The Prevalence of IS$_{Aba}$1 and IS$_{Aba}$4 in *Acinetobacter baumannii* Species of Different International Clone Lineages Among Patients With Burning in Tehran, Iran

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Background: Multidrug resistant strains of *Acinetobacter baumannii* (MDR-AB) have emerged as alarming nosocomial pathogens among patients with burning.

Objectives: The current study aimed to determine the susceptibility of *A. baumannii* species, carbapenems resistance patterns, and their association with IS$_{Aba}$1 and IS$_{Aba}$4 elements upstream of the *bla*$_{OXA}$-like genes, and the distribution of international clone (IC) of *A. baumannii* isolates among patients with burning in Tehran, Iran.

Materials and Methods: In the current study, 62 *A. baumannii* species isolates from patients with burning in Tehran, Iran, in 2012 were evaluated for the antimicrobial susceptibility, genetic relationships, ICs, carbapenemase encoding genes, and insertion elements IS$_{Aba}$ upstream of *bla*$_{OXA}$-like genes.

Results: The highest rates of susceptibility were observed with colistin (88.7%) and tigecycline (82.2%). The extensively drug-resistance and pan drug-resistance were observed in 37.1% and 8.1% of the isolates, respectively. About 98.3% of 17 genotypes categorized into three distinct clusters. Thirty-six of the 62 isolates (58%) belonged to the IC II lineage. The most prevalent acquired OXA-type carbapenemase was *bla*$_{OXA-23}$-like (62.9%). IS$_{Aba}$1 and IS$_{Aba}$4 were detected upstream of *bla*$_{OXA-23}$-like genes in 45.1% and 12.9% of isolates, respectively. In 32.2% of all isolates, IS$_{Aba}$1 laid upstream of *bla*$_{OXA-51}$-like genes. The PCR results were negative for carbapenemase genes of Ambler class A and B, except *bla*$_{VIM-2}$. (1.6%).

Conclusions: It was the first study that attempted to detect the insertion elements IS$_{Aba}$ and IC lineages in MDR-AB species isolated from patients with burning in Iran.

Keywords: Drug Resistance; beta-Lactamases; Molecular Epidemiology; Carbapenemase; IS elements; *Acinetobacter baumannii*

1. Background

Acinetobacter baumannii is an aerobic Gram-negative organism widely distributed in the environment and is also an important nosocomial pathogen. *Acinetobacter baumannii* outbreak, involving multidrug-resistant (MDR) strains, occurs worldwide. In hospitalized patients, *A. baumannii* frequently colonize the skin and upper respiratory tract and is isolated from human sputum, urine, feces, and blood. This bacterium also persist for a long time on inanimate hospital environment such as surfaces and medical equipment and is isolated from various locations, including tap water faucets, air, bedside urinals, gloves, ventilators, and catheters (1). Multidrug Drug-Resistant (MDR) and Extensively Drug-Resistant (XDR) strains of *A. baumannii* emerge as formidable nosocomial pathogens among patients with burning (1, 2).

In the developing countries such as Iran, clinicians face serious challenges to manage patients with burning and MDR-*A. baumannii* (MDR-AB) infections, which present significant healthcare challenges by prolonging hospitalization, treatment failure, and increased mortality (3).

Carbapenems are currently the drug of choice; however, clonal outbreaks of carbapenems resistant *A. baumannii* (CR-AB) have led to an inadequacy of therapeutic choices in treating MDR-AB infections among patients in the developing countries (4). While expression of *bla*$_{OXA}$-like genes play a major role in carbapenem resistance among CR-AB (5); *bla*$_{OXA}$–mediated carbapenem resistance requires enhancement of gene expression either by insertion of element IS$_{Aba}$1 (upstream of *bla*$_{OXA-51}$-like genes), or IS$_{Aba}$1 and/or IS$_{Aba}$4 (upstream of *bla*$_{OXA-23}$-like genes)

(6-8). $IS_{Aba}1$ and/or $IS_{Aba}4$ (upstream of bla_{OXA}-like genes) are increasingly reported worldwide in the strains of the epidemic international clones (IC), the so-called European Clones (EC) I, II and III (9, 10). Despite a few reports on the distribution and/or frequency of resistant genes among CR-AB isolated from patients with burning in Iran (11, 12); the scarcity of molecular epidemiologic data renders the national efforts to control the spread of CR-AB infections unsuccessful. To date, no study has addressed spreading IS_{Aba} and distribution of the dominant ICs in MDR-AB strains that cause infection in patients with burning in Iran.

2. Objectives

The current study investigated (I) the susceptibility of *A. baumannii* isolates, (II) the carbapenems resistance patterns, and their association with $IS_{Aba}1$ and $IS_{Aba}4$ elements upstream of the bla_{OXA}-like genes, and (III) the distribution of IC of *A. baumannii* species isolated among patients with burning in Tehran, Iran.

3. Materials and Methods

3.1. Bacterial Isolates

In 2012, a total of 62 non-repetitive clinical species of *A. baumannii* were collected from the patients with burning of a tertiary burn center in Tehran, Iran (1).

3.2. Identification of Acinetobacter Species

Initially, isolates were identified as *A. baumannii* using API-20NE system (bioMérieux, Marcy-l'Etoile, France), and were later confirmed by detection of bla_{OXA-51} like carbapenemase gene and gyrB-multiplex Polymerase Chain Reaction (PCR) (13, 14). All the clinical Isolates were stored at -20°C in CRYOBANK™ (Copan Diagnostics Inc., Canada) until further use.

3.3. Susceptibility Testing

The Clinical and Laboratory Standards Institute (CLSI, 2013) disk agar diffusion (DAD) method (15) and CLSI breakpoint interpretation were used to assess susceptibility of *A. baumannii* isolates to the current antimicrobial agents used in the treatment of MDR-AB infections. Minimum Inhibitory Concentration (MIC) values of all isolates for colistin and tigecycline were determined by the microbroth dilution method as described by the CLSI guidelines. MICs of colistin were interpreted according to the CLSI breakpoints. However, no breakpoints for tigecycline were available in the CLSI guidelines; therefore, the criteria for interpretation of the MIC values of tigecycline were determined according to the European committee on antimicrobial susceptibility testing (EU-CAST) for members of the Enterobacteriaceae spp. All antimicrobial agents were purchased from Mast Co. Di-

agnostics Ltd., except tigecycline (purchased from Sigma-Aldrich). During each test, *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853 were used as quality control microrganisms to ensure accuracy of the antimicrobial susceptibility assays. Isolates of *A. baumannii* were defined as multidrug-resistant (MDR) when the organism was resistant to at least one agent in three or more antimicrobial categories. Species were considered extensively drug-resistant (XDR) when they were non-susceptible to one or more of the agents in all but two or less categories. Pandrug-Resistant (PDR) was defined as non-susceptibility to all antimicrobial agents (16).

3.4. Detection of Carbapenemase Encoding Genes

A multiplex-PCR, referred to as hexaplex-PCR (h-PCR), was optimized for simultaneous detection of frequent carbapenemase genes of Ambler class A (bla_{KPC}, bla_{GES}) and B metallo-β-lactamases (bla_{IMP-1}, bla_{VIM-2}, bla_{NDM-1}, and bla_{SPM-1}) in positive controls (Figure 1). PCR primers (Table 1) were designed using Primer 3 software (version 4.0; http://primer3.wi.mit.edu/; accessed 05.06.11). Reference gene sequences were extracted from Gen-Bank (http://www.ncbi.nlm.nih.gov/GenBank [bla_{KPC}: GQ140348, bla_{GES}: GU207844, bla_{IMP-1}: EF375699, bla_{VIM-2}: GQ288396, bla_{NDM-1}: JN794561, and bla_{SPM-1}: HM370523; accessed 04.06.11]).

The h-PCR was performed using Master Mix reagents (Ampliqon, Denmark) and primers, shown in Table 1, (SinaClon, Tehran, Iran) under the following conditions: preheat at 95°C for five minutes; 35 cycles of 94°C for one minute, 52°C for 40 seconds, and 72°C for 45 seconds; followed by 72°C for five minutes. Figure 1 shows representative agarose gel analysis of specific bla_{IMP-1}, bla_{SPM-1}, bla_{GES}, bla_{KPC}, bla_{NDM-1} and bla_{VIM-2} target sequence amplicons. The Ambler Class D type carbapenemase genes ($bla_{OXA-23, 40, 51, 58, 143}$-like genes) were detected (17).

3.5. Detection of $IS_{Aba}1$ and $IS_{Aba}4$ Sequences

To determine the frequency of $IS_{Aba}1$ and $IS_{Aba}4$ elements upstream of the bla_{OXA-23}-like genes, as well as $IS_{Aba}1$ upstream of the bla_{OXA-51}-like genes novel sets of primers ($IS_{Aba}1F/OXA-23R$ and $IS_{Aba}4F/OXA-23R$) and $IS_{Aba}1F/OXA-51R$ were used, respectively (Table 1). PCR primers were designed using Primer 3 software (version 4.0; http://primer3.wi.mit.edu/; accessed 05.06.11). Reference gene sequences were extracted from GenBank (http://www.ncbi.nlm.nih.gov/GenBank [$IS_{Aba}1F/OXA-23R$: AB849270.1, $IS_{Aba}4F/OXA-23R$: EF059914.1, and $IS_{Aba}1F/OXA-51R$: HQ637467.1; accessed 04.06.11]). PCR assays were performed under standard conditions (18) with modifications in annealing temperatures, as per Table 1.

3.6. AFLP Genomic Fingerprint Analysis

Epidemiological types of all *A. baumannii* isolates were determined by Amplified Fragment Length Polymorphism (AFLP) (19). Briefly, genomic DNA from isolates

were double digested with *Mbo* I and *Mse* I (Fermentas, Lithuania) and ligated to their corresponding adaptors. Ligation products served as templates for preliminary-PCR amplification using PreAmp-Mbo and PreAmp-Mse primers. Diluted preliminary-PCR products were then used for selective PCR amplification to generate AFLPs profiles. To determine the AFLP genotypes, the selective PCR products were separated by electrophoresis on 2% (w/v) agarose gels. Gel images were analyzed by BioNumerics version 5.10 (Applied Maths) using *A. baumannii* NCTC 12156 as a normalization reference. The similarity between the band patterns was calculated using the Dice coefficient (with an optimization of 0.5% and a position tolerance of 1%). The AFLP clusters and type identification were defined by groups formed at 60% and 90% Dice similarity cutoffs, respectively, on a dendrogram constructed by the unweighted-pair group method using average linkages (UPGMA) (19).

3.7. Determination of Clonal Type

International clone (IC) lineages were determined using three-locus dual assay multiplex PCR (M-PCR) (20). The M-PCR assays selectively amplified the outer-membrane protein A (*ompA*), chaperone–subunit usher E (*csuE*) and intrinsic carbapenemase (*bla*$_{OXA-51}$-like) genes of *A. baumannii* isolates. Identification of an isolate as a member of IC I or IC II needed the amplification of all three fragments in the corresponding M-PCR, and an absence of any amplification by the other M-PCR. IC III strains were defined as the amplification of only the *ompA* fragment in the IC II M-PCR, and the amplification of only the *csuE* and *bla*$_{OXA-51}$-like fragments in the IC I M-PCR. Standard *A. baumannii* strains of IC type I, II, and III served as controls. Strains double negation either IC type I, II, or type III were reported as variant (V) clonal type.
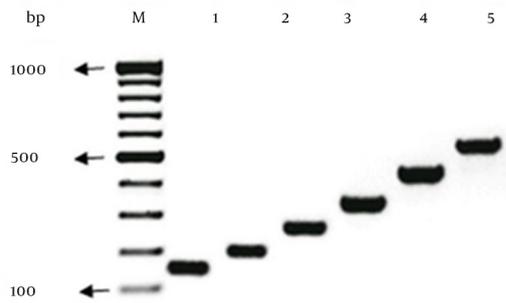
3.8. Statistical Analysis

To determine associations between *bla*$_{OXA}$-like genes and carbapenems resistant phenotype, statistical analysis including the chi-squire and Fisher's exact tests were performed by the SPSS software (version 18, Chicago, IL, USA). In all of the experiments, P-values < 0.05 were considered significant.

Table 1. Applied Primers Sequences [a]

Name/Primer	Sequence (5' - 3')	Annealing Temperature, °C	Size of Ampelicon, bp	Use (s)
hexaaplex PCR (h- PCR)		52		**Detection of carbapenemase in the molecular classes A and B**
IMP-1F	CTATCCCCACGTATGCATCTGA		174	
IMP-1R	TTTCAGGCAACCAAACCACTA			
VIM-2F	TGGTCTATTTGACCGCGTCTAT		210	
VIM-2R	CATCACCATCACGGACAATGAG			
NDM-1F	ACTGGATCAAGCAGGAGATCAA		279	
NDM-1R	CGGTGATATTGTCACTGGTGTG			
KPCF	ATATCTGACAACAGGCATGACG		315	
KPCR	GTCGTGTTTCCCTTTAGCCAAT			
bla$_{GES}$F	TCGAAAACTTTCATATGGGCCG		411	
bla$_{GES}$R	TCTTTAGGAAAACCCGCTCGTA			
SPM-1F	ATTGATTCGGACGTTTTCGTCG		540	
SPM-1R	CAGATAACCAAGTTCCTTCGGC			
ISAba1F/OXA-51 R		51	227	**ISABa1detection upstream of *bla*$_{OXA-51}$-like genes**
IS$_{Aba}$1F	AAGCATGATGAGCGCAAAG			
OXA-51 R	GGTGAGCAGGCTGAAATAAAA			
ISAba1F/OXA-23 R		44	321	**ISABa1 detection upstream of *bla*$_{OXA-23}$-like genes**
IS$_{Aba}$1F	TGAGATGTGTCATAGTATTC			
OXA-23 R	AGAGCATTACCATATAGATT			
ISAba4F/OXA-23 R		41	327	**ISABa4detection upstream of *bla*$_{OXA-51}$-like genes**
IS$_{Aba}$4F	CACAATTTCTGATAAAGATA			
OXA-23 R	TTTATTAAATTATGCTGAAC			

[a] All References are belong to this study.

Ambler; bla_{KPC}, bla_{GES}, bla_{IMP-1}, bla_{VIM-2}, bla_{NDM-1}, and bla_{SPM-1} by multiplex PCR. Clinical isolates harboring bla_{IMP-1} (*Pseudomonas aeruginosa*; lane 2), bla_{VIM-2} (*Pseudomonas aeruginosa*; lane 3), NDM-1 (*Klebsiella pneumonia*; lane 4), KPC (*Klebsiella pneumonia*; lane 5), bla_{GES} (*Pseudomonas aeruginosa*; lane 6) and bla_{SPM-1} (*Pseudomonas aeruginosa*; lane 7) were used as PCR controls. The molecular size marker (lane 1) was a 100 bp ladder.

4. Results

4.1. Isolates

In the current study, all 62 non-repetitive isolates identified as *A. baumannii* in the API 20NE system were also positive for intrinsic bla_{OXA-51}-like genes.

4.2. Susceptibility Testing

In the antimicrobial susceptibility test, *A. baumannii* isolates showed resistance to phenotype (including intermediate susceptibility) 5 - 12 (median of 6) of all 12 test antimicrobials; all isolates were resistant to ceftriaxone and cefepime (Table 1). All isolates were MDR. In the current study, 23 (37.1%), and 5 (8.1%) of *A. baumannii* isolates were XDR and PDR, respectively. The highest rates of susceptibility were observed to colistin (88.7%; n = 55), tigecycline (82.2%; n = 51), and minocycline (72.5%; n = 45). All colistin resistant isolates were susceptible to tigecycline and/or tobramycin. Fortunately, despite the alarming rates of imipenem resistance, only 3.2% of imipenem-resistant *A. baumannii* isolates were resistant to colistin. Two imipenem- and colistin-resistant *A. baumannii* isolates were sensitive to *tigecycline*. The drug resistance pattern showed that the MICs of colistin and tigacycline ranged between 0.25 - 16 and 0.5 – 8 μg/mL, with MIC 90 of 8 and 4 μg/mL, respectively. According to the results of MIC for imipenem, 4 isolate was intermediate and 38 isolates were resistant to imipenem. The MICs for imipenem varied between 0.5 and 128 μg/mL.

4.3. Detection of Carbapenemase Encoding Genes

All 62 CRAB isolates included in the study were positive for bla_{OXA-51}-like genes. The most prevalent acquired OXA-

type carbapenemase were bla_{OXA-23}-like genes isolated from 39 (62.9%) burning wounds (Table 2). Distribution of OXA-23 was not significantly different between AFLP clusters (P > 0.05) and bla_{OXA-23}-like harboring isolates belonging to all AFLP-types. Another acquired OXA carbapenemase, bla_{OXA-58}-like, was less common and isolated from 9 (14.5%) burning wounds. Despite OXA-23 encoding genes, bla_{OXA-58}-like genes were more commonly isolated from burning wounds in clusters A, and D (P < 0.05). The bla_{OXA-51}-like and bla_{OXA-23}-like genes were detected among 20 (32.2%) and 6 (9.6%) imipenem non-susceptible isolates, respectively. Co-existence of bla_{OXA-23}-like/$bla_{OXA-23-40}$-like, $bla_{OXA-23-23}$-like/$bla_{OXA-23-58}$-like, $bla_{OXA-23-40}$-like/$bla_{OXA-23-58}$-like, $bla_{OXA-23-23}$-like/$bla_{OXA-23-40}$-like/$bla_{OXA-23-58}$-like and $bla_{OXA-23-23}$-like/$bla_{OXA-23-40}$-like/$bla_{OXA-23-58}$-like/$bla_{OXA-23-40}$-like/$bla_{OXA-23-58}$-like/$bla_{OXA-23-143}$-like genes were detected in 19.3%, 4.8%, 1.6%, 3.2% and 1.6% of isolates, respectively which correlated with imipenem MICs > 32 μg/mL (P < 0.05). The PCR results were negative for bla_{KPC}, bla_{GES}, bla_{IMP-1}, bla_{NDM-1}, and bla_{SPM-1} type carbapenemase genes for all of the evaluated isolates, and bla_{VIM-2} encoding gene was observed in 1.6% of the isolates.

4.4. Detection of ISAba1 and ISAba4

Since it is also reported that $IS_{Aba}4$ raises MIC of isolates against carbapenems, in order to represent a more complete analysis of the bla_{OXA}-like genes harboring *A. baumannii* isolates from Iran, detection of $IS_{Aba}4$ as well as $IS_{Aba}1$ by PCR were included. $IS_{Aba}1$ and $IS_{Aba}4$ were detected as upstream of bla_{OXA-23}-like genes in 45.1% and 12.9%, respectively (Figure 2). In 32.2% of all isolates $IS_{Aba}1$ laid upstream of bla_{OXA-51}-like genes. In all AFLP genotypes of the current study, ISAba1 and ISAba4 imparting imipenem resistance in *A. baumannii* isolates except two AFLP genotypes, E and S, which they were imipenem susceptible genotypes. One (1.6%) of *A. baumannii* isolates harboring bla_{OXA-23}-like genes with high MIC for imipenem (16 μg/mL) was negative for $IS_{Aba}1$ and $IS_{Aba}4$ genes in the upstream of bla_{OXA-23}-like genes; 11 (17.7%) of the isolates with high MIC for imipenem (64 - 128 μg/mL) harbored $IS_{Aba}1$ in both upstream of bla_{OXA-51}-like genes and bla_{OXA-23}-like genes. Eight (13%) *A. baumannii* isolates harboring bla_{OXA-23}-like genes with high MIC for imipenem (32 - 128 μg/mL) also harbored $IS_{Aba}4$ genes in the upstream of bla_{OXA-23}-like genes.

4.5. AFLP Genomic Fingerprint Analysis

Dendrogram constructed the following AFLP pattern preparation and the results of AFLP analysis of isolates were shown in Figure 2 using a similarity index of ≥ 60% since the threshold categorized 98.3% of the isolates into three distinct clusters (A, B and D) (Figure 2). Cluster D was more heterogeneous with six AFLP types (C, E, K, P, S, and T). AFLP represented 17 distinct AFLP types (A-Q) with

Table 2. AFLP-based Dendogram and Resistance Profiles of Isolated *Acinetobacter baumannii*

	AFLP Type (No.)																
IC and Variants of Isolates (No.)	Q (1)	M (4)	A (4)	H (5)	I (14)	B (1)	N (7)	R (1)	F (5)	G (1)	O (7)	P (1)	T (2)	E (4)	C (2)	K (1)	S (2)
	II (1)	I (1), II (3)	I (1), II (2), V2 (1)	I (1), V1 (1), V4 (3)	I (3), II (11)	I (1)	I (2), II (5)	II (1)	I (2), V3 (1), V4 (2)	I (1)	I (1), II (6)	I (1)	I (1), II (1)	I (1), II (3)	I (1), II (1)	II (1)	I (1), II (1)
No. of Isolates with bla_{OXA}-like Genes																	
Only OXA-51					3				1		1				1		
OXA-51, OXA-23	1	4	3	2	6		5		3	1	5		2	3	1		2
OXA-51, OXA-40			1	2	4	1	3		1	1	1		1	1		1	
OXA-51, OXA-58	1	1		1	2			1		1		1		1		1	1
OXA-51, OXA143					1												
OXA-51, OXA-23, OXA-40			1	1	3		2		1		1		1	1	1	1	
OXA-51, OXA23, OXA-58				1	1									1			
OXA-51, OXA-40, OXA-58					1												
OXA-51, OXA-23, OXA-40, OXA-58					1									1			
No. of Isolates With Insertion Elements																	
OXA-51, OXA-23, OXA-40, OXA-58, OXA143					1												
IS$_{Aba}$1 on bla_{OXA-23}-like	1	2	1	2	6		4		3	1	3	1	1	3	1	1	2
IS$_{Aba}$1 on bla_{OXA-51}-like	1			2	3	1	2	1	1	1	1	1	1	1	2	1	1
IS$_{Aba}$1 on bla_{OXA-23}-like and bla_{OXA-51}-like	1			1	2		2		1	1				1	1		
IS$_{Aba}$4 on bla_{OXA-23}-like			1		2	1	1				1	1				1	
Without IS$_{Aba}$		2	2	2	5		2		2		2		1	1			
MIC of IPM, µg/mL																	
0.5 - 4			3	2	8		3	1	2	1	4			1	1		
8-Apr			1		1								1	1		1	1
≥ 8	1	4	1	3	5	1	4		3	1	3	1	1	2	1	1	2

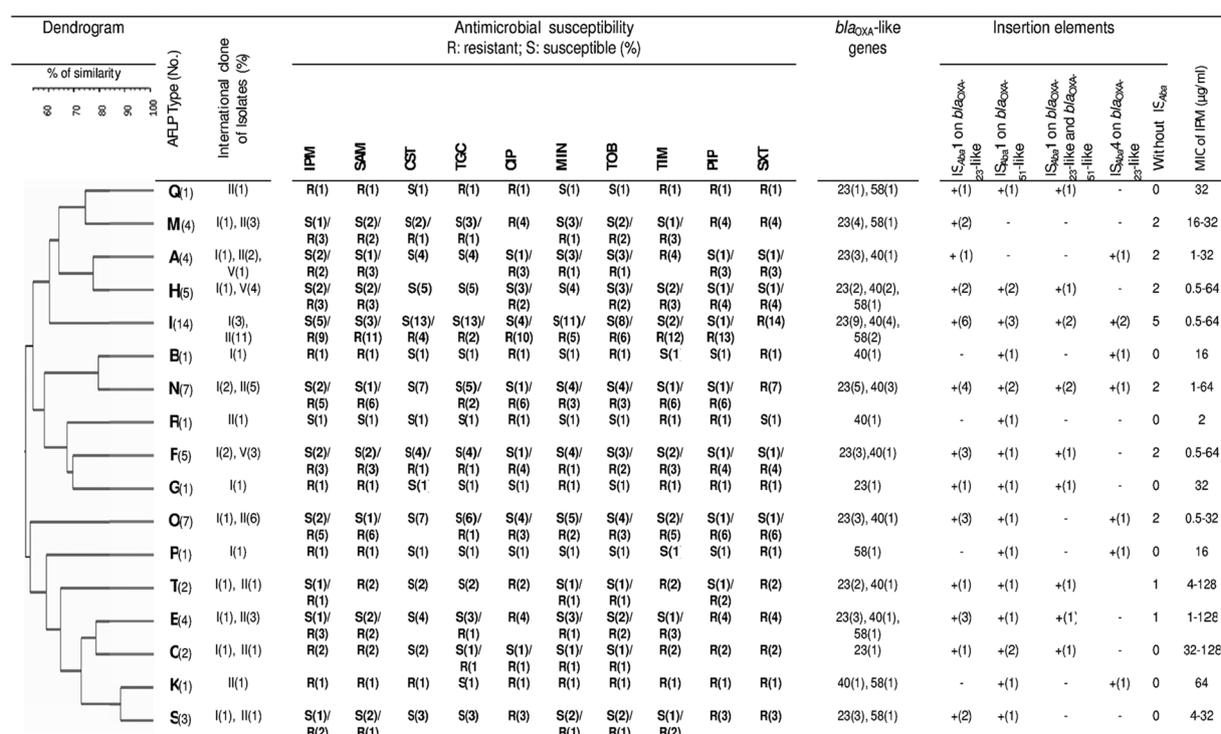| Dendrogram (% of similarity)	AFLP Type (No.)	International clone of isolates (%)	IPM	SAM	CST	TGC	CIP	MIN	TOB	TIM	PIP	SXT	bla_{OXA}-like genes	ISAba1 on bla_{OXA-23}-like	ISAba1 on bla_{OXA-51}-like	ISAba1 on bla_{OXA-23}-like and bla_{OXA-51}-like	ISAba4 on bla_{OXA-23}-like	Without ISAba	MIC of IPM (µg/ml)
	Q(1)	II(1)	R(1)	R(1)	S(1)	R(1)	R(1)	S(1)	S(1)	R(1)	R(1)	R(1)	23(1), 58(1)	+(1)	+(1)	+(1)	-	0	32
	M(4)	I(1), II(3)	S(1)/R(3)	S(2)/R(2)	S(2)/R(1)	S(3)/R(1)	R(4)	S(3)/R(1)	S(2)/R(2)	S(1)/R(3)	R(4)	R(4)	23(4), 58(1)	+(2)	-	-	-	2	16-32
	A(4)	I(1), II(2), V(1)	S(2)/R(2)	S(1)/R(3)	S(4)	S(4)	S(1)/R(3)	S(3)/R(1)	S(3)/R(1)	R(4)	S(1)/R(3)	S(1)/R(3)	23(3), 40(1)	+ (1)	-	-	+(1)	2	1-32
	H(5)	I(1), V(4)	S(2)/R(3)	S(2)/R(3)	S(5)	S(5)	S(3)/R(2)	S(4)	S(3)/R(2)	S(2)/R(3)	S(1)/R(4)	S(1)/R(4)	23(2), 40(2), 58(1)	+(2)	+(2)	+(1)	-	2	0.5-64
	I(14)	I(3), II(11)	S(5)/R(9)	S(3)/R(11)	S(13)/R(4)	S(13)/R(2)	S(4)/R(10)	S(11)/R(5)	S(4)/R(6)	S(2)/R(12)	S(1)/R(13)	R(14)	23(9), 40(4), 58(2)	+(6)	+(3)	+(2)	+(2)	5	0.5-64
	B(1)	I(1)	R(1)	R(1)	S(1)	S(1)	R(1)	S(1)	R(1)	S(1	S(1)	R(1)	40(1)	-	+(1)	-	+(1)	0	16
	N(7)	I(2), II(5)	S(2)/R(5)	S(1)/R(6)	S(7)	S(5)/R(2)	S(1)/R(6)	S(4)/R(3)	S(4)/R(3)	S(1)/R(6)	S(1)/R(6)	R(7)	23(5), 40(3)	+(4)	+(2)	+(2)	+(1)	2	1-64
	R(1)	II(1)	S(1)	S(1)	S(1)	S(1)	R(1)	S(1)	S(1)	R(1)	R(1)	S(1)	40(1)	-	+(1)	-	-	0	2
	F(5)	I(2), V(3)	S(2)/R(3)	S(2)/R(3)	S(4)/R(1)	S(4)/R(1)	S(1)/R(4)	S(4)/R(1)	S(3)/R(2)	S(2)/R(3)	S(1)/R(4)	S(1)/R(4)	23(3), 40(1)	+(3)	+(1)	+(1)	-	2	0.5-64
	G(1)	I(1)	R(1)	R(1)	S(1	R(1)	R(1)	S(1)	R(1)	R(1)	R(1)	R(1)	23(1)	+(1)	+(1)	+(1)	-	0	32
	O(7)	I(1), II(6)	S(2)/R(5)	S(1)/R(6)	S(7)	S(6)/R(1)	S(4)/R(3)	S(5)/R(2)	S(4)/R(3)	S(2)/R(5)	S(1)/R(6)	S(1)/R(6)	23(3), 40(1)	+(3)	+(1)	-	+(1)	2	0.5-32
	P(1)	I(1)	R(1)	R(1)	S(1)	S(1)	S(1)	S(1)	S(1)	S(1	S(1)	R(1)	58(1)	-	+(1)	-	+(1)	0	16
	T(2)	I(1), II(1)	S(1)/R(1)	R(2)	S(2)	S(2)	R(2)	S(1)/R(1)	S(1)/R(1)	R(2)	S(1)/R(2)	R(2)	23(2), 40(1)	+(1)	+(1)	+(1)		1	4-128
	E(4)	I(1), II(3)	S(1)/R(3)	S(2)/R(2)	S(4)	S(3)/R(1)	R(4)	S(3)/R(1)	S(2)/R(2)	S(1)/R(3)	R(4)	R(4)	23(3), 40(1), 58(1)	+(3)	+(1)	+(1)	-	1	1-128
	C(2)	I(1), II(1)	R(2)	R(2)	S(2)	S(1)/R(1	S(1)/R(1)	S(1)/R(1)	S(1)/R(1)	R(2)	R(2)	R(2)	23(1)	+(1)	+(2)	+(1)	-	0	32-128
	K(1)	II(1)	R(1)	R(1)	R(1)	S(1)	R(1)	R(1)	R(1)	R(1)	R(1)	R(1)	40(1), 58(1)	-	+(1)	-	+(1)	0	64
	S(3)	I(1), II(1)	S(1)/R(2)	S(2)/R(1)	S(3)	S(3)	R(3)	S(2)/R(1)	S(2)/R(1)	S(1)/R(2)	R(3)	R(3)	23(3), 58(1)	+(2)	+(1)	-	-	0	4-32

Figure 2. Dendrogram of AFLP analysis of genomic DNA from 62 non-repetitive A. baumannii isolates. Susceptibility to select antimicrobials, frequency of blaOXA-like genes, international clones, ISAba presence and MIC against imipenem is also indicated.

predominance of genotype I, in 14 patients (Figure 2). AFLP types were between 1 and 14 isolates. Each of the AFLP types B, G, K, P, Q, and R was observed in one patient solely. Strain O also displayed a very low level of similarity (55%) to the others and was clearly far less related to any of the other 61 isolates.

4.6. Determination of International Clone Types

International Clone (IC) I, II and four other different variants were identified among the 62 *A. baumannii* isolates (Figure 2). thirty-six isolates (58%) belonged to the IC II and 18 (29%) isolates belonged to IC I. Eight (13%) isolates pertained to variants of clonal types, defined according to the new combination of amplified products obtained from the two separate multiplex PCRs that did not correspond to the previously defined IC I, II, and III (European Clone I, II, and III, respectively). *Acinetobacter baumannii* isolates with the variant of ICs were assigned to various AFLP types (A, F and H) (Figure 2). In the current study, no IC III was found in the isolates. According to the best of the authors' knowledge, it is the first time that the prevalence of ICs is described in the bacteria isolated from burning wounds of Iranian patients.

4.7. Correlation of Acinetobacter baumannii Genotypes with Antibiotype and IC Lineages

The AFLP type analysis of all colistin-resistant *A. bau-mannii* (CoR-AB) strains revealed that five out of seven (71.4%) isolates belonged to the cluster A (AFLP types F, I, K and M). Among seven CoR-AB strains, three (42.8%) included in the study had distinct AFLP type (I) and antimicrobial resistance profiles (IPM, SAM, CST, TGC, CIP, MIN, TOB, TZP, PIP and SXT). Two CoR-AB strains had the same AFLP type (M) with distinctive antibiotypes (ampicillin-sulbactam, ciprofloxacine, colistin, imipenem, minocycline, piperacillin, piperacillin-tazobactam, tigecycline, tobramicin, and trimethoprim-sulfamethoxazole and ampicillin-sulbactam, ciprofloxacine, colistin, piperacillin, piperacillin-tazobactam, tobramicin, and trimethoprim-sulfamethoxazole). The obtained data revealed that PDR strains belonged to the cluster A, and B; three of five (60%) PDR isolates were assigned to AFLP type I. AFLP types B, G, K, P, Q, and R corresponded to a unique IC comprising IC1 and GC2 strains. The other AFLP types were present in more than one GC (Figure 2). The AFLP types of the CoR-AB strains identified in the three clusters A, B, and D were further investigated, and the results were shown in Figure 2. Four of 7 (57.1%) CoR-AB isolates which belonged to cluster A, were categorized to the same genotype (I)

5. Discussion

Nosocomial infections caused by multidrug resistant strains of *A. baumannii* (MDR-AB) are currently among the most difficult to treat, and they continue to present

serious challenges to clinicians' empirical and therapeutic decisions in patient with burning (1). Outbreaks of extensively, and pan drug-resistant *A. baumannii* (XDR, and PDR, respectively) are currently reported worldwide. In the current study, the high prevalence of XDR and PDR *A. baumannii* isolates (37.1% and 8.1%, respectively) from patient with burning, is consistent with the previous reports (1, 11). The increasing prevalence of XDR and PDR *A. baumannii* strains and limited treatment options prompt the use of antibiotics combinations like tigecycline and colistin as therapeutic regimens (21-23).

The present study revealed that 17.8% vs. 41.8% and 11.3% vs. 0% of *A. baumannii* isolates from patient with burning are resistant to tigecycline and colistin, respectively, compared to the other studies in Iran (11, 12). In a timely manner, antimicrobial resistance surveillance and strict infection control strategies are still absent in burn wards in Iran, despite the alarming emergence of MDR-AB strains, particularly among the isolates that are not susceptible to colistin. The emergence of colistin-resistant *A. baumannii* in the current study might be due to differences in *A. baumannii* strains available in the study or excessive use of colistin. However, it is still unknown whether this obvious resistance to colistin in *A. baumannii* isolates of patient with burning in Iran was due to increase of *A. baumannii* virulence or other intervening factors. Interestingly, all colistin resistant isolates were susceptible to *Tigecycline* and/or tobramycin. It is very important to treat serious infections caused by *Colistin* resistance isolates. However, this combination still needs to be validated in animal model and clinical trials. The results of the current study were consistent with a recent report in which a number of combinations exhibited potent activity against Multidrug resistant strains of *A. baumannii* (MDR-AB) (24).

It is well known that the bla_{OXA-23}-like genes are among the most prevalent acquired carbapenemase-encoding genes worldwide, which can be on the chromosome or plasmids in different genetic structures (25). Analysis of carbapenemase encoding genes prevalence demonstrated that 30 MDR-AB isolates (62.9%) were positive for bla_{OXA-23}-like genes. It was consistent with the results of the study by Pajand et al. (11), which showed that bla_{OXA-23}-like genes were the most common genes encoding carbapenemase in patients with burning, suggesting that to prevent the spread of bla_{OXA-23}-like genes in *A. baumannii* will be a major concern for both clinicians and local communities. In contrast to the study by Shahcheraghi et al. (26), in the current study bla_{SPM-1} and bla_{GES-1} were not found among the clinical strains of *A. baumannii*. This dissimilarity might be due to differences in locations of sample collection. The NDM-1 metallo-β-lactamase detected recently in *A. baumannii*, especially in patients from India and Pakistan (25), the neighboring countries of Iran, was not detected in any of the current study isolates. It is clearly demonstrated that $IS_{Aba}1$ in

the upstream of bla_{OXA}-like genes provides a promoter sequence with enhancing properties on OXA–enzymes expression (27). In contrast to the study by Pajand et al. (11) in northwest of Iran, $IS_{Aba}1$ was found upstream of the bla_{OXA-51}-like genes in 32.2% of the isolates in the present study. This dissimilarity might be due to differences in the locations of sample collection and *A. baumannii* strains available for the study.

Most IC1 and IC II *A. baumannii* isolates are MDR, and are important opportunistic pathogens associated with life threatening nosocomial infections and hospital outbreaks worldwide (28, 29). In the current study, 36 of 62 (58%) MDR- AB strains belonged to the IC II lineage, indicating the clonal relationship of MDR-AB isolates in patients with burning in Tehran, Iran. In the current study, V4 had the same pattern of a variable group described in the previous studies (19), which yielded *ompA* fragment in the Group 1 PCR and *csuE* fragment in the Group 2 PCR. Although the presence of IS_{Aba} in the IC lineages seem to play a substantial role in antimicrobial resistance in MDR-AB isolates, according to the authors' best knowledge there is no data on the presence of IS_{Aba} in MDR-AB strains isolated from patients with burning in Iran. However, further investigation is required to assess the prevalence of IS_{Aba} in MDR-AB strains isolated from Iran.

AFLP analysis grouped the obtained isolates from patients with burning into 17 distinct AFLP types. Cluster analysis of the AFLP patterns suggested that the outbreak MDR-AB isolates were quite diverse, and that outbreak strains of *A. baumannii* were low clonal, with a high diversity that may be caused by movement of insertion elements, a feature already recognized as being important in MDR-AB isolates (30). These data support the view that the Iranian MDR-AB isolates are more diverse than those of the other parts of the world (9, 22). Finally, a number of important limitations need to be considered. First, the number of patients was relatively small. Second, the isolates were collected from teaching hospitals, which may not represent the epidemiology of MDR, XDR and PDR *A. baumannii* at private hospitals. Based on the current study findings, studies should be planned to further explain the number and types of IS_{Aba} in carbapenems resistant strains of *A. baumannii*.

In conclusion, the obtained data support the idea that tigecycline alone or plus tobramycin exhibited a potent activity against colistin-resistant *A. baumannii* species isolated from patients with burning. *Acinetobacter baumannii* strains isolated from Iranian patients with burning are heterogeneous and this is the first report on spreading the IS_{Aba} -type genomic island and IS_{Aba} in the upstream of bla_{OXA}-like genes in MDR-AB isolates belonging to different IC lineages among patients with burning in Iran. It was also shown that MDR-AB IC I and II were present among patients with burning in Iran at almost the same time that they were described worldwide.

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Authors' Contributions

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