Original Article

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Microbiology Section

Molecular Analysis of *Oxacillinase* Genes and Identification of Drug Resistance Pattern in MDR Strains of *Acinetobacter baumannii* Isolated from Burn Wound Samples in Kermanshah, Iran

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ABSTRACT

Introduction: Carbapenem Resistant *Acinetobacter Baumannii* (CRAB) is a dangerous nosocomial pathogen that can cause high mortality in patients. This bacterium has a remarkable ability to acquire various resistance mechanisms due to this it is considered as one of the health priorities.

Aim: To investigate the prevalence of the *OXA-23, OXA-24*, and *OXA-58* genes in *Acinetobacter baumannii* isolates collected from burn wound samples in Kermanshah, Iran.

Materials and Methods: A cross-sectional study was done during 11 months period from December 2018 to October 2019, 74 *A. baumannii* isolates were collected from those admitted to the Burns Unit of Imam Khomeini Hospital in Kermanshah, Iran. The 74 *A. baumannii* isolates were detected using particular bacteriological methods. Following determination of the antibiotic sensitivity of the specimens using the disk diffusion technique, polymerase chain reaction was performed to determine the frequency of the *OXA-23*, *OXA-24*, and *OXA-58* genes using their specific primers. Data were analysed using Fisher's-exact test and Chi-squared test in Statistical Package for the Social Sciences (SPSS) version 20.0. A p-value <0.05 was considered statistically significant.

Results: All the 74 *A. baumannii* isolates were Multidrug-Resistant (MDR) (41 from males and 33 from females). The highest drug resistance was against cefotaxime (100%) and piperacillin (98.6%), while all the isolates were sensitive to polymyxin B and colistin. *Oxacillinase* genes with the highest and lowest frequencies were *OXA-23* (64.7%) and *OXA-58* (3.5%), respectively. The highest frequency of isolates with two genes were related to *OXA-23* and *OXA-24*. A significant relationship was observed among the existence of *oxacillinase* genes and resistance to some antibiotics.

Conclusion: The results of this study indicated the significance of *OXA* carbapenemase genes in burn patients. Due to the high drug resistance of *A. baumannii* isolates collected from wound samples, the identification of carbapenemase-producing *A. baumannii* isolates is paramount in developing prevention and control programs for these drug-resistant isolates.

Keywords: Antibiotic sensitivity, Carbapenemase genes, Multidrug resistance

INTRODUCTION

Acinetobacter is a gram negative, aerobic, forced aerobic coccobacillus capable of growing in a variety of environments [1]. *A. baumannii*, as the most common species of this bacterium, can cause urinary tract infections, pneumonia, sepsis, skin and wound infections, meningitis, endocarditis, and peritonitis [2]. Infections caused by this bacterium pose a serious challenge to the treatment process followed in burn patients due to the increased resistance to various antimicrobial agents [3,4]. In recent years, *A. baumannii* has been reported to be a major cause of nosocomial infections in burn patients. Burn cases are sensitive to infections because of damage to the skin and, subsequently, immune system disorders [5,6]. A.*baumannii* has been introduced as the second MDR bacterium causing nosocomial infections in burn patients [7].

Long-term hospitalisation, Intensive Care Unit (ICU) admission, surgery, burns, serious illness, immunosuppression, exposure to antimicrobial agents, use of central venous catheter, and other factors can lead to colonisation or infection caused by this bacterium [8]. *Acinetobacter baumannii* has a high resistance to a variety of antibiotics; this resistance is either inherent or acquired through resistance genetic factors, including resistance genes present on mobile genetic elements such as transposons and integrons [9]. Carbapenems are among the beta-lactam antibiotics with extensive activity in the treatment of bacterial infections, especially severe and life-threatening infections [10]. In recent years, the presence of Carbapenem-Resistant Acinetobacter Baumannii (CRAB) isolates, including imipenem, has increased significantly, and most of these isolates are Multidrug-Resistant (MDR). The frequency of CRAB isolates is a serious problem in burn patients [11,12]. Lack of proper management in the antibiotic treatment of infections caused by these isolates can cause strains with Extensively Drug Resistant (XDR) and Pandrug Resistant (PDR). The ability of these drug-resistant isolates to hydrolyse carbapenems through carbapenemase enzymes is one of the most common and significant underlying mechanisms of their resistance to carbapenems, with Ambler class D enzymes called *oxacillinases* (*OXA*-type) being the most common among all *A. baumannii* isolates [5,7,13].

The *OXA*-type carbapenemases can be divided into eight subgroups or branches: *OXA-23, OXA-24, OXA-40, OXA-58, OXA-143, OXA-235,* and *OXA-51*, which are the most commonly identified subclasses of *OXA* in *A. baumannii* isolates [14,15]. Only *OXA-51* is naturally present in *A. baumannii*, and other OXA genes are acquired by the bacterium [16]. Thus, the identification of *A. baumannii* isolates producing carbapenemase genes is paramount in developing prevention and control programs for these drug-resistant isolates.

Considering the importance of determining carbapenem-resistant *A. baumannii* isolates and the fact that in recent years no study has been performed in this field in Kermanshah, Iran to determine the frequency of *OXA* genes of this bacterium, the present study aimed at determining the frequency of the *OXA-58, OXA-24,* and *OXA-23* genes and antibiotic-resistance pattern in MDR *A. baumannii* isolates separated from clinical samples of Imam Khomeini Hospital.

MATERIALS AND METHODS

This cross-sectional descriptive study was conducted in a period of 11 months (December 2018 to October 2019), all burn wound samples (374 burn wound samples) were collected from patients admitted to the burn ward of Imam Khomeini Hospital in Kermanshah, Iran. Then, after microbiological studies and culture of the samples, 74 isolates of *A. baumannii* were isolated. Informed consent was obtained from patients in this study. The Ethics Committee of Kermanshah University of Medical Sciences, Kermanshah, Iran (approval code no.: 1395.621) confirmed this study.

Inclusion criteria: All MDR isolates of *A.baumannii* separated from patients who had not consumed any antibiotic a week before being hospitalised based on their report and file in given time period were included in the study.

Exclusion criteria: Other bacterial isolates separated from patient's burn samples and other strains *Acinetobacter* separated from the samples were excluded from the study [17].

Procedure

All the samples in this study were the specimens of the burn wound. After collection, these samples were cultured on MacConkey agar and blood agar media (Indian media) and incubated for 1-2 days at 37°C under laboratory conditions. Then, to identify *A. baumannii* isolates, biochemical tests, including the growth of slant/alkaline butt pattern on Triple Sugar Iron (TSI) medium (Merck, Germany), oxidase and catalase negative test, immobility on Sulfide Indole Motility (SIM) medium (Merck, Germany), and no pigment production, were performed. Polymerase Chain Reaction (PCR) of the OXA-51 gene was used for the final confirmation of possible isolates of *A. baumannii* using its specific primer. A total of 74 *A. baumannii* isolates were identified. According to the Clinical and Laboratory Standards Institute (CLSI) instructions, antibiotic-sensitivity evaluation was performed by the disk diffusion method (Kirby-Bauer), by bacterial suspension equivalent to half McFarland turbidity (1.5×10^8 CFU/mL) and Müller-Hinton agar culture medium (media India) for antibiotic discs (MAST.UK), including amikacin (30 µg), gentamycin (10 µg), ceftazidime (30 µg), tobramycin (10 µg), ciprofloxacin (5 µg), meropenem (10 µg), levofloxacin (10 µg), imipenem (10 µg), cefepime (5 µg), polymyxin B (300 units), piperacillin (100 µg), colistin (25 µg), ampicillin-sulbactam (10 µg), and cefotaxime (30 µg). The suspension of isolated bacteria was first cultured on Müller-Hinton agar medium after comparison with the McFarland 0.5 standard. After placing the antibiotic disks and incubating at 37°C for one day, the diameter of their growth inhibition zone was evaluated and compared to that mentioned in the CLSI tables [18].

For quality control, standard strains of *A. baumannii* ATCC 19606 and *Escherichia coli* ATCC 25922 were used. *Acinetobacter* isolates with resistance to three or more groups of antibiotics were determined to be MDR strains. The PCR was performed to identify the presence of the *OXA-58*, *OXA-24*, and *OXA-23* genes using specific primers (Takapou Zist Co., Iran) and according to [Table/Fig-1] [19]. After that, the standard strains *A. baumannii* NCTC 13304, NCTC 13302 and NCTC 13305 were utilised as positive controls to detect the *OXA-23*, *OXA-24* and *OXA-58* genes, respectively. The boiling method was used to extract chromosomal Deoxyribonucleic Acid (DNA) of the isolates. In doing so, after culturing *A. baumannii*, we dissolved several pure bacterial colonies in 0.5 mL of sterile distilled water, and after 5 min of boiling and cooling in the next step, they were centrifuged at 7000 gm for 1 min. Afterward, the solution was transferred to new Eppendorf tubes as bacterial DNA to perform PCR. Then, using NanoDrop Synergy HTX (Bio Tek Instrument, Inc. Highland Park, USA), concentrations of DNA were measured at the Optical Density (OD) of 260 nm to be 33 pmol/µL, and DNA purity at the OD of 260/280 nm was calculated to be 1.85. PCR was performed with a final volume of 25 µL, including 12.5 µL of master mix, 1 µL of each primer, 3 µL of bacterial DNA, and sterile distilled water up to a volume of 25 µL. PCR reaction was performed separately for each of the *oxacillinase* genes. The PCR reaction temperature included primary denaturation at 94°C for 5 minutes, and followed by 35 main cycles, according to [Table/Fig-1] and the eventual extension at 72°C for 6 minutes [19]. Finally, using 1.5% agarose gel and ethidium bromide staining under UV radiation in a gel doc device with a voltage of 80 V for 50 minutes, the PCR products were evaluated.

Genes	Sequence (ś-ǵ)	35 cycles			
		Denaturation 94°C	Annealing 45 seconds	Extension 72°C	Product size (bp)
OXA-51	TAA TGC TTT GAT CGG CCT TG TGG ATT GCA CTT CAT CTT GG	40 seconds	59 °C	60 s	353
OXA-23	GAT CGG ATT GGA GAA CCA GA ATT TCT GAC CGC ATT TCC AT	40 seconds	50 °C	60 s	501
OXA-24	GGT TAG TTG GCC CCC TTA AA AGT TGA GCG AAA AGG GGA TT	40 seconds	52 °C	60 s	249
OXA-58	AAG TAT TGG GGC TTG TGC TG CCC CTC TGC GCT CTA CAT AC	40 seconds	51 °C	60 s	599

[Table/Fig-1]: The primers and temperature cycles used in the PCR reaction.

STATISTICAL ANALYSIS

Data were analysed using Chi-square test in Statistical Package for the Social Sciences (SPSS) version 20.0. A p-value ≤0.05 was considered statistically significant.

RESULTS

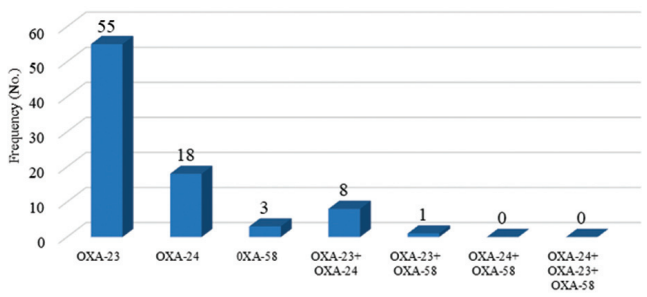
In this study, 74 isolates of *A. baumannii* were investigated {41 (55.4%) from males and 33 (44.6%) from females} in the age group of 8-71 years, with a mean age of 44.22±14.55 years. All isolates of this bacterium were collected from burn wound samples. According to [Table/Fig-2], the highest drug resistance of *A. baumannii* isolates was against cefotaxime (100%) and piperacillin (98.6%), and all samples were susceptible to polymyxin B and colistin (0). All the 74 *A. baumannii* isolates (100%) were found to be MDR. In addition, total 85 *OXA* genes were found from 74 isolates of *A. baumannii*. The highest and lowest frequencies of OXA genes were related to *OXA-23* 55 (64.7%) and *OXA-58* 3 (3.5%), respectively.

Antibiotic	Resistance (n,%)	Intermediate (n,%)	Sensitive (n,%)
Ceftazidime	69 (93.2%)	1 (1.4%)	4 (5.4%)
Cefotaxime	74 (100%)	0	0
Cefepime	64 (86.5%)	2 (2.7%)	8 (10.8%)
Ciprofloxacin	70 (94.6%)	0	4 (5.4%)

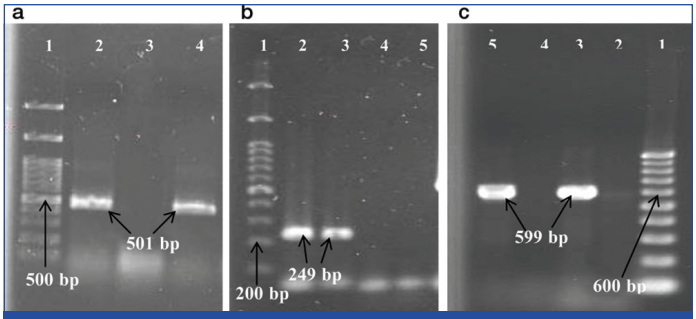
Levofloxacin	68 (91.9%)	1 (1.4%)	5 (6.7%)
Meropenem	69 (93.2%)	0	5 (6.7%)
Imipenem	71 (95.9%)	0	3 (4.1%)
Ampicillin/sulbactam	32 (43.2%)	3 (4.1%)	39 (52.7%)
Tobramycin	64 (86.5%)	3 (4.1%)	7 (9.5%)
Gentamycin	65 (87.9%)	5 (6.7%)	4 (5.4%)
Amikacin	66 (89.2%)	5 (6.7%)	3 (4.1%)
Polymyxin B	0	0	74 (100%)
Colistin	0	0	74 (100%)
Piperacillin	73 (98.6%)	0	1 (1.4%)

[Table/Fig-2]: Results of antibiotic resistance of *A. baumannii* isolates (N=74).

The frequency of the *OXA-24* gene was 21.2% (18 isolates). The total number of isolates with two genes simultaneously was (9, 10.6%), among which the highest frequency was related to isolates with two genes *OXA-23* and *OXA-24* with a frequency of eight cases. None of the isolates had all the three genes present simultaneously [Table/Fig-3]. The presence of *OXA* genes and resistance to some antibiotics, including the presence of the *OXA-23* gene and resistance to carbapenems, amikacin, and ampicillin/sulbactam, as well as the presence of the *OXA-58* gene and resistance to levofloxacin and amikacin, showed a significant relationship [Table/Fig-4]. The PCR results for *OXA* genes are shown in [Table/Fig-5].



[Table/Fig-3]: Frequency of *Oxacillinase* genes in *A. baumannii* isolates.



[Table/Fig-5]: (Gel electrophoresis of PCR products of *OXA*). a): *OXA-23*: 1- Lader (100 bp), 2-Positive control NCTC 13304 (501 bp), 3- Negative control, 4- Positive sample (501 bp); b): *OXA-24*: 1- Lader (100 bp), 2-Positive control NCTC 13302 (249 bp), 3-Positive sample (249 bp), 4,5- Negative control; c): *OXA-58*: 1- Lader (100 bp), 2- Negative control, 3- Positive control NCTC 13305 (599 bp), 5- Positive sample (599 bp).

the present study, all 74 (100%) *A. baumannii* isolates were found to be MDR. In studies conducted in Iran, the prevalence of MDR in burn patients was reported to be between 97.9% and 100%, which was consistent with the current study findings [21-26]. Similar results related to these MDR *A. baumannii* isolates have been reported in other studies abroad. In 2020, a study was performed on burn samples by Mabrouk A et al., where in all 21 *A. baumannii* isolates were identified to be MDR, which was consistent with present study [27]. However, in few other studies, the prevalence of MDR was found to be lower than the present study [28-30].

Among all MDR isolates examined in the current study, cefotaxime (100%) presented the maximum antibiotic resistance, followed by piperacillin (98.6%), while all samples were susceptible sensitive to colistin and polymyxin B. Other national and international research reported enhanced resistance in MDR samples to various antibiotics such as cefotaxime, piperacillin, imipenem, and meropenem [28,29,31]. In the study by Sarhaddi N et al., all MDR isolates were found to be sensitive to colistin and polymyxin B [32]. According to these results, it can be concluded that these antibiotics are still effective in the treatment of infections caused by this bacterium, so their use should be controlled.

	OXA-23 (n=55)				*OXA-24* (n=18)				*OXA-58* (n=3)			
Antibiotics	Resistance	Intermediate	Sensitive	p-value	Resistance	Intermediate	Sensitive	p-value	Resistance	Intermediate	Sensitive	p-value
Cefepime	45	2	8	0.085	17	0	1	0.320	3	0	0	0.875
Levofloxacin	52	1	2	0.069	17	0	1	0.354	1	0	2	0.030*
Ciprofloxacin	51	0	4	0.058	17	0	1	0.192	3	0	0	0.327
Tobramycin	46	2	7	0.142	16	1	1	0.682	3	0	0	0.125
Ceftazidime	52	1	2	0.325	16	0	2	0.213	3	0	0	0.371
Gentamycin	51	3	1	0.061	17	0	1	0.095	3	0	0	0.254
Amikacin	53	0	2	0.039*	17	1	0	0.362	2	0	1	0.148
Meropenem	55	0	0	0.05*	18	0	0	0.123	3	0	0	0.786
Colistin	0	0	55	0.175	0	0	18	0.118	0	0	3	0.625
Polymyxin B	0	0	55	0.117	0	0	18	0.087	0	0	3	0.451
Cefotaxime	55	0	0	0.058	18	0	0	0.369	3	0	0	0.382
Ampicillin-Sulbactam	27	3	25	0.041*	8	0	10	0.286	2	0	1	0.085
Piperacillin	54	0	1	0.398	18	0	0	0.075	3	0	0	0.675
Imipenem	55	0	0	0.048*	17	0	1	0.410	3	0	0	0.490

[Table/Fig-4]: Association between the frequency of *Oxacillinase* genes and antibiotic resistance.
*Significant p-value (Chi-square test)

DISCUSSION

A.*baumannii* is a major hospital pathogens, found especially in burn patients, which causes a high mortality in these patients [16]. The reason for this is the ability of this microorganism to survive in hospital environments and to acquire a mechanism of resistance against antimicrobial agents [20]. The prevalence of MDR *A. baumannii* isolates has been regarded as a serious concern worldwide [9]. In Recently, the prevalence of carbapenem-resistant isolates, including imipenem, has increased significantly, and most of these isolates are MDR. A frequent and principal cause of resistance to carbapenem antibiotics is their ability to hydrolyse carbapenems through carbapenemase enzymes, with Ambler class D enzymes called oxacillin (OXA-type) being the most common among *A. baumannii* isolates [13].

The most common *A. baumannii* carbapenemase genes involved in carbapenem resistance are *OXA-23*, *OXA-24*, and *OXA-58*. The frequencies of the *OXA-23*, *OXA-24*, and *OXA-58* genes among the 74 isolates of *A. baumannii* collected from burn samples were determined to be 64.7%, 21.2%, and 3.5%, respectively. In two studies, conducted in Iran, including the present study, the prevalence of *OXA-23* was higher than *OXA-24* and *OXA-58* [6,11,21].

Tafreshi N et al., reported the prevalence of these three genes at 53.57%, 41.66%, and 30.59% in *A. baumannii* isolates collected from burn samples, respectively [22]. Mohajeri P et al., reported the frequencies of 77.9%, 19.2%, and 0% for *OXA-23*, *OXA-24*, and *OXA-58*, respectively, indicating that the prevalence of *OXA-23* was much higher than the other genes, which was consistent with the present study findings [33]. The results of the current study were compared with other previous studies in Iran and other countries [Table/Fig-6] [11,22-24,28,29,31,32,34-37]. In the present study, the number of isolates carrying the two genes *OXA-23* and *OXA-24*, which had the highest frequencies, was equal to 8 (9.4%) out of 85, and the combination of these two genes always showed resistance or reduced sensitivity to antibiotics [30].

Authors and year of publication of study	MDR (%)	Resistance to Carbapenems (%)	*Oxacillinase* Genes (%)		
			OXA-23	*OXA-24*	*OXA-58*
Vahhabi A et al., (2021) [11]	100%	100%	82.1%	-	-
Tafreshi N et al., (2019) [22]	100%	27.35%	53.57%	41.66%	30.59%
Tarafdar F et al., (2020) [23]	100%	94%	-	-	-
Abbasi E et al., (2020) [24]	97.9%	98%	100%	74%	0
Biglari S et al., (2017) [28]	77.2%	79%	82%	0	0
Banerjee T et al., (2018) [29]	88.02%	77.2%	93%	-	-
Josheghani B et al., (2017) [31]	100%	100%	90%	40%	0
Sarhaddi N et al., (2017) [32]	100%	100%	66.7%	68.5%	0
Alavi-Moghaddam M et al., (2020) [34]	90%	80.5%	75.7%	14.1%	0
Nureen Z et al., (2021) [35]	100%	21.65%	73%	0	0
Mortazavi SM et al., (2020) [36]	91.25%	86.9%	68.75%	20%	-
Nojookambari NY et al., (2021) [37]	83.3%	88.33%	93%	36.67%	3.33%
Present study	100%	95.6%	64.7%	21.2%	3.5%

[Table/Fig-6]: Comparison of the results this study with the results of other similar studies [11,22-24,28,29,31,32,34-37].

Among the reasons for the differences in the reports on the frequency of these genes include the diversity in the pattern of antibiotic use and appropriate control strategies in different wards of hospitals. The *OXA-58* gene produces a broad-spectrum class D beta-lactamase that can hydrolyse penicillin, oxacillin, and imipenem. The results of this study showed a significant relationship between the presence of the *OXA-58* gene and resistance to levofloxacin and amikacin. Of the 55 strains resistant to imipenem and meropenem, all carried the *OXA-23* gene. The *OXA-23* gene produces a carbapenem-hydrolysing beta-lactamase that promotes resistance to imipenem and meropenem [14]. Therefore, the reason for carbapenem resistance can be the high prevalence of these carbapenem genes. There is a significant link between the presence of the *OXA-23* gene and resistance to carbapenems, amikacin, and ampicillin/sulbactam.

Limitation(s)

The limitations of this study was the sample small size examined and and lack of access to patient files.

CONCLUSION(S)

In this study, *A. baumannii* showed resistance to most of the available antibiotics and it also appears that colistin and polymyxin B are currently the only antibiotics effective in treating infections caused by this bacterium. Therefore, it is necessary to pay more attention to controlling the use of these antibiotics in nosocomial infections. The results of this study show the importance of OXA-type carbapenemases in treating burn patients. Therefore, the identification of *A. baumannii* isolates producing carbapenemase genes is paramount in the development of prevention and control programs for these drug-resistant isolates.

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REFERENCES

[1] Azizi M, Mortazavi SH, Etemadimajed M, Gheini S, Vaziri S, Alvandi A, et al. Prevalence of extended-spectrum β-lactamases and antibiotic resistance patterns in *Acinetobacter Baumannii* isolated from clinical samples in Kermanshah, Iran. Jundishapur J Microbiol. 2017;10(12):e61522.
[2] El-Shazly S, Dashti A, Vali L, Bolaris M, Ibrahim AS. Molecular epidemiology and characterization of multiple drug-resistant (MDR) clinical isolates of *Acinetobacter baumannii.* Int J Infect Dis. 2015;41:42-49.
[3] Moradi J, Hashemi FB, Bahador A. Antibiotic resistance of Acinetobacter baumannii in Iran: A systemic review of the published literature. Osong Public Health Res Perspect. 2015;6(2):79-86.
[4] Madadi-Goli N, Moniri R, Bagheri-Josheghani S, Dasteh-Goli N. Sensitivity of levofloxacin in combination with ampicillin-sulbactam and tigecycline against multidrug-resistant *Acinetobacter baumannii.* Iran J Microbiol. 2017;9(1):19.
[5] Azimi L, Talebi M, Pourshafie MR, Owlia P, Lari AR. Characterization of carbapenemases in extensively drug resistance *Acinetobacter baumannii* in a burn care center in Iran. Int J Mol Cell Med. 2015;4(1):46.
[6] Mohammadi F, Goudarzi H, Hashemi A, Nojookambari NY, Khoshnood S, Sabzehali F. Detection of ISAba1 in *Acinetobacter baumannii* strains carrying OXA genes isolated from iranian burns patients. Arch Pediatr Infect Dis. 2017;5(2):e39307.
[7] Farshadzadeh Z, Hashemi FB, Rahimi S, Pourakbari B, Esmaeili D, Haghighi MA, et al. Wide distribution of carbapenem resistant *Acinetobacter baumannii* in burns patients in Iran. Front Microbiol. 2015;6:1146.
[8] Fishbain J, Peleg AY. Treatment of Acinetobacter infections. Clin Infect Dis. 2010;51(1):79-84.
[9] AlAmri AM, AlQurayan AM, Sebastian T, AlNimr AM. Molecular surveillance of multidrug-resistant *Acinetobacter baumannii*. Curr Microbiol. 2020;77(3):335-42.
[10] Hsu LY, Apisarnthanarak A, Khan E, Suwantarat N, Ghafur A, Tambyah PA. Carbapenem-resistant *Acinetobacter baumannii* and Enterobacteriaceae in south and southeast Asia. Clin Microbiol Rev. 2017;30(1):01-22.
[11] Vahhabi A, Hasani A, Rezaee MA, Baradaran B, Hasani A, Kafil HS, Soltani E. Carbapenem resistance in *Acinetobacter baumannii* clinical isolates from northwest Iran: High prevalence of OXA genes in sync. Iran J Microbiol. 2021;13(3):282-93.
[12] Abdollahi A, Alirameza A, Salehi M, Norouzi Shadehi M, Ghourchian S, Douraghi M. Co-infection of ST2IP carbapenem-resistant *Acinetobacter baumannii* with SARS-CoV-2 in the patients admitted to a Tehran tertiary referral hospital. BMC Infect Dis. 2021;21(1):01-07.
[13] Schultz MB, Thanh DP, Do Hoan NT, Wick RR, Ingle DJ, Hawkey J, et al. Repeated local emergence of carbapenem-resistant *Acinetobacter baumannii* in a single hospital ward. Microb Genom. 2016;2(3):e000050.
[14] Karmostaji A, Najar Peerayeh S, Hatef Salmanian A. Distribution of OXA-type class D β-lactamase genes among nosocomial multi drug resistant *Acinetobacter baumannii* isolated in Tehran hospitals. Jundishapur J Microbiol. 2013;6(5):e8219.
[15] Rezaei A, Fazeli H, Moghadampour M, Halaji M, Faghri J. Determination of antibiotic resistance pattern and prevalence of OXA-type carbapenemases among *Acinetobacter baumannii* clinical isolates from inpatients in Isfahan, central Iran. Infez Med. 2018;26(1):61-66.
[16] Higgins PG, Pérez-Llarena FJ, Zander E, Fernández A, Bou G, Seifert H. OXA-235, a novel class D β-lactamase involved in resistance to carbapenems in *Acinetobacter baumannii*. Antimicrob Agents Chemother. 2013;57(5):2121-26.
[17] Vaziri S, Afsharian M, Mansouri F, Azizi M, Nouri F, Madadi-Goli N, et al. Frequency of qnr and aac(6')Ib-cr Genes Among ESBL-Producing Klebsiella pneumoniae Strains Isolated from Burn Patients in Kermanshah, Iran. Jundishapur J Microbiol. 2020;13(7):e100348.
[18] CLSI. Performance standards for antimicrobial susceptibility testing, CLSI supplement M100. Wayne, PA: Clinical and Laboratory Standards Institute; 2019.

[19] Woodford N, Ellington MJ, Coelho JM, Turton JF, Ward ME, Brown S, et al. Multiplex PCR for genes encoding prevalent OXA carbapenemases in Acinetobacter spp. Int J Antimicrob Agents. 2006;27(4):351-53.
[20] Shoja S, Moosavian M, Rostami S, Farahani A, Peymani A, Ahmadi K, et al. Dissemination of carbapenem-resistant *Acinetobacter baumannii* in patients with burn injuries. J Chin Med Assoc. 2017;80(4):245-52.
[21] Ku WW, Kung CH, Lee CH, Tseng CP, Wu PF, Kuo SC, et al. Evolution of carbapenem resistance in *Acinetobacter baumannii*: An 18-year longitudinal study from a medical center in northern Taiwan. J Microbiol Immunol Infect. 2015;48(1):57-64.
[22] Tafreshi N, Babaeekhou L, Ghane M. Antibiotic resistance pattern of *Acinetobacter baumannii* from burns patients: Increase in prevalence of blaOXA-24-like and blaOXA-58-like genes. Iran J Microbiol. 2019;11(6):502.
[23] Tarafdar F, Jafari B, Azimi T. Evaluating the antimicrobial resistance patterns and molecular frequency of blaOXA-48 and blaGES-2 genes in Pseudomonas aeruginosa and *Acinetobacter baumannii* strains isolated from burn wound infection in Tehran, Iran. New Microbes New Infect. 2020;37:100686.
[24] Abbasi E, Goudarzi H, Hashemi A, Chirani AS, Ardebili A, Goudarzi M, et al. Decreased carO gene expression and OXA-type carbapenemases among extensively drug-resistant *Acinetobacter baumannii* strains isolated from burn patients in Tehran, Iran. Acta Microbiol Immunol Hung. 2020;68(1):48-54.
[25] Kooti S, Motamedifar M, Sarvari J. Antibiotic resistance profile and distribution of oxacillinase genes among clinical isolates of *Acinetobacter baumannii* in Shiraz teaching hospitals, 2012-2013. Jundishapur J Microbiol. 2015;8(8):e20215.
[26] Sedaghat A, Khadem-Rezaiyan M, Ahmadabadi A, Abbaspour H, Youssefi M, Shirzad MM, et al. Antibacterial resistance pattern of *Acinetobacter baumannii* in burn patients in Northeast of Iran. Jundishapur J Microbiol. 2019;12(10):e94668.
[27] Mabrouk A, Chebbi Y, Raddaoui A, Krir A, Messadi AA, Achour W, et al. Clonal spread of PER-1 and OXA-23 producing extensively drug resistant *Acinetobacter baumannii* during an outbreak in a burn intensive care unit in Tunisia. Acta Microbiol Immunol Hung. 2020;67(4):222-27.
[28] Biglari S, Hanafiah A, Mohd Puzi S, Ramli R, Rahman M, Lopes BS. Antimicrobial resistance mechanisms and genetic diversity of multidrug-resistant *Acinetobacter baumannii* isolated from a teaching hospital in Malaysia. Microb Drug Resist. 2017;23(5):545-55.
[29] Banerjee T, Mishra A, Das A, Sharma S, Barman H, Yadav G. High prevalence and endemicity of multidrug resistant Acinetobacter spp. in intensive care unit of a tertiary care Hospital, Varanasi, India. J Pathog. 2018.
[30] Noori M, Mohsenzadeh B, Bahramian A, Shahi F, Mirzaei H, Khoshnood S. Characterization and frequency of antibiotic resistance related to membrane porin and efflux pump genes among *Acinetobacter baumannii* strains obtained from burn patients in Tehran, Iran. J Acute Dis. 2019;8(2):63.
[31] Josheghani SB, Moniri R, Firoozeh F, Sehat M, Dastehgoli K, Koosha H, et al. Emergence of bla OXA-carrying carbapenem resistance in multidrug-resistant *Acinetobacter baumannii* in the intensive care unit. Iran Red Crescent Med J. 2017;19(5):e27327.
[32] Sarhaddi N, Soleimanpour S, Farsiani H, Mosavat A, Dolatabadi S, Salimizand H, et al. Elevated prevalence of multidrug-resistant *Acinetobacter baumannii* with extensive genetic diversity in the largest burn centre of northeast Iran. *J Glob Antimicrob Resist*. 2017;8:60-66.
[33] Mohajeri P, Farahani A, Feizabadi MM, Ketabi H, Abiri R, Najafi F. Antimicrobial susceptibility profiling and genomic diversity of *Acinetobacter baumannii* isolates: A study in western Iran. Iran J Microbiol. 2013;5(3):195.
[34] Alavi-Moghaddam M, Dolati M, Javadi A, Saki BG, Karami-Zarandi M, Khoshnood S. Molecular detection of oxacillinase genes and typing of clinical isolates of *Acinetobacter baumannii* in Tehran, Iran. J Acute Dis. 2020;9(1):33-39.
[35] Nureen Z, Zeshan B, Qadri MMA, Ishaq M, Afzal M, Ahmed N. Phenotypic and genotypic evaluation of antibiotic resistance of *Acinetobacter baumannii* bacteria isolated from surgical intensive care unit patients in Pakistan. Jundishapur J Microbiol. 2021;14(4):e113008.
[36] Mortazavi SM, Farshadzadeh Z, Janabadi S, Musavi M, Shahi F, Moradi M, et al. Evaluating the frequency of carbapenem and aminoglycoside resistance genes among clinical isolates of *Acinetobacter baumannii* from Ahvaz, south-west Iran. New Microbe and New Infect. 2020;38:100779.
[37] Nojookambari NY, Sadredinamin M, Dehbanipour R, Ghalavand Z, Eslami G, et al. Prevalence of β-lactamase-encoding genes and molecular typing of *Acinetobacter baumannii* isolates carrying carbapenemase OXA-24 in children. Ann Clin Microbiol Antimicrob. 2021;20(1):01-08.

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