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RESEARCH ARTICLE

Molecular Detection of OXA-type Carbapenemases among *Acinetobacter baumannii* Isolated from Burn Patients and Hospital Environments

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Abstract:

Background:

Acinetobacter baumannii is known as one of the major causes of nosocomial infections, especially in intensive care units and burn patients. The emergence of antimicrobial resistance in burn wound bacterial pathogens is a severe health crisis. Detection of carbapenem resistance and genetic elements in *A. baumannii* associated with burn patients and hospital environments play a key role in the control and alerting in clinical settings.

Purpose:

In this study, the prevalence of OXA-type carbapenemases was investigated in *A. baumannii* strains isolated from burn patients and from a hospital environment in Tehran, 2021.

Methods:

A total of 85 non-duplicate *A. baumannii* isolates (53 from various surfaces of the hospital environment and 32 from burn patients) were recovered in the Burns Hospital in Tehran. The *A. baumannii* isolates were screened for antibiotic susceptibility and the presence of the most common OXA-type carbapenemase genes.

Results:

A. baumannii was isolated from 38.5% of hospital patient burn wounds and 22.1% of surfaces, including burn units (15.6%) and intensive care units (52.4%). Antibiotic susceptibility results showed that (100%) of burn patient isolates were resistant to imipenem, while (100%) of ICU isolates and (96.8%) of burn isolates were resistant to imipenem. All clinical isolates were identified as MDR and XDR, whereas all (100%) and 98.1% of environmental isolates were identified as MDR and XDR, respectively. All studied *A. baumannii* isolates carried $bla_{\text{OXA-51-like}}$ gene. Moreover, 50 (94.3%) and 49 (92.5%) of environmental isolates, 32 (100%) and 30 (93.7%) of burn patient isolate harbored $bla_{\text{OXA-23-like}}$ and $bla_{\text{OXA-24/40-like}}$ genes, respectively. None of the isolates carried the $bla_{\text{OXA-58}}$ or $bla_{\text{OXA-143}}$ genes and all isolates had at least 2 OXA-type carbapenemase genes.

Conclusion:

Our results suggest that surfaces in the hospital environment, particularly in ICUs, are contaminated with MDR or XDR *A. baumannii* strains. They may be considered a potential reservoir for the colonization of hospital patients. In addition, OXA-type carbapenemases, including OXA-23-like and OXA-24/40-like, appear to be one of the major mechanisms of carbapenem resistance in the clinical and environmental *A. baumannii* strains.

Keywords: *Acinetobacter baumannii*, Hospital environment, OXA-type carbapenemases, Burns centers, Molecular detection, Burn units.

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1. INTRODUCTION

Acinetobacter baumannii is known as a major cause of nosocomial infections, especially in intensive care units (ICUs) and in patients with burns [1, 2]. The ability of this bacterium to acquire antibiotic resistance and its high persistence on hospital surfaces over long periods of time have made this bacterium a dangerous hospital pathogen [3, 4]. Patients with burns are susceptible to colonization with the organism in the hospital environment and are also considered a source of environmental contamination [5]. One of the most important features of this bacterium is the high potential for the acquisition of antibiotic resistance genes by mobile genetic elements (MGEs) such as plasmids, transposons, and integrons, leading to the formation of multidrug-resistant (MDR) strains [6]. Treatment of infections caused by MDR *A. baumannii* is one of the major health problems. Due to increasing antibiotic resistance, carbapenems have been proposed as the treatment of choice for infections caused by MDR *A. baumannii* strains [6]. Unfortunately, increasing resistance of *A. baumannii* strains to carbapenems has been reported in various parts of the world over the past decade [7, 8].

Resistance to carbapenems arises by several mechanisms, the most important of which is the production of carbapenemase enzymes [9]. The most common carbapenemases in *A. baumannii* belong to the Carbapenem-Hydrolyzing Class D β-lactamases (CHDLs) are known as oxacillinases or OXA-type enzymes [9, 10]. OXA-type carbapenemases (OTCs) are classified into several phylogenetic subgroups, the most common of which are detected in *A. baumannii*, including OXA-23-like, OXA-24/40-like, OXA-58-like, OXA-51-like, and OXA-143-like [11 - 13]. The first OTC (OXA-23) in *A. baumannii* was isolated in 1985 in a Scottish hospital [14]. The gene encoding this enzyme is $bla_{\text{OXA-23,}}$ mainly located on the plasmid [15]. The OXA-51-like is the largest subgroup and contains enzymes encoded by chromosomes [16]. It has been shown that $bla_{\text{OXA-51-like}}$ genes are ubiquitous in *A. baumannii*. Incorporation of *ISAba1* elements upstream of $bla_{\text{OXA-51-like}}$ genes may act as promoters to increase gene expression and ultimately increase resistance to carbapenems [17]. Enzymes grouped in the subgroup OXA-58-like can be located on plasmids, which explains their widespread distribution [18]. OXA-24/40-like, originally known as OXA-24, is another OTC subgroup associated with both chromosomes and plasmids, and finally OXA-143-like is a plasmid-encoded mutant of the OXA-24/40 subgroup [12, 15].

Several studies have been performed on the molecular investigation of OXA-type carbapenemases in clinical isolates of *A. baumannii*. However, there is limited information on strains of environmental origin and their possible association with patient isolates [19, 20]. The aim of the study was to investigate the prevalence of OXA-type carbapenemases in *A. baumannii* strains isolated from burn patients and from the hospital environment.

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2. METHODS

2.1. Bacterial Isolates

A total of 85 non-duplicated *A. baumannii* isolates (53 from environmental surfaces and 32 from burn patients) were collected from Burns Hospital in Tehran between April 2020 and May 2021. The environmental *A. baumannii* isolates were collected by swabbing the hospital surfaces in burn wards and ICUs, including medical devices, bed surfaces, bedside tables, door and window handles and personal belongings of patients. Burn wound swabs were taken from patients during the same period. Swabs obtained were transferred to Brain Heart Infusion (BHI) media (Merck, Germany) and incubated at 37°C. After overnight incubation, samples were inoculated onto blood agar (Merck, Germany) and MacConkey agar (Merck, Germany) and incubated at 37°C for 24 hours. Identification of *A. baumannii* isolates was made using standard biochemical tests. The isolates were finally confirmed as *A. baumannii* by PCR -sequencing of *the rpoB* gene [21].

2.2. Susceptibility Testing

The antimicrobial susceptibility of *A. baumannii* was determined using the disk diffusion method as recommended by Clinical and Laboratory Standards Institute (CLSI) 2020 guidelines [22]. The antibiotic plates tested (Mast, UK) contained: imipenem (10 µg), ciprofloxacin (5 µg), ceftazidime (30µg), gentamicin (10 µg), minocycline (30 µg), ampicillin-sulbactam (20 µg), doxycycline (30 µg), and trimethoprim/sulfamethoxazole (1.25/23.75 µg). Susceptibility to colistin and tigecycline was measured, and resistance to imipenem was confirmed by determining minimum inhibitory concentrations (MIC) using the broth microdilution method according to CLSI guidelines [22]. Tigecycline resistance was interpreted according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST) (tigecycline resistance was defined as a MIC of > 2 µg/ml) [23]. MDR, extensively drug-resistant (XDR) and pan-drug resistant (PDR) *A. baumannii* isolates were defined as acquired non-susceptible to at least one agent in three or more antimicrobial classes, non-susceptible to at least one agent in all but two or fewer antimicrobial classes and non-susceptibility to all antimicrobial agents, respectively [24]. The control strain was *Escherichia coli* (ATCC 25922) and *Pseudomonas aeruginosa* (ATCC 27853).

2.3. Detection of OXA-type Carbapenemases Genes

Genomic DNA of *A. baumannii* isolates was extracted by boiling method and polymerase chain reaction (PCR) assays were performed to detect OXA-23-like, OXA-24/40 -like, OXA-58-like, OXA-51-like, and OXA-143-like genes as described previously [25]. Primers used to amplify targeted genes are listed in Table **1**. The amplification programs of $bla_{\text{OXA-23-like}}$, $bla_{\text{OXA-24 /40 -like}}$, $bla_{\text{OXA-58-like}}$, $bla_{\text{OXA-51-like}}$ genes were as follows: one cycle initial denaturation temperature of 94°C for 5 min; 30 cycles of amplification with a denaturation temperature of 94°C for 25 s; annealing temperature of 54°C for 40 s and extension temperature of 72°C for 50 s, ending with a final extension temperature of 72ºC for 6 min. The amplification of the $bla_{\text{OXA-143-like}}$ gene was performed using

PCR conditions as described previously [23]. The PCR products were visualized under an Ultraviolet (UV) transilluminator (Bio-Rad, UK) after electrophoresis on 1% gel agarose stained with GelRed®.

2.4. Statistical Analysis

Data were statistically analyzed using Statistical Package for the Social Sciences (SPSS) version 21 software (SPSS, Inc). Differences between variables were compared using chi-square ($\chi 2$) or Fisher's exact test, and P values less than 0.05 were considered significant.

3. RESULTS

A. baumannii was identified in 53 (22.1%) of the 240 samples collected from hospital environments as follows: environmental surfaces of intensive care units (22/42), including (15 medical equipment, 4 bed surfaces, 1 door handle, and 2 other sites) and burn units (31/198), including (4 medical equipment, 10 bed surfaces, 11 bedside Table **2** door and window handles, and 4 other sites). On the other hand, 32 (38.5%) *A. baumannii* strains were isolated from burn wounds of 83 hospitalized patients. Antimicrobial susceptibility testing showed that the strains were highly resistant to ceftazidime, trimethoprim/sulfamethoxazole, imipenem, ciprofloxacin, and gentamicin (Table **2**). MIC determination indicated that all (100%) of ICU and (96.8%) of burn unit from environmental isolates and 100% clinical isolates were resistant to imipenem, whereas all (100%) environmental and clinical isolates were sensitive to colistin and tigecycline. All clinical isolates were identified as MDR and XDR, whereas all (100%) and 52(98.1%) of environmental *A. baumannii* isolates showed MDR and XDR phenotypes, respectively. None of the isolates (environmental and clinical isolates) were also found to be a PDR strain. The $bla_{\text{OXA-51-like}}$ gene was detected in all (100%) environmental and clinical *A. baumannii* isolates. Fifty (94.3%) and 49 (92.5%) of isolates from hospital environments, and 32 (100%) and 30 (93.7%) of isolates from burn patients possessed OXA-23-like and OXA-24/40–like, respectively. In addition, the OXA-143-like and OXA-58-like were not identified in any isolates. All strains carried more than one OTCs gene. The characteristics of the isolates that carried more than one gene are shown in Table **3**. Statistical analysis of antibiotic resistance rates and OTCs gene transmission showed no significant association between the clinical and environmental isolates ($P > 0.05$). In addition, no significant differences in the presence of OTCs genes and antibiotic resistance were also found between ICUs and burn units ($P > 0.05$), except for resistance to ciprofloxacin, which was significantly higher in isolates from ICUs ($P = 0.035$).

Table 1. Primers to detect genes encoding OXA-type carbapenemases.

Primer Name	Sequence (5′-3′)	Product (bp)	References
OXA-23-like F	GAT CGG ATT GGA GAA CCA GA	501	[21]
OXA-23-like R	ATT TCT GAC CGC ATT TCC AT		
OXA-24 /40 -like F	GGT TAG TTG GCC CCC TTA AA	246	[21]
OXA-24 /40 -like R	AGT TGA GCG AAA AGG GGA TT		
OXA-51-like F	TAA TGC TTT GAT CGG CCT TG	353	[21]
OXA-51-like R	TGG ATT GCA CTT CAT CTT GG		
OXA-58 F	GGA ATA GAG TGG CTT AAY TCT C	599	[21]
OXA-58 R	GGT TTA AYA AAA CAA CCA CC		
OXA-143 F	TGGCACTTTCAGCAGTTCCT	149	[19]
OXA-143 R	TAATCTTGAGGGGGCCAACC		

Table 2. Antimicrobial resistance of environmental and clinical *Acinetobacter baumannii* isolates by disk diffusion method.

Antibiotic	ICUs			Burn Wards			*P-value*	Environmental Isolates			Burn Patients			*P-value*
	R, N (%)	I, N (%)	S, N (%)	R, N (%)	I, N (%)	S, N (%)		R, N (%)	I, N (%)	S, N (%)	R, N (%)	I, N (%)	S, N (%)	
Imipenem	22 (100.0)	0 (0.0)	0 (0.0)	28(90.3)	2(6.5)	1(3.2)	> 0.05	50 (94.3)	2 (3.8)	1 (1.9)	32(100.0)	0 (0.0)	0 (0.0)	> 0.05
Ceftazidime	22 (100.0)	0 (0.0)	0 (0.0)	31 (100.0)	0 (0.0)	0 (0.0)	> 0.05	53 (100.0)	0 (0.0)	0 (0.0)	32(100.0)	0 (0.0)	0 (0.0)	> 0.05
Gentamicin	19 (86.3)	2 (9.1)	1 (4.5)	22 (70.9)	4 (13.0)	5 (16.1)	> 0.05	41 (77.4)	6 (11.3)	6 (11.3)	29 (90.6)	1 (3.1)	2 (6.3)	> 0.05
Doxycycline	3 (13..6)	0 (0.0)	19 (86.3)	0 (0.0)	0 (0.0)	31 (100.0)	> 0.05	3 (5.7)	0 (0.0)	50 (94.3)	2 (6.3)	0 (0.0)	30 (93.7)	> 0.05
Minocycline	2 (9.1)	0 (0.0)	20 (90.9)	0 (0.0)	0 (0.0)	31 (100.0)	> 0.05	2 (3.8)	0 (0.0)	51 (96.2)	1 (3.1)	1 (3.1)	30 (93.7)	> 0.05
Ciprofloxacin	22 (100.0)	0 (0.0)	0 (0.0)	25 (80.7)	1 (3.2)	5 (16.1)	0.035*	47 (88.7)	1 (1.9)	5 (9.4)	30 (93.7)	0 (0.0)	1 (3.1)	> 0.05

(Table 2) contd.....

Ampicillin-Sulbactam	0 (0.0)	2 (9.1)	20 (90.9)	0 (0.0)	0 (0.0)	31 (100.0)	> 0.05	0 (0.0)	2 (3.8)	51 (96.2)	1 (3.1)	0 (0.0)	30 (93.7)	> 0.05
Trimethoprim/Sulfamethoxazole	22 (100.0)	0 (0.0)	0 (0.0)	30 (96.8)	1 (3.2)	0 (0.0)	> 0.05	52 (98.1)	1 (1.9)	0 (0.0)	32 (100.0)	0 (0.0)	0 (0.0)	> 0.05

R: Resistant; I: Intermediate; S: Susceptible based on CLSI (2020) criteria. * Statistically significant ($P \leq 0.05$).

Table 3. Prevalence of OXA-type carbapenemase genes in *A. baumannii* isolates from Burns Hospital environmental surfaces and burn patients.

OXA-type Genes	ICUs, N (%)	Burn Wards, N (%)	*P* value	Hospital Surfaces, N (%)	Burn Patients, N (%)	*P* value
OXA-51-like + OXA-24/40-like	2 (9.1)	1 (3.2)	> 0.05	3 (5.7)	-	-
OXA-51-like + OXA-23-like	1 (4.5)	3 (9.7)	> 0.05	4 (7.5)	2 (6.2)	> 0.05
OXA-51-like +OXA-23-like + OXA-24/40-like	19 (86.4)	27 (87.1)	> 0.05	46 (86.8)	30 (93.8)	> 0.05
Total	22 (100)	31 (100)		53 (100)	32 (100)	

4. DISCUSSION

The ability of *A. baumannii* to survive for long periods of time on hospital surfaces and medical equipment, as well as its ability to acquire antibiotic resistance genes, have made this bacterium one of the most important antibiotic-resistant bacteria causing nosocomial infections [26].

In the current study, the presence of *A. baumannii* was investigated in clinical isolates from burn patients and in environmental samples collected from burn units and intensive care units. Also, the antimicrobial resistance patterns and OXA-type carbapenemase genes of the studied isolates were compared. The results of our study showed that *A. baumannii* was isolated from a wide variety of surfaces in the hospital environment, especially from intensive care units (52.4%), indicating the wide distribution of this organism in the hospital and can be considered as a potential risk of colonization in hospital patients, especially patients in intensive care units.

The association between outbreaks of *A. baumannii* and environmental sources in hospitals, including patient beds and medical equipment, has been documented [27]. In a study conducted by Aygun *et al*. [28], *A. baumannii* was detected in 39.3% of samples from environmental surfaces in ICUs. In a previous study, 17% of patient beds were contaminated with *A. baumannii*, with ICU beds having the highest level of contamination [27].

Our results showed that medical equipment was the most contaminated surface in IUCs, suggesting that it could be a conceivable source of *A. baumannii* infection in these units. These findings underscore the importance of disinfecting medical instruments and surfaces based on standard protocols to prevent and reduce the transmission of *A. baumannii* from these sources.

It has been reported that surfaces in the hospital environment are a reservoir for MDR bacteria that can be transmitted to patients [29]. Considering that all of our clinical and environmental *A. baumannii* were MDR bacteria, this finding is alarming. Similar to ours, 98.8% and 100% of *A. baumannii* isolates from the hospital environment were identified as MDR in the studies by Raro *et al*. [26] and Nazari *et al*. [30], respectively. In severe infections caused by XDR strains of *A. baumannii*, such as bacteremia and ventilator-associated pneumonia (VAP), a mortality rate of 50% has been reported [31]. Since the high percentage of *A. baumannii* strains studied were XDR strains, their transmission from the environment to patients is considered a serious threat.

All *A. baumannii* strains tested in the current study carried the $bla_{\text{OXA-51-like}}$ carbapenemase genes, which are considered part of the microorganism's genetic content [32]. We also found an extremely high prevalence of $bla_{\text{OXA-23-like}}$ carbapenemase genes in *A. baumannii* strains, especially in clinical isolates, which is consistent with other studies in the world showing the global distribution of these genes [33]. Wu *et al.* [33], and Minandri *et al.* [34], have been found that $bla_{\text{OXA-23-like}}$ became significantly more prevalent and replaced other bla_{OXA} carbapenemases genes such as $bla_{\text{OXA-58-like}}$ in *A. baumannii* strains [33, 34]. OXA-23 is also identified as the most common OXA type carbapenemase in *A. baumannii* isolates from various hospital environments in Isfahan, Iran [27]. The $bla_{\text{OXA-24/40–like}}$ was another high prevalent gene detected in the present study and its prevalence in *A. baumannii* isolates was much higher than in previous studies in Iran [27]. In a study of an outbreak of MDR *A. baumannii*, the $bla_{\text{OXA-24}}$ carbapenemase was detected in all isolates and the surface of a serum container was identified as a source of infection [35]. OXA-24-producer *A. baumannii* strains have been documented as endemic in Spain [36, 37]. The prevalence of OXA-24/40-like in our study could indicate that the OXA-24/40-like producing *A. baumannii* strains are increasing. Similar to other studies, the $bla_{\text{OXA-143-like}}$ and $bla_{\text{OXA-58-like}}$ were not found among any of the clinical and environmental *A. baumannii* isolates [30]. Other studies in Northwest Iran in 2012 and in Tehran in 2008, have been reported a prevalence of 3.2% and 9% of $bla_{\text{OXA-58-like}}$ among clinical samples of *A. baumannii* respectively [38, 39]. It can be concluded that the prevalence of $bla_{\text{OXA-58-like}}$ in our region is decreasing over time and has been replaced by other OXA-type carbapenemases genes. Another interesting finding was that all *A. baumannii* isolates carried more than one gene encoding OXA-type carbapenemase, such that 93.8% of clinical isolates and 86.8% of environmental isolates had three of the above genes simultaneously. The co-existence of $bla_{\text{OXA-51-like}}$, $bla_{\text{OXA-23-like}}$ and/$bla_{\text{OXA-24/40-like}}$ has been reported in other studies which can be associated with more effective resistance to carbapenems [40, 41].

CONCLUSION

Our results suggest that surfaces in the hospital environment, especially in the intensive care unit, are contaminated with MDR or XDR *A. baumannii* strains, which may be considered a potential reservoir for colonizing hospital patients. In addition, OXA-type carbapenemases, including OXA-23-like and OXA-24/40-like, appear to be the main mechanisms of carbapenem resistance in the clinical and environmental *A. baumannii* strains.

LIST OF ABBREVIATIONS

ATCC	=	American Type Culture Collection
BHI	=	Brain Heart Infusion
CLSI	=	Clinical and Laboratory Standards Institute

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The study was approved by the ethical clearance committees of the Alborz University of Medical Sciences (IR.ABZUMS.REC.1398.201).

HUMAN AND ANIMAL RIGHTS

No animals were used in this research. All procedures performed in studies were in accordance with the ethical standards of the institutional and/or research committee and with the 1975 Declaration of Helsinki, as revised in 2013.

CONSENT FOR PUBLICATION

Participants provided written informed consent.

STANDARD OF REPORTING

STROBE guidelines were followed in this study.

AVAILABILITY OF DATA AND MATERIALS

All data is included in the manuscript, and there is no additional data to deposit.

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None.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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