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Distribution of Class D Carbapenemase and Extended-Spectrum β-Lactamase Genes among *Acinetobacter Baumannii* Isolated from Burn Wound and Ventilator Associated Pneumonia Infections

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ABSTRACT

Introduction: Resistance to *Acinetobacter baumannii* is dramatically on the rise in Iran. Therefore, it is important to study resistance pattern among *Acinetobacter* isolates which is a common cause of nosocomial infections.

Aim: To investigate antibiotic resistance patterns and the role of resistant genes and biofilm formation in the induction of resistance among *Acinetobacter baumannii* isolated from burn wound and ventilator associated pneumonia infections.

Materials and Methods: Total 103 isolates such as 33 burn samples from Rasool Akram Hospital and 70 isolates from ventilated patients in Shahid Motahhari Hospital were identified with *A. baumannii* using biochemical method, and then identified to species level with PCR of *gyrB* and bla_{OXA-51} gene. Antibiotic sensitivity pattern for β-lactam and carbapenem antibiotics was assessed using Agar disc diffusion test and E-test. The presence of different carbapenemase and metalo-β-lactamase ($bla_{OXA-51-like}$, *gyrB*, $bla_{OXA-23-like}$, $bla_{OXA-24-like}$, bla_{OXA-58}, bla_{VEB}, bla_{PER}, bla_{GIM}, bla_{SIM}, bla_{IMP}, bla_{VIM}), extended-spectrum β-lactamases (bla_{TEM}, bla_{SHV}) and two insertion sequences genes (IS_{aba1}, IS_{1113}) was assessed. Biofilm formation of all isolates was then assessed. Chi-square analysis or Fisher's-exact tests were used for statistical analysis. A p-value <0.05 was considered statistically significant.

Results: Colistin was the most effective antimicrobial agents, although 10.7% (11/103) of the isolates were resistant. The high rate of resistance to meropenem (93.2%) and imipenem (90.3%) was determined. Also, with exception of ampicillin-sulbactam, surprisingly the resistant rate was 28.2%, the resistance to β-lactam antibiotic was dramatically increased. Co-existence of two and three blaOXA genes was also determined. The blaOXA-58 was detected in only one isolate. The blaTEM and blaOXA-23 was the most prevalent Extended-Spectrum β-Lactamases (ESBL) gene. All isolates were biofilm producers.

Conclusion: Antibiotic resistance is increasing among A. baumannii isolates which is due to excessive use of antibiotics and also acquired resistant genes and biofilm production. Resistance to nearly all antimicrobial agents especially colistin as end choice for treatment of multiple drug resistance A. baumannii is a big concern.

Keywords: β–lactams, Insertion elements, Nosocomial infections

INTRODUCTION

Genus *Acinetobacter* are cause of infections in hospitalised patients and especially those in Intensive Care Unit (ICU) [1]. Due to *Acinetobacter* spp. biofilm producing ability, they survive longer on dry surfaces or on instruments and disseminate in hospital environments and cause nosocomial infections [2]. This organism is the cause of hospital infections which occur in most disabled ICU patients. Presence of resistance genes among in *Acinetobacter* spp. make them more prevalent in healthcare settings [3]. The most common *Acinetobacter* spp. isolated from human samples is *Acinetobacter baumannii* [4].

This bacterium is resistant to many available antibiotics because it has been in contact with other gram-negative bacteria in hospital environments and also exposed to extensive bombardment with antibiotics, so most strains of *A. baumannii* are resistant to ampicillin, tetracycline, rifampin, amoxicillin/clavulanic acid, macrolides, anti-staphylococcal penicillin, and wide range cephalosporins except for cefepime, ceftazidime, and chloramphenicol [5]. Therefore, it can acquire resistance mechanisms from plasmids, integrons, transposons, and other gram-negatives, in addition to its inherent tendency to acquire resistance [3].

Today, Multiple Drug Resistance (MDR) and especially Extensively-Drug Resistant (XDR) pathogen are global issues [1]. Usually it is called MDR *Acinetobacter* when it is resistant to three or more groups of antibiotics or to one key treatment antibiotic, and is Pan Drug-Resistant (PDR) *Acinetobacter* when it is resistant to all available groups of antibiotics for the experimental treatment of its infections [6].

Emergence of β-lactamases among bacteria has caused resistance in many bacteria responsible for hospital infections, and has therefore caused serious problems in treating bacterial infections [7]. Production of carbapenemase is mostly the mechanism for resistance to carbapenems. Carbapenem resistance in *A. baumannii* is mediated by presence of bla_{OXA-23}, $bla_{OXA-24-}$, and bla_{OXA-58} type of class D family of serine β-lactamases and IMP/VIM class B of metallo-β-lactamases [8]. Mostly the isolates of *Acinetobacter* spp. have multiple copies of Insertion Sequence (IS) [9]. IS_{Aba1} and other *IS*, are on the upstream of OXA type class D carbapenemases and modulates the expression and transfer of OXA-type carbapenemase genes [10]. Resistance of antimicrobial agents among clinical isolates may add to the burden of treating infections and also negatively affect clinical results and treatment costs [2,11].

In addition to the ability to acquire resistance indicators by strains of *A. baumannii*, another issue adding to the clinical importance of these bacteria in the last 15 years which threatens antibiotic therapy is their ability to form biofilm [12]. In fact, biofilm formation ability is an important strategy in their survival, and increases their resistance to antimicrobial compounds under stress such as host defense or antibiotic use [13].

The present study was conducted in Rasool Akram and Shahid Motahhari Hospitals in order to determine the frequency of resistance to β-lactam antibiotics; the prevalence of different β-lactamases genes and relationship between expression of antibiotic resistance and biofilm formation in strains of *A. baumannii* isolated from burn wounds and Ventilator Associated Pneumonia (VAP) infections.

MATERIALS AND METHODS

Studied Population, Phenotypic and Genotypic Confirmation of Isolates

This prospective study was conducted from April 2015 to March 2016. Total 103 *Acinetobacter* isolates consisting of 33 burn samples (swab) from Rasool Akram Hospital (Tehran city) and 70 isolates from ventilated patients in Shahid Motahhari Hospital (Tehran city) were collected. After culturing isolates on nutrient media (Conda, Spain), routine tests such as growth in 45°C and 37°C and producing acid in Oxidative/Fermentation glucose (OF glucose) (Conda, Spain) were conducted to identify *A. baumannii* species. Inherent genes of *Acinetobacter* strains including $bla_{OXA-51-like}$ and *gyrB* were tested using PCR for genotypic confirmation of isolates. After genotypic and phenotypic confirmation of samples, isolates were moved to an environment of 15% glycerol and 5% liquid Brain-Heart Infusion (BHI) medium (Conda, Spain) for storage and preservation at -70°C.

Antimicrobial Susceptibility Testing

Antibiotic sensitivity pattern with antibiotic disks (Mast, UK) imipenem (10 µg), meropenem (10 µg), cefepime (30 µg), piperacillin/tazobactam (100/10 µg) , ampicillin/sulbactam (10/10 µg), piperacillin (100 µg), ticarcillin/clavulanic acid (75/10 µg), ceftazidime (30 µg), and ceftriaxone (30 µg) was assessed using agar disc diffusion test according to the recommendations and definitions of the manufacturers and CLSI 2015 guidelines [14]. Minimum Inhibitory Concentration (MIC) against Colistin was determined using E-test (AB BIODISK, Sweden). *Escherichia coli* ATCC® 25922™ and ATCC® 35218™ and *Pseudomonas aeruginosa* ATCC® 27853™ were used as quality controls in each susceptibility determination.

Determining of Antibiotic Resistant Genes and Insertion Elements

PCR for determining the presence of 15 different β-lactamases, $bla_{OXA-51-like}$, *gyr*B, IS_{aba1}, $bla_{OXA-23-like}$, $bla_{OXA-24-like}$,bla_{OXA-58},bla_{TEM}, bla_{SHV}, bla_{VEB}, bla_{PER}, bla_{GIM}, bla_{SIM}, bla_{IMP}, bla_{VIM}, IS_{1113} genes were carried out. Primers used to identify genes were listed in [Table/Fig-1]. *A. baumannii* NCTC 12156, NCTC 13302, NCTC 13303, NCTC 13304 were used as standard control for bla_{OXA-51}, bla_{OXA-23}, bla_{OXA-24} and bla_{OXA-58} genes respectively. For IS_{1113} gene was repeated twice. For all other PCR amplification, the product obtained were considered positive based on amplification size and direct sequencing of selected amplicons. In negative result, PCR amplification was repeated at least twice for these genes.

PCR was carried out with 50 ng of the template DNA, 10 pmol of each primer, 1 X PCR buffer, 2.5 mM $MgCl_2$, 0.2 mM dNTP mix, and 1U of Taq DNA polymerase (Fermentas, Lithuania) in a total volume of 25 µl. PCR amplification was carried out under the following conditions: 30 cycles of denaturation at 95°C for 30 seconds, annealing at primer set specific temperatures for one minute, and extension at 72°C for one minute followed by a final extension cycle at 72°C for 10 minute. PCR products were resolved on 1.0% agarose gels (Roche, Switzerland), stained with ethidium bromide and photographed with UV illumination.

Biofilm Formation Assay

First, an 18-24 hour colony was added to a tube with Lysogeny Broth medium (LB medium) (Conda, Spain). After 18-24 hour of incubation, its concentration was regulated to use to spectrophotometer at 650 nm, 0.1-0.08. About 190 µl of LB medium and 10 µl of microbial suspension were added into each well of a 96-well microplate and incubated in 37°C for 24-48 hour.

Biofilm formation assay was conducted three times for each bacterium. Negative control for each bacterium had 200 µl of LB medium. After rinsing microplates with distilled water, each well was stained using 0.1% crystal violet for 10 minute at room temperature, and then rinsed three more times with distilled water.

In the last step, 200 µl of 95% ethanol was added to each well, and light absorption at 492 nm was assessed using ELISA reader. Light absorption values were considered as indicators of bacteria's link to the surface and formation of biofilm. For quantitative analysis of biofilm formation, mean light absorption of three wells (A) was calculated, compared with that of a control well (Ac), and then assessed as: no biofilm formation, $A \leq Ac$; weak biofilm formation, $Ac < A \leq (2\times Ac)$; average biofilm formation, $(2\times Ac) < A \leq (4\times Ac)$; and strong biofilm formation, $(4\times Ac) < A$.

STATISTICAL ANALYSIS

Chi-square analysis and Fisher's exact test using SPSS, version 21.0. were employed for statistical analyses. A p-value < 0.05 were employed as statistically significant.

RESULTS

Antimicrobial Susceptibility Test

All 103 samples had inherent *gyrB* and bla_{OXA-51} genes, identified as *A. baumannii*. These isolates were 93.2% resistant to meropenem, 90.3% to imipenem, 88.3% to cefepime, 87.4% to ceftazidime, 82.4% to ceftriaxone. The highest resistance in clinical isolates of *A. baumannii* was against meropenem (93.2%), and the lowest resistance was against ampicillin/sulbactam (28.2%). MIC range for colistin was 0.064 µg/ml to 1024 µg/ml of concentration, and MIC50 for ventilated patients and MIC90 for burn patients were 0.125 µg / ml and 0.5 µg /ml for this antibiotic respectively [Table/Fig-2].

Frequency of Antibiotic-resistant and Insertion Elements Genes

Frequencies of bla_{OXA-23}, bla_{OXA-24}, bla_{OXA-58}, bla_{TEM}, and bla_{PER} genes were 90.3%, 38.9%, 1%, 60.2%, and 18.5% respectively. The bla_{TEM} and bla_{OXA-23} genes had the highest frequencies. In this study, bla_{GIM}, bla_{VIM}, bla_{IMP}, bla_{SIM}, bla_{VEB}, and bla_{SHV} genes were not identified [Table/Fig-3].

Based on statistical analysis, there is a significant relationship between the presence of bla_{TEM} gene and resistance to ceftriaxone and ceftazidime (p=0.01). A high level of resistance to these two antibiotics was observed in strains where this gene was present. Statistical analysis show a significant relationship between the presence of bla_{OXA-23} and resistance to imipenem (p=0.04) and meropenem (p=0.01). Analysis also show a significant relationship between the presence of $bla_{OXA-24}$and resistance to Imipenem (p=0.02), while no significant relationship was observed between the presence of this gene and resistance to Meropenem (p=0.08).

A 69.9% and 56.3% of strains had IS_{Aba-1} and IS_{1113} insertion elements respectively. Statistical analysis demonstrated a significant relationship between the presence of IS_{Aba1} insertion elements and resistance to imipenem, meropenem, and ceftazidime (p=0.01).

Gene		Nucleotide sequence (5'-3')	Amplicon size (bp)	Annealing temp (°C)	References
bla$_{\text{OXA-51-like}}$	F	TAATGCTTTGATCGGCCTTG	353	53	[15]
	R	TGGATTGCACTTCATCTTGG			
gyrB	F	CACGCCGTAAGAGTGCATTA	294	57.2	[16]
	R	AACGGAGCTT-GTCAGGGTTA			
		TGG CAC TTC ACT ATC AAT AC	490		
IS$_{\text{Aba1}}$	F	ATGCAGCGCTTCTTTGCAGG	393	55	[15]
	R	AATGATTGGTGACAATGAAG			
bla$_{\text{OXA-23-like}}$	F	TCTGGTTGTACGGTTCAGC	501	53	[17]
	R	AGTCTTTCCAAAAATTTTG			
bla$_{\text{OXA-24-like}}$	F	ATGAAAAAATTTATACTTCC	246	53	[18]
	R	TTAAATGATTCCAAGATTTTC			
bla$_{\text{OXA-58}}$	F	ATGAAATTATTAAAAATATTGAGTTTAG	599	53	[18]
	R	TTATAAATAATGAAAAACACCCAAC			
bla$_{\text{TEM}}$	F	GCACGAGTGGGTTACATCGA	310	51	[15]
	R	GGTCCTCCGATCGTTGTCAG			
bla $_{\text{SHV}}$	F	ATGCGTTATATTCGCCTGTG	753	45	[15]
	R	TGCTTTGTTATTCGGGCCAA			
bla$_{\text{VEB}}$	F	ATGAAAATCGTAAAAAGGATATT	780	47	[15]
	R	TTATTTATTCAAATAGTAATTCC			
bla$_{\text{PER}}$	F	ATGAATGTCATTATAAAAG	927	44	[15]
	R	TTGGGCTTAGGGCAG			
bla$_{\text{GIM}}$	F	ATATTACTTGTAGCGTTGCCAGC	729	53	[15]
	R	TTAATCAGCCGACGCTTCAG			
bla$_{\text{SIM}}$	F	TACAAGGGATTCGGCATCG	741	59	[19]
	R	TAATGGCCTGTTCCCATGTG			
bla$_{\text{IMP}}$	F	GTTTATGTTCATACWTCG	432	56	[15]
	R	GGTTTAAYAAAACAACCAC			
bla$_{\text{VIM}}$	F	TTTGGTCGCATATCGCAACG	500	62	[15]
	R	CCATTCAGCCAGATCGGCAT			
IS $_{\text{1113}}$	F	ATGACACATCTCAATGAGTTATAT	543	58	[18]
	R	TTAACACGAATGCAGAAGTTGATG			

[Table/Fig-1]: Sequences of the primers used in the study.

Colistin	MIC ranges (µg/ mml)	MIC50 (µg/ mml)	MIC90 (µg/ mml)	Resistant MIC≥ 4µg/ ml		Susceptible MIC≤ 2µg/ ml		Total	
				No	%	No	%	No	%
Burn	0.19- 8	0.38	0.5	1	3	32	97	33	100
Ventilator	0.125-8	0.125	8	10	14.2	60	85.7	70	100
Total	0.125-8	0.38	8	11	10.7	92	89.3	103	100

[Table/Fig-2]: Results of MIC determination for colistin using E-test method.

Genes Sample	*bla*$_{\text{OXA-23}}$	*bla*$_{\text{OXA-24}}$	*bla*$_{\text{OXA-58}}$	*bla*$_{\text{PER}}$	*bla*$_{\text{TEM}}$	*IS*$_{\text{aba1}}$	*IS*$_{\text{1113}}$
Burn	32%	7.8%	1%	4.9%	30.1%	28.2%	22.3%
VAP	58.3%	31.1%	0%	13.6%	30.1%	41.7%	34%

[Table Fig-3]: Percentage of antibiotic-resistant genes and insertion elements according to sample origin.

Oxacillinase gene	Frequency (%)	Only Resistant to Imipenem	Only Resistant to Meropenem	Resistance to both	Total %
bla$_{\text{OXA-51}}$	3 (2.9)	0 (0)	0 (0)	0 (0)	0
bla$_{\text{OXA-23}}$	59 (57.3)	0 (0)	5 (4.9)	51 (49.5)	54.4
bla$_{\text{OXA-24}}$	7 (6.8)	0 (0)	2 (1.9)	5 (4.9)	6.8
bla$_{\text{OXA-58}}$	0 (0)	0 (0)	0 (0)	0 (0)	0
bla$_{\text{OXA-23/24like}}$	33 (32)	1 (1)	3 (4.9)	27 (26.2)	31.1
bla$_{\text{OXA-23/58like}}$	1 (1)	0 (0)	0 (0)	1 (1)	1

[Table/Fig-4]: Frequency of oxacillinase genes in carbapenem-resistant strains of *A. baumannii*

Antibiotic	Type of sensitivity	Strong biofilm	Average biofilm	Weak biofilm
Imipenem	Resistant	32%	35%	23.2%
	Intermediate	1%	0%	0%
	Sensitive	4.9%	1%	2.9%
Meropenem	Resistant	%35	0%	23.3%
	Intermediate	0%	35%	0%
	Sensitive	2.9%	1%	2.9%
Cefepime	Resistant	32%	35%	21.4%
	Intermediate	0%	1%	1%
	Sensitive	5.8%	0%	3.9%
Piperacillin	Resistant	31.1%	31.1%	18.4%
	Intermediate	1%	0%	4.2%
	Sensitive	5.8%	4.9%	2.9%
Ceftazidime	Resistant	31.1%	34%	22.3%
	Intermediate	3.9%	0%	2.9%
	Sensitive	2.9%	1.9%	%1
Ceftriaxone	Resistant	29.1%	32%	21.4%
	Intermediate	3.9%	1.9%	1.9%
	Sensitive	4.9%	1.9%	2.9%
Tazobactam	Resistant	30.1%	34%	18.4%
	Intermediate	1%	0%	0%
	Sensitive	6.8%	1.9%	7.8%
Ampicillin/ sulbactam	Resistant	16.5%	7.8%	3.2%
	Intermediate	1%	25.2%	%1
	Sensitive	20.4%	2.9%	21.4%
Clavulanic acid-tazobactam	Resistant	29.1%	31.1%	18.4%
	Intermediate	1%	0%	1.9%
	Sensitive	7.8%	4.9%	5.8%
Colistin	Resistant	1.9%	1.9%	6.8%
	Intermediate	0%	0%	0%
	Sensitive	35%	34%	19.4%

[Table/Fig-5]: Relationship between the amount of biofilm formation using microtiter plate and resistance to studied antibiotics.

Investigating the frequency of oxacillinase genes in *A. baumannii* strains resistant to carbapenem showed there were only three samples with *bla*$_{\text{OXA-51}}$ gene and without other carbapenemase genes which did not show any resistance to carbapenem. Here, 57.3% of strains with only *bla*$_{\text{OXA-23}}$ oxacillinase gene and without other oxacillinase genes were resistant to both imipenem or meropenem and one of them which indicates the prominent role of this gene in resistance to carbapenem in *A. baumannii*. In 32%, both *bla*$_{\text{OXA-24}}$ and *bla*$_{\text{OXA-23}}$ were present; 31.1% of these strains were resistant to both or one of carbapenems. The simultaneous presence of *bla*$_{\text{OXA-58}}$ and *bla*$_{\text{OXA-23}}$ was seen in 1 strain which was resistant to both carbapenems. No simultaneous presence of *bla*$_{\text{OXA-58}}$ and *bla*$_{\text{OXA-24}}$ was observed [Table/ Fig-4].

Biofilm Formation

Results of biofilm formation study showed that all (100%) isolated strains tended to form biofilm. Frequency of ability of strong biofilm, average biofilm and weak biofilm formation in clinical *A. baumannii* strains were 37.8%, 37.9% and 24.3% respectively. In this study, biofilm formation was investigated in resistant, intermediate and sensitive strains; based on results and on statistical analysis, there was no significant relationship between the amount of biofilm formation using microtiter plate and resistance to studied antibiotics ($p=0.13$). Nevertheless, the amount of biofilm formation was higher in resistant strains compared with sensitive strains [Table/Fig-5].

DISCUSSION

A. baumannii shows resistance to most β-lactam antibiotics and quinolones due to its ability to survive in hospital environments, create resistance mechanism, and cause severe infections in patients. Its resistance to aminoglycosides is also emerging [19,20]. Extensive use of antibiotics has increased antibiotic resistance in this bacterium, so it is reported to be resistant to β-lactamases all over the world [21]. Wide range β-lactamases have different prevalence rates not only among different countries, but also in various geographical regions of one country [22]. Results of bla_{OXA-51} gene proliferation in this study showed that it existed in all isolates. Prevalence of bla_{OXA-51} among strains of *A. baumannii* has been reported by various studies to be 84.37% in Iran [23], 50% in Taiwan [24], 82.94% in UK [25] and 100% in a study from Iran [26]. In addition to the bla_{OXA-51} gene PCR, results of *gyrB* gene PCR were also used in this study for genotypic confirmation. Results of *gyrB* gene proliferation demonstrated that it was present in all isolates, perfectly compatible with Higgins P et al., [16]. According to our results, PCR detection of *gyrB* gene can be a cheaper, easier, and more accurate method compared to other genotypic-based diagnosis methods.

The highest antibiotic resistance observed in this study was against Imipenem (90.3%) and Meropenem (93.2%) which increased over time. Moradi J et al., in their review reported resistance to Imipenem and Meropenem in Iran in 2012-2014 to be 76.5% and 81.5% respectively which was increased over time [27]. Their study showed that many species of *A. baumannii* have at least two carbapenemase genes simultaneously: bla_{OXA-51} which is inherent to this bacterium; bla_{OXA-58}, bla_{OXA-24}, and bla_{OXA-23} which are acquired.

In the present study, bla_{OXA-23} gene was present in 90.3% of *A. baumannii* strains. In another study done by Sohrabi N et al., bla_{OXA-23} was reported in 88.7% of imipenem resistant strains, which was closer to our results [28]. The high prevalence of bla_{OXA-23} is compatible with global reports which estimate it to be 70-100% [26,29,30].

In our study, 38.9% of strains had bla_{OXA-24} and 1% had bla_{OXA-58}. 32% carried both bla_{OXA-24} and bla_{OXA-23}, and 1% carried bla_{OXA-58} and bla_{OXA-23}. All resistant or sensitive strains carried bla_{OXA-51}. In another study, none of the strains had $bla_{OXA-24/40}$, and $IS_{Aba1-OXA-51}$ was found in four strains without any bla_{OXA-58}, bla_{OXA-23}, or bla_{OXA-40} genes [31].

Different results of studies can be attributed to assessment of different hospitals. Studies from all over the world show that numerous geographical differences have been observed in molecular epidemiology of carbapenemase genes. In the present study, 54.4% of strains with only bla_{OXA-23} oxacillinase gene and without other oxacillinase genes were resistant to both imipenem or meropenem or one of them indicating the prominent role of this gene in resistance or decreasing sensitivity of *A. baumannii* against carbapenems. Of course, we must consider three strains which were sensitive to both carbapenems in spite of carrying carbapenemase gene, similar to a study from China in which, 14 carbapenem sensitive *A. baumannii* strains harboring the $bla_{OXA–51}$ like gene showed no resistance to carbapenem drugs [32]. Since resistance to Ceftazidime, Imipenem, and Meropenem was also high in this study strains were investigated for the presence of insertion elements as well. Results showed that 69.9% and 56.3% of strains had IS_{Aba1} and IS_{1113} insertion elements respectively.

Sohrabi N et al., reported the prevalence of IS_{Aba1} gene to be 90% in Iran [28]. Moreover, its prevalence was reported to be 69% by Rezaee MA et al., [33]. In addition, a 2010 study in 10 hospitals in Taiwan showed that 40.2% of 291 *A. baumannii* isolates included IS_{Aba1}, and the prevalence of bla_{OXA-51} IS_{Aba1} insertion element differed between 6.7% to 64.3% in different hospitals [34].

In the present study, $bla_{GIM, IMP, SIM}$ and bla_{VIM} genes were assessed using PCR in order to investigate the role of metallo-β-lactamases and determine the frequency of productive strains. As expected, none of these genes were identified.

In a study from Tehran, Shahcheraghi F et al., reported that 9% of *Acinetobacter* produced metallo-β-lactamase (using combined disk method), yet no gene was separated in PCR [35]; no class B metallo-β-lactamase genes, including $bla_{, IMP,}$ bla_{VIM} were observed [36], compatible with our results, showing the low frequency of this class of β-lactamases.

In the present study, biofilm-forming strains had, 93.2% to Meropenem, 90.3% resistance to Imipenem, 88.3% to cefepime, 87.4% to ceftazidime, 82.4% to ceftriaxone, and 82.5% to tazobactam, and it seems that biofilm-forming strains show a high resistance. Nonetheless, no significant relationship was seen between biofilm formation and level of resistance, in contrast to results of Rodríguez Baño J et al., who had found a low resistance in biofilm-forming strains [37].

A similar study on the relationship between biofilm formation ability and level of resistance was carried out in 2013 in Bangladesh. A total 66% of strains could form biofilm and were 81%, 100%, 100%, and 7% resistant to Imipenem, Ceftazidime, Ceftriaxone, and Colistin respectively [38].

The present study showed a decreased sensitivity to most available antimicrobial agents for the treatment of *Acinetobacter* infections, except for Colistin and Ampicillin/sulbactam, which can be introduced as choice drugs for treating resistant strains. Among Imipenem- and Meropenem-resistant strains, 54.4% carried bla_{OXA-23}; therefore, the high prevalence of class D carbapenemases among these isolates may be responsible for resistance to studied carbapenems.

Investigating biofilm formation pattern showed that all strains could form biofilm. Since ventilators are highly involved in *Acinetobacter* infections, disinfection and sterilization of respiratory equipment and devices can be one way to prevent dissemination of these infections.

LIMITATION

In present study, the sample size was very small to conclude significant results also, only burn and VAP cases were included which do not reveal much about the resistant genes distribution in other infections caused by *Acinetobacter baumannii*. Also, pattern of spread of resistance among isolates was not studied in current study which would help in infection control. Thus, further studies are needed to be done to study resistance spread pattern among isolates to control such infections in healthcare settings.

CONCLUSION

The present study showed that the presence of insertion elements, co-existence of two or more resistance genes and biofilm producing genes, increases the resistance of isolates. bla_{OXA-23} and bla_{TEM} are major resistance gene among *Acinetobacter* isolates. Colistin and ampicillin/sulbactam are treatment choices left for such resistant isolates. Finally, due to extension of antibiotic resistance, identified by the current study, performance of a precise and regular national program to control the immethodical consumption of antibiotics is suggested.

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