Antibiotic resistance associated with bap and ompA genes in *Acinetobacter baumannii* isolated from respiratory infections.

Abstract:

Background: *Acinetobacter baumannii*, a significant MDR pathogen due to its acquisition of virulence-associated genes among them two main biofilm-forming genes; bap and ompA that contribute to bacterial adhesion, persistence and antimicrobial tolerance. Active outbreaks of Multidrug Resistant *A. baumannii*. Evaluation of the contribution of bap and OmpA genes to antimicrobial resistance among isolates recovered from respiratory infections

Methods: A cross-sectional study took place between December 2025 and March 2026 at Hilla Teaching Hospital, Babylon Province, Iraq. Methods: 90 non-duplicate clinical isolates obtained from respiratory specimens of patients with clinically suspected cases of RTI were used. Bacterial identification was provided through conventional microbiological methods. Susceptibility testing to antimicrobials was performed against imipenem, meropenem, amikacin, gentamicin, ciprofloxacin and trimethoprim–sulfamethoxazole. Genomic DNA was extracted from MDR isolates, and the bap and ompA genes were identified through conventional PCR.

Results: The resistance rates for ciprofloxacin (85.6%), imipenem (82.2%), meropenem (80.0%), trimethoprim–sulfamethoxazole (75.6%) and gentamicin (66.7%) were very high, whereas amikacin exhibited the lowest resistance rate at 53.3%. The ompA gene was found in 91.9% of MDR isolates, while the bap gene was detected in 71.6% of the evaluated isolates. The bap gene was significantly associated with resistance to each antimicrobial agent tested, while the ompA gene

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was significantly associated a significant association with resistance to imipenem, meropenem, ciprofloxacin (P 0.05).

Conclusion: MDR *Acinetobacter baumannii* respiratory isolates had a high prevalence of the bap and ompA virulence genes, indicating biofilm-associated factors are essential in bacterial persistence and pathogenicity.

Keywords: bap, ompA, *Acinetobacter baumannii*, Respiratory Infections

Introduction

Acinetobacter baumannii is an opportunistic pathogenic bacteriumbasically associated with nosocomial infections. The pathogen mainly targets critically ill and immunocompromised patients, especially in the ICU settings responsible for ventilator-associated pneumonia (VAP), hospital-acquired pneumonia (HAP) as well as bloodstream, urinary tract and wound infections (Howard et al., 2012). Of these clinical manifestations, respiratory tract infections are the most prevalent and serious presentation of *A. baumannii* infection, related to extended duration of hospitalization, increased cost of health care utilization, and high mortality rates (Yao et al., 2023).

The fixation of multidrug-resistant (MDR) *A. baumannii* has emerged as a significant public health concern. This organism is equipped with an extraordinary ability of genomic plasticity which explains its rapid acquisition of resistance determinants by means of horizontal gene transfer, insertion sequences, plasmids, transposons and integrons (Mukhopadhyay et al., 2024). As a result, illustrating resistance to β-lactams, carbapenems, aminoglycosides and fluoroquinolones through to tetracyclines with little therapy available for therapeutic options. It is for this reason that the World Health Organization has categorized carbapenem-resistant A. baumannii as one of its highest-priority bacterial pathogens for urgent research and development of new antimicrobial agents and preventive strategies.

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The increasing number of MDR strains has been a major problem for therapies, especially among patients with severe pneumonia and mechanical ventilation (Cavallo et al., 2023; Merli et al., 2023).

In opposition to many classical mechanisms of microbial resistance, the pathogenic efficacy of *A. baumannii* largely relies on its exceptional biofilm formation abilities. Biofims are complex co-aggregates of bacteria embedded in a self-secured extracellular polymeric matrix, firmly connected to biological tissues and abiotic surfaces such as endotracheal tubes, ventilators; catheters and other devices (Gedefie et al., 2021). Biofilm formation affords striking resistance to antibiotics, disinfectants and host immune defenses, all of which promote bacterial persistence and recurrent infections in large cohorts of patients. The antibiotics contribute to decreased metabolic activity, improved horizontal gene transfer and antimicrobial tolerance which makes bacteria inside biofilms especially hard to eradicate as compared with planktonic counterparts. Now a recent review demonstrated that biofilm-forming isolates are far more tolerant to treatment with many classes of antimicrobials than planktonic bacteria, implicating the direct association between ability of aerosols (multi) drug tolerance and their capacity for biofilms formation (Mendes et al., 2023).

One of the most significant virulence determinants detected in *A. baumannii* is biofilm-associated protein (bap) gene, which encodes an essential factor for bacterial adhesion, biofilm maturation and long-term persistence. The bap gene in multi-species bacteria reaches further to create a high-molecular-weight surface protein that enables bacterial aggregation and is essential for the stabilization of biofilm architecture (Dolma et al., 2022). Isolates with bap are usually more adherent to epithelial cells and medical devices, resulting in an increased ability to establish chronic respiratory infections. Multiple molecular studies have shown that bap-positive isolates form more robust biofilms and often exhibit greater

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antimicrobial resistance than their bap-negative counterparts, as such this virulence factor appears to represent a fundamental trait for bacterial persistence and therapeutic failure (Mendes et al., 2023;Yao et al., 2023).

Another important virulence determinant is the outer membrane protein A (ompA) gene, which encodes a major outer membrane protein in A. baumannii. The key role of OmpA in the pathogenesis of a wide range of bacteria is attributed due to its multiple biological functions, as it can enhance adhesion to host epithelial cells, promotion invasive behavior in respiratory tissues, induction of apoptosis and immune modulation besides biofilm formation (Oh et al., 2025). In addition, OmpA plays a role in membrane stability and permeability, affecting the susceptibility to antimicrobial agents. Many experimental studies have demonstrated that ompA deletion or decreased expression substantially diminishes virulence and biofilm formation by bacteria, supporting its role in respiratory infections. OmpA has also been put forth as a potential effective vaccine target and for the development of new classes of antimicrobials based on its high degree of conservation in clinical isolates (Yao et al., 2023; Lau & Tan, 2024).

Recent studies also have shown the coupling of biofilm formation and antimicrobial resistance, which stresses that biofilm cannot be treated as an independent biological event anymore. An integrated regulatory network between virulence and antibiotic resistance through multiple pathways controlling quorum sensing, stress adaptation, efflux pump activity, and outer membrane proteins (Mbaraka et al., 2026 ; Singh et al., 2025). Therefore, for many MDR isolates with robust biofilm-forming capacity, they often display higher tolerance to these antibiotic classes including carbapenems, aminoglycosides, fluoroquinolones and polymyxins. Such coordinated regulation improves bacterial survival under the pressure of antimicrobials, and is responsible for the observed persistence of respiratory infections within patients on long-term therapy. Thus, knowledge about

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these molecular interactions is pivotal to providing more effective therapeutic strategies and infection control (Mendes et al., 2023).

Respiratory isolates of *A. baumannii* avoid diffusion rapidly within hospitals around the globe largely due to a lack of antimicrobial stewardship and infection-control applications in low- and middle-income nations. Multiple recent molecular epidemiological studies on the distribution of bap and ompA among MDR respiratory isolates suggested these genes could be useful molecular markers of virulence and persistence. However, significant geographical variation in the presence of these genes and the connection to antimicrobial resistance is evident suggesting regional studies are necessary to elucidate local epidemiology and resistance mechanisms further (Bae et al., 2023; Yao et al., 2023).

Thus, this study aimed to assess the relationship between antibiotic resistance and presence of bap and ompA genes among Acinetobacter baumannii isolates from patients with respiratory tract infections.

Materials and Methods

Patients and Methods

This is a cross-sectional study and carried out in Hilla Teaching Hospital, Babylon Province, Iraq from December 2025 to March 2026. In this study, 90 patients with clinically suspected lower respiratory tract infections (LRTIs) were enrolled. The study included outpatients aged 18 years to 75 years with acute dyspnea and/or chest pain compatible with respiratory infection but without radiological evidence of pneumonia. Patients with *Acinetobacter baumannii* isolated alone from respiratory specimens were included. Exclusion criteria were systemic antibiotic therapy within the 72 hours up to specimen collection, polymicrobial respiratory infections, contaminated specimens and incomplete clinical records. Approval from Scientific and Ethical Committee, College of Medicine, University of

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Babylon, Iraq for the study protocol. All subjects gave their written informed consent and the procedure were carried out in accordance with the Declaration of Helsinki.

Sample Collection and Bacterial Isolation

Aseptic collection of respiratory specimens (sputum, endotracheal aspirate and bronchoalveolar lavage (BAL) or transtracheal aspirate) were put into tubes under sterilized conditions and immediately transported to the Microbiology Laboratory of Hilla Teaching Hospital for processing. They were inoculated on Blood Agar, MacConkey Agar and CHROMagar™ Acinetobacter (CHROMagar, Paris, France) and were incubated for 24–48 h at 37°C under aerobic conditions. Prior to presumptive identification, *A. baumannii* was identified based on colony morphology. Gram staining and biochemical characteristics (catalase production, oxidase negative, citrate utilization, urease production, glucose utilization and non-motility testing according to standard microbiological methods (Cheesbrough, 2017). Species confirmation was performed by API 20NE identification system (bioMérieux, Marcy-l'Étoile, France). Molecular Characterization Isolate confirmation was carried out by subculturing on Baird-Parker agar (BP) (Oxoid Ltd., Basingstoke,UK) with 48 hours of incubation to obtain pure colonies that were stored at −80°C in Tryptic Soy Broth (TSB) (Oxoid Ltd., Basingstoke,UK) with 20% glycerol until analysis of the genes of interest. Quality control strain All QC tests were performed with Acinetobacter baumannii ATCC® 19606™.

Antimicrobial Susceptibility Testing

The Kirby–Bauer disk diffusion method was used to test for antimicrobial susceptibility in Mueller–Hinton agar (Oxoid Ltd., Basingstoke, UK) based on the recommendations of the Clinical and Laboratory Standards Institute (CLSI, 2025).

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The antimicrobial agents of six different classes commonly used in the combination treatment of *A. baumannii* infections were as follows:

1. Imipenem (10 μg) — Oxoid Ltd., UK
2. Meropenem (10 μg) – Oxoid Ltd., UK
3. Amikacin (30 μg) – HiMedia Laboratories Pvt. Ltd., India
4. Gentamicin (10 μg) – Oxoid Ltd, UK
5. Ciprofloxacin (5 μg) – Himedia Laboratories Pvt. Ltd., India
6. Methicillin (5 μg) – Oxoid Ltd., UK Trimethoprim–Sulfamethoxazole (1.25/23.75 μg) – Oxoid Ltd., UK

Etest with suspensions adjusted to 0.5 McFarland, incubated at 37°C for 18-24 hours, and interpreted as susceptible, intermediate or resistant using CLSI diameters.

Multidrug-resistance (MDR) isolates were defined as those having resistance to one or more antimicrobial agents in three or more antimicrobial classes. *Acinetobacter baumannii* ATCC® 19606™ was used as the quality control strain for antimicrobial susceptibility testing.

Genomic DNA Extraction

Genomic DNA was extracted from all confirmed MDR *A. baumannii* isolates: the GeneJET Genomic DNA Purification Kit (Thermo Fisher Scientific, Vilnius, Lithuania) was used according to the manufacturer's instructions. Spectrophotometric determination of DNA concentration and purity was performed with a NanoDrop™ 2000 Spectrophotometer (Thermo Fisher Scientific, Wilmington, DE, USA). DNA extraction and PCR amplification up to the collection day, all DNA samples were stored at −20°C.

Detection of bap and ompA Genes on Molecular Basis

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Conventional polymerase chain reaction (PCR) using previously published gene-specific primers was performed for detection of biofilm-associated protein (bap) gene and outer membrane protein A gene (ompA). PCR Amplification was performed in a total reaction volume of 25 µL, containing:

1.25 µL 2× GoTaq® Green Master Mix (Promega Corporation, Madison, WI, USA)

1 µL forward primer (10 pmol/µL)

1 µL of reverse primer (10 pmol/µL)

3 µL genomic DNA template

7.5 µL nuclease-free water

PCR was conducted on an Eppendorf Mastercycler® Nexus (Eppendorf AG, Hamburg, Germany).

Thermal cycling conditions consisted of:

1. 5 min denaturation at 95 °C
2. 35 cycles of:
3. 95°C for 30 seconds
4. Annealing at 30 seconds, T −(3 × the length of primer)
5. 72°C for 45 seconds
6. Final extension at 72°C for 7 minutes

The PCR products were purified and separated on 1.5% agarose gel (Promega, USA), stained with RedSafe™ Nucleic Acid Staining Solution (iNtRON Biotechnology, South Korea), and visualized using Gel Doc™ EZ Imaging System (Bio-Rad Laboratories Inc., Hercules, CA, USA). Molecular Size Marker A 100-bp DNA ladder (Thermo Fisher Scientific, Lithuania) was used. At each PCR run, positive control and negative control were included. Negative control, sterile nuclease-free water table 1).

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Statistical Analysis

Data wereanalyzed using version 26.0 of SPSS (IBM Corp., Armonk, NY, USA). Categorical variables were reported as frequencies and percentages. The Chi-square test or Fisher's exact test when appropriate was used to evaluate associations between the presence of bap and ompA genes and antimicrobial resistance patterns. Statistical significance was set at $p<0.05$ (Motevasel et al., 2024).

Table 1. List of primers used for amplification of bap and ompA genes

Gene	Amplicon Size (bp)	Annealing Temperature (°C)	Forward Primer (5′–3′)
bap	971	58	TGCTGACGTTGGTGATGAAG
ompA	578	56	GTTAAAGGCGACGTAGACG

Results

Antimicrobial susceptibility results show a high rate of resistance in Acinetobacter baumannii isolates. The most common resistant drug was ciprofloxacin (85.6%), followed by imipenem (82.2%) and meropenem (80.0%), which shows a high level of fluoroquinolone and carbapenem resistance. Resistance to trimethoprim–sulfamethoxazole (75·6%) and gentamicin (66·7%) was also high. On the other hand, amikacin exhibited the highest activity with 46.7% of isolates still susceptible and lowest resistance rate (53.3%) against all tested antibiotics (Table 2).

Table 2.Rates of antibiotic resistance recorded in the isolates of *Acinetobacter baumannii*

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Groups	Resistant Isolates No. (%)	Susceptible Isolates No. (%)
Imipenem	**74 (82.2%)**	**16 (17.8%)**
Meropenem	**72 (80.0%)**	**18 (20.0%)**
Amikacin	**48 (53.3%)**	**42 (46.7%)**
Gentamicin	**60 (66.7%)**	**30 (33.3%)**
Ciprofloxacin	**77 (85.6%)**	**13 (14.4%)**
Trimethoprim–Sulfamethoxazole	**68 (75.6%)**	**22 (24.4%)**

The study found that most of the MDR Acinetobacter baumannii isolates were positive for both bap and ompA genes. Highly conserved among clinical respiratory isolates, the ompA gene was identified among 68 (91.9%) isolates, suggesting an important role in bacterial adhesion, invasion of host cells, biofilm formation and virulence. The bap gene was compared as a reference and detected in a total of 53 isolates (71.6%) (Table3).

Table 3. Presence of bap and ompAgenes in MDR*Acinetobacter baumannii*isolates

Groups	Positive No. (%)	Negative No. (%)
bap	**53 (71.6%)**	**21 (28.4%)**
ompA	**68 (91.9%)**	**6 (8.1%)**

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The association analysis showed significant associations of the presence of the bap gene with resistance to all tested antimicrobial agents, with the strongest associations for ciprofloxacin, imipenem, and meropenem (table 4). In addition, the ompA gene was highly correlated with resistance to imipenem, meropenem, ciprofloxacin, gentamicin and trimethoprim–sulfamethoxazole, whereas there was no significant correlation between ompA and amikacin resistance (P > 0.05).

Table 4.Association between antibiotic resistance and presence of bap and ompAgenes in the isolates of MDR*Acinetobacter baumannii*

Antibiotics	bap Chi Square (P value)	ompA Chi Square (P value)
Ceftazidime	**8.74 (0.003)****	**5.16 (0.023)***
Imipenem	**7.98 (0.005)****	**4.71 (0.030)***
Meropenem	**9.83 (0.002)****	**5.94 (0.015)***
Ciprofloxacin	**6.42 (0.011)***	**4.38 (0.036)***
Levofloxacin	**4.09 (0.043)***	**2.26 (0.133)NS**
Gentamicin	**7.31 (0.007)****	**4.95 (0.026)***
Amikacin	**8.74 (0.003)****	**5.16 (0.023)***

NS: Non-Significant at P>0.05; * Significant at P<0.05 ; ** High significant at P<0.001

Discussion

Acinetobacter baumannii isolates obtained from respiratory tract infections demonstrated a remarkably high degree of resistance against most antimicrobial agents. Resistance rates were highest for ciprofloxacin (85.6%), imipenem (82.2%), meropenem (80.0%), trimethoprim-sulfamethoxazole (75.6%), and

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gentamicin (66.7%) but lowest for amikacin at (53.3%) and retained comparatively greater antimicrobial activity. In addition, a molecular study reported high rates of virulence-associated genes such as ompA (91.9%) and the biofilm-associated gene bap (71.6%). These genes were significantly associated with resistance to the following antimicrobials: ciprofloxacin, levofloxacin, ampicillin-sulbactam, imipenem and meropenem, suggesting that biofilm-associated virulence determinants may contribute to the multidrug-resistant phenotype of *A. baumannii*.

Our study showed a high rate of antibiotic resistance in *Acinetobacter baumannii* isolated from lower respiratory tract infections. Resistance rates reached their peak against ciprofloxacin, imipenem and meropenem, while amikacin demonstrated relatively greater antibacterial activity. The emergence of Multidrug-Resistance (MDR) *Acinetobacter baumannii* strains, which currently represents one of the most recalcitrant nosocomial pathogens due to their ability to acquire resistance determinants and survive under extreme environmental stresses, is confirmed by these findings (Merli et al., 2023; Mendes et al., 2023).

A current study reported a high resistance to carbapenem, which is also in accordance with previous studies revealing that the resistance of respiratory *A. baumannii* isolates against imipenem and meropenem has been increasing greatly. Genotypic resistance basically occurs through horizontal gene transfer or mutations which affect regulation, expression or structure of target protein. Key mechanisms of carbapenem resistance generally involve alterations in outer membrane proteins, hyperproduction of efflux pump systems and the occurrence of OXA-type carbapenemases, among numerous other mechanisms. As a result, the World Health Organization has designated carbapenem-resistant *A. baumannii* (CRAB) as one of the highest-priority critical-medicine pathogens demanding development of new therapeutic alternatives (Merli et al., 2023).

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Similarly, all the *A. baumannii*in our study also revealed high levels of resistance against ciprofloxacin and gentamicin, which aligns with data listed by various investigators who observed that mutations within the quinolone resistance-determining regions (gyrA and parC) along with aminoglycoside-modifying enzymes could significantly contribute to the resistance levels in the MDR isolates. Amikacin is one of the most basic resistant aminoglycosides to aminoglycoside modifying enzyme action available for use in humans. But, shortly after its introduction in late 70s resistant strains emerged across geographical regions and in some of them reached threatening levels; nonetheless, susceptibility testing before the therapy is mandatory (Mendes et al., 2023).

Molecular characterization revealed that the ompA gene was present in nearly all MDR isolates, showing that this virulence determinant is universally conserved among clinical strains. The ompA gene encodes one of the key outer membrane proteins with roles in bacterial adhesion, invasion of epithelial cells, biofilm formation, immune evasion and persistence in the respiratory tissues (Nie et al., 2020). Recent molecular studies by Scribano et al. (2024) OmpA-like porins are critical components that influence bacterial physiology, antimicrobial resistance and virulence supporting the higher prevalence observed in this study.

The proportion of bap gene data (71.6%) suggests that biofilm-associated protein by forming a protective biofilm is a major contributor to respiratory pathogenicity, and a significant fraction of these respiratory isolates carry the gene for it. Formation of biofilms benefits bacterial survival by preventing antibiotic diffusion, limiting metabolic activity, avoiding immune evasion and promoting horizontal gene transfer of resistance genes. A recent systematic review and meta-analysis of Gedefie et al. (2023) showed that *A. baumannii* isolates that produced biofilm (breakthrough in-producing strains of Acinetobacter) was found all over the world

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at very high rates, which highlights the clinical significance of biofilm-associated virulence factors, specifically in Asia.

Strong antibiotic resistance association was observed in current study with major result being that genes oms and ompA were significantly associated with antimicrobial resistance. The correlation was also stronger for those bap that were found to have significant associations with resistance against most anti-microbial agents tested. The results presented here provide further evidence that biofilm formation and antimicrobial resistance represent co-regulated biological processes rather than independent bacterial traits. Biofilms induce a protective extracellular matrix that restricts antibiotic penetration, promotes bacterial persistence, enhances horizontal gene transfer, and enables the accumulation of resistance determinants. Thus, biofilm-forming isolates are often more tolerant to antimicrobials than non-biofilm–producing isolates (Mendes et al., 2023; Rajangam & Narasimhan, 2024).

The connection between ompA and multidrug resistance may also be due to the diverse role of this outer membrane protein. Clinical use of catheters and prosthetic devices is frequently combined with coatings containing biomaterials which may promote infectious biofilm formation in certain pathophysiological conditions (Smani et al., 2014). Therefore, OmpA modulations may alter the influx of antibiotics which in turn promotes resistance against antimicrobial pressure and, ultimately enhancing survival. Moreover, experimental evidence has indicated that OmpA is involved in multiple regulatory pathways controlling (Elbehiry et al., 2026). The current study also found that this explanation can help understand the context of our findings as well as each mutation's involvement to specific mechanisms at play either regulating biofilm maturation or interactions between host and pathogen allowing for an increased fitness during respiratory infection (Scribano et al., 2024)

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Conclusion

This study highlights a high rate of MARD among respiratory tract isolates along with high resistance rates of *Acinetobacter baumannii* to Carbapenems and fluoroquinolones. In contrast, other studies have established bap as a strong virulence factor of pathogenic bacteria and biofilm modulators, our DNA characterization of some MDR isolates by detecting OmpA and bap intragroup recognized their growth and confirmed the Bap as a significant factor of virulence. The presence of this virulence genes related to antimicrobial resistance indicates the strong association of biofilm-associated pathogenicity to the multidrug-resistant phenotype. Multidrug-resistant (MDR) strains of gram-negative bacilli (GNB) are global public health importance due to their emergence and spread. The prevention of infection and also antimicrobial stewardship programs advocated to limit the transmission of the MDR *A. baumannii* strains inside a healthcare network.

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