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Expression of *bap* Gene in Clinical *Acinetobacter baumannii* Isolates in Khorramabad, Iran

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Abstract

Background: Biofilm-associated protein (Bap) in *Acinetobacter baumannii* is an essential factor in biofilm production and persistence in the hospital environment.
Objectives: This study aimed to detect the *bap* gene in *A. baumannii* by real-time polymerase chain reaction (PCR) from clinical specimens in Khorramabad, Iran.
Methods: This cross-sectional study was performed during April 2017 - April 2018 on 43 *A. baumannii* strains from clinical samples collected and identified by microbiological and biochemical tests. The expression of the *bap* gene was evaluated by real-time PCR. Data were analyzed with SPSS version 24.
Results: Out of 43 *A. baumannii* strains, 23, 8, 3, 3, 3, and 3 samples were isolated from chest sputum, wounds, urine, tissues, blood, and, respectively. According to the PCR results, all isolates had the *bap* gene except one. Real-time PCR showed significant differences in the expression of the *bap* gene between *A. baumannii* isolates from diverse clinical samples. The highest expression of the *bap* gene was found in chest sputum and wound samples and had a significant difference with other samples ($P < 0.0001$).
Conclusions: We observed the *bap* gene in most strains, with the high expression of this gene in chest sputum and wound samples. Therefore, further studies are recommended to find strategies to inhibit the expression of this gene and biofilm formation, which help treat infections caused by biofilm-forming *A. baumannii* strains.

Keywords: *Acinetobacter baumannii*, Biofilm, Clinical Specimens

1. Background

Acinetobacter baumannii is a gram-negative, aerobic, nonfermenting, and rod-shaped ubiquitous in the medical environment and is generally regarded as a significant opportunistic pathogen (1). One of the public health threats that have recently been considered in the United States, Europe, Asia, and the Middle East is the rapid increase in the antibiotic-resistant isolates of *A. baumannii* (2). This pathogen is responsible for many infections, such as bacteremia, urinary tract infection (UTI), and respiratory tract infections, especially in immunocompromised patients (3). *Acinetobacter baumannii* transmits and survives in the hospital by attaching to different surfaces, such as cerebrospinal fluid shunts and vascular catheters. Catheter-acquired UTIs (CAUTIs) are among the most common nosocomial infections. In the previous study, forming *A. baumannii* biofilms along the catheter surface was the most important cause of bacteriuria (4).

Acinetobacter baumannii has several virulence factors, among which the ability to form biofilm is one of the most important factors (5). Biofilms are complex bacterial communities attached to surfaces, created by an extracellular matrix produced by bacteria. This matrix comprises polysaccharides, DNA, and proteins (5). Biofilm formation is a complex process that requires many factors, including aggregation, collagen adhesion, pili expression, and iron uptake (6, 7). Among the diverse factors effective in biofilm formation, the biofilm-associated protein (*Bap*, high-molecular-weight proteins) encoded by the *bap* gene has a significant role in attachment to bronchial cells, structural integrity, and water channel formation in the biofilm (8). This protein is located on the outer surface of bacteria and consists of a central core of the successive iterations of similar sequences (9). Disruption of the *bap* gene reduces the thickness and volume of biofilm and interbacterial cell adhesion (10).

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2. Objectives

As mentioned, the *bap* gene is essential in forming *A. baumannii* biofilm. Therefore, our investigation aimed to assess the expression of the *bap* gene in *A. baumannii* using real-time polymerase chain reaction (PCR) in clinical samples from Khorramabad, Iran.

3. Methods

3.1. Sample Collection

This cross-sectional study was conducted during April 2017 - April 2018 on 43 *A. baumannii* strains from clinical samples, including urine, blood, wound, tissues, and chest sputum collected from teaching hospitals in Khorramabad (west of Iran). All strains were identified by microbiological and biochemical tests, such as oxidase, oxidation-fermentation (OF), triple sugar iron (TSI) test, motility in sulfur, indole, motility (SIM), and growth at 44°C (11). After identification, isolates were cultured in tryptic soy broth (TSB) containing 15% glycerol and were stored at -70°C.

3.2. Evaluation of bap Gene by PCR

Bacterial genomic DNA was extracted from all isolates according to the kit protocol (Sinaclon Co, Iran). *bap* gene was detected by PCR using specific primers (Table 1). Electrophoresis was performed on 1.5% agarose gels and was visualized by an ultraviolet gel documentation system (Bio-Rad, USA). *Acinetobacter baumannii* ATCC19606 was used as a positive control.

3.2. Evaluation of bap Gene Expression by Real-Time PCR

RNA was extracted from *A. baumannii* isolates to study the expression of the *bap* gene. RNA extraction was performed according to the manufacturer's instructions (GeneAll Co., South Korea). The concentration of used RNA was considered to be about 1 - 2 μg. For this purpose, light absorption was measured at a wavelength of 260 nm. Moreover, light absorption at 280/260 nm was assessed to ensure the lack of protein contamination, and light absorption at 260/230 nm was measured to ensure the lack of salt contamination. The cDNA was synthesized from the extracted RNA after DNase I treatment. *DNA gyrase A* was used as an internal control to study the expression of the *bap* gene. The sequences of the forward and reverse primer pairs of the two *bap* and *DNA gyrase A* genes are shown in Table 1. The temperature program and volume of each material used in the reaction are presented in Table 2.

3.3. Statistical Analysis

The expression of target genes normalized by housekeeping genes was $\log_2$ transformed before analysis. Data were analyzed using a one-way analysis of variance on the linear 2-$\Delta\Delta$CT dataset and the least significant difference method to analyze the differences between outcome groups. Data were analyzed utilizing the SPSS software version 24 (SPSS Inc., Chicago, IL, USA), and differences were deemed significant where $P < 0.05$.

4. Results

Out of 43 *A. baumannii* clinical strains from teaching hospitals in Khorramabad, Iran, 23, 8, 3, 3, 3, and 3 specimens were isolated from chest sputum, wounds, urine, tissues and blood respectively. According to the PCR results, all isolates except one had the *bap* gene. Following the real-time PCR results, 42 isolates expressed the *bap* gene. The results of real-time PCR for the *bap* gene of *A. baumannii* isolates in various clinical samples are presented in Figure 1.

Our findings showed that the relative expression of the *bap* gene was not significantly different between the control and blood ($P = 0.713$), sputum and blood ($P = 0.997$), control and sputum ($P = 0.401$), while the differences between other groups were significant ($P < 0.0001$). There was no significant difference in *bap* expression between urine and tissue groups ($P = 0.998$), while other groups were significantly different ($P < 0.0001$). Relative expression of the *bap* gene in the chest sputum group had the significantly highest value compared to all groups ($P < 0.0001$). Moreover, the relative expression of the *bap* gene in the wound group was significantly different from all groups ($P < 0.0001$) (Figure 1).

5. Discussion

Biofilm formation is one of the most important factors in the pathogenicity of *A. baumannii* and is effective in bacterial survival in various conditions by binding to substrates (3). For example, biofilm formation in ventilator-associated pneumonia and CAUTIs associated with non-living substrates plays a role in bacterial survival (13). Several factors, including bap protein, are involved in producing biofilms in *A. baumannii* (14). In the current study, the *bap* gene was present in all strains except one. Fallah et al. (15) and Mahmoudi Monfared et al. (16) showed that the frequency of the *bap* gene was 92% and 70.3%, respectively. In the study by Ghasemi et al., the *bap* gene was detected in 14.2% of *A. baumannii* isolates, which is not in line with our findings (17). Goh et al. reported a high prevalence of

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Table 1. Characteristics of Sequence Primers of *bap* and *DNA Gyrase A* Genes

Target Genes	Primer Sequences	Size (bp)	Ref
***bap* gene**	R: 5'-TGCAACTAGTGGAATAGCAGCCCA-3'	121	(12)
	F: 5'-TGCTGACAGTGACGTAGAACCACA-3'		
DNA gyraseA	R: 5'-AACCGTACCAGAAGCTGTC<G-3'	110	(12)
	F: 5'-AAGGCCGTCCAATCGTGAA<T>-3'		

Table 2. Temperature Program and the Volume of Each Material Used for Detecting *bap* Gene

Target Gene	Conditions	Volume Reactions
bap	1 cycle: 95°C (10 min); 40 cycle: 95°C (20 s), 58°C (40 s), 95°C (15 s), 60°C (30 s), 95°C (15 s)	2 μL of cDNA, 10 pM of forward and reverse primers for both *bap* and *DNA gyrase A* genes, 10 μL of master-mix. H_2O up to 25 μL

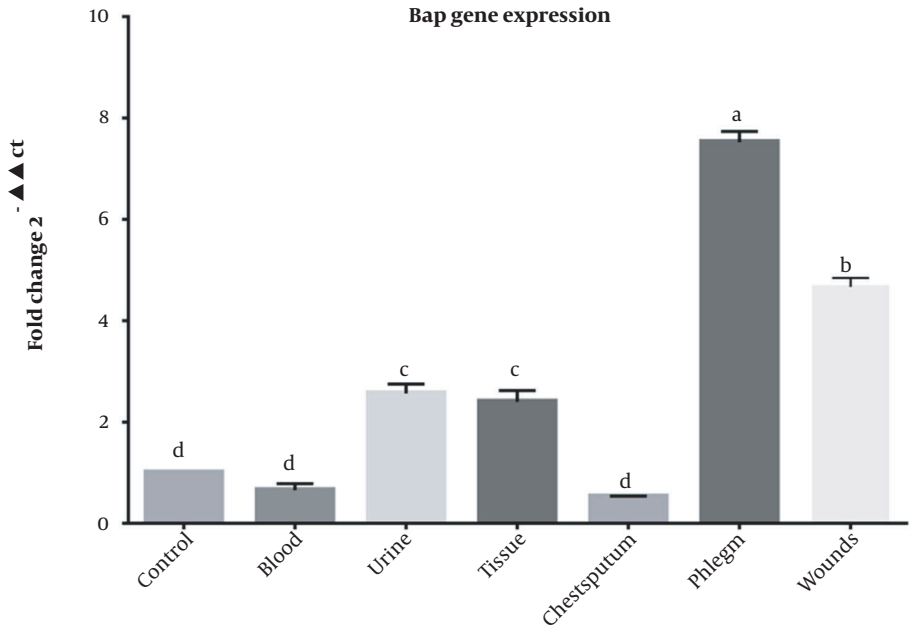

Figure 1. Result of real-time PCR for the *bap* gene in *Acinetobacter baumannii* isolates in different clinical samples; A - D, Within a row, different superscripts indicate the differences between groups ($P \leq 0.05$).

the *bap* gene (91.7%) in *A. baumannii*, which is consistent with the results of the present study (18). Ghasemi et al. attributed the difference in the frequency of the *bap* gene between different studies to the variations in the source and the number of studied isolates. In the research by Ghasemi, 120 *A. baumannii* was isolated from clinical and environmental samples (17). Therefore, in other studies, the small number of strains and the isolation of strains from clinical samples cause the increasing frequency of the *bap* gene.

Several studies indicated a strong association between the *bap* gene and resistance to different classes of antibiotics (15, 19, 20). However, in the current study, the expression of the *bap* gene was compared between distinct clinical samples, including urine, blood, wounds, tissue, and chest sputum for the first time. Our results confirm that the amount of *bap* gene expression can also depend on the type of clinical specimen as the highest expression of the *bap* gene was observed in chest sputum and wound samples and had a significant difference with other samples ($P < 0.0001$). The latter finding may result from biofilm formation in wound and chest sputum samples more eas-

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ily than in blood. Chest sputum was collected from a hospitalized patient under a ventilator. Furthermore, our results revealed the need for further investigations on a large number of *A. baumannii* samples isolated from different clinical and environmental specimens over a more extended period. In addition, the relationships between gene expression and other variables, such as the parts of the hospital and resistance to different classes of antibiotics, need to be evaluated.

5.1. Conclusion

According to the results of the current study, there is a relationship between sample type and the presence of the *bap* gene, which is one of the main factors in forming biofilms by *A. baumannii* isolates. Therefore, due to the importance of biofilm in bacterial virulence, detecting the *bap* gene by molecular assay in hospitalized patients, especially in ventilator-associated pneumonia infections and CAUTIs, which have suitable conditions for biofilm formation, can be helpful in infection control. Considering the prevalence of biofilm-producing *A. baumannii* isolates and the importance of biofilms in antibiotic resistance, the results could provide a perspective for further research to prevent infections by biofilm-forming *A. baumannii* strains.

Footnotes

Authors' Contribution: Study conception and design, Delfani. S; Performed experiments, Shakib. P.; Analysis and interpretation of results, Halimi. Sh.; Initial draft preparation, Shakib. P.; Draft review and editing, Rezaei. F.; Validation and supervision, Delfani. S.

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