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Emergence of *bla*$_{OXA}$-Carrying Carbapenem Resistance in Multidrug-Resistant *Acinetobacter baumannii* in the Intensive Care Unit

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Abstract

Background: Emergence of multidrug-resistant (MDR) *Acinetobacter baumannii* infections is becoming a worldwide threat to hospitalized patients, particularly in intensive care units.
Objectives: The aim of this study was to investigate the antimicrobial susceptibility patterns and prevalence of *bla*$_{OXA}$-type carbapenemases of *A. baumannii* isolates in a teaching hospital in Iran.
Patients and Methods: The study included a total of 40 isolates of carbapenem-resistant *A. baumannii*, obtained from 103 tracheal tubes in hospitalized ICU patients. Antimicrobial susceptibility testing was performed according to CLSI guidelines. The *bla*$_{OXA}$-51, *bla*$_{OXA}$-23, *bla*$_{OXA}$-24, *bla*$_{OXA}$-58, and IS*Aba1* genes were detected by PCR.
Results: All of the *A. baumannii* isolates were resistant to imipenem and meropenem, and 100% of the isolates were MDR. The *bla*$_{OXA-51}$ and IS*Aba1* genes were detected in 100% of the isolates. *bla*$_{OXA-23}$ and *bla*$_{OXA-24}$ were detected in 90% and 40% of the isolates, respectively, but *bla*$_{OXA-58}$ was absent in the *A. baumannii* isolates. In addition, 32.5% of carbapenem-resistant strains contained at least three genes encoding *bla*$_{OXA}$-type carbapenemase. Colistin and polymyxin B were the most effective antibiotics. The sole risk factor for infection of hospitalized patients with carbapenem-resistant Acinetobacter strains was age over 40 years (P = 0.042). The mortality rate was 27.5%.
Conclusions: These findings signify the alarming spread of OXA genes in *A. baumannii* strains in our intensive care unit. The spread of carbapenem-resistant *Acinetobacter* strains has serious health implications and requires the application of strict infection-control measures.

Keywords: Carbapenem, Antibiotic Resistance, Intensive Care Unit, *Acinetobacter baumannii*

1. Background

Multidrug-resistant (MDR) *Acinetobacter baumannii* has been identified as an important worldwide cause of nosocomial infections. Ventilation-associated pneumonia (VAP) caused by *A. baumannii* is primarily an opportunistic nosocomial infection, mainly acquired in intensive care units (ICUs) (1). VAP is associated with an extensive morbidity rate due to the increased length of ICU stay and prolonged mechanical ventilation (2, 3). Carbapenems have been used as the most appropriate choice for treatment of infections due to MDR strains of *A. baumannii*. Unfortunately, the prevalence of carbapenem-resistant isolates is increasing (4). Since carbapenems play a vital role in the treatment of nosocomial infections caused by *A. baumannii*, evaluation of the sensitivity rate of *A. baumannii* to carbapenem is crucial for proper antibiotic therapy and for preventing the emergence of MDR strains (5). Extensive administration of broad-spectrum cephalosporins and/or carbapenems is a significant risk factor for development of colonization or infection with carbapenemase-producing *A. baumannii*.

Four types of eight known clusters of OXA-type carbapenemases have been identified in *A. baumannii*: *bla*$_{OXA-23}$-like, *bla*$_{OXA-24}$, *bla*$_{OXA-58}$, and *bla*$_{OXA-51}$ (6, 7). The genes encoding the *bla*$_{OXA-51}$ and *bla*$_{OXA-23}$ enzymes have been found to be adjacent to IS*Aba1*, suggesting that IS*Aba1* provides the promoter for these genes (8). The higher carbapenem hydrolysis rates may occur due to acquisition of the IS*Aba1* element upstream of the *bla*$_{OXA-51}$ gene (9).

Carbapenemase-producing *A. baumannii* may lead to clinical infections or asymptomatic colonization (10). Patients infected or colonized with carbapenemase-producing *A. baumannii* should be placed under contact protection. Screening of high-risk patients to detect colonization may also be helpful in controlling transmission.

2. Objectives

The aim of this study was to investigate carbapenem susceptibility rates and a determination of the four subgroups of OXA carbapenemase in carbapenem-resistant *A. baumannii* isolates from the tracheal tubes of patients in the ICU.

3. Patients and Methods

3.1. Sample Collection

This cross-sectional study was performed at Beheshti Hospital, a tertiary care center in Kashan, Iran, from October 2012 to March 2014. The ethics committee of Kashan University of Medical Sciences approved the study protocol. The specimens were collected from the tracheal tubes of patients hospitalized in the ICU. The *A. baumannii* strains were isolated and identified by using Microgen® GNA (Microgen Bioproducts Co., UK).

3.2. Determination of Antibiotic Resistance

Antibiotic susceptibility of the isolates was determined with imipenem, meropenem, piperacillin, ampicillin-sulbactam, piperacillin-tazobactam, ceftazidime, cefepime, cefotaxime, ceftriaxone, amikacin, gentamicin, levofloxacin, ciprofloxacin, tetracycline, trimethoprim-sulfamethoxazole, colistin, and polymyxin B disks (MAST, Merseyside, UK), using the Kirby-Bauer disk-diffusion breakpoint assay on Mueller-Hinton agar (Merck, Germany). Bacteria were classified as susceptible, intermediate, or resistant, according to the Clinical Laboratory Standard Institute (CLSI) recommendations (11). *Escherichia coli* ATCC 25922 was used as a quality control for each susceptibility test. The DNA extraction was performed from fresh cultures using boiling techniques, and stored at 4°C until PCR amplification was performed.

3.3. PCR Amplification of the Carbapenemase-Encoding Gene

All primers used in this study are listed in Table 1. The primers used for these tests were purchased from Bioneer Co. (Korea). All isolates were subjected to multiplex PCR to detect the $bla_{\text{OXA-51}}$, $bla_{\text{OXA-23}}$, $bla_{\text{OXA-24}}$, and $bla_{\text{OXA-58}}$ genes. The IS*Aba1* element was identified using the primers as described in a previous study (10). The thermocycler (Eppendorf, Hamburg, Germany) was programmed at 94°C for 5 minutes, followed by 30 cycles of 25 seconds at 94°C, 40 seconds at 53°C, and 50 seconds at 72°C, with a final cycle of 6 minutes at 72°C (12).

The amplification conditions to detect IS*Aba1* were as follows: initial denaturation at 95°C for 5 minutes, 35 cycles of 95°C for 45 seconds, 56°C for 45 seconds, 72°C for 3 minutes, and a final elongation at 72°C for 5 minutes (8). PCR products were set on 1.0% agarose gels, stained with ethidium bromide, and photographed by UV illumination (Ingenius, Syngene). The sizes of the PCR products were compared with a 100-bp DNA ladder (Bioneer, Korea). *A. baumannii* ATCC 19606 was used as the reference strain.

Sequencing of the *bla*OXA-type β-lactamase genes was also performed. *BLAST* was used for sequence analysis.

3.4. Statistical Analysis

The statistical analysis was performed with SPSS (version 19, Chicago, IL, USA). The Chi-square test or Fisher's exact test was used to compare proportions. P values of < 0.05 were considered statistically significant.

4. Results

The mean age of the studied patients was 53.88 ± 22.62 years. Fifty-three of the 103 enrolled patients in this study were male (51.5%). *A. baumannii* was isolated from 38.8% of the patients (40 out of 103). The other isolated microorganisms in this study were as follows: *S. aureus* (12.6%), *Klebsiella* spp. (11.7%), *Staphylococcus* coagulase-negative (9.7%), *Pseudomonas aeruginosa* (8.7%), *E. coli* (3.9%), *Citrobacter* and *Enterobacter* (each 2.9%), *Enterococcus* (1.9%), and *Streptococcus pyogenes* (1%). The median length of hospitalization was 38.1 days (SD: 24.53), and the median length of ICU stay was 30 days. The maximum and minimum lengths of hospitalization were 98 days and 2 days, respectively. The duration of hospitalization in *Acinetobacter*-positive patients increased to 9.8 days after adjusting for sex and age. In the unadjusted model, the duration of hospitalization decreased to 9.48 days. The characteristics of the patients are summarized in Table 2. The mortality rate for *Acinetobacter*-positive ICU patients was 27.5% (11 out of 40). The average age at death was 61.5 ± 23.8 years.

The frequency rates of antibiotic resistance patterns of the 40 carbapenem-resistant *A. baumannii* strains are shown in Table 3. All of the isolates were resistant to at least three classes of antibiotics (MDR). All of the *A. baumannii* isolates from the ICU were resistant to imipenem and meropenem. Colistin and polymyxin B

Table 1. Primers Used in This Study for Amplification of Genes from *A. baumannii* Isolates

Primer	Nucleotide Sequence (5' to 3')	Amplicon Size (bp)	Annealing (°C)	Reference
OXA51LF	TAA TGC TTT GAT CGG CCT TG	353	53	(12)
OXA51LR	TGG ATT GCA CTT CAT CTT GG			
OXA23LF	GAT CGG ATT GGA GAA CCA GA	501	53	(12)
OXA23LR	ATT TCT GAC CGC ATT TCC AT			
OXA24LF	GGT TAG TTG GCC CCC TTA AA	246	53	(12)
OXA24LR	AGT TGA GCG AAA AGG GGA TT			
OXA58LF	AAGTATTGGGGCTTGTGCTG	599	53	(12)
OXA58LR	CCCCTCTGCGCTCTACATAC			
IS*Aba1*F	CACGAATGCAGAAGTTG	549	56	(8)
IS*Aba1*R	CGACGAATACTATGACAC			

Table 2. Demographic Characteristics of the Study Population

Parameter	No. (%) of Patients		Mean ± SD (Range[Min, Max])		P Value
	***A. baumannii* (n = 40)**	**Other (n = 63)**	***A. baumannii***	**Other**	
Age (yrs)					0.042
≤ 40	12 (30)	31 (49.2)	57.25 ± 20.76	51.75 ± 23.63	
40	28 (70)	32 (50.8)	(70 [25,95])	(92 [5,97])	
Length of hospital stay (days)					0.247
≤ 30	18 (45)	34 (54)	43.9±22.78	34.41±25.07	
30	22 (55)	29 (46)	(87 [11,98])	(182 [2,184])	
Sex (male/female)	18/22	35/28			0.200
Death ratio	11/29	10/53			0.120

remained the most effective studied antibiotics. Unfortunately, all of the isolated *A. baumannii* strains were resistant to piperacillin, piperacillin-tazobactam, ceftazidime, cefepime, cefotaxime, ceftriaxone, meropenem, imipenem, ciprofloxacin, levofloxacin, and trimethoprim-sulfamethoxazole. The highest rates of resistance were observed for tetracycline (92.5%), ampicillin-sulbactam (90%), amikacin (87.5%), and gentamicin (82.5%). The PCR results based on the β-lactamase gene are shown in Table 4. The *A. baumannii* isolates were 100% positive for the $bla_{\text{OXA-51}}$ and IS*Aba1* genes, 90% positive for the $bla_{\text{OXA-23}}$ gene, and 40% positive for the $bla_{\text{OXA-24}}$ gene. None were positive for $bla_{\text{OXA-58}}$.

Analysis of the incidence of the OXA-encoding gene in the ICU isolates demonstrated that the majority of the strains, 23 out of 40 (57.5%), were simultaneously positive for the $bla_{\text{OXA-51}}$ and $bla_{\text{OXA-23}}$ genes, although 3 out of 40 isolates (7.5%) had both $bla_{\text{OXA-51}}$ and $bla_{\text{OXA-24}}$ genes. In addition, 13 out of 40 isolates (32.5%) had the $bla_{\text{OXA-51}}$, $bla_{\text{OXA-23}}$, and $bla_{\text{OXA-24}}$ genes simultaneously.

The resistance rate of *A. baumannii* to imipenem and meropenem in Iran is shown in Figure 1.

5. Discussion

A. baumannii is an important cause of nosocomial infections in many hospitals around the world, which complicates both treatment and control, due to its prolonged environmental survival time and its ability to develop resistance to multiple antimicrobial agents. *A. baumannii* infections mainly occur in special individuals with various risk factors, such as mechanical ventilation, hospitalization in the ICU, and a previous history of antibiotic therapy, chiefly with broad-spectrum types, such as third-generation cephalosporins and carbapenems. *A. baumannii* has a tendency to develop antimicrobial resistance very quickly, causing severe therapeutic problems. Carbapenems are frequently the antibiotic of choice for the treatment of serious infections. The results of the present study

Table 3. Antimicrobial Susceptibility of 40 Carbapenem-Resistant *A. baumannii* Isolates[a]

No.	PRL	SAM	PTZ	CAZ	CPM	CTX	CRO	MEM	IMI	AK	GM	LEV	CIP	T	TS	CO	PB
1	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	S	S
2	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	S	S
3	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	S	S
4	R	R	R	R	R	R	R	R	R	S	R	R	R	R	R	S	S
5	R	I	R	R	R	R	R	R	R	R	R	R	R	I	R	S	S
6	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	S	S
7	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	S	S
8	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	S	S
9	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	S	S
10	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	S	S
11	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	S	S
12	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	S	S
13	R	I	R	R	R	R	R	R	R	I	R	R	R	R	R	S	S
14	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	S	S
15	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	S	S
16	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	S	S
17	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	S	S
18	R	R	R	R	R	R	R	R	R	S	S	R	R	R	R	S	S
19	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	S	S
20	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	S	S
21	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	S	S
22	R	I	R	R	R	R	R	R	R	R	S	R	R	R	R	S	S
23	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	S	S
24	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	S	S
25	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	S	S
26	R	S	R	R	R	R	R	R	R	R	R	R	R	R	R	S	S
27	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	S	S
28	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	S	S
29	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	S	S
30	R	R	R	R	R	R	R	R	R	R	I	R	R	R	R	S	S
31	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	S	S
32	R	R	R	R	R	R	R	R	R	I	R	R	R	R	R	S	S
33	R	R	R	R	R	R	R	R	R	R	S	R	R	R	R	S	S
34	R	R	R	R	R	R	R	R	R	R	S	R	R	I	R	S	S
35	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	S	S
36	R	R	R	R	R	R	R	R	R	R	S	R	R	I	R	S	S
37	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	S	S
38	R	R	R	R	R	R	R	R	R	R	S	R	R	R	R	S	S
39	R	R	R	R	R	R	R	R	R	I	R	R	R	R	R	S	S
40	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	S	S

[a] Abbriviations: AK: amikacin; CAZ: ceftazidime; CIP: ciprofloxacin; CO: colistin; CPM: cefepime; CRO: ceftriaxone; CTX: cefotaxime; GM: gentamicin; I: intermediate; IMI: imipenem; LEV: levofloxacin; MEM: meropenem; PB: polymyxin B; PRL: piperacillin; S: susceptible; PTZ: piperacillin-tazobactam; R: resistant; SAM: ampicillin-sulbactam; T: tetracycline, TS: trimethoprim-sulfamethoxazole.

Table 4. PCR Results Based on β-lactamase Gene

No. (%)	$bla_{OXA\text{-}23No}$. (%)	$bla_{OXA\text{-}24}$ No. (%)	$bla_{OXA\text{-}51}$ No. (%)	*bla*oxa-58no. (%)	IS*Aba1*No. (%)
23 (57.5)	+	-	+	-	+
3 (7.5)	-	+	+	-	+
13 (32.5)	+	+	+	-	+
Total	40 (100)	0 (0)	40 (100)	16 (40)	36 (90)

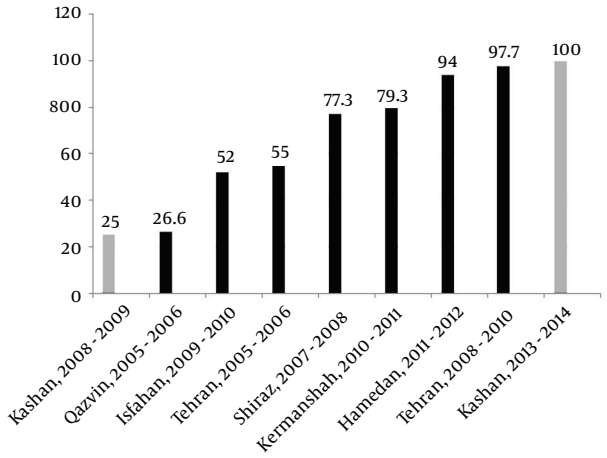

Figure 1. Distribution of Carbapenem Resistance of *A. baumannii* in Iran (2005-2014)

show that there was an extreme increase at our hospital in the resistance rate of *A. baumannii* to imipenem, which is the drug of choice, from 25% in 2008 to 100% in 2014 (13). In addition, the resistance rate of *A. baumannii* to meropenem was 100%, which is higher than in previous reports in Iran (14-20). The present study showed low susceptibility rates of the most clinically applicable antibiotics for the treatment of infections caused by *A. baumannii*, except for polymyxin B and colistin, which may be used as the final options in the management of infections caused by this bacterium. In this study, the high resistance rate of *A. baumannii* against carbapenems may indicate the outcome of overuse and misuse of carbapenems in our hospital. Several studies on *A. baumannii* in different parts of the world have also shown huge drug-resistance rates; for example, there is 70% resistance to imipenem in Egypt, 64.3% resistance to meropenem in Nigeria, 32.6% resistance to carbapenem in Saudi Arabia, and 17.6% resistance to imipenem in Chinese hospitals (21-24). Variations in reports between the studies could be influenced by ecological factors and different administration of antimicrobial plans.

Multiple mechanisms are reported for carbapenem resistance in *A. baumannii*, including active efflux of drugs, enzymatic inactivation, and modification of target sites. The results of our study showed that all carbapenem-resistant *A. baumannii* were positive for the $bla_{\text{OXA-51}}$ gene. Similar results were reported by Heritier et al., Woodford et al., and Taherikalani et al. (5, 25, 26). The present study confirmed a previous report that detection of $bla_{\text{OXA-51}}$ can be used as a reliable way to identify *A. baumannii* (27). Bali et al. reported that 77% of carbapenem-resistant Acinetobacter isolates elaborated $bla_{\text{OXA-23}}$ and $bla_{\text{OXA-51}}$ genes in a temperate northern Indian state (28). Mohajeri et al. reported that 100% of the Acinetobacter isolates from the western part of Iran were positive for $bla_{\text{OXA-51}}$, 77.9% for $bla_{\text{OXA-23}}$, and 19.2% for $bla_{\text{OXA-24}}$, but none were positive for $bla_{\text{OXA-58}}$. They also stated that $bla_{\text{OXA-23}}$ was the predominant gene, which is similar to the results of the present study (16). The $bla_{\text{OXA-23}} + bla_{\text{OXA-51}}$ combination was predominant between the carbapenem-resistant isolates in our study. $bla_{\text{oxa-23}}$, $bla_{\text{OXA-24}}$, and the combination of these two genes were always associated with resistance or at least with reduced susceptibility to antibiotics (29).

The results of this study showed that 12 isolates that contained the $bla_{\text{OXA-51}}$, $bla_{\text{OXA-23}}$, and $bla_{\text{OXA-24}}$ genes had MDR to carbapenems, broad-spectrum cephalosporins, aminoglycosides, ampicillin/sulbactam, piperacillin-tazobactam, tetracycline, trimethoprim-sulfamethoxazole, and ciprofloxacin. The $bla_{\text{OXA-58}}$ gene is an extensively spread carbapenem-hydrolyzing class D β-lactamase that has been reported in *A. baumannii*

isolates from France, Argentina, Kuwait, the United States, and many Asian-Pacific nations (30-33). This gene can hydrolyze penicillins, oxacillin, and imipenem, but is not able to hydrolyze expanded-spectrum cephalosporins. The $bla_{\text{OXA-58}}$ gene was not isolated in our study. In previous studies, carbapenem resistance was associated only with isolates in which ISAba1 was upstream of $bla_{\text{OXA-51}}$. $bla_{\text{OXA-51}}$ chromosomally encoded enzymes are intrinsic for *A. baumannii* (9). In this study, all 40 carbapenem-resistant isolates were PCR positive for IS*Aba1*. It has been reported that possession of the ISAba1-encoded gene increases the hydrolysis of carbapenems (24). Recently, the significance of ISAba1 for carbapenem resistance caused by OXAs in *A. baumannii* has been described many times (34).

The presence of class D carbapenemase-encoding genes within mobile elements, as well as the genetic potential of *A. baumannii* for acquiring foreign DNA from the environment, facilitates the distribution of resistant isolates and/or resistance genes in the ICU. These findings also provide strong evidence that the ICU environment has become the major reservoir of these resistance genes, which may help in the DNA exchange among nosocomial pathogens. Proper implementation of infection-control procedures and introduction of a sophisticated antibiotic therapy policy is suggested. This study demonstrated a high distribution of carbapenemase-encoding genes, mainly $bla_{\text{OXA-23}}$, in *A. baumannii* isolates in the ICU, while $bla_{\text{OXA-24}}$ was less common. Polymyxin B and colistin were the most active antimicrobial agents tested. *A. baumannii* infections associated with mechanical ventilation frequently develop as nosocomial infections, and are associated with a high mortality rate. The need for mechanical ventilation, and infection with carbapenem-resistant strains, were risk factors associated with a higher mortality rate in patients with *A. baumannii* infections in our study.

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Footnotes

Authors' Contribution: Rezvan Moniri, Farzaneh Firoozeh, Sareh Bagheri, and Mojtaba Sehat: study concept and design, acquisition of data, statistical analysis, analysis and interpretation of data, and drafting of the manuscript; Kamran Dastehgoli: critical revision of the manuscript for important intellectual content; Reza Khaltabadi Farahani and Hasan Koosha: assistance in acquisition of data and with all experiments, and technical and material support.

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