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Brief Report

Evaluation of Antimicrobial Resistance and Frequency of *bap* Gene Among *Acinetobacter baumannii* Strains Isolated from Hospitalized Patients

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Abstract

Background: *Acinetobacter baumannii* has emerged as a major cause of hospital-acquired infections, posing a significant threat, especially in critical care environments, with mortality rates reaching up to 35%. Its ability to form biofilm on various surfaces within healthcare settings further facilitates the transmission of infections, raising growing concerns. This study was designed to evaluate antimicrobial resistance and the frequency of *bap* gene among *A. baumannii* collected from hospitalized patients in Mazandaran Province.

Methods: This cross-sectional study was conducted on 100 clinical isolates of *A. baumannii* recovered from hospitalized patients. In accordance with Clinical and Laboratory Standards Institute (CLSI) guidelines, the antibiotic susceptibility profiles of the isolates were evaluated using the disk diffusion method. For colistin, the minimum inhibitory concentration (MIC) was determined by the broth microdilution method. The presence of the *bap* gene was assessed using conventional polymerase chain reaction (PCR).

Results: The findings revealed that *A. baumannii* strains demonstrated high resistance to several antibiotics, particularly carbapenems and cephalosporins, with limited effective treatment options. Notably, colistin, tobramycin, and gentamicin remained effective against these isolates. Importantly, the *bap* gene was detected in all *A. baumannii* isolates, highlighting its widespread prevalence.

Conclusion: This study highlights the substantial burden *A. baumannii* imposes on healthcare systems and patients due to its multidrug-resistant nature. The continued effectiveness of colistin, tobramycin, and gentamicin suggests the need to reconsider reliance on traditional antibiotics such as carbapenems. The prevalence of the *bap* gene underscores the urgency of addressing this virulent pathogen. These findings provide valuable insights for local healthcare strategies to combat *A. baumannii* infections and ultimately improve patient outcomes.

Keywords: Biofilm, *Acinetobacter baumannii*, *bap* gene

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Introduction

Acinetobacter baumannii is a Gram-negative, aerobic bacterium with multidrug resistance (MDR) and multiple virulence factors. It possesses various antibiotic resistance mechanisms and causes serious problems in immunocompromised patients (1,2). Clinical isolates of *A. baumannii* can form biofilms on diverse surfaces. Notably, the emergence of biofilm-producing, antibiotic-resistant Gram-negative bacteria has become a growing concern, particularly among pathogens commonly encountered in healthcare environments (3).

A large 854-kDa protein known as biofilm-associated protein (BAP) is a key factor in biofilm production and adhesion to host cells (4). In recent years, only a few studies have examined the prevalence of the *bap* gene and its association with antibiotic-resistant *A. baumannii* strains.

Given the limited data on biofilm-forming ability and the prevalence of the biofilm-associated *bap* gene in *A. baumannii*, as well as its connection to antibiotic resistance

in northern Iran, this study aimed to investigate the antibiotic susceptibility profiles and conduct a molecular evaluation of the *bap* gene in *A. baumannii* strains isolated from hospitalized patients.

Materials and Methods

Sample Collection

This cross-sectional study (February 2022 to June 2023), approved under the ethics code IR.MAZUMS.REC.1401.240, included 100 clinical samples collected from teaching hospitals in Sari County. The samples were obtained from pulmonary secretions, blood, wounds, urine, broncho-alveolar lavage (BAL), abscess, urinary catheters, pleural fluid, and cerebrospinal fluid (CSF).

Isolation and Identification of Acinetobacter baumannii Isolates

All samples were transferred to the microbiology laboratory and cultured on blood agar and MacConkey agar media. The inoculated plates were subsequently incubated at 37 ˚C for 24 hours to allow optimal bacterial growth. Preliminary identification of the isolates was performed using standard biochemical tests (5). Final confirmation of the *A. baumannii* strain was achieved through a standard polymerase chain reaction (PCR) assay targeting the bla-*OXA-51* gene (bla-*OXA-51*-F: 5′-TAA TGC TTT GAT CGG CCT TG-3′, bla-OXA_{51}-R: 5′-TGG ATT GCA CTT CAT CTT GG -3′) (6).

Antibiotic Susceptibility

Antibiotic susceptibility of all isolates was evaluated using the Kirby-Bauer disk diffusion method on Mueller-Hinton agar, following the 2021 Clinical and Laboratory Standards Institute (CLSI) guidelines. The susceptibility of the isolates was assessed using the following antimicrobial disks: gentamicin (30 µg), tetracycline (5 µg), imipenem (10 µg), tobramycin (10 µg), trimethoprim-sulfamethoxazole (30 µg), ceftazidime (30 µg), doripenem (30 µg), piperacillin-tazobactam (10 µg), ciprofloxacin (5 µg), cefepime (30 µg), piperacillin (30 µg), cefazolin (30 µg), amikacin (30 µg), and ampicillin (30 µg) (MAST, UK).

The minimum inhibitory concentration (MIC) for colistin against *A. baumannii* (0.25–128 µg/mL) was determined using the CLSI broth microdilution (BMD) method. MIC values were evaluated based on CLSI guidelines (7). *Escherichia coli* ATCC 25922 was employed as the standard reference strain for quality verification.

Identification of bla-OXA-51 Gene in Acinetobacter baumannii Isolates

In this study, the genome of 100 isolates was obtained using the boiling extraction method. PCR amplification was conducted under the following conditions: initial denaturation at 95 ˚C for five minutes, 30 cycles of 95 ˚C for 60 seconds, 50 ˚C for 50 seconds, 72˚C for 60 seconds, followed by a final extension at 68˚C for two minutes. DNA presence, concentration, and quality were assessed using agarose gel electrophoresis.

Polymerase Chain Reaction for Detection of the bap Gene

In the present study, the primers used for *bap* gene detection were: *bap*-F: 5′-TAGGGAGGGTACCAATGCAG-3′ and *bap*-R: 5′-TCATGATTTGATGCTGCAGCG-3′, applied in the PCR method (8).

Results

Descriptive Epidemiology

In the current study, 100 *A. baumannii* isolates were collected from different clinical specimens, including pulmonary secretions (46%), blood (30%), wound (10%), urine (5%), BAL (3%), abscess (2%), urinary catheter (2%), pleural fluid (1%), and CSF (1%). Of the isolates identified as *Acinetobacter* spp. phenotypically, all were confirmed as *A. baumannii* using bla-*OXA-51* primers, which yielded the expected 353 bp bands on agarose gel electrophoresis.

Antimicrobial Susceptibility Testing

The MIC results indicated that out of the 100 *A. baumannii* strains, 97 were susceptible and 3 were resistant to colistin. Susceptibility to other antibiotics was assessed using the standard disk diffusion assay. The detailed susceptibility profiles of other tested antibiotics are presented in Figure 1.

Presence of bap

All isolates involved in this study carried the *A. baumannii* *bap* gene (Figure 2).

Discussion

Over the past decade, *A. baumannii* has attracted increasing research interest as a major multidrug-resistant pathogen (9-11). Among its virulence-related genes, the *bap* gene has received considerable attention due to its critical role in biofilm formation, which enhances the persistence of *A. baumannii* in hospital environments where exposure to disinfectants is frequent (12,13).

In Iran, several studies have reported that the frequency of the *bap* gene among *A. baumannii* isolates ranges from 43% (14) to 100% (15). These high frequencies highlight the worldwide distribution and dissemination of this important virulence factor in *A. baumannii* strains. Similarly, in a study conducted on 53 *A. baumannii* clinical isolates collected from inpatients in Iraq, the frequency of the *bap* gene was reported to be 79% (16). The lowest frequency (43%) was reported by Zeighami et al, who examined 100 *A. baumannii* strains recovered from immunocompromised patients hospitalized in the intensive care units (ICUs) (14). The low frequencies observed in some studies may be due to geographical variations and differences in the source of isolates. For example, one of our reference hospitals (Shahid Zare) is a burn-specialized hospital where *A. baumannii* strains

Antibiotic	COL	TOB	GEN	TE	AK	AMS	TPZ	SXT	PRL	IPM	DOR	CPM	CIP	CFZ	CAZ
Resistant	3	31	41	19	85	91	100	90	100	100	100	100	100	100	100
Intermediate	0	0	1	43	5	2	0	8	0	0	0	0	0	0	0
Susceptible	97	69	59	38	10	7	0	2	0	0	0	0	0	0	0

Figure 1. Antibiotic Resistance Patterns of *A. baumannii* Isolates. *Note:* AK: Amikacine; AMS: Ampicillin-sulbactam; CAZ: Cefazolin; CFZ: Ceftazidime; CIP: Ciprofloxacine; COL: Colistin; CPM: Cefepime; DOR: Doripenem; GEN: Gentamicin; IPM: Imipenem; PRL: Piperacillin; SXT: Trimethoprim-sulfamethoxazole; TE: Tetracycline; TOB: Tobramycin; TPZ: Piperacillin-tazobactam

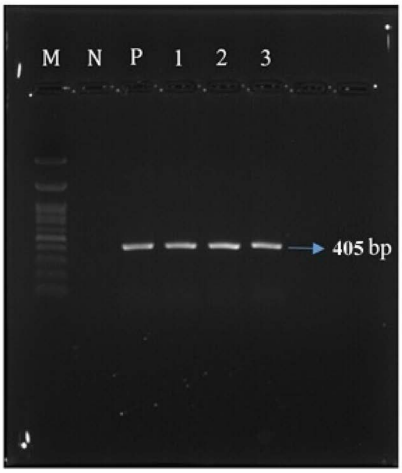

Figure 2. PCR Image of Three *A. baumannii* Isolates (1, 2, 3) Showing 405 bp DNA Bands Representing the bap Gene. *Note.* M: DNA ladder; N: Negative control; P: Positive control

are exposed to extreme amounts of disinfectants and prophylactic antibiotics. The capacity of *A. baumanni*i to form biofilms has been closely linked to the development of an MDR phenotype. Consistent with our observations, previous studies have reported a strong association between the presence of the *bap* gene and biofilm formation, as well as increased biofilm production in MDR isolates, highlighting the critical role of *bap*-related biofilms in enhancing antimicrobial resistance mechanisms in *A. baumannii* (17)

However, carbapenems (imipenem and doripenem) and cephalosporins (ceftazidime, cefepime, and cefazoline) were found to be completely ineffective against *A. baumannii* isolates, as none of the isolates exhibited susceptibility to these drugs. Interestingly, all isolates were resistant to imipenem and doripenem, as these drugs (Carbapenem family) are the first-line treatment for *A. baumannii* infections in many areas. Consistent with our findings, multiple studies have reported similarly high resistance rates of *A. baumannii* isolates to carbapenems (16,18-21).

These results suggest that carbapenems should be decommissioned as the drugs of choice for *A. baumannii* infections. In contrast, colistin and aminoglycosides (tobramycin and gentamicin) showed excellent activity against *A. baumannii* isolates. Several other studies have similarly reported a high susceptibility profile of *A. baumannii* isolates to colistin and aminoglycosides (16,18), supporting their use as potential therapeutic options for *A. baumannii* infections in Mazandaran province and northern Iran.

Conclusion

In conclusion, our study highlights the significant burden that *A. baumannii* imposes on healthcare systems and patients, primarily due to its MDR nature. We identified colistin, tobramycin, and gentamicin as effective treatment options, underscoring the need to reconsider reliance on traditional antibiotics such as carbapenems.

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Authors' Contribution

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Competing Interests

The authors declare no conflict of interests.

Ethical Approval

This study was approved by the Research Ethics Committees of Mazandaran University of Medical Sciences (MAZUMS) (Approval ID. IR.MAZUMS.REC.1401.240).

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