Research in Molecular Medicine

Prevalence of β-lactamase-encoding Genes in Isolated *Acinetobacter baumannii* From Clinical Samples in Sari, Iran

Golnar Rahimzadeh[1], Sasan Sarli[2], Mohammad Ahanjan[3*]

1. Pediatric Infectious Diseases Research Center, Communicable Diseases Institute, Mazandaran University of Medical Sciences, Sari, Iran.
2. Student Research Committee, Faculty of Medicine, Mazandaran University of Medical Sciences, Sari, Iran.
3. Department of Microbiology, Molecular and Cellular Biology Research Center, Faculty of Medicine, Mazandaran University of Medical Sciences, Sari, Iran.

Use your device to scan and read the article online

Citation Rahimzadeh G, Sarli S, Ahanjan M. Prevalence of β-lactamase-encoding Genes in Isolated Acinetobacter baumannii From Clinical Samples in Sari, Iran. Research in Molecular Medicine. 2023; 11(1):67-72. https://doi.org/10.32598/rmm.11.1.1292.1

https://doi.org/10.32598/rmm.11.1.1292.1

Article Type:
Research Paper

Article info:
Received: 01 Jan 2024
Revised: 28 Jan 2024
Accepted: 16 Feb 2024

Keywords:
Nosocomial infections, Multiple drug resistance, β-lactamases, Metallo-β-lactamases, *Acinetobacter baumannii*

ABSTRACT

Background: The prevalence of *Acinetobacter baumannii* as a causative agent of hospital-acquired infections, particularly in burn units and intensive care units, is a major concern due to its innate and acquired resistance to several antibiotics. The presence of beta-lactamase-encoding genes in this bacterium has made it resistant to carbapenems as the last-resort antibiotics for treating infections caused by *A. baumannii*. This study aims to determine the prevalence of β-lactamase-encoding genes and antibiotic resistance of *A. baumannii* isolates from burn patients in northern Iran.

Materials and Methods: In this descriptive cross-sectional study, *A. baumannii* isolates were obtained from clinical samples of patients in Zare Burn Hospital in Sari City, from 2013 to 2015. The isolates' antibiotic sensitivity was determined using the disk diffusion method. To investigate the prevalence of β-lactamase genes (*blaVIM*, *blaIMP*, and *INT*), the PCR test was conducted.

Results: Of 150 patients, 54.7% were men and 45.3% were women. The highest resistance rates were against ceftazidime, cefepime, meropenem, imipenem, ciprofloxacin, amikacin, gentamicin, and colistin in order. It was observed that 31% of the isolates produced metallo-β-lactamase enzyme. The genes *blaVIM*, *blaIMP*, and *INT* were detected in 35%, 45%, and 60% of the isolates, respectively.

Conclusion: *A. baumannii* isolates have significant resistance to cephalosporins and carbapenems. It is recommended to avoid the irrational prescription of cephalosporins and carbapenems for infections caused by *A. baumannii*.

*** Corresponding Author:**
Mohammad Ahanjan, Associate Professor.
Address: *Pediatric Infectious Diseases Research Center, Communicable Diseases Institute, Mazandaran University of Medical Sciences, Sari, Iran.*
Phone: *+98 (911) 3546204*
E-mail: *ahanjan2007@gmail.com*

Research in Molecular Medicine

Introduction

Acinetobacter baumannii is a gram-negative bacterium and a well-known human opportunistic pathogen, which is an important cause of hospital-acquired infections (HAIs) such as bacteremia- and ventilator-associated pneumonia (VAP), surgical site (SSIs) and burn infections, meningitis, and urinary tract infections (UTIs) in adults and children worldwide [1-3], especially in people with a weak immune system and patients admitted to the intensive care units (ICU) and people with cancer, neutropenia and burns. The mortality rates of ventilator-associated pneumonia and blood infection caused by *A. baumannii* have been reported as 40%-70% and 28%-43%, respectively [3]. The survivability of *A. baumannii* and resistance to drought have made it a successful pathogen for dissemination in healthcare settings. Recently, *A. baumannii*, with multiple drug resistance (MDR), has emerged as a challenging pathogen with high morbidity and mortality [4]. The MDR can be caused by decreased expression of outer membrane proteins, the addition of insertion sequences (ISs), genome rearrangement, horizontal expansion of resistance genes, differential expression of intrinsic resistance genes, and increased expression of secretory pumps [5]. Several groups of beta-lactamases such as class A (such as TEM, SHV, CTX-M, PER, VEB, and GES), class B (such as IMP, VIM, GIM, and NDM), and class D carbapenemases/oxacillinases (such as OXA-23- like, OXA-24-like, OXA-51-like, and OXA-58-like) have often been described in *A. baumannii* [6, 7]. Carbapenemases are the last antibiotic options against *A. baumannii* infections. However, carbapenem resistance limits treatment options [8]. Due to the irrational and experimental prescription of antibiotics, the growing reports of *A. baumannii* strains with MDR, the increase in treatment costs, the long stay of patients in the hospital, and the increase in patient mortality [8], it is necessary to investigate the prevalence and patterns of antibiotic resistance in *A. baumannii* strains with MDR, causing hospital infections, especially in the burn unit of hospitals, to minimize the spread of microbial resistance, mortality from hospital-acquired infections, and treatment costs in patients. Therefore, this study aims to investigate the antibiotic resistance and the frequency of beta-lactamase genes in *A. baumannii* isolates from the samples of patients admitted to a burn hospital in Sari, northern Iran.

Materials and Methods

Sampling and collection of *A. baumannii* strains

In this descriptive cross-sectional study, 150 clinical samples (trachea, sputum, urine, blood, wound culture, ascites fluid, pleural fluid, catheter, and cerebrospinal fluid) were collected from patients admitted to Zare Burn Hospital in Sari from 2013 to 2015. The patients with burns and those hospitalized due to nosocomial infections (NIs) were included. The characteristics of the patients, including age, sex, and antibiotic treatments, were recorded using a demographic form. After transferring the clinical samples to the laboratory, the samples were cultured on blood agar (BA) and eosin methylene blue (EMB) (OUELAB, USA). Then, the plates were incubated in aerobic conditions at 37°C for 24 hours. Subsequently, conventional microbial tests were performed to confirm *A. baumannii* isolates.

Determining the antibiotic sensitivity pattern

The antibiotic sensitivity of *A. baumannii* strains was determined using the Kirby-Bauer method and according to the Clinical Laboratory Standard Institute (CLSI) guidelines (2011). The studied antibiotics included ciprofloxacin, imipenem, meropenem, cefepime, ceftazidime, gentamicin, amikacin. Also, to determine the resistance of the isolates to colistin, the minimum inhibitor concentration (MIC) was determined according to the CLSI guidelines (2011) by the macrodilution method. In 10 sterile tubes, serial dilutions of antibiotics were prepared. Tube numbers 11 and 12 were used as positive control and negative control, respectively. Then, *A. baumannii* was added to each tube with a concentration equivalent to 0.5 McFarland. The tubes were incubated at 37°C for 24 hours. A tube with no turbidity was reported as MIC value [9, 10].

Phenotypic study of metallo-beta-lactamase production by the combined disk test

In the combined disk test (CDT), imipenem disc (10 µg) alone and imipenem-EDTA combined disc (10 µg/750 µg) were used. The isolates were cultured on Mueller-Hinton agar with a concentration equivalent to 0.5 McFarland and incubated at 37°C for 24 hours. An increase ≥7 in the diameter of the non-growth zone around the imipenem-EDTA combination disc compared to the imipenem disc alone indicated beta-lactamase production [9, 10].

Table 1. Sequence of primers and amplification conditions of beta-lactamase producing genes

Gene	Primer Sequences (5′-3′)	PCR Product Size (bp)	Initial Denaturation	Denaturation	Annealing	Extension	Cycles	Final Extension
INT1 F *INT1* R	5′-GGTGTGGCGGGCTTCGTG-3′ 5′-GCATCCTCGGTTTTCTGG-3′	457	95°C/2 min	93°C/30 S	60°C/30 S	72°C/45 S	30	72°C/10 min
VIM F *VIM* R	5′-GTTTGGTCGVATATCGCAAC-5-2′ 5′-AATGCGCAGCACCAGGATAG-3′	382	95°C/2 min	93°C/30 S	60°C/30 S	72°C/45 S	30	72°C/10 min
IMP F *IMP* R	5′-GAAGGCGTTTATGTTCATAC-5-1′ 5′-GTATGTTTCAAGAGTGATGC-3′	594	95°C/2 min	93°C/30 S	60°C/30 S	72°C/45 S	30	72°C/10 min

DNA extraction and determination of beta-lactamase genes

After culturing *A. baumannii* isolates for 24 hours in a tryptic soy broth (TSB) (QUELAB, USA), the bacterial genome was extracted using the boiling method. In this way, 2-3 bacterial colonies were removed from the culture medium and dissolved in 500 µL of sterile distilled water inside the microtube. After vortexing, the resulting solution was boiled in a bain-marie for 10 minutes, and the microtubes were then microfuged for 10 minutes at 12,000×g. The supernatant solution was used for the PCR test. The primer sequence of *blaVIM*, *blaIMP*, and *INT* genes and the conditions of reproduction of the mentioned genes are shown in Table 1. To amplify the studied genes, a final 25 µL volume of the PCR mixture was prepared. To confirm the *balVIM* and *INT* genes, the genome of the standard strain of *Pseudomonas aeruginosa* (ATCC 27853) was used. To confirm the *blaIMP* gene, the genome of the standard strain of *Klebsiella pneumoniae* (ATCC 7881) was used [9, 10].

Results

Clinical samples were collected from 82 male (54.7%) and 68 female (45.3%) patients aged 14-75 years. The highest frequency of isolates according to the type of sample was related to trachea, urine, sputum, pleural fluid, and wound culture. Based on the results of the antibiogram, the highest resistance of the obtained strains to ceftazidime (100%), cefepime (94%), meropenem (91%), imipenem (83%), ciprofloxacin (80%), amikacin (78%), and gentamicin (63%) was observed. Based on the MIC assessment results by the macrodilution method and based on the CLSI guideline (interpretive categories and MIC breakpoints µg/mL: Resistant ≥4 and sensitive ≥2), the frequency of colistin-resistant strains (28%) was determined.

In the investigation of metallo-beta-lactamases production in 150 isolates using the CDT, it was shown that 31% of the isolates produced metallo-beta-lactamase enzymes. The results of the PCR test to find *INT*, *blaVIM*, *bla IMP* genes showed that 35% of the isolates carried the *blaVIM* gene, 45% carried the *blaIMP* gene, and 60% carried the *INT* gene (Figure 1).

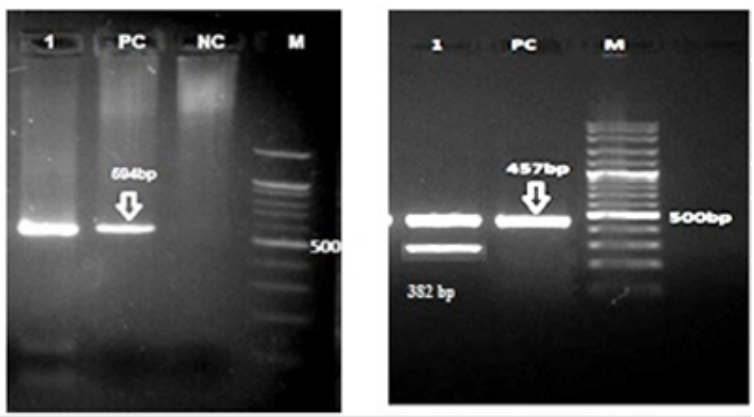

Figure 1. Gel electrophoresis results of *balVIM* (382 bp), *blaIMP* (594 bp), *INT* (457 bp) genes using 1.5% agarose with voltage 100V

Note: SM: Ladder (100-3000 bp); PC: Positive control; NC: Negative control; 1: *A. baumannii* isolate.

Discussion

A. baumannii is an opportunistic pathogen. The ability of this pathogen to acquire genetic factors causing antibiotic resistance has led to the emergence of strains with MDR. In recent years, strains with MDR have become widespread in the world and have caused major problems in infection control and treatment in different hospital departments such as the burn unit [11, 12]. Resistance to carbapenems as the last option for treating infections caused by *A. baumannii* is a major concern in medical centers. The increase of strains resistant to carbapenems is a serious challenge since it is involved in increasing the mortality of patients by 30% [13-15].

In the present study, the highest frequency of *A. baumannii* isolates from the clinical samples of patients with burns and nosocomial infections was related to the trachea. Therefore, it seems that the respiratory system is the most involved part in infections caused by *A. baumannii* [16]. Therefore, disinfection and sterilization of respiratory equipment and devices is one of the ways to prevent the spread of this infection. In the present study, the results showed that 31% of the *A. baumannii* isolates produced metallo-beta-lactamase enzymes. This is consistent with the studies by Shahcheraghi et al. in Tehran [17] and Sinha et al. in India [18].

In this study, the results of the PCR test showed that 35% of the strains carried the *blaVIM* gene, 45% carried the *blaIMP* gene, and 60% carried the *INT* gene. Comparison of this result with the findings of other studies in Iran shows the high prevalence of *balVIM*, *blaIMP*, and *INT* genes among the clinical isolates of *A. baumannii* in Zare Burn Hospital in Sari City. The *blaIMP* gene had a relatively high prevalence, which is consistent with other studies conducted in Iran, indicating that among the metallo-beta-lactamase genes, *blaIMP* is more prevalent in Iran [19, 20]. The results of this study compared to other studies showed the high presence of integrons and high resistance in *A. baumannii* strains. In the study by Taherkalani et al. in Ilam, 58% of *A. baumannii* isolates had class I integron. In the study by Japoni et al., the prevalence of class I and class II integrons was 47.7% and 3.4%, respectively, while there was no class III integron [21, 22].

In this study, the resistance of the *A. baumannii* isolates to ceftazidime, cefepime, meropenem, imipenem, ciprofloxacin, amikacin, gentamicin, and colistin was 100%, 94%, 91%, 88%, 80%, 78%, 63%, and 28%, respectively. Therefore, the highest rate of resistance was to cephalosporins and carbapenems. The results of the studies have shown that, due to the wide spectrum use of beta-lactams, cephalosporins are not suitable drugs for treating hospital infections caused by *A. baumannii*. The results of a study [23] and the results of the present study indicate an 88% resistance to imipenem. This high resistance seems to be due to the excessive administration of imipenem to treat infections caused by *A. baumannii*. The rate of resistance to imipenem has been increasing rapidly in recent years. In Iran, the resistance rate to this antibiotic has been reported as 16-68 % from 2005 to 2010 [24, 22]. In Safari et al.'s study, the antibiotic sensitivity of *A. baumannii* isolates to imipenem, meropenem, ciprofloxacin, amikacin, piperacillin, and cefotaxime was reported as 85, 94, 97, 84, 95, and 98%, respectively, and 99% of all strains were metallo-beta-lactamase producers [25]. In the present study, a high antibiotic sensitivity of *A. baumannii* isolates to colistin was observed. In other countries, the sensitivity rate to colistin was 100% in Algeria, 70.9% in Saudi Arabia, and 92.5% in Kuwait [26]. The possible reason for the high sensitivity to this antibiotic is its low prescription in the recent period.

According to the results of this study, beta-lactamase-producing strains are considered as an increasing problem in medical centers, especially in the burn units. Many of these strains have become resistant to all available antibiotics. Considering the high prevalence of resistance to beta-lactams, especially carbapenems, there is a serious need for proper management of the rational prescription of antibiotics, identifying the pattern of microbial resistance to prevent the spread of microbial resistance genes, and determining the beta-lactamase-producing strains, especially metallo-beta-lactamase- and oxacillinase-producing strains in medical centers.

Ethical Considerations

Compliance with ethical guidelines

This study was approved by he Ethics Committee of Mazandaran University of Medical Sciences (Code: IR.MAZUMS.REC.1393.1011).

Funding

This research did not receive any grant from funding agencies in the public, commercial, or non-profit sectors.

Authors contribution's

Study design, data collection, and final approval: Mohammad Ahanjan and Sasan Sarli; Writing: Golnar Rahimzadeh.

Conflict of interest

The authors declared no conflict of interest.

Acknowledgements

The authors would like to thank the laboratory staff of Zare Burn Hospital and the Microbiology Laboratory staff of the School of Medicine, Mazandaran University of Medical Sciences for their cooperation in this study.

References

[1] Alsan M, Klompas M. Acinetobacter baumannii: An emerging and important pathogen. J Clin Outcomes Manag. 2010; 17(8):363-9. [PMID]

[2] Rahimzadeh G, Rezai MS. [Detection extended spectrum beta lactamase and carbapenemase producing Enterobacteriaceae isolates from clinical samples; Narrative review (Persian)]. J Isfahan Med Sch. 2022; 40(688):743-58. [DOI:10.48305/JIMS.V40.I688.0743]

[3] Rahimzadeh G, Rezai MS, Farshidi F. Genotypic patterns of multidrug resistant acinetobacter baumannii: A systematic review. Adv Biomed Res. 2023; 12:56. [DOI:10.4103/abr.abr_434_22] [PMID]

[4] Logan LK, Gandra S, Trett A, Weinstein RA, Laxminarayan R. Acinetobacter baumannii resistance trends in children in the United States, 1999-2012. J Pediatr Infect Dis Soc. 2019; 8(2):136-142. [DOI:10.1093/jpids/piy018] [PMID]

[5] Vijayakumar S, Anandan S, Ms DP, Kanthan K, Vijayabaskar S, Kapil A, et al. Insertion sequences and sequence types profle of clinical isolates of carbapenem-resistant A. baumannii collected across India over four year period. J Infect Public Health. 2020; 13(7):1022-8. [DOI:10.1016/j.jiph.2019.11.018] [PMID]

[6] Bonomo RA. β-Lactamases: A focus on current challenges. Cold Spring Harb Perspect Med. 2017; 7(1):a025239. [DOI:10.1101/cshperspect.a025239] [PMID]

[7] Tooke CL, Hinchliffe P, Bragginton EC, Colenso CK, Hirvonen VHA, Takebayashi Y, et al. β-Lactamases and β-lactamase inhibitors in the 21st century. J Mol Biol. 2019; 431(18):3472-500. [DOI:10.1016/j.jmb.2019.04.002] [PMID]

[8] Sawa T, Kooguchi K, Moriyama K. Molecular diversity of extended-spectrum β-lactamases and carbapenemases, and antimicrobial resistance. J Intensive Care. 2020; 8:13. [DOI:10.1186/s40560-020-0429-6] [PMID]

[9] Azimi L, Talebi M, Pourshafie MR, Owlia P, Rastegar Lari A. Characterization of carbapenemases in extensively drug resistance Acinetobacter baumannii in a burn care center in Iran. Int J Mol Cell Med. 2015; 4(1):46-53. [PMID]

[10] Benmahmod AB, Said HS, Ibrahim RH. Prevalence and mechanisms of carbapenem resistance among acinetobacter baumannii clinical isolates in Egypt. Microb Drug Resist. 2019; 25(4): 480-8. [DOI:10.1089/mdr.2018.0141] [PMID]

[11] Guerrero DM, Perez F, Conger NG, Solomkin JS, Adams MD, Rather PN, et al. Acinetobacter baumannii-associated skin and soft tissue infections: Recognizing a broadening spectrum of disease. Surg Infect (Larchmt). 2010; 11(1):49-57. [DOI:10.1089/sur.2009.022] [PMID]

[12] Zubair M, Malik A, Ahmad J, Rizvi M, Farooqui KJ, Rizvi MW. A study of biofilm production by gram-negative organisms isolated from diabetic foot ulcer patients. Biol Med. 2011; 3(2):147-57. [Link]

[13] Teethaisong Y, Nakouti I, Evans K, Eumkeb G, Hobbs G. Nitro-Carba test, a novel and simple chromogenic phenotypic method for rapid screening of carbapenemase-producing Enterobacteriaceae. J Glob Antimicrob Resist. 2019; 18:22-5. [DOI:10.1016/j.jgar.2019.01.012] [PMID]

[14] Correa AAF, Fortaleza CMCB. Incidence and predictors of health care-associated infections among patients colonized with carbapenem-resistant Enterobacteriaceae. Am J Infect Control. 2019; 47(2):213-6. [DOI:10.1016/j.ajic.2018.08.007] [PMID]

[15] Jean SS, Lee NY, Tang HJ, Lu MC, Ko WC, Hsueh PR. Carbapenem-resistant enterobacteriaceae infections: Taiwan aspects. Front Microbiol. 2018; 9:2888. [DOI:10.3389/fmicb.2018.02888] [PMID]

[16] Goudarzi H, Hashemi A, Fatemeh F, Noori M, Erfanimanesh S, Yosefi N, et al. [Detection of blaDIM, blaAIM, blaGIM, blaNDM and blaVIM Genes among Acinetobacter baumannii strains isolated from hospitalized patients in Tehran hospitals, Iran (Persian)]. Iran J Med Microbiol. 2016; 9(4):32-9. [Link]

[17] Shahcheraghi F, Abbasalipour M, Feizabadi M, Ebrahimipour G, Akbari N. Isolation and genetic characterization of metallo-β-lactamase and carbapenamase producing strains of Acinetobacter baumannii from patients at Tehran hospitals. Iran J Microbiol. 2011; 3(2):68-74. [PMID]

[18] Sinha M, Srinivasa H, Macaden R. Antibiotic resistance profile & extended spectrum beta-lactamase (ESBL) production in Acinetobacter species. Indian J Med Res. 2007; 126(1):63-7. [PMID]

[19] Fallah F, Borhan RS, Hashemi A. Detection of bla (IMP) and bla (VIM) metallo-β-lactamases genes among Pseudomonas aeruginosa strains. Int J Burns Trauma. 2013; 3(2):122-4. [PMID]

[20] Hakemi Vala M, Hallajzadeh M, Hashemi A, Goudarzi H, Tarhani M, Sattarzadeh Tabrizi M, et al. Detection of Ambler class A, B and D ß-lactamases among Pseudomonas aeruginosa and Acinetobacter baumannii clinical isolates from burn patients. Ann Burns Fire Disasters. 2014; 27(1):8-13. [PMID]

[21] Taherikalani M, Maleki A, Sadeghifard N, Mohammadzadeh D, Soroush S, Asadollahi P, et al. Dissemination of class 1, 2 and 3 integrons among different multidrug resistant isolates of Acinetobacter baumannii in Tehran hospitals, Iran. Pol J Microbiol. 2011; 60(2):169-74. [DOI:10.33073/pjm-2011-024]

[22] Japoni S, Japoni A, Farshad S, Ali AA, Jamalidoust M. Association between existence of integrons and multi-drug resistance in Acinetobacter isolated from patients in southern Iran. Pol J Microbiol. 2011; 60(2):163-8. [DOI:10.33073/pjm-2011-023] [PMID]

[23] Lee SO, Kim NJ, Choi SH, Hyong Kim T, Chung JW, Woo JH, et al. Risk factors for acquisition of imipenem-resistant Acinetobacter baumannii: A case-control study.Antimicrob Agents Chemother. 2004; 48(1):224-8. [DOI:10.1128/AAC.48.1.224-228.2004] [PMID]

[24] Hashemizadeh Z, Emami A, Rahimi MJ. [Acinetobacter antibiotic resistance and frequency of ESBL-producing strains in ICU patients of Namazi Hospital (2008- 2009) (Persian)]. J Inflamm Dis. 2010; 14(2): 47-53. [Link]

[25] Safari M, Mozaffari Nejad AS, Bahador A, Jafari R, Alikhani MY. Prevalence of ESBL and MBL encoding genes in Acinetobacter baumannii strains isolated from patients of intensive care units (ICU). Saudi J Biol Sci. 2015; 22(4):424-9. [DOI:10.1016/j.sjbs.2015.01.004] [PMID]

[26] Al-Agamy MH, Khalaf NG, Tawfick MM, Shibl AM, El Kholy A. Molecular characterization of carbapenem-insensitive Acinetobacter baumannii in Egypt. Int J Infect Dis. 2014; 22:49-54. [DOI:10.1016/j.ijid.2013.12.004] [PMID]