Expression of \textit{bap} Gene in Clinical \textit{Acinetobacter baumannii} Isolates in Khorramabad, Iran

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Abstract

\textbf{Background:} Biofilm-associated protein (Bap) in \textit{Acinetobacter baumannii} is an essential factor in biofilm production and persistence in the hospital environment.

\textbf{Objectives:} This study aimed to detect the \textit{bap} gene in \textit{A. baumannii} by real-time polymerase chain reaction (PCR) from clinical specimens in Khorramabad, Iran.

\textbf{Methods:} This cross-sectional study was performed during April 2017 - April 2018 on 43 \textit{A. baumannii} strains from clinical samples collected and identified by microbiological and biochemical tests. The expression of the \textit{bap} gene was evaluated by real-time PCR. Data were analyzed with SPSS version 24.

\textbf{Results:} Out of 43 \textit{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 \textit{bap} gene except one. Real-time PCR showed significant differences in the expression of the \textit{bap} gene between \textit{A. baumannii} isolates from diverse clinical samples. The highest expression of the \textit{bap} gene was found in chest sputum and wound samples and had a significant difference with other samples (P < 0.0001).

\textbf{Conclusions:} We observed the \textit{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 \textit{A. baumannii} strains.

\textbf{Keywords:} \textit{Acinetobacter baumannii}, Biofilm, Clinical Specimens

1. Background

\textit{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 \textit{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). \textit{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 \textit{A. baumannii} biofilms along the catheter surface was the most important cause of bacteriuria (4).

\textit{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, pilus 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 \textit{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 \textit{bap} gene reduces the thickness and volume of biofilm and interbacterial cell adhesion (10).
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 (BioRad, 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_{10} transformed before analysis. Data were analyzed using a one-way analysis of variance on the linear 2-∆∆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
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'  
F: 5'-TGCTGACAGTGACGTAGAACCACA-3' | 121 | (12) |
| DNA gyraseA  | R: 5'-AACCGTACCAGAAGCTGTCG-3'  
F: 5'-AAGGCCGTCCAATCGTGAA-3' | 110 | (12) |

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, H₂O 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 ≤ 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
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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### References

1. Perez F, Hujer AM, Hujer KM, Decker BK, Rather PN, Bonomo RA. Global challenge of multidrug-resistant Acinetobacter baumannii. Antimicrob Agents Chemother. 2007;51(10):3471-84. doi: 10.1128/AAC.00464-06. [PubMed: 17646421]. [PubMed Central: PMC2041292].

2. Luo LM, Wu LJ, Xiao YI, Zhao D, Chen ZX, Kang M, et al. Enhancing pilus assembly and biofilm formation in Acinetobacter baumannii ATCC9606 using non-native acyl-homoserine lactones. BMC Microbiol. 2015;15:62. doi: 10.1186/s12866-015-0397-5. [PubMed: 25888221]. [PubMed Central: PMC4381447].

3. Peleg AV, Seifert H, Paterson DL. Acinetobacter baumannii: emergence of a successful pathogen. Clin Microbiol Rev. 2008;21(3):338-82. doi: 10.1128/CMR.00058-07. [PubMed: 18625667]. [PubMed Central: PMC2493088].

4. Longo F, Vaotto C, Donelli G. Biofilm formation in Acinetobacter baumannii. New Microbiol. 2014;37(2):119-27. doi: 24858639.

5. Pakhurakova N, Tuittila M, Paavilainen S, Malmi H, Pariloiva O, Tenenberg S, et al. Structural basis for Acinetobacter baumannii biofilm formation. Proc Natl Acad Sci U S A. 2018;115(21):5558-63. doi: 10.1073/pnas.1800961115. [PubMed: 29735695]. [PubMed Central: PMC5003481].

6. Gaddy JA, Actis IA. Regulation of Acinetobacter baumannii biofilm formation. Future Microbiol. 2009;4(3):273-8. doi: 10.2217/fmb.09.5. [PubMed: 19327814]. [PubMed Central: PMC224675].

7. Gentile V, Frangipani E, Bonchi C, Minandri F, Runci F, Visca P, Iron and Acinetobacter baumannii Biofilm Formation. Pathogens. 2014;3(1):704-19. doi: 10.3390/pathogens3010704. [PubMed: 25438009]. [PubMed Central: PMC4243436].

8. Azziri O, Shachcheraghi F, Salimizand F, Shahkibaie MR, Mansouri S, et al. Molecular Analysis and Expression of bap Gene in Biofilm-Forming Multi-Drug-Resistant Acinetobacter baumannii. Rep Biochem Mol Biol. 2016;5(1):52-72. [PubMed: 28070537]. [PubMed Central: PMC5214666].

9. Brossard KA, Campagnari AA. The Acinetobacter baumannii biofilm-associated protein plays a role in adherence to human epithelial cells. Infect Immun. 2012;80(1):228-33. doi: 10.1128/IAI.00591-11. [PubMed: 22083703]. [PubMed Central: PMC3525684].

10. Loehfelm TW, Luke NR, Campagnari AA. Identification and characterization of an Acinetobacter baumannii biofilm-associated protein. J Bacteriol. 2008;190(1):1036-44. doi: 10.1128/JB.00416-07. [PubMed: 18024522]. [PubMed Central: PMC2223572].

11. Forbes BA, Sahm DF, Bailey WR, Weissfeld AS, Scott EG, Forbes BA, et al. Bailey & Scott's Diagnostic Microbiology, 12 ed. Elsevier Mosby: Missouri, USA; 2007.

12. Bahador A, Saghii H, Ataei R, Emami D. The Study of Inhibition Effects Satureja khuzestaniea Essence against Gene Expression bap in Acinetobacter baumannii with Real time PCR Technique. Iran J Med Microbiol. 2015;9(1):34-2.

13. Eijkelkamp BA, Hassan KA, Pausen JT, Brown MH. Investigation of the human pathogen Acinetobacter baumannii under iron limiting conditions. BMC Genomics. 2011;12:126. doi: 10.1186/1471-2164-12-126. [PubMed: 21342532]. [PubMed Central: PMC3053841].

14. Luo TL, Rickard AH, Srivivasan U, Kaye KS, Foxman B. Association of blaOXA-23 and AgP with the persistence of Acinetobacter baumannii within a major healthcare system. Front Microbiol. 2015;6:182. doi: 10.3389/fmicb.2015.00082. [PubMed: 25814985]. [PubMed Central: PMC4357298].

15. Fallah A, Rezaee MA, Hasani A, Baraghhi MHS, Kafil HS. Frequency of bap and cpaA virulence genes in drug resistant clinical isolates of Acinetobacter baumannii and their role in biofilm formation. Iran J Basic Med Sci. 2017;20(8):849-55. doi: 10.22038/ijbms.2017.9015. [PubMed: 29085575]. [PubMed Central: PMC565469].

16. Mahmoudi Monfared A, Rezaei A, Pourisna F, Faghihi J. Detection of Genes Involved in Biofilm Formation in MDR and XDR Acinetobacter baumannii Isolated from Human Clinical Specimens in Isfahan, Iran. Arch Clin Infect Dis. 2019;14(2):e5766. doi: 10.5822/archcid.85766.

17. Ghasedi E, Ghalavand Z, Goudarzi H, Yeganeh F, Hashemi A, Davabi H, et al. Phenotypic and genotypic investigation of biofilm formation in clinical and environmental isolates of Acinetobacter baumannii. Arch Clin Infect Dis. 2018;13(4):e12914. doi: 10.5812/archcid.12914.
18. Goh HM, Beatson SA, Totsika M, Moriel DG, Phan MD, Szubert J, et al. Molecular analysis of the Acinetobacter baumannii biofilm-associated protein. *Appl Environ Microbiol*. 2013;79(21):6535–43. doi: 10.1128/AEM.01402-13. [PubMed: 23956398]. [PubMed Central: PMC3811493].

19. Lee HW, Koh YM, Kim J, Lee JC, Lee YC, Seol SY, et al. Capacity of multidrug-resistant clinical isolates of Acinetobacter baumannii to form biofilm and adhere to epithelial cell surfaces. *Clin Microbiol Infect*. 2008;14(1):49–54. doi: 10.1111/j.1469-0691.2007.01842.x. [PubMed: 18005176].

20. Rodriguez-Bano J, Marti S, Soto S, Fernandez-Cuenca F, Cisneros JM, Pachon J, et al. Biofilm formation in Acinetobacter baumannii: associated features and clinical implications. *Clin Microbiol Infect*. 2008;14(3):276–8. doi: 10.1111/j.1469-0691.2007.01916.x. [PubMed: 18190568].