Arch Pediatr Infect Dis. 2017 April; 5(2):e39307. doi: 10.5812/pedinfect.39307.
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Detection of *ISAba1* in *Acinetobacter baumannii* Strains Carrying *OXA* Genes Isolated From Iranian Burns Patients

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Abstract

Background: The presence of carbapenemase-producing *Acinetobacter baumannii* has become a growing concern in patients who are hospitalized in burns centers.
Objectives: The aims of this study were to determine the antimicrobial susceptibility patterns and prevalence of *blaOXA* carbapenemases, as well as to detect the presence of *ISAba1*, in *A. baumannii* strains carrying *OXA* genes obtained from burns patients at Shahid Motahari hospital, Tehran, Iran.
Methods: From August 2013 to March 2014, 100 clinical *A. baumannii* isolates were collected from patients who were admitted to the burns ward at Shahid Motahari hospital. Antimicrobial susceptibility was determined using a disc diffusion test. PCR, sequencing, and multiplex PCR were used for the detection of *blaOXA-23*-like, *blaOXA-51*-like, blaOXA-24-like, and *blaOXA-58*-like genes, which were then sequenced. The *ISAba1* gene was detected, and PCR was performed to detect the presence of *ISAba1*/*blaOXA-51*-like and *ISAba1*/*blaOXA-23*-like genes.
Results: The results showed that 93% of the strains were multi-drug resistant, while 82% of them were extensively drug resistant. Additionally, all the strains carried *blaOXA-23*-like and *blaOXA-51*-like genes, while 74% and 0% of the strains harbored *blaOXA-24*-like and *blaOXA-58* genes, respectively. *ISAba1* was detected in all the strains except for one. The co-existence of *ISAba1*/*blaOXA-51*-like genes and ISAba1/blaOXA-23-like genes was detected in 65% and 80% of strains, respectively.
Conclusions: The results of this study indicate that the emergence of *OXA*-type carbapenemases in *A. baumannii* causing nosocomial infections in burns patients could be of importance for hospital infection control systems in Iran.

Keywords: Carbapenemase, Isaba1, Multi-Drug Resistance, Extensively Drug Resistance, Burn, *Acinetobacter baumannii*

1. Background

Burns patients are at risk of acquiring infection due to their damaged skin and impaired immune system (1). *Acinetobacter baumannii* is an opportunistic pathogen that appears to have become one of the most important causes of nosocomial infections in hospitalized patients, particularly those in burns units, in recent years (2-5). During the past decade, this pathogen has been reported to be the second most common cause of nosocomial infections in burns patients (3, 6-8). The nosocomial infection strains of *A. baumannii* are multi-drug resistant (MDR) and extensively drug resistant (XDR) due to increased rates of resistance to the most commonly available antibiotics, including carbapenemas, which is the drug of choice for treating infection with *A. baumannii* (9-12). One of the most common mechanisms in carbapenem resistance is the production of carbapenem-hydrolyzing β-lactamase enzymes (carbapenemases) by these strains (2, 10, 11, 13-15). Two molecular classes of carbapenemases, namely Ambler class B (metallo-β-lactamase) and Ambler class D (oxacillinase), have been identified, with class D being the most prevalent enzyme among strains of *A. baumannii* (2, 6, 13, 16, 17). The genetic analysis of *OXA*-type enzymes (encoded by *blaOXA* genes) has categorized them into eight distinctive subgroups. Four of them *OXA-51*-like, *OXA-23*-like, *OXA-24*-like, and *OXA-58*-like have been found in *A. baumannii* (2, 11, 14, 18, 19). According to reports from different countries, *blaOXA-51*-like genes are intrinsically harbored by *A. baumannii* isolates, although their expression varies according to the presence of an insertion sequence such as *ISAba1* on the upstream of the gene (11, 20, 21). In addition, other OXA carbapenemase genes that are not part of the normal genome of the species can inactivate carbapenems, albeit less efficiently, and their presence or activation by ISAba1 is correlated with resistance (21-23).

2. Objectives

The aims of this study were to survey the drug resistance patterns of *A. baumannii* strains isolated from burns patients with nosocomial infections at Shahid Motahari hospital in Tehran, Iran, as well as to identify the genes encoding the *ISAba1* element and the four subgroups of OXA-type carbapenemases among these isolates. The study also sought to determine the co-existence of *ISAba1*/*blaOXA-51*-like genes and *ISAba1*/*blaOXA-23*-like genes.

3. Methods

3.1. Bacterial Strains

This study was conducted at Shahid Motahari hospital, a burns center in Tehran, Iran. One hundred samples of *A. baumannii* isolated from patients' burn wounds were collected between August 2013 and March 2014. Prior to sampling, the wounds were washed using physiological serum. The samples were transferred to Stuart media, cultured on blood agar and MacConkey agar, and then incubated at 37°C for 24 hours. Conventional biochemical tests such as oxidase, triple sugar iron (TSI), sulfide indole motility (SIM), and growing at 44°C were used for the primary identification of *A. baumannii* strains. The identification of the isolates was confirmed by blaOXA-51-like gene detection (5).

3.2. Antimicrobial Susceptibility Testing

The antimicrobial susceptibility testing was performed using the disk agar diffusion method on Mueller-Hinton agar according to Clinical and Laboratory Standards Institute (CLSI) guidelines (24). Disks containing cefepime (30 μg), ceftazidime (30 μg), ciprofloxacin (5 μg), ceftriaxon (30 μg), cefotaxime (30 μg), imipenem (10 μg), meropenem (10 μg), piperacillin-tazobactam (100/10 μg), gentamicin (10 μg), amikacin (30 μg), tetracycline (30 μg), trimethoprim-sulfamethoxazole (5 μg), and piperacillin (100 μg) were placed on a plate. Then, the samples were incubated at 37°C for 24 hours. The minimum inhibitory concentrations (MICs) of the imipenem, meropenem, ceftazidime, ciprofloxacin, and colistin were determined according to the microdilution broth method. The standard antibiotic disks and powders used in this study were obtained from Mast Diagnostics (Mast Group Ltd., Bootle, UK). *P. aeruginosa* ATCC 27853 was used as the control strain in each susceptibility test.

3.3. PCR Amplification for the blaOXA Genes and ISAba1 Element

DNA was extracted from the strains using a PrimePrep Genomic DNA Isolation Kit (Cat. No. K-3000, GENET-BIO Inc., Daejeon, South Korea) according to the manufacturer's instructions. The 25 μL PCR mixture contained 2 μL of bacterial DNA, 10 pM of each primer, 250 μM of each dNTP, 1.5 mM of $MgCl_2$, 10 Mm of Tris-HCL, 30 Mm of KCL, and 1 U of Taq DNA polymerase (Cat. No. K-2012, Bioneer Company, Korea). The reactions were performed in a thermal cycler (Mastercycler Gradient, Eppendorf, Hamburg, Germany). To amplify the genes encoding the carbapenemases, PCR assays were run for the *blaOXA-23*-like, *blaOXA-51*-like, *blaOXA-24*-like, and *blaOXA-58*-like genes and the *ISAba1* element. In addition, a set of multiplex PCRs for the *blaOXA-23*-like, *blaOXA-51*-like, and *blaOXA-24*-like genes were designed using an internal positive control for each gene. The primers used to amplify these genes are listed in Table 1, while the PCR system conditions for the amplification of each gene are presented in Table 2. Furthermore, PCRs were performed to detect the *ISAba1*/*blaOXA-51*-like and *ISAba1*/*blaOXA-23*-like sequences using a combination of the *ISAba1* forward primers and the *blaOXA-51*-like and *blaOXA-23*-like reverse primers (Table 1). The PCR products were analyzed using 1.5% agarose gel electrophoresis with ethidium bromide staining.

3.4. Sequencing Technique

The PCR products were purified using a PCR purification kit (Bioneer Co., Korea). The direct sequencing of the amplicons was performed by the Bioneer Company (Korea). The nucleotide sequences were analyzed using FinchTV software and BLAST in NCBI.

3.5. Statistical Analysis

MINITAB 16 software was used for all the statistical analyses in this study. This proposal was accepted by Medical Ethics in Shahid Beheshti University of Medical Sciences, (IR.SBMU.RAM.REC.1394.213).

4. Results

One hundred strains of *A. baumannii* were isolated from burns patients admitted to Shahid Motahari hospital. Of the 100 strains obtained from patients' burn wounds, 26 strains were isolated from female patients (26%) and 74 strains from males (74%). The patients were aged from 1- to 90-years-old (Table 3).

Table 1. Sequence of Primers

Primer	Sequence (5' to 3')	Target	References
OXA-23-likeF	GATCGGATTGGAGAACCAGA	*blaOXA-23*-like	(25)
OXA-23-likeR	ATTTCTGACCGCATTTCCAT		
OXA-24-likeF	GGTTAGTTGGCCCCCTTAAA	*blaOXA-24*-like	(25)
OXA-24-likeR	AGTTGAGCGAAAAGGGGATT		
OXA-58-likeF	AAGTATTGGGGCTTGTGCTG	*blaOXA-58*-like	(25)
OXA-58-likeR	CCCCTCTGCGCTCTACATAC		
IS*Aba1*F	CACGAATGCAGAAGTTG	*ISAba1*	(26)
IS*Aba1*R	CGACGAATACTATGACAC		
IS*Aba1*F1	AGGCTATAAAGCGTTGA	*ISAba1*/*blaOXA-51*-like	(27)
OXA-51-likeR1	CTTCTGTGGTGGTTGC		
IS*Aba1*F2	AACGATTGCGAGCATC	*ISAba1*/*blaOXA-23*-like	(27)
OXA-23-likeR2	GTCAACCAGCCCACTT		

Table 2. PCR Conditions for the *bla* Genes Amplification

Factor	Temperature (°C)							Time						
	blaOXA-23	*Blaoxa-51*	*Blaoxa-24*	*Blaoxa-58*	*ISAba1*	*ISAba1/blaOXA-23*	*ISAba1/blaoxa-51*							
Initial denaturation	94	94	94	94	94	94	94	5 min	5 min	5 min	7 min	5 min	5 min	5 min
Denaturation	94	94	94	94	94	94	94	30 s	30 s	30 s	1 min	45 s	45 s	45 s
Annealing	53	53	54	53	41	54	52	45 s	45 s	45 s	1 min	1 min	1 min	1 min
Extension	72	72	72	72	72	72	72	45 s	45 s	45 s	1 min	45 s	45 s	45 s
Final extension	72	72	72	72	72	72	72	5 min	5 min	5 min	5 min	5 min	5 min	5 min
Cycle	36	36	36	36	36	36	36							

Table 3. Distribution of Patients Infected with *A. baumannii* by Age

Age	Number of Patients (%)
1 - 15	5 (5)
16 - 30	5 (5)
31 - 45	31 (31)
46 - 60	36 (36)
61 - 75	17 (17)
76 - 90	6 (6)
Total	100 (100)

4.1. Antimicrobial Susceptibility

Based on the antimicrobial susceptibility testing, high rates of resistance to cefotaxime (100%), ceftriaxone (100%), ceftazidime (100%), meropenem (98%), imipenem (98%), gentamicin (93%), amikacin (90%), ciprofloxacin (100%), cefepime (100%), piperacillin/tazobactam (100%), tetracycline (82%), piperacillin (99%), and trimethoprim/sulfamethoxazole (95%) were observed (Table 4). Some 93% of strains were resistant to all the tested antibiotics, while 98% of them showed resistance to all the tested carbapenems. The MIC values of imipenem, meropenem, colistin, ceftazidime, and ciprofloxacin in relation to the *A. baumannii* isolates are shown in Table 5. Colistin was found to be the most effective drug against *A. baumannii* strains, with a 100% susceptibility rate being reported. In this study, all the isolates that had OXA-type genes were carbapenem resistant. The results showed that 93% of the strains were MDR, while 82% of them were XDR.

4.2. Carbapenemases of A. baumannii

The *blaOXA-51*-like gene was identified in all the *A. baumannii* strains (100%) by PCR and multiplex PCR. While it was not necessarily related to the increased resistance among these isolates, the gene was associated with the insertion of *ISAba1*, overexpression, and reduced susceptibility to carbapenems. Although the prevalence of *blaOXA-23*-like and *blaOXA-24*-like genes was 100% and 74%, respectively, the blaOXA-58-like gene was not detected in any of these isolates (Figure 1). The ISAba1 element was identified in all the isolates except for one (99%). The co-

Table 4. Antimicrobial Susceptibility Pattern of *A. baumannii* Isolates According to the Disc Diffusion Method

Antimicrobials	Susceptible No. (%)	Intermediate No. (%)	Resistant No. (%)
Imipenem	0	2 (2)	98 (98)
Meropenem	1 (1)	1 (1)	98 (98)
Ceftriaxon	0	0	100 (100)
Cefotaxime	0	0	100 (100)
Ceftazidime	0	0	100 (100)
Cefepime	0	0	100 (100)
Ciprofloxacin	0	0	100 (100)
Trimetoprim-sulfametoxazole	4 (4)	2 (2)	94 (94)
Amikacin	5 (5)	5 (5)	90 (90)
Tetracycline	10 (10)	8 (8)	82 (82)
Gentamycin	5 (5)	2 (2)	93 (93)
Piperacillin	0	0	100 (100)
Piperacillin Tazobactam	0	1 (1)	99 (99)

Table 5. The MIC Values of the Antibiotics Against *A. baumannii*

Antibiotic	Susceptible No. (%)	Intermediate No. (%)	Resistant No. (%)
Imipenem	3 (3)	3 (3)	94 (94)
Meropenem	6 (6)	5 (5)	89 (89)
Ceftazidime	0	0	100 (100)
Ciprofloxacin	0	2 (2)	98 (98)
Colistin	100 (100)	0	0

existence of ISAba1/blaOXA-51-like genes and *ISAba1*/*blaOXA-23*-like genes was detected in 65% and 80% of strains, respectively.

4.3. Sequencing

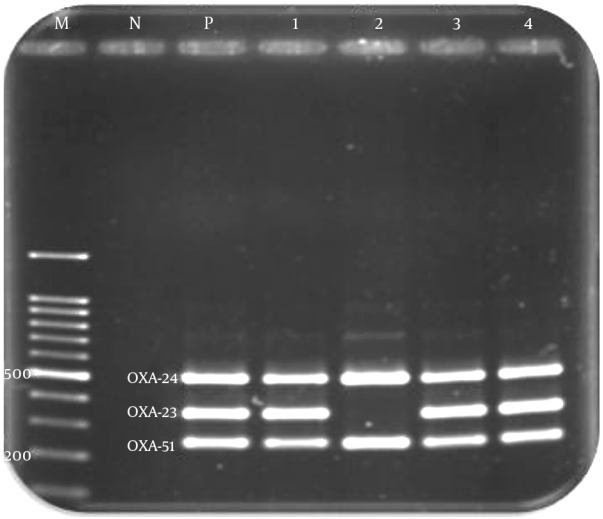
The sequencing of the PCR products showed conserved regions for the *blaOXA-23*-like, *blaOXA-51*-like, *blaOXA-24*-like, and *ISAba1* genes, which were confirmed with the basic local alignment search tool (BLAST) of the national center for biotechnology information (NCBI). The nucleotide sequence data reported in this paper have been submitted to the GenBank sequence database, and accession numbers KT313639 and KT313638 have been assigned for the *OXA-23* and *OXA-24* encoding genes, respectively.

5. Discussion

According to previous studies, *A. baumannii* is an emerging nosocomial pathogen in burns patients due to the potential for acquiring resistance to multiple antimicrobial agents, which often leads to mortality and morbidity in infected patients (28, 29). A broad spectrum of antimicrobial resistance, including carbapenems, is an important challenge for Iranian burns patients infected with *A. baumannii*, since treating them is difficult due to the limited number of antibiotics that remain effective against *OXA*-type producing *A. baumannii* strains such as polymyxin B and colistin. Hence, the emergence of resistance to these agents and the neurological risks arising from them should be considered (6, 10).

The antibiotic resistance of *A. baumannii* strains is increasing day by day, which leads to the emergence of MDR and XDR strains because of the indiscriminate use of a broad spectrum of antimicrobial agents and the high ability of these isolates to acquire multiple resistant genes. The results of this study showed that 93% of the strains isolated from burns patients were MDR, while 82% of them were XDR. The results of several prior studies have shown that antimicrobial resistance is increasing worldwide (30,

Figure 1. Multiplex PCR Amplification of the *OXA-24*, *OXA-23*, and *OXA-51* Genes of *A. baumannii* Isolates



Lane M, DNA size marker; Lane N, negative control; Lane P, positive control; Lanes 1, 2, 3, and 4, positive isolates.

31). Further, an increasing trend of carbapenem resistance from 2009 until the present day is visible (30, 32).

OXA-type carbapenemases are the dominant enzymes involved in antibiotic-resistant *A. baumannii* strains, especially the *OXA-23*-like genes, which have been reported worldwide (27, 33). *OXA-51*-like encoded genes naturally occur in *A. baumannii* isolates, and they have also been identified worldwide (34, 35). In our study, all the strains had intrinsically *blaOXA-51*-like genes, and the identification of the isolates as *A. baumannii* has been confirmed. The presence of *blaOXA-23*-like genes was observed in all the strains. In 2013, *OXA-23*-like encoded genes were detected in 94% of isolated *A. baumannii* in China (36). Additionally, the results of a study conducted in Iran in 2012 indicated that blaOXA-23-like genes were detected in all the strains (37), which is consistent with our results.

The *OXA-24* group has been reported in Portugal, Spain, Belgium, France, and the United States (38). In addition, strains producing blaOXA-58-like genes were found in isolates recovered from Italy, Belgium, France, Greece, the United States, and Argentina (34). Although *blaOXA-24*-like genes were detected in 74% of the *A. baumannii* isolates in our study, this rate was lower than that reported in previous studies (34, 37, 39). The differing results may be due to the different geographical locations, different clinical samples, and various antibiotype patterns in the different studies.

The absence of *OXA-58*-like encoded genes in all the isolates in our study is consistent with the findings of similar studies (34, 37, 40). The important thing is that an ISAba1 element was detected in all the *A. baumannii* strains. The presence of the *ISAba1* insertion upstream of the *OXA-51* and *OXA-23* was seen in 63% and 80% of the isolates, respectively. This co-existence has been shown to confer high levels of carbapenem resistance (20). In 2012, the incidence of co-existence with ISAba1 was 80% and 66% for *blaOXA-51* and *blaOXA-23*, respectively, in Turkey (32). Further, the *ISAab1* element was detected in the upstream of 85% of *blaOXA-51* genes and 80% of *blaOXA-23* genes in Egypt in 2012 (39). All these results demonstrate a high level of similarity to the findings of our study, and they emphasize the presence of ISAba1 upstream of *blaOXA-23*-like genes in instances of resistance enhancement.

The results of this study indicate the emergence of *OXA*-type carbapenemases in *A. baumannii* causing nosocomial infections in burns patients, which could be of importance for hospital infection control systems in Iran. Additionally, *A. baumannii* exhibiting the co-existence of *OXA* genes and *ISAba1* are extremely prevalent in Iran, which may cause serious problems during the treatment of *A. baumannii* infections using antibiotics

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Footnotes

Authors' Contribution: All the authors cooperated in conducting the isolation, denaturation, and PCRs, as well as in writing the article.

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