



# Prevalence of *bla*<sub>TEM</sub> and *bla*<sub>VEB</sub> Genes in Multidrug-Resistant *Acinetobacter baumannii* Strains Isolated from Burn Wound Infection in Isfahan, Iran

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## ABSTRACT

**Aims:** *Acinetobacter baumannii* is an opportunistic pathogen that is resistant to many antibiotics including beta-lactams. Production of  $\beta$ -lactamases is the main mechanism of  $\beta$ -lactam resistance in *A. baumannii* strains. The aim of this study was to determine the frequency of *bla*<sub>TEM</sub> and *bla*<sub>VEB</sub> genes in clinical isolates of *A. baumannii* and the relationship between the antibiotic resistance and the presence of ESBL genes in strains isolated from burn wound infection in Isfahan.

**Materials & Methods:** In this study, 123 MDR *A. baumannii* strains were isolated from burn wound infection. After antibiotic resistance evaluation using the Kirby-Bauer disc-diffusion method, all the isolates were evaluated with polymerase chain reaction (PCR) technique to detect ESBL genes, followed by statistical analysis by the end.

**Findings:** Out of 123 *A. baumannii* isolates, 77 (62.60%) strains were ESBL positive according to the PCR results. The frequency of *bla*<sub>TEM</sub> and *bla*<sub>VEB</sub> genes was 52 (42.3%) and 67 (54.5%), respectively. There was a significant relationship between the antibiotic resistance and the presence of ESBL genes (*bla*<sub>TEM</sub> and *bla*<sub>VEB</sub>) in *A. baumannii* strains.

**Conclusion:** The high prevalence of *bla*<sub>TEM</sub> and *bla*<sub>VEB</sub> genes in *A. baumannii* strains found in this study is the major concern about burn wound infections in Isfahan and Iran because of the complexity in treating infections caused by these strains. This study results highlighted the need for infection control measures to prevent the spread of resistant isolates and ESBL genes, especially in burn hospitals.

**Keywords:** *Acinetobacter baumannii*, Antibiotic resistance, *bla*<sub>TEM</sub> and *bla*<sub>VEB</sub>

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## Introduction

The skin is the largest organ and the first line of defense against microorganisms in the body. Skin burns are one of the most serious injuries [1]. Annually, 1.3 and 0.14 per 100000 people die from burn infections in underdeveloped and developed countries, respectively [2]. Also, 75% of post-burn deaths are due to infection, and 70% of infections in burn patients are caused by bacteria [3-4]. One of the most common Gram-negative bacteria causing burn wound infection in hospitalized patients is *Acinetobacter baumannii*. Multidrug resistant (MDR) *A. baumannii* is one of the most important leading causes of burn wound infections [5-6]. This organism causes many infections including septicemia, pneumonia, urinary tract infections, meningitis, and wound infections and could be transmitted by direct or indirect contact between patients [7-8]. *A. baumannii* has been recognized by the World Health Organization as one of the three pathogens with high antibiotic resistance. Prominent features of this bacterium are its inherent resistance to many antibiotics and its ability to resist new antibiotics when exposed to and acquiring genes associated with them. Today, MDR has become a major problem in most hospitals around the world [9-10]. Beta-lactamase enzymes cause the hydrolysis of beta-lactam ring in beta-lactam antibiotics, resulting in bacterial resistance to beta-lactam antibiotics, including penicillins [11]. These resistant strains could be caused by overuse of new beta-lactam antibiotics in recent decades. Extended-spectrum  $\beta$ -lactamase enzymes are located on the plasmid, and the strains producing these enzymes are inhibited by tazobactam and clavulanate antibiotics. Gram-negative bacteria have the ability to produce beta-lactamase enzymes, including *A. baumannii* isolates which are able to produce  $bla_{TEM}$  and  $bla_{VEB}$  genes (ESBL genes).  $bla_{TEM}$  causes the

hydrolysis of a wide range of beta-lactam antibiotics other than carbapenems; it has been identified for the first time in a patient called Temoneira [11]. This gene has been detected in many *A. baumannii* strains and other Gram-negative bacteria.  $bla_{VEB}$  gene has also been isolated from *A. baumannii* species in Europe and Asia but has not yet been reported in America. The ESBL genes ( $bla_{TEM}$  and  $bla_{VEB}$ ) belonging to Class A beta-lactamases belong to the BJM (Bush Jacoby Medeiros) classification. Because ESBL genes are located on moving genetic elements, their transfer is very easy, thereby increasing the spread of antibiotic resistance. Thus, identifying strains with ESBL genes could help treat infections caused by them. [12-13].

**Objectives:** This study aimed to investigate the frequency of ESBL genes ( $bla_{TEM}$  and  $bla_{VEB}$  genes) in MDR *A. baumannii* strains isolated from burn wound infections and their relationship with antibiotic resistance.

## Materials and Methods

**Sampling:** This descriptive cross-sectional study was performed on 123 MDR *A. baumannii* strains isolated from burn wounds of hospitalized patients in Imam Musa Kazim hospital in Isfahan during 2017-2018. All samples were detected using biochemical tests, including TSI (Triple sugar iron agar), oxidase, and OF (oxidative fermentative), and then confirmed by a molecular method; in addition,  $bla_{oxa-51}$  genes were also identified, which are endogenous to *A. baumannii* [14].

**Antibiotic susceptibility determination:** The antibiotic resistance pattern was determined using the Kirby-Bauer method as follows: after obtaining a concentration of 0.5 McFarland from the bacteria, they were cultured on the surface of Mueller-Hinton agar culture medium, and antibiotic disks were placed on the culture medium

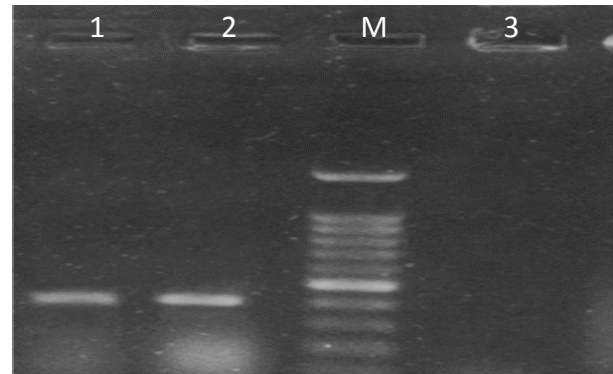
surface. After 24 hours, according to the CLSI standards (2018), the results were interpreted. These disks included: gentamicin (10 µg), ceftazidime (30 µg), imipenem (10 µg), tobramycin (10 µg), levofloxacin (5 µg), ciprofloxacin (10 µg), trimethoprim (1.25 µg), sulfamethoxazole (32.75 µg), cefepime (30 µg), ceftriaxone (30 µg), piperacillin (100 µg), and tazobactam (10 µg) [15].

**DNA extraction and polymerase chain reaction:** DNA was extracted using the phenol-chloroform method and used to perform the PCR reaction. The sequence of primers used to identify *bla*<sub>TEM</sub> and *bla*<sub>VEB</sub> genes are shown in Table 1. The PCR was performed in a final volume of 25 µL containing 12 µm master mix, 1 µL of each primer, 1 µL of DNA, and 11 µL of deionized water. The timing program used for *bla*<sub>TEM</sub> gene was as follows: an initial denaturation step at 95 °C for 7 min, denaturation at 95 °C for 1 min, annealing at 58.5 °C for 1 min, 35 cycles of extension at 72 °C for 2 min, and a final extension step at 72 °C for 6 min. The program used for *bla*<sub>VEB</sub> gene was as follows: an initial denaturation step at 94 °C for 3 min, denaturation at 94 °C for 45 sec, annealing at 58 °C for 30 sec, 35 cycles of extension at 72 °C for 1 min, and a final extension step at 72 °C for 5 min. At the end, the PCR product was observed by 1% electrophoresis gel [16-17].

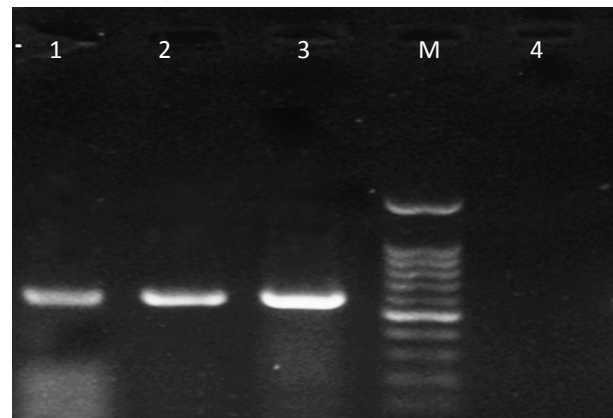
## Findings

Out of 123 MDR resistant *A. baumannii*

strains isolated from burn wound infections, 77 (62.60%) isolates were beta-lactamase-producing strains. PCR results showed that 67 (54.5%) isolates harbored *bla*<sub>VEB</sub> gene, and 52 (42.3%) isolates harbored *bla*<sub>TEM</sub> gene, while 37 (30.08%) strains simultaneously harbored



**Figure 1)** Electrophoresis of PCR products for identification of *bla*<sub>TEM</sub> genes in *A. baumannii* isolates. Lane 1: Positive control, Lane 2: Clinical sample, M: 100 bp marker and lane 3: Negative control.



**Figure 2)** Electrophoresis of PCR products for identification of *bla*<sub>VEB</sub> genes in *A. baumannii* isolates. Lane 1: Positive control, Lanes 2-3: Clinical samples, M: 100 bp marker and Lane 4: Negative control.

**Table 1)** The primers used in this study to detect *bla*<sub>OXA51</sub>, *bla*<sub>TEM</sub> and *bla*<sub>VEB</sub> in *A. baumannii* strains.

Target Gene	Primer Sequence (5'-3')	Amplicon Size (bp)	References
<i>TEM</i> <sub>bla</sub>	TEM-F TTTCGTGTCGCCCTTATTCC	403 bp	(16)
	TEM-R ATCGTTGTCAGAAGTAAGTTGG		
<i>bla</i> <sub>VEB</sub>	VEB-F CGACTTCCATTTCCCGATGC	585bp	(20)
	VEB-R GGACTCTGCAACAAATACGC		

**Table2)** Antibiotic resistance pattern based on the presence of *bla*<sub>TEM</sub> gene in all *A. baumannii* strains.

Antibiotic	Resistant NO (%)	Intermediate NO (%)	Susceptible NO (%)	P-value
Imipenem	47(90.38)	0	5(9.61)	<i>P</i> >.05
Ceftriaxone	50(96.15)	2(3.84)	0	<i>P</i> >.05
Ceftazidime	52(100)	0	0	<i>P</i> >.05
Gentamicin	29(55.76)	1(1.92)	22(42.30)	<i>P</i> <.05
Cefepime	45(86.53)	3(5.76)	4(7.69)	<i>P</i> <.05
Tobramycin	23(44.23)	6(11.53)	23(44.23)	<i>P</i> <.05
Piperacillin-Tazobactam	47(90.38)	4(7.69)	1(1.92)	<i>P</i> >.05
Trimethoprim- sulfamethoxazole	49(94.23)	2(3.84)	1(1.92)	<i>P</i> >.05
Ciprofloxacin	51(98.07)	0	1(1.92)	<i>P</i> >.05
Levofloxacin	37(71.15)	10(19.23)	5(9.61)	<i>P</i> >.05

**Table3)** Antibiotic resistance pattern based on the presence of *bla*<sub>VEB</sub> gene in all *A. baumannii* strains.

Antibiotic	Resistant	intermediate	Susceptible	P-value
Imipenem	62(92.53)	0	5(7.46)	<i>P</i> >.05
Ceftriaxone	60(89.55)	7(10.44)	0	<i>P</i> <.05
Ceftazidime	67(100%)	0	0	<i>P</i> >.05
Gentamicin	44(65.67)	1(1.49)	22(32.83)	<i>P</i> >.05
Cefepime	45(67.16)	13(19.40)	9(13.43)	<i>P</i> <.05
Tobramycin	40(59.70)	1(1.49)	26(38.80)	<i>P</i> >.05
Piperacillin-Tazobactam	57(85.07)	9(13.43)	1(1.49)	<i>P</i> <.05
Trimethoprim- sulfamethoxazole	62(92.53)	2(2.98)	3(4.47)	<i>P</i> >.05
Ciprofloxacin	64(95.52)	0	3(4.47)	<i>P</i> >.05
Levofloxacin	41(61.19)	21(31.34)	5(7.46)	<i>P</i> >.05

both  $bla_{VEB}$  and  $bla_{TEM}$  genes (Figures 1, 2). Among the strains harboring  $bla_{VEB}$  gene, the highest resistance was observed to ceftazidime (67, 100%), followed by ciprofloxacin (64, 95.52%) and imipenem (62, 92.53%), whereas the highest susceptibility was observed to tobramycin (17, 30.35%) (Table 2). Among the strains containing no  $bla_{VEB}$  gene, the highest antibiotic resistance was observed to ceftriaxone (55, 98.21%), ceftazidime (55, 98.21%), piperacillin-tazobactam (53, 94.64%), ciprofloxacin (50, 89.28%), imipenem (49, 87.5%), trimethoprim-sulfamethoxazole (49, 87.5%), levofloxacin (44, 78.57%), cefepime (44, 78.57%), gentamicin (38, 67.85%), and tobramycin (34, 60.71%), respectively. Among the strains harboring  $bla_{TEM}$  gene, the highest resistance was observed to ceftazidime (52, 100%), followed by ciprofloxacin (51, 98.07%) and ceftriaxone (50, 96.15%), whereas the highest susceptibility was observed to tobramycin (23, 44.23%) (Table 3). Among the strains containing no  $bla_{TEM}$  gene, the highest antibiotic resistance was observed to ceftazidime (70, 98.59%), ceftriaxone (65, 91.54%), imipenem (64, 90.14%), piperacillin-tazobactam (63, 88.73%), ciprofloxacin (63, 88.73%), trimethoprim-sulfamethoxazole (63, 88.73%), gentamicin (53, 74.64%), tobramycin (50, 70.42%), levofloxacin (48, 67.60%), and cefepime (44, 61.91%), respectively.

**Statistical analysis results:** Using the Chi-square and Fisher tests, the relationship between the presence of  $bla_{VEB}$  and  $bla_{TEM}$  genes and resistance to various antibiotics was investigated. A significant relationship was found between the presence of  $bla_{VEB}$  gene and resistance to piperacillin-tazobactam, cefepime, and ceftriaxone antibiotics, as well as between the presence of  $bla_{TEM}$  gene and resistance to tobramycin, cefepime, and gentamicin antibiotics ( $p < .05$ ).

## Discussion

*A. baumannii* is an opportunistic pathogen

with high pathogenicity and the common cause of burn wounds infection. Today, the treatment of infections caused by beta-lactamase-producing strains has become a major problem due to their widespread resistance to various antibiotics, especially the third generation of cephalosporins and aztreonam (monobactam). The prevalence of these strains is increasing due to the widespread and uncontrolled consumption of various antibiotics [18]. In this study, 62.60% of the strains harbored beta-lactamase genes, among which 54.5% harbored a  $bla_{VEB}$  gene, and 42.3% harbored a  $bla_{TEM}$  gene. The highest resistance of *A. baumannii* strains harboring both  $bla_{TEM}$  and  $bla_{VEB}$  genes was against cefepime, followed by levofloxacin, and the lowest resistance was against tobramycin and cephalosporins. There was a significant relationship between the presence of  $bla_{VEB}$  gene and resistance to piperacillin-tazobactam, cefepime, and ceftriaxone, as well as between the presence of  $bla_{TEM}$  gene and resistance to tobramycin, cefepime, and gentamicin. In a study conducted by Mousavian and his colleagues (2017) in Ahvaz, 36% of the isolates harbored  $bla_{TEM}$  genes [19]. In another study by Bali and colleagues (2010) in Turkey, 73% of the strains contained  $bla_{TEM}$  genes [20]. Also, in Salih's (2019) study in Iraq, 32% of the isolates had  $bla_{TEM}$  genes [21]. Adbar (2019) in Tehran found that 42% of *A. baumannii* strains had a  $bla_{TEM}$  gene, while no isolate had  $bla_{VEB}$  gene [22]. In a study by Ahmadikiya (2017) in Yazd, 31.6 and 13.7% of the isolates had  $bla_{TEM}$  and  $bla_{VEB}$  genes, respectively [23]. In Fazeli's (2014) study in Isfahan, the presence of  $bla_{VEB}$  gene in *A. baumannii* isolates was reported to be 26.6% (14), while in another study by Azizi (2017) in Tehran, this value was reported to be 35.3% [24]. Emami (2014) in Qom also found that 26.6% of the isolates had  $bla_{VEB}$  genes [18]. In Shahcheraghi's study (2009) in

Tehran, there was a significant relationship between the presence of  $bla_{TEM}$  gene and resistance to cefotaxime [25]. In another study by Safari (2015) in Hamadan, 20% of the isolates had  $bla_{TEM}$  genes, and there was no significant relationship between the presence of  $bla_{TEM}$  gene and resistance to various antibiotics [26]. Various studies have shown the presence of beta-lactamase-producing strains in different cities of Iran and other countries. Comparing the present and previous studies results shows that strains containing beta-lactamase genes are rapidly increasing. The difference in the prevalence of ESBL producing strains could be related to the availability of transmission conditions in some areas compared to other areas. In this study, many strains containing beta-lactamase genes showed greater resistance to different antibiotics than the strains containing no beta-lactamase gene, which could be attributed to the presence of genes related to beta-lactamases.

### Conclusion

Burn wound infection is one of the most important complications which occur in the burn area. Multidrug-resistant *A. baumannii* strains have a high frequency in the burn section. Given that one of the main reasons for antibiotic resistance, especially in Gram-negative bacteria, is the presence of beta-lactamases, an increase in the prevalence of these strains contributes to concerns about the treatment of patients with infections associated with beta-lactamase-producing strains. Due to the resistance of these strains to beta-lactam antibiotics, the use of these antibiotics is not recommended for the treatment of patients. The presence of relevant genes on moving genetic elements and the possibility of rapid transfer of these genes to different bacterial strains makes it possible to increase resistance. Investigating the frequency of  $bla_{VEB}$  and

$bla_{TEM}$  genes in this study indicated an increase in the number of strains containing the relevant genes in comparison with the previous studies in Iran. It is essential to provide special conditions in hospital settings to prevent the release of beta-lactamase-producing strains.

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**Authors Contribution:** Conceptualization: AAR, Data curation: EH, NK; Formal analysis: EH, NK; Funding acquisition: EH; Investigation: EH, NK; Methodology: AAR, EH; Project administration: AAR; Resources: EH; Software: EH; Supervision: AAR; Validation: EH; Visualization: EH, AAR; Writing-original draft: EH; Writing-review and editing: EH, AAR.

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**Consent to participate:** Authors declare that all patients have signed the participation form.

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