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Original Article

Dissemination of carbapenem-resistant *Acinetobacter baumannii* in patients with burn injuries

Saeed Shoja [a,*], Mojtaba Moosavian [b], Soodabeh Rostami [c], Abbas Farahani [b,d], Amir Peymani [e], Khadijeh Ahmadi [b], Nasim Ebrahimifard [b]

[a] *Infectious and Tropical Diseases Research Center, Hormozgan Health Institute, Hormozgan University of Medical Sciences, Bandar Abbas, Iran*
[b] *Infectious and Tropical Diseases Research Centre, Department of Microbiology, Faculty of Medicine, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran*
[c] *Infectious Diseases and Tropical Medicine Research Center, Isfahan University of Medical Sciences, Isfahan, Iran*
[d] *Student Research Committee, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran*
[e] *Department of Microbiology, Qazvin University of Medical Sciences, Qazvin, Iran*

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Abstract

Background: Carbapenem-resistant *Acinetobacter baumannii* has emerged as an important cause of infection in burn patients. This study aimed to characterize the antimicrobial susceptibility pattern, determine the prevalence of oxacillinase and metallo-beta-lactamase (MBL) genes, and type the *A. baumannii* isolates obtained from burn patients.

Methods: During a 1-year period, a total of 40 nonduplicated isolates of *A. baumannii* were obtained from burn patients who were hospitalized in the Taleghani Burn Hospital in Ahvaz, in the southwest of Iran. Testing for antimicrobial susceptibility was carried out by disk diffusion and E-test. To screen MBL production, a double disk synergy and MBL E-test were performed. The presence of $bla_{\text{OXA-23-like}}$, $bla_{\text{OXA-24-like}}$, $bla_{\text{OXA-51-like}}$ and $bla_{\text{OXA-58-like}}$, bla_{VIM}, bla_{IMP} and bla_{SPM}, and bla_{NDM} was sought by polymerase chain reaction (PCR). Repetitive extragenic palindromic sequence-based PCR was carried out for determination of isolates clonality.

Results: Overall, 92.5% of isolates were carbapenem-resistant. Polymyxin B, colistin, and ampicillin-sulbactam were the most effective agents *in vitro*, with a susceptibility rate of 100%, 97.5%, and 72.5%, respectively. According to the double disk synergy and E-test, 55.6% and 97.3% of isolates were MBL producers, respectively. Furthermore, 70% of isolates harbored $bla_{\text{OXA-23-like}}$ and 20% were positive for $bla_{\text{OXA-24-like}}$. However, no encoding genes were detected for bla_{VIM}, bla_{IMP} and bla_{SPM}, bla_{NDM}, and $bla_{\text{OXA-58-like}}$. Repetitive extragenic palindromic sequence-based PCR revealed that carbapenem-resistant isolates belonged to four clones, including A, B, C, and D; the predominant clones were B and C.

Conclusion: The rate of carbapenem resistance was high, and it appeared that $bla_{\text{OXA-23-like}}$ and $bla_{\text{OXA-24-like}}$ contributed to the carbapenem resistance of *A. baumannii* isolates. This result suggests that the two predominant clones of *A. baumannii* were spread among burn patients. In order to prevent future dissemination of resistant isolates among burn patients, an effective infection control plan is necessary.

Keywords: Acinetobacter baumannii; burns; carbapenem- resistant

Conflicts of interest: The authors declare that they have no conflicts of interest related to the subject matter or materials discussed in this article.

* Corresponding author: Dr. Saeed Shoja, Infectious and Tropical Diseases Research Center, Hormozgan Health Institute, Hormozgan University of Medical Sciences, Shahid Mohammadi Hospital, Jomhuri Blvd, Bandar Abbas, P. O. Box: 7919693116, Iran.

E-mail address: Shojasaeed@gmail.com (S. Shoja).

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1. Introduction

In patients with burn injuries, infection is the most common cause of death.[1] This is due to the fact that burn patients are susceptible and have a high risk for infection.[2] *Acinetobacter baumannii* has become an important and troubling pathogen in hospitals worldwide, and nosocomial outbreaks have been reported due to multidrug-resistant strains, particularly in the intensive care units and burn units.[3–6] At patient burn sites, it is difficult to distinguish between colonization and infection with *A. baumannii.* In fact, this organism is one of the very important causes of infection in these patients, and is isolated with increasing frequency from burn patients.[7,8] Furthermore, outbreaks of *A. baumannii* are common in burn units, and the affected patients show a reservoir for this organism.[9] Most typically, carbapenems are the drugs of choice for treatment of *A. baumannii* infections, but the increased clinical use of these drugs has led to the progress of resistant strains.[10] Carbapenem-resistant *A. baumannii* has become a problematic nosocomial pathogen globally.[11] In the case of *A. baumannii,* different mechanisms, including change of penicillin binding protein, loss of outer membrane protein, efflux pump, and carbapenem-hydrolyzing enzymes are involved in carbapenem resistance.[3] It should be noted that carbapenem-inactivating enzymes belonging to the Class D carbapenemase (OXA enzymes) are the main mechanism of resistance in *A. baumannii* worldwide.[12] However, metallo-beta-lactamase (MBL) production is less common in this organism.[11] For effective control and prevention of *A. baumannii* in hospitals, molecular typing is necessary to determine genomic fingerprinting.[13] According to previous studies of repetitive extragenic palindromic sequence-based polymerase chain reaction (REP-PCR), in most cases, one or more epidemic clones of *A. baumannii* are circulating in the studied hospital or burn units.[8,14,15] In this study, we decided to investigate antimicrobial resistance pattern, the prevalence of oxacillinase and MBL, and typing of *A. baumannii* isolates from burn patients.

2. Methods

2.1. Bacterial collection and phenotypic tests

During the period from August 2011 to August 2012, a total of 40 nonrepetitive *A. baumannii* isolates (colonization or infection) were obtained from burned wound samples of 40 patients [31 (77.5%) female and 9 (22.5%) male] who were hospitalized with burn injuries in Taleghani Hospital in Ahvaz, in the southwest of Iran. Of the noted isolates, 18 were recovered from wounds, 18 were recovered from skin biopsy, and four isolates were recovered from blood samples. *A. baumannii* initially was identified and characterized by biochemical tests such as Triple Sugar Iron (TSI) agar medium and oxidase test, API 20 NE kit (version 6.0, bioMerieux, Marcy L Etoile, France), and growth in 42°C, to ensure that the isolates were *A. baumannii*.[16] Genomic DNA for PCR was extracted by proteinase K, and sodium dodecyl sulfate method as previously described.[17] Presumptive *A. baumannii* isolates were confirmed to the species level by PCR amplification for $bla_{\text{OXA-51-like}}$.[18]

2.2. Antimicrobial susceptibility testing

Testing of susceptibility to antimicrobial agents was performed by disk diffusion in Mueller-Hinton agar and was interpreted according to guidelines of the Clinical Laboratory Standard Institute (CLSI 2011).[19] Bacterial suspension at a turbidity equivalent to a 0.5-McFarland standard was inoculated on a Mueller-Hinton agar plate (Merck, Frankfurt, Germany). The antimicrobial agents tested included: imipenem 10μg, meropenem 10μg, polymyxin B 300 units, gentamicin 10μg, ceftriaxone 30μg, colistin 10μg, piperacillin 100μg, piperacillin-tazobactam 100/10μg, cefepime 30μg, tobramycin 10μg, tigecycline 15μg, amikacin 30μg, tetracycline 30μg, ciprofloxacin 5μg, trimethoprim-sulfamethoxazole 1.25/23.75μg, ceftazidime 30μg, rifampin 5μg, aztreonam 30μg, and ampicillin-sulbactam 10/10μg (MAST, Group Ltd., Merseyside, UK).[20] Reference strains, including *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853 were used for quality control testing.[19] Minimum inhibitory concentrations (MICs) of imipenem, meropenem, colistin, and tigecycline were determined by the E-test strips (Liofilchem, Roseto degli Abruzzi, Italy) according to the manufacturer's instructions. The European Committee on Antimicrobial Susceptibility Testing criteria for the technical note on tigecycline in *Acinetobacter* spp. and other Gram-negative bacteria, was utilized for the tigecycline breakpoint.[21]

2.3. Phenotypic screening of MBL production

For the identification of MBL production, a double disk synergy (DDS) was performed. Briefly, bacterial suspension at a turbidity equivalent to 0.5 McFarland standards was inoculated on a Mueller-Hinton agar plate (Merck). Thereafter, two 10μg of imipenem disks were placed on the surface of an inoculated plate. A 0.5M EDTA solution was prepared and 10μL was added to one of the imipenem disks to obtain a desired concentration of 750μg. After overnight incubation, the inhibition zone of imipenem and imipenem plus EDTA disks were compared, and the enhancement in the inhibition zone of imipenem plus EDTA disk was considered as positive for MBL production.[22,23] E-test MBL strips (Liofilchem, Italy) were used to evaluate MBL production. The results were interpreted according to the manufacturer's instructions.

2.4. PCR amplification of OXA-type genes

A multiplex PCR analysis was performed to determine the presence of OXA-type carbapenemase, $bla_{\text{OXA-23-like}}$, $bla_{\text{OXA-24-like}}$ and $bla_{\text{OXA-58-like}}$. Amplification reaction was carried out in a final volume of 25μL with 1X PCR buffer, 1 U Taq polymerase, 2mM $MgCl_2$, 200μM of Deoxynucleotide triphosphate (dNTP) (Sinaclon, Tehran, Iran), 0.2μM of

each primer (TAG, Copenhagen A/S Denmark), and 1μL of template DNA. PCR conditions were programmed in a Mastercycler (Eppendorf, Hamburg, Germany) as follows: initial denaturation at 94°C for 5 minutes, followed by 30 cycles at 94°C for 30 seconds, 53°C for 40 seconds, and 72°C for 50 seconds, and a final extension at 72°C for 6 minutes. PCR products were separated with electrophoresis on 1.5% agarose gel (Sinaclon), and after staining with ethidium bromide, visualized under UV gel documentation system. *A. baumannii* reference strains, including NCTC 13304, NCTC 13302, and NCTC 13305 were used as positive control for $bla_{\text{OXA-23-like}}$, $bla_{\text{OXA-24-like}}$ and $bla_{\text{OXA-58-like}}$, respectively. A negative control was included in each PCR reaction, containing all components except the DNA template, which was replaced by distilled water.[24] For each gene, one amplicon was sequenced (Bioneer, Daejeon, South Korea).

2.5. Detection of MBL genes

To detect MBL genes, bla_{VIM} and bla_{IMP} were sought by PCR with specific primers that are listed in Table 1, with a protocol used in two previous studies.[20,25] Two clinical isolates of *P. aeruginosa*, harbor bla_{IMP} and bla_{VIM}, were sequenced by (Bioneer) and used as positive control for identification of these genes. DNA from a bla_{SPM} positive *P. aeruginosa* was purchased from the Pasteur Institute of Iran and used as positive control for bla_{SPM} in each PCR reaction. In addition, bla_{NDM} was sought by PCR with standard conditions, but we do not have any positive control for this gene because the first report of New Delhi MBL-1 gene found in 2013 by Shahcheraghi et al[26] and our study was conducted from August 2011 to August 2012. Prior to 2012, the authors could not find any positive control for bla_{NDM} in Iran.

2.6. REP-PCR

To determine clonal relatedness, REP-PCR was performed on all isolates with specific primers previously described,[27] that are listed in Table 1. Amplification conditions were performed using previously established protocols with some modifications.[28] Each reaction mixture for PCR contained 1X PCR buffer, 3.5mM of $MgCl_2$, 300μM of Deoxynucleotide triphosphate (dNTP), 3% Dimethyl sulfoxide (DMSO) (Sinaclon), 0.5μM of each primer (TAG Copenhagen A/S, Denmark), and 1 U of Taq polymerase and 1μL of genomic DNA in a final volume of 25μL. PCR conditions included: 94°C for 10 minutes; 30 cycles of 94°C for 1 minute, annealing temperatures 45°C for 1 minute, 72°C for 2 minutes, and 72°C for 16 minutes. Amplification products were separated by electrophoresis on 1.2% agarose gel (Sinaclon); after staining with ethidium bromide, they were observed in a UV gel documentation system, then they were photographed and compared together by visual inspection.[28] All fingerprints were observed by one observer, and fingerprints were interpreted according to previous studies.[27,29]

2.7. Statistical analysis

The results were analyzed using the IBM SPSS (Version.20 IBM, Chicago, IL, USA) version 16.0 to obtain frequencies and comparison among clones.

3. Results

3.1. Bacterial collection

Forty isolates of *A. baumannii* were studied, of which 20 (50%) were from the women's ward, 13 (32.5%) were from the intensive care unit, six (15%) were from the men's ward, and

Table 1
Sequences of primers used in this study.

Primer names	Sequence (5′-3′)	Amplicon size (bp)	Reference
$bla_{\text{OXA-51- like}}$ - F	TAATGCTTTGATCGGCCTTG	353	(14)
$bla_{\text{OXA-51- like}}$ -R	TGGATTGCACTTCATCTTGG		
$bla_{\text{OXA-23- like}}$ -F	GATCGGATTGGAGAACCAGA	501	
$bla_{\text{OXA-23- like}}$ -R	ATTTCTGACCGCATTTCCAT		
$bla_{\text{OXA-24- like}}$ -F	GGTTAGTTGGCCCCCTTAAA	249	
$bla_{\text{OXA-24- like}}$ -R	AGTTGAGCGAAAAGGGGATT		
$bla_{\text{OXA-58- like}}$ F	AAGTATTGGGGCTTGTGCTG	599	
$bla_{\text{OXA-58- like}}$ -R	CCCCTCTGCGCTCTACATAC		
bla_{IMP}-F	TCGTTTGAAGAAGTTAACGG	568	(18)
bla_{IMP}-R	ATGTAAGTTTCAAGAGTGATGC		
bla_{VIM}-F	GGTGTTTGGTCGCATATCGCAA	502	
bla_{VIM}-R	ATTCAGCCAGATCGGCATCGGC		
bla_{NDM}- F	GGTTTGGCGATCTGGTTTTC	624	(15)
bla_{NDM}-R	CGGAATGGCTCATCACGATC		
bla_{SPM}-F	AAAATCTGGGTACGCAAACG	271	
bla_{SPM}-R	ACATTATCCGCTGGAACAGG		
REP-1	IIIGCGCCGICATCAGGC		(13)
REP-2	ACGTCTTATCAGGCCTAC		

Table 2
Results of antimicrobial susceptibility test by disk diffusion.

Different antibiotics	Resistant (%)	Intermediate (%)	Sensitive (%)
Aztreonam	100	—	—
Ciprofloxacin	97.5%	—	2.5
Meropenem	92.5	—	7.5
Ceftazidime	92.5	—	7.5
Cefepime	92.5	—	7.5
Piperacillin	92.5	—	7.5
Piperacillin-tazobactam	92.5	—	7.5
Ceftriaxone	92.5	—	7.5
Rifampin	92.5	—	7.5
Tobramycin	87.5	—	12.5
Gentamicin	85	2.5	12.5
Amikacin	80	7.5	12.5
Imipenem	67.5	—	32.5
Tetracycline	57.5	12.5	30
Ampicillin sulbactam	17.5	10	72.5
Polymyxin	0	—	100
Colistin	0	—	100

one (2.5%) from the repair ward. All isolates were positive for $bla_{\text{OXA-51-like}}$. Partial sequencing of the PCR product confirmed the presence of $bla_{\text{OXA-51-like}}$. GenBank Accession number: HG937621.

3.2. Antimicrobial susceptibility test

The results of antimicrobial susceptibility testing of the 40 isolates are shown in Table 2. According to the findings, colistin and polymyxin B were the most effective antimicrobials *in vitro* against the majority of isolates of *A. baumannii*. Moreover, determination of MIC by the E-test showed that 92.5% of isolates were resistant to imipenem. The lowest level of MIC for imipenem was 0.38μg/mL, and the highest level was 256μg/mL. Most of the imipenem-resistant isolates (30%) had an imipenem MIC = 256μg/mL. The meropenem MIC was similar to those for imipenem, and 92.5% of isolates were resistant to meropenem with the lowest value of MIC = 0.19μg/mL, and the highest value was 32μg/mL. Most of the meropenem-resistant isolates (85%) had a meropenem MIC = 32μg/mL. Furthermore, based on E-testing, 97.5% of isolates were susceptible to colistin and one isolate (2.5%) had MIC = 3μg/mL. In the CLSI guideline, there is no breakpoint for colistin MIC = 3μg/mL and we could not categorize this isolate as intermediate. No colistin-resistant isolates were detected. Tigecycline MIC showed that 55% of the isolates were sensitive, 10% intermediate, and 35% were resistant. The obvious difference was seen for imipenem MIC compared to the disk diffusion results. When the E-test was undertaken, 92.5% of isolates were resistant to imipenem, whereas imipenem disk identified 67.5% of isolates as resistant. For meropenem, the result of the E-test was consistent with the disk diffusion method and 92.5% of isolates were resistant by two methods.

3.3. Detection of OXA-type carbapenemase

PCR experiments with primers specific for $bla_{\text{OXA-23-like}}$, $bla_{\text{OXA-24-like}}$, and $bla_{\text{OXA-58-like}}$ were performed on all 40 isolates. $Bla_{\text{OXA-23-like}}$ was positive in 70% of isolates (28/40 isolates). Eight of the 40 isolates (20%) were $bla_{\text{OXA-24-like}}$ positive (Fig. 1). No isolates were positive for $bla_{\text{OXA-58-like}}$. Co-existence of $bla_{\text{OXA-23-like}}$ and $bla_{\text{OXA-24-like}}$ was not detected. Interestingly, one isolate was carbapenem-resistant, but only had $bla_{\text{OXA-51-like}}$ and was negative for $bla_{\text{OXA-23-like}}$, $bla_{\text{OXA-24-like}}$, and $bla_{\text{OXA-58-like}}$. Additionally, carbapenem susceptible isolates were negative for $bla_{\text{OXA-23-like}}$, $bla_{\text{OXA-24-like}}$, and $bla_{\text{OXA-58-like}}$.

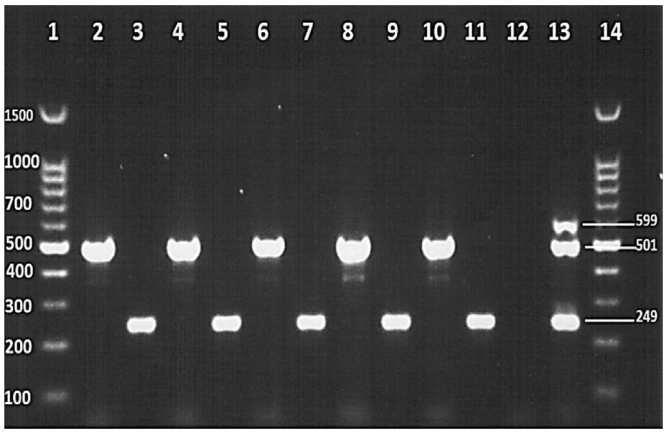

Fig. 1. Results of multiplex polymerase chain reaction (PCR). Lanes 1 and 14: 100bp DNA ladder. Lanes 2, 4, 6, 8, and 10: isolates with $bla_{\text{OXA-23-like}}$ in 501bp. Lanes 3, 5, 7, 9, and 11: isolates with $bla_{\text{OXA-24-like}}$ in 249bp, Lane 12: negative control (distilled water), and Lane 13: positive control (*Acinetobacter baumannii* NCTC 13304, NCTC 13302, NCTC 13305) used for $bla_{\text{OXA-23-like}}$, $bla_{\text{OXA-24-like}}$ and $bla_{\text{OXA-58-like}}$, respectively.

3.4. MBL

To study the MBL production, carbapenem-resistant isolates were evaluated by DDS and E-test. The results obtained with DDS and the E-test indicated that 55.6% and 97.3% of carbapenem-resistant *A. baumannii* isolates produce MBL, respectively. No amplicon was obtained with primer targeting

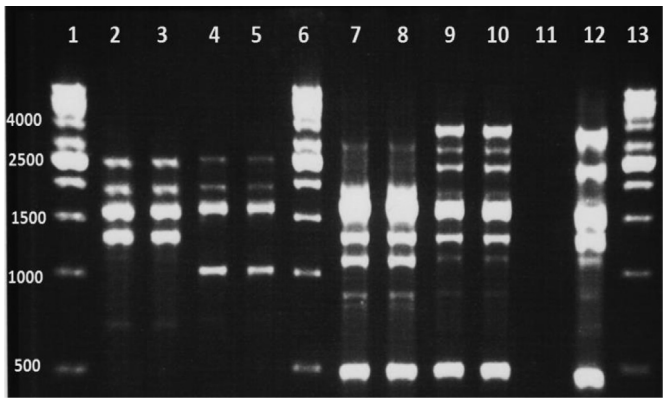

Fig. 2. The result of repetitive extragenic palindromic sequence-based polymerase chain reaction (REP-PCR) on carbapenem-resistant isolates. Double fingerprints have been shown for each isolate; Lanes 1, 6, 13 1 kb DNA ladder. Lanes 2, 3 (Clone A), Lanes 4, 5 (Clone B), Lanes 7, 8 (Clone C), Lanes 9, 10 (Clone D), Lane 11 negative control, and Lane 12, *Acinetobacter baumannii* NCTC 12156 (ATCC 19606).

the bla_{IMP}, bla_{VIM}, and bla_{SPM}. Despite standard PCR conditions, all isolates were negative for the bla_{NDM} gene, and we were unable to interpret this negative result.

3.5. REP-PCR results

REP-PCR was carried out for evaluation of clonality of the isolates. Analysis of the REP-PCR results demonstrated that the isolates belonged to four genotypes (Fig. 2). Among those genotypes, 12.5% (5 of 40 isolates) belonged to genotype A, 32.5% (13 of 40 isolates) belonged to genotype B, 32.5% (13 of 40 isolates) belonged to genotype C, and 22.5% (9 of 40 isolates) belonged to genotype D.

3.6. Nucleotide sequence accession number

The nucleotide sequences obtained in this study were submitted to the GenBank nucleotide sequence database under the accession numbers: HG937619 for $bla_{OXA\text{-}23\text{-}like}$, HG937620 for $bla_{OXA\text{-}24\text{-}like}$, and HG937621 for $bla_{OXA\text{-}51\text{-}like}$.

4. Discussion

A. baumannii remains one of the most common nosocomial pathogens and frequent causes of outbreaks in burn wound infections.[2,4,23] Unfortunately, most of the isolates are resistant to antimicrobial agents. β-Lactamases are microbial enzymes that hydrolytically inactivate β-lactam antibiotics and these enzymes, due to the emergence of pathogenic bacteria resistant to β-Lactamases are microbial enzymes that hydrolytically inactivate β-lactam antibiotics and these enzymes can cause resistance to β-lactam antibiotics, including carbapenems, penicillins and cephalosporins. Based on Ambler classification, these enzymes are belonging to four molecular classes: A, B, C, and D.[30,31] Class D serine carbapenemases belong to the OXA (oxacillinase) family, and are most prevalent in *A. baumannii* isolates. The ability of OXA enzymes to hydrolyze the isoxazolyl penicillin oxacillin is faster than classical penicillins (benzylpenicillin).[32] Provisionally, they can be grouped into six subclasses; however, today, based on amino acid sequence analysis, Class D carbapenemases are reclassified into 12 subgroups: OXA-23, OXA-24/40, OXA-48, OXA-51, OXA-58, OXA-134a, OXA-143, OXA-211, OXA-213, OXA-214, OXA-229, and OXA-235. In addition, they include 232 enzymes with few variants and the same carbapenemase activity.[31,33] In burn patients, the resistance rate of *A. baumannii* against carbapenems has been reported from 33.33% to 98%.[15,23,34,35] In our study, according to susceptibility test results, a considerable proportion of *A. baumannii* isolates (92.5%) were resistant to carbapenems, and this finding suggests that carbapenems are not appropriate for the treatment of *A. baumannii* infections in burn patients hospitalized at Taleghani hospital. In the present study, there was a difference between imipenem E-test results and imipenem disk diffusion. This indicated that imipenem disk could not detect 25% of resistant isolates and use of this disk for antimicrobial susceptibility could lead to false susceptible results. In contrast to imipenem, meropenem disk was the same as using the E-test, and detected all resistant isolates. It seems that meropenem disk is more appropriate than imipenem disk for identification of resistant isolates. Among the tested antibiotics, colistin and polymyxin B were the most effective agents *in vitro*, and 97.5% and 100% of isolates were susceptible, respectively. While colistin-resistant isolates have been reported worldwide,[36] no colistin-resistant isolates were obtained from burn patients. Ampicillin-sulbactam is another treatment option, and 72.5% of the isolates were susceptible to this drug *in vitro*. It has been proposed that ampicillin-sulbactam is an effective and inexpensive treatment for nosocomial infection due to ampicillin-sulbactam susceptible *A. baumannii*.[37] However, it has been suggested that OXA carbapenemase plays an important role in carbapenem resistance in *A. baumannii*.[15]

Outbreaks of carbapenem-resistant *A. baumannii* carrying $bla_{OXA\text{-}23\text{-}like}$ were discovered in different countries,[38–41] and the range of $bla_{OXA\text{-}23\text{-}like}$ has been reported from 31% to 100% worldwide.[11,13,40,42–46] In our study, the vast majority of isolates (70%) were positive for $bla_{OXA\text{-}23\text{-}like}$. Moreover, we found that 20% of isolates harbor $bla_{OXA\text{-}24\text{-}like}$, and this rate is inconsistent with previous studies globally, which reported the rate of $bla_{OXA\text{-}24\text{-}like}$ from 0% to 85.43%.[11,42,45–48] While several studies demonstrated the $bla_{OXA\text{-}58\text{-}like}$ gene in *A. baumannii*, it has been detected from 2% to 84.92% in different parts of the world,[13,42–44,46,48] In contrast, however, in our study, no isolate was positive for the $bla_{OXA\text{-}58\text{-}like}$ gene. Based on the DDS and E-test results, 55.6% and 97.3% of isolates were MBL producers. In spite of phenotypic test, no amplicon was detected for studied MBL genes. While MBL genes, bla_{IMP}, bla_{VIM}, bla_{NDM}, and bla_{SPM} have been reported sporadically in *A. baumannii*,[49–53] we could not detect any of these genes among our isolates. Similar to our results, different studies have been reported that *A. baumannii* isolates were MBL producers by phenotypic tests, but no MBL encoding genes were detected.[13,45,47,54–56] One probable reason for these positive MBLs may be due to the bactericidal activity of EDTA, which may lead to an increased inhibition zone, but this increase is false positive and not associated with true MBL production.[57] By contrast, these results may be true positive associated with another MBL gene. Other studies have indicated that false-negative results often occurred, in particular when a low level of resistance is observed and phenotypic detection tests are time-consuming.[58] Furthermore, one isolate of *A. baumannii* was carbapenem -resistant, but it had only the $bla_{OXA\text{-}51\text{-}like}$ gene, and other genes were not detected. The mechanism of carbapenem-resistance in this isolate may be in relation to the modification of penicillin binding proteins, loss of porins and decreased permeability, AmpC stable derepression, overexpression of efflux pump, or insertion of IS*Aba1* in upstream of $bla_{OXA\text{-}51\text{-}like}$.[14,59–61] In addition, further research is needed to identify different carbapenem-resistant mechanisms in this isolate. Since we found only $bla_{OXA\text{-}23\text{-}like}$ and $bla_{OXA\text{-}24\text{-}like}$, we therefore suppose that carbapenem resistance in our isolates is mostly due to these genes. REP-PCR is a useful tool for typing of *A. baumannii* and provides

information about the epidemiology of this bacterium in the hospitals. In order to investigate the clonality of *A. baumannii* in different wards, REP-PCR was done and analysis of REP-PCR revealed that four clones (A, B, C, and D) are circulating in different wards. Consistent with our study, in a study on burn isolates of *A. baumannii*, Mahdian et al[62] reported that 11 clones were detected, of which four occurred most frequently. In the present study, Clones B and C were the most epidemic ones and it seems these clones were possibly interward spread and caused infection in burn patients.

In conclusion, we observed that the rate of carbapenem-resistant *A. baumannii* was high among burn patients. In addition, the most effective drugs against *A. baumannii in vitro* were colistin and polymyxin B. Since resistant isolates only had $bla_{\text{OXA-23-like}}$ and $bla_{\text{OXA-24-like}}$, it seems that carbapenem resistance in our isolates is more associated with these genes. Based on the information obtained from REP-PCR, two clones are more prevalent among burn patients. Information about clonality and type of resistance is useful in epidemiology of *A. baumannii* and further research is needed on burn patients with more isolates. We conclude that infection control policies and programs will be considered in order to control the dissemination of *A. baumannii* in burn units. Monitoring and management of *A. baumannii* can lead to control transmission of this organism in burn units.

4.1. Limitations

In this study, the results had certain limitations. The major limitations were the absence of a positive control for the NDM gene, the short period of our study, sample size, single center, and inadequate demographic data about patients from the different wards. Additionally, MIC for polymyxin B was not done.

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