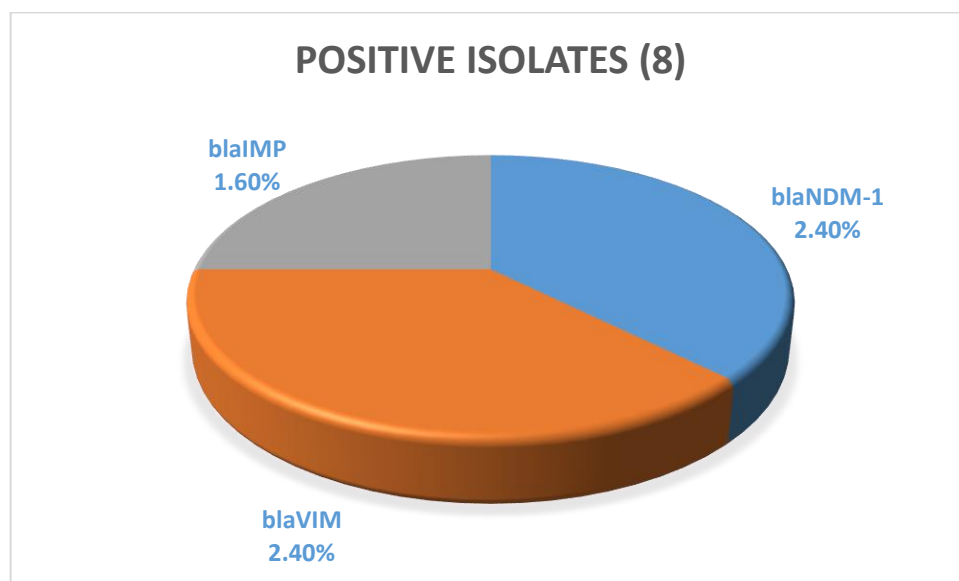


Figure 2-Detection of MBL Genes in *A. baumannii*

Discussion

In this study, all 126 *Acinetobacter baumannii* isolates tested positive for the **blaOXA-51 gene (100%)**, confirming species identification. The *blaOXA-51* gene is an intrinsic carbapenemase gene and is widely regarded as a definitive molecular marker for *A. baumannii*. The universal detection in our isolates is consistent with several previous studies. Gupta et al. reported 100% detection of *blaOXA-51* in ICU isolates from a tertiary hospital in India, highlighting its reliability for species confirmation.⁶ Similarly, Ababneh et al. found *blaOXA-51* in all clinical isolates studied in Jordan, further confirming its ubiquitous presence among *A. baumannii*.⁷ The consistent presence of this gene across different geographic regions supports its use as a gold-standard molecular marker for species-level identification, especially in epidemiological surveillance and outbreak investigations.

Regarding **biofilm formation**, approximately 63% of isolates in our study were biofilm producers, with 18.3% being strong producers, 26.9% moderate, and 17.5% weak producers. Biofilm formation is a key virulence factor in *A. baumannii*, contributing to persistence on medical devices and resistance to antimicrobial therapy. Our findings are comparable to Ababneh et al., 2024⁸ who reported that ~80% of ICU *A. baumannii* isolates were biofilm producers, with a substantial proportion showing strong biofilm activity.⁹ Similarly, Nowak et al. documented biofilm production in 68.8% of clinical *A. baumannii* isolates in Poland, emphasizing that biofilm formation is a common trait across isolates from diverse hospital settings.¹⁰ The presence of biofilm-producing strains is particularly concerning in ICU environments, where indwelling devices such as catheters and ventilators provide surfaces for colonization. Biofilms not only protect bacteria from host immune responses but also reduce antibiotic penetration, contributing to the persistence of infections and treatment failure. This may partly explain the high rates of multidrug-resistant isolates observed in our study.

The **antimicrobial resistance profile** of our isolates revealed that 92 isolates (73%) were multidrug-resistant (MDR), while 48 isolates (38.1%) were extensively drug-resistant (XDR). These findings align with global reports indicating high MDR and XDR prevalence in ICU-associated *A. baumannii* infections. Gupta et al. similarly reported high rates of MDR and XDR *A. baumannii* in tertiary care hospitals in India, where antibiotic pressure and prolonged ICU stays contributed to selection of resistant strains. Ababneh et al.¹¹ reported even higher XDR prevalence (96.7%) among ICU isolates, demonstrating that the burden of antimicrobial resistance varies geographically but remains a consistent concern in critical care settings. The correlation between biofilm formation and MDR/XDR phenotypes has been highlighted by Patil et al. (2023)¹² suggesting that biofilm formation may facilitate the survival of resistant strains by providing a protective niche that reduces antibiotic efficacy. This dual combination of biofilm capability and drug resistance poses significant challenges

in ICU infection management, often requiring last-resort antibiotics such as polymyxins and tigecycline.

The prevalence of **metallo- β -lactamase (MBL) genes** among our isolates was relatively low. *bla*NDM-1 and *bla*VIM were each detected in 3 isolates (2.4%), while *bla*IMP was found in 2 isolates (1.6%), giving an overall MBL-positive rate of 6.3%. These findings indicate that while MBL genes contribute to carbapenem resistance, they are less prevalent than OXA-type carbapenemases in this population. Gupta et al.(2021)^{13,14} reported a higher *bla*NDM-1 prevalence (21.4%) in Indian ICU isolates, whereas Kumar et al. documented *bla*VIM and *bla*IMP at lower frequencies, similar to our findings. The variability in MBL gene prevalence may reflect regional differences in antibiotic usage, infection control practices, and genetic diversity of circulating strains. Moreover, the low frequency of MBL genes in our study suggests that carbapenem resistance is primarily driven by intrinsic OXA-type carbapenemases and other resistance mechanisms rather than MBL production.

Conclusion - our study demonstrates a high burden of MDR/XDR *A. baumannii* in ICU settings, accompanied by significant biofilm-forming capacity. These findings are in concordance with global reports.emphasizing the persistent challenge posed by this pathogen in critical care. The combination of intrinsic resistance determinants, acquired resistance genes, and biofilm formation highlights the adaptive capabilities of *A. baumannii* in nosocomial environments. Effective management of ICU infections requires robust infection control measures, strict antimicrobial stewardship, and continuous molecular surveillance to track resistance trends and prevent the dissemination of these difficult-to-treat strains. Furthermore, understanding the relationship between biofilm formation and drug resistance could inform the development of novel therapeutic strategies, such as biofilm-disrupting agents or combination therapy, to improve clinical outcomes in ICU patients.

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