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Original Article

Molecular monitoring of Carbapenem-resistant, risk Factors and mortality rates of multidrug-resistant clinical isolates of *Acinetobacter baumannii* isolates in ICU Patients of Tehran

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Abstract

Introduction & Objective: Infections caused by multidrug-resistant (MDR) and carbapenemase-producing *Acinetobacter baumannii* present significant challenges for healthcare systems. These infections lead to increased hospitalizations, longer lengths of stay, and higher mortality rates. The objective of this study was to evaluate the proportion of carbapenem-resistant *A. baumannii* isolates, examine antibiotic resistance patterns, and investigate the relationship between clinical risk factors and mortality.

Materials & methods: This cross-sectional study analyzed 185 samples from ICU patients in Tehran hospitals between October and December 2024. Bacterial identification utilized biochemical and molecular methods, while antimicrobial susceptibility was examined using disc diffusion and microdilution techniques. Carbapenemase production was assessed with the carbapenem inactivation test, and binary logistic regression analyzed risk factors for mortality.

Results: *Acinetobacter baumannii* was isolated from 49 samples (30.26%); 51% of infections were isolated, and the remaining were associated with pathogens such as *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Escherichia coli*, and *fungi*. The mean age of the patients was 57 years, and 67.3% of the cases had underlying diseases. The mortality rate of the study subjects was 65.3% and was significantly associated with the length of hospital stay and mechanical ventilation. Logistic regression showed that hospital length of stay and diabetes were the main predictors of mortality. The highest antibiotic resistance was related to cephalosporins and carbapenems (100%). Also, 20 isolates (40.81%) were carbapenemase producers, all of which were multidrug-resistant.

Conclusion: The study highlights the association between mortality and factors such as age, underlying diseases, and hospitalization duration, underscoring the urgent need for strategies to address carbapenem-resistant and multidrug-resistant *A. baumannii* infections.

Keywords: *Acinetobacter baumannii*; Carbapenem resistance, Nosocomial infections, Intensive Care Unit (ICU), Risk Factors.

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Introduction

Acinetobacter baumannii, a key member of the *Acinetobacter calcoaceticus–baumannii* complex, is increasingly recognized for its ability to survive and thrive in clinical settings, contributing to higher infection rates among critically ill hospitalized patients (1). The World Health Organization (WHO) has identified this pathogen as a high-priority organism for antimicrobial research, and it is also categorized as an ESKAPE (*Klebsiella pneumoniae*, *Enterococcus faecium*, *Staphylococcus aureus*, *A. baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter species*) pathogen, a group of multidrug-resistant organisms responsible for challenging hospital-acquired infections (2). This organism is linked to a variety of clinical presentations, with the most frequent being ventilator-associated pneumonia, bloodstream infections, wound and burn infections, and, in less common cases, meningitis (3). Over time, the organism has developed extensive antimicrobial resistance (4, 5), making it a growing global public health concern (6). Reports indicate that antibiotic-resistant strains particularly those resistant to multiple drug classes-have been increasingly documented across various regions over the past few decades (7). Highly resistant strains first emerged in the early 1990s, spreading to the Middle East by 2006 and later throughout Europe by 2015. In certain regions, mortality rates have been reported as high as 30–75%. (8). Resistance to carbapenems has been particularly problematic and is often associated with the proliferation of multidrug-resistant (MDR), extensively drug-resistant (XDR), and pan-drug-resistant (PDR) phenotypes. These categories represent the increasing loss of treatment options. Multidrug-resistant (MDR) strains show resistance to at least three classes of antimicrobials. Extensively drug-resistant (XDR) strains are only susceptible to one or two classes, while pan-drug-resistant (PDR) strains are resistant to all routinely tested antibacterial agents (9).

A. baumannii isolates that are resistant to carbapenems, cephalosporins, aminoglycosides, and fluoroquinolones are classified as extensively drug-resistant (XDR). Strains that demonstrate resistance to both polymyxins and tigecycline are referred to as pan-drug-resistant (PDR). The increasing incidence of antibiotic resistance in *A. baumannii* has become a significant global health concern, leading to approximately 7,300 infections and 500 deaths each year (7).

Currently, The carbapenem-resistant *A.baumannii* (CRAB) isolates is listing among the most urgent threats due to its high mortality rates, propensity to spread in vulnerable healthcare environments, and limited available treatments (1). CRAB infections pose a considerable challenge for healthcare systems, resulting in higher rates of ICU admissions, prolonged hospital stays, and increased mortality rates. These issues contribute to escalating costs for healthcare systems that are already under strain (3). Recent years have intensified therapeutic challenges, leading to the investigation of new treatment options like cefiderocol and sulbactam-durlobactam, although their accessibility is limited in many healthcare settings (1), Moreover, the growing identification of metallo -beta-lactamase–producing strains has made treatment more difficult and raised worldwide concerns (9). This study investigates the escalating occurrence of CRAB infections globally by looking at the frequency of CRAB isolates among ICU patients in Tehran. It also examines resistance patterns and identifies clinical factors that may influence patient mortality.

Material and Methods

A) Study design: In a cross-sectional study, 185 unique samples, including both blood and respiratory specimens, were collected from adult patients (excluding pregnant women and children) admitted to the ICU of two hospitals in Tehran. These samples were collected between October 2024 and December 2024 and subsequently transferred to the microbiology laboratory for potential isolation of *A. baumannii* isolates.

B) Patient data gathering: Patient data was collected by reviewing medical and nursing records. Information included age, gender, underlying conditions, ICU stay duration, need for mechanical ventilation, prescribed antibiotics, and outcomes (discharge or death).

C) Identification of microbial isolates: The collected samples were cultured on both general and selective-differential media, including blood agar, chocolate agar, and MacConkey agar. After an incubation period of 18 to 24 hours at 37°C, the suspected *A. baumannii* colonies, which appeared bright pink, smooth, and mucoid, were subject to Gram staining to confirm their morphology as gram-negative coccobacilli. Following this, the colonies were phenotypically identified using a series of standard biochemical tests, such as catalase, urease, Simon citrate, oxidase, the trisaccharide-iron agar test (TSI), the indole sulfide mobility test (SIM), lysine decarboxylase, and the methyl red/Voges-Proskauer (MR/VP) test. Finally, bacterial cultures were preserved in freshly prepared tryptone soy broth (TSB) containing 15% glycerol at -20°C for future analysis.

D) Molecular Identification of A.baumannii isolates: Identification of *A. baumannii* isolates was confirmed by amplification of the *A. baumannii*-specific *gluconolactonase* and the *OXA-51-like* carbapenemase genes using PCR (10). Chromosomal DNA was extracted using the thermal cell lysis method and quantified for concentration and purity with a Nanodrop spectrophotometer. The PCR reaction was prepared in a total volume of 20 µL, containing 5 µL of 5X Master Mix, 2 µL of extracted genomic DNA, 1 µL each of the forward and reverse primers (10 pmol), and 11 µL of sterile deionized water. *A. baumannii* ATCC19606 and sterile deionized water were used as positive and negative controls. All reaction products were analyzed on a 1% agarose gel at 100 V for 70 minutes, and putative bands were visualized under a UV transilluminator.

E) The antimicrobial susceptibility testing: Antimicrobial susceptibility testing of *A. baumannii* isolates was performed using the Kirby-Bauer disc diffusion test and the broth microdilution test. Kirby-Bauer results were grouped as resistant, susceptible, or intermediate per the Clinical and Laboratory Standards Institute (CLSI) protocol, which measures the inhibitory zone diameter around each antibiotic disc (11).

Antibiotics tested included: cefepime (CPM, 30 µg), cefotaxime (CTX, 30 µg), ceftriaxone (CRO, 30 µg), ceftazidime (CAZ, 30 µg), imipenem (IPM, 10 µg), meropenem (MER, 10 µg), gentamicin (GM, 10 µg), tobramycin (TM, 10 µg), amikacin (AN, 30 µg), doxycycline (DO, 30 µg), trimethoprim-sulfamethoxazole (SXT, 1.25/23.75 µg), piperacillin-tazobactam (TZP, 100/10 µg), ciprofloxacin (CP, 5 µg), and ampicillin-sulbactam (SAM, 10/10 µg). Colistin efficacy was tested by broth MIC, according to CLSI guidelines. For colistin, MIC ≤ 2 µg/ml is intermediate; MIC ≥ 4 µg/ml is resistant. Control isolates were *Escherichia coli* (ATCC 25922) and *Pseudomonas*

aeruginosa (ATCC 27853).

F) Determination of carbapenem-resistant A. baumannii (CRAB) isolates: To screen and identify CRAB strains, each confirmed *A. baumannii* isolate had a phenotypic test for carbapenemase using the carbapenem inactivation method (sCIM) from Jing et al (20). For this, a 0.5 McFarland standard turbidity was prepared with *E. coli* ATCC 25922, diluted 1:10 in sterile Ringer's solution, and streaked on Mueller-Hinton Agar (MHA). After drying, 2-4 colonies from each test isolate were streaked onto one side of a 10 µg imipenem disk (Rosco, Denmark). The disk was then placed on an MHA plate seeded with *E. coli* ATCC 25922, ensuring that the bacterial side of the disk contacted the agar. MHA plates were incubated for 16–18 hours at 35 ± 2 °C. Carbapenemase-producing strains hydrolysed imipenem, rendering the drug ineffective and allowing growth of the reference strain. A positive result was a 6–20 mm inhibition zone or colonies within ≤22 mm. An inhibition zone ≥26 mm was negative, and ≥23–25 mm was indeterminate. An uninoculated imipenem disk served as a positive control.

G) Determination of MDR and XDR A. baumannii Isolates: The MDR strains are defined as being non-susceptible to at least one agent in three different antimicrobial classes. XDR strains: These are nonsusceptible to at least one agent in all but two antimicrobial classes.

H) Statistical Analysis: To compare the means of continuous and discrete quantitative variables, t-test, chi-square test, or Fisher's exact test were used, respectively. Also, to examine the effect of effective predictors on mortality, the Binary logistic regression analyses were used. All analyses were conducted using IBM SPSS Statistics version 26.0.

Results

A) Frequency of A. baumannii isolates: A total of 185 clinical specimens were collected. Of these, 160 contained viable microorganisms. Among the 160 specimens, 49 (30.62%) were identified as containing *A. baumannii* isolates. Biochemical testing revealed suspicious, pale pink, mucoid colonies on MacConkey agar. These were selected as potential *A. baumannii*. Gram staining showed red gram-negative coccobacilli. Definitive identification of the colonies as *A. baumannii* was based on several characteristics. They were gram-negative, catalase positive, oxidase-negative, indole negative, urease-negative, non-fermenting, non H2S-producing, lysine decarboxylase-negative, citrate-positive, and non-motile. Additionally, PCR targeting the gluconolactone gene confirmed that all 49 samples tested positive. Bands of 185 bp appeared on an agarose gel (Figure 1). Notably, only twenty of the samples contained the bla-OXA-51-like gene.

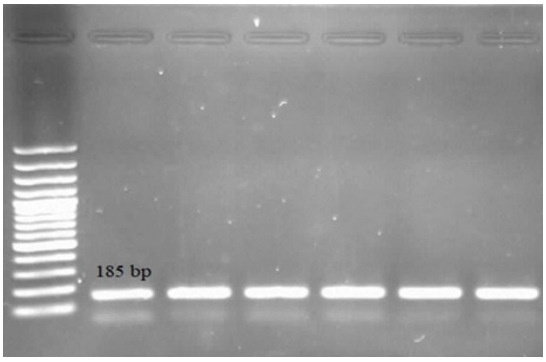

Fig 1. Molecular confirmation of identified *A. baumannii* isolates. From left, the first well is a molecular marker (1 kbp); wells 2-7 represent the gluconolactone gene, with bands of 185 bp.

B) Clinical and demographic data of patients: Among patients with *A. baumannii* infection, there was an equal proportion of males and females. The mean age was 57 years (maximum age: 80, minimum age: 22), with a median of 62 years. About 67.3% (n = 35) of

patients had underlying diseases, including diabetes (23.1%), hypertension (34.6%), heart disease (9.6%), and cancer (15.4%). 73.5% (n=36) of hospitalized patients were on mechanical ventilation. The mean intensive care unit stay was 26.3 days, with a median of 17 days. The overall mortality rate (n = 32) was 65.3%. Mortality was significantly associated with hospitalization duration (p = 0.04); 78.1% (n=25) of those who died were on mechanical ventilators (p=0.00). The most commonly prescribed drugs were carbapenems (75.6%), colistin (53.3%), quinolones (37.8%), and piperacillin tazobactam (10.2%). Binary logistic regression showed that increased age (p=0.47), hospitalization duration (p=0.03), and diabetes (p=0.04) are predictors of mortality.

C) Infections Associated with A. baumannii: Out of the 49 samples that contained *A. baumannii*, 25 samples (51%) had *A. baumannii* isolates present alone. In contrast, 24 samples contained *A. baumannii* alongside other bacterial and fungal isolates, including *Klebsiella pneumoniae, Pseudomonas aeruginosa, Escherichia coli*, and various fungi. Notably, among the patient samples, six contained two different isolates associated with *A. baumannii*: five samples had both *K. pneumoniae* and *P. aeruginosa*, while one sample had *K. pneumoniae* and *E. coli*. Furthermore, 18 samples contained only one isolate, all of which were *A. baumannii*; within this group, 12 were *K. pneumoniae*, 2 were *P. aeruginosa*, 1 was *E. coli*, and 3 contained a fungal isolate. The list of frequencies and numbers of isolates associated with *A. baumannii* is presented in Table 1.

D) Antimicrobial Susceptibility Results: Antibiotic resistance and susceptibility pattern results of all identified *A. baumannii* isolates was assessed against 15 different antibiotics from eight classes: cephalosporins, aminoglycosides, polymyxins, beta-lactam inhibitors, tetracyclines, fluoroquinolones, sulfonamides, and carbapenems. High resistance rates were observed across most classes. Cephalosporins showed the highest resistance: 95.9% for cefepime, 100% for cefotaxime, 100% for ceftriaxone, and 98% for ceftazidime. Carbapenem resistance was also high, with 98% for imipenem and 100% for meropenem. Piperacillin-tazobactam had a resistance rate of 98%. In contrast, colistin exhibited the lowest resistance rate at 12.2%, as shown in Figure 2. All isolates were classified as MDR, with 71% categorized as XDR. Full resistance to carbapenems was detected using the Kirby-Bauer method. Additionally, 20 isolates (81.4%) tested positive for carbapenemase production through phenotypic methods.

Table 1. Types of bacterial and fungal infections associated with *A. baumannii* isolates obtained from patient samples.

Isolated infections	Total number (%)
K.pneumoniae	(60) 18
P. aeruginosa	(35) 7
Various fungi	(15) 3
E. coli	(10) 2

Fig 2. The pattern of antibiotic resistance and susceptibility of *A.baumannii* isolates.
CTX: cefotaxime, CRO: ceftriaxone, CAZ: ceftazidime, CPM: cefepime, MER: meropenem, IPM: imipenem, GM: gentamicin, AN: amikacin, TM: tobramycin, CP: ciprofloxacin, SXT: trimethoprim-sulfamethoxazole, TZP: piperacillin-tazobactam, SAM: ampicillin-sulbactam, DO: doxycycline, and COL: colistin.

Discussion

CRAB is a global concern because these strains are often resistant to commonly used antibiotics (12). As a result, eradicating infections caused by MDR *A. baumannii* is becoming more difficult. Most studies report a higher incidence of *A. baumannii* infection among male patients. However, our study found no difference between male and female patients. In a study by Panahi et al. and Cerniauskiene *et al*., *A. baumannii* prevalence was higher in men than in women (13, 14). Similarly, Xiaoxuan Liu *et al*. found that men are nearly twice as likely (65.9%) to acquire *A. baumannii* infection. The carbapenem resistance rate was also significantly higher in men. The potential effects of genital hormones and immune system reactions should be investigated as risk factors for sex differences and mortality (15). A high mortality rate was observed among patients, especially elderly individuals with comorbidities such as diabetes, long-term hospitalization, and the need for mechanical ventilation. Mortality among ICU patients with carbapenem-resistant Gram-negative infections may be influenced by underlying health conditions and inflammatory responses (16). n our study, patients with diabetes mellitus had more severe outcomes and higher mortality rates. Similarly, Ching-Hsiang Leung et al. reported that 46% of CRAB patients also had diabetes, with an average age of 62 years. These patients showed significantly higher mortality than those without diabetes. Multivariate analyses identified septic shock as a contributor to mortality in diabetic patients (17). In a retrospective multicenter study by Alidoost *et al*. (2024), 143 patients were infected with *A. baumannii*. Of these, 37 (25.9%) died, most of whom were 45–65 years old. Intensive care unit hospitalization and carbapenem resistance were significant mortality risk factors (18). In a study by Cerniauskiene *et al*. (2025), 136 (69.4%) patients were male and 60 (30.6%) were female. The mean age was 16.6 ± 61.7 years (range, 52–74). Most patients (76.5%) had at least one underlying disease. The mortality rate was 58.7%. More than 6 days of effective antibiotic therapy, invasive mechanical ventilation, combination therapy, age over 58 years, and absence of co-infection were independent predictors of in-hospital mortality, as retrieved from regression analyses (13). El-Rahmani et al. examined mortality factors among patients infected with *A. baumannii*. Advancing age, male gender, underlying medical conditions, and longer hospital stays increased the risk of death (19). Our study confirms the alarming prevalence of CRAB as a major threat in the ICUs of two Tehran hospitals. Pogue et al. conducted a comparative cohort study of patients with CRAB- and CSAB-related infections from 250 US hospitals (2014–2019). CRAB was more often associated with respiratory infections. These patients had longer hospital stays and a higher mortality rate compared to CSAB-infected patients (20). Our study found high resistance to cephalosporins and piperacillin-tazobactam, showing that beta-lactam antibiotics are ineffective against *A. baumannii* isolates here. In a meta-analysis by Beigi et al. across 80 countries (1995–2023), rising carbapenem resistance was reported, peaking during 2021–2023(21). Rania Itani et al. in Lebanon found that all *A. baumannii* isolates from 111 patients (59%) were carbapenem-resistant; about 43% were XDR (22). Our findings also align with those of Sannathimmappa *et al*., who reported increased piperacillin-tazobactam resistance in Oman. Despite our results, aminoglycoside and

quinolone resistance remain low. Unlike our findings, which showed universal MDR, their samples had an MDR rate of 67% (23). Folasade Muibat Adeyemi et al. found that 42 of 143 samples (29.4%) were *A. baumannii*, with 100% resistance to imipenem and 88.1% resistance to ceftriaxone. Of those, 54.8% were MDR and 92.9% carried multiple carbapenem resistance genes (9). Mostafavi et al. studied resistance in *A. baumannii* complex isolates from three hospitals in Isfahan over 2 years. They reported a 75% increase in resistance to several antibiotic classes. The MDR rate was 88.7%, while colistin resistance was low (0%) (24). Boustanghadiri *et al*. reported a low but rising colistin resistance rate of 4% in clinical isolates. South America had the highest colistin resistance (25). Chienhsiu Huang et al. concluded there is no clear advantage of colistin monotherapy over colistin-meropenem combination therapy for MDR *A. baumannii* infections. Proper clinical trial monitoring may reveal comparable effectiveness (26).

This study has several limitations. First, we collected samples from two hospitals, but the cross-sectional design and the limited sampling period (October–December 2024) limit generalizability and preclude trend analysis. Second, we relied on phenotypic methods (sCIM) for carbapenemase detection. The observed discrepancy with resistance results highlights the need for molecular detection to identify the full range of carbapenemase genes (*OXA-23*, *OXA-24*, *VIM*, *NDM*, and *GES*). Future research should focus on molecular surveillance to map the resistance genes present in Tehran hospitals. Some *A. baumannii* samples were linked to other hospital-acquired bacteria. These associations may affect patient outcomes and mortality, including hospitalization length, mechanical ventilation needs, and pre-existing health conditions. Thus, co-infections with *A. baumannii* should be considered when analyzing risk factors.

Conclusion

This study revealed a high prevalence of multidrug-resistant CRAB isolates among ICU patients. The observed high mortality rate, along with a significant association between prolonged hospital stays, advanced age, and diabetes mellitus, highlights the increased risk of mortality in patients with CRAB infections. These conditions emphasize the critical need for surveillance and preventive measures against these infections, especially in intensive care units.

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Authors' Contribution

MMQ drafted the manuscript, analyzed all statistical analyses, collected samples, wrote the manuscript, and more.

Conflict of interests

The authors declare no competing interests.

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