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Characterization of Oxacillinase and Metallo-β-Lactamas Genes and Molecular Typing of Clinical Isolates of *Acinetobacter baumannii* in Ahvaz, South-West of Iran

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Abstract

Background: Carbapenem resistant *Acinetobacter baumannii* is an important nosocomial pathogen associated with a variety of infections.
Objectives: The current study aimed to characterize the antimicrobial susceptibility, analyze the prevalence of oxacillinase and metallo-β-lactamase (MBL) genes and molecular typing of clinical isolates of *A. baumannii*.
Materials and Methods: A total of 124 non-repetitive isolates of *A. baumannii* were collected from various clinical specimens in two teaching hospitals in Ahvaz, south-west of Iran. Antimicrobial susceptibility test was carried out by disk diffusion method. The minimum inhibitory concentrations (MICs) of imipenem, meropenem, colistin and tigecycline were determined using E-test. To screen for MBL production, double disk synergy (DDs) test and MBL E-test were performed. The presence of $bla_{\text{OXA-23-like}}$, $bla_{\text{OXA-24-like}}$, $bla_{\text{OXA-51-like}}$, $bla_{\text{OXA-58-like}}$, bla_{VIM}, bla_{IMP} and bla_{SPM} genes was assessed by polymerase chain reaction (PCR). To identify clonal relatedness, all isolates were subjected to repetitive sequence-based PCR (rep-PCR)
Results: Based on disk diffusion results, the highest rate of resistance was observed in rifampin (96.8%). Colistin and polymyxin-B were the most effective agents in vitro. According to the MIC results, the rate of resistance to imipenem, meropenem, colistin and tigecycline were 78.2%, 73.4%, 0.8% and 0, respectively. Metallo-β-lactamase production was positive in 42.3% and 79.4% of the isolates by DDs test and E-test, respectively. All isolates (100%) carried $bla_{\text{OXA-51-like}}$ gene. According to the results of multiplex PCR, $bla_{\text{OXA-23-like}}$ and $bla_{\text{OXA-24-like}}$ genes were detected in 85.6% and 6.2% of carbapenem resistant isolates, respectively. No $bla_{\text{OXA-58- like}}$, bla_{VIM}, bla_{IMP} and bla_{SPM} were detected. By rep-PCR, carbapenem resistant isolates were separated into six genotypes (A to F). Genotype A (30.9%) was the most prevalent (P value < 0.001). Genotypes B and C were found in 28.9% and 26.8% of the isolates, respectively.
Conclusions: The rate of carbapenem resistant *A. baumannii* isolates were high in this study. Since, $bla_{\text{OXA-58-like}}$ or MBL genes were not detected, it seems that resistance to carbapenems is related to $bla_{\text{OXA-23-like}}$ and $bla_{\text{OXA-24-like}}$. Moreover, $bla_{\text{OXA-23-like}}$ was the most prevalent oxacillinase (OXA) gene. Most of the isolates belonged to one of the four dominant genotypes indicating clonal dissemination in the hospitals under study. In order to control the spread of carbapenem-resistant *A. baumannii*, infection- control strategies are needed.

Keywords: Acinetobacter baumannii, Carbapenems, Oxacillinase, Typing

1. Background

In recent years, *Acinetobacter baumannii* has emerged as an important pathogen in nosocomial infections and especially infects critically ill patients admitted to the intensive care units (ICUs) (1, 2). Septicemia, pneumonia, urinary tract infection, wound infection and meningitis are among the infections caused by this pathogen (3). In the hospital environment, resistance of *A. baumannii* to antimicrobial agents raises concerns (4). Carbapenem resistant *A. baumannii* are great concerns for physicians because carbapenems are common choice to treat infections caused by this pathogen (4, 5). In addition, therapeutic efficacy of carbapenems is limited due to spread of carbapenem resistant *A. baumannii* (4, 6). Carbapenem resistance is now observed worldwide in *A. baumannii* and these isolates are usually resistant to all classes of antimicrobial agents. A plenty of outbreak due to carbapenem resistant *A. baumannii* are reported from different countries and this situation had a worrying trend (4).

Carbapenem resistance in *A. baumannii* is mediated by combined different mechanisms including: reduced per-

meability, changes in penicillin binding protein, AmpC stable derepression, efflux pumps and mostly by production of oxacillinases (OXAs) and less common by metallo-β-lactamase (MBLs) genes (7-9). Clonal transmission of drug resistant *A. baumannii* is reported globally (10). Inter hospital transmission of carbapenem resistant *A. baumannii* is demonstrated (4). It is well documented that in the nosocomial outbreaks, in most of the cases, one or two epidemic clones are involved in a given hospital (4). For epidemiological purposes and to control the spread of resistant iaolates, rapid diferrentiation of epidemic strains from the numerous incidental strains is necessary (11, 12). Molecular typing such as repetitive sequence-based polymerase chain reaction (rep-PCR) is required to determine the clonal relatedness of *A. baumannii* (12). The rep-PCR is beneficial for the molecular typing of *A. baumannii* (11).

2. Objectives

The current study aimed to determine the antimicrobial susceptibility pattern, prevalence and types of oxacillinase and metallo-β-lactamase genes and molecular typing by rep-PCR in the clinical isolates of *A. baumannii*.

3. Materials and Methods

3.1. Collection and Identification of Acinetobacter baumannii Isolates

From July 2011 to January 2013, a total of 124 non-duplicated *A. baumannii* isolates were collected from various clinical specimens in two teaching hospitals in Ahvaz, south-est of Iran. Bacterial isolates were initially identified as *A. baumannii* by biochemical tests (13). Suspected isolates were confirmed by PCR to identify $bla_{\text{OXA-51-like}}$ gene with specific primers (listed in Table 1) to amplify a 353 base pair sequence (14). DNA template for PCR was obtained by boiling method (15). Each reaction was carried out in a final volume of 25 μL containing 1x PCR buffer, 1 U Taq polymerase, 1.5 mM $MgCl_2$, 200 μM of dNTP (SinaClon, Iran), 10 pmol of each primer (Eurofins MWG Operon, Germany) and 1 μL of the extracted DNA. PCR conditions were programmed in Mastercycler Eppendorf (Eppendorf, Germany) as follows: Initial denaturation at 94°C for 3 minutes; 35 cycles of 94°C for 45 seconds, annealing 57°C for 45 seconds, extension 72°C for 1 minute and final extension 72°C for 5 minutes. PCR products were separated on 1.5% agarose gel (SinaClon, Iran) by electrophoresis, stained with ethidium bromide (SinaClon, Iran) and then visualized under UV illumination (Syngene GeneGenius gel documentation system). *Acinetobacter baumannii* ATCC 19606 was used as positive control (14).

3.2. Antimicrobial Susceptibility Testing

Antimicrobial susceptibility testing of all isolates was performed using Kirby-Bauer method according to the clinical and laboratory standard institute (CLSI, 2011) guidelines. The following antimicrobial agents were tested: imipenem 10 μg, meropenem 10 μg, polymyxin-B 300 U, gentamicin 10 μg, ceftriaxone 30 μg, colistin 10 μg, piperacillin 100 μg, piperacillin-tazobactam 100/10 μg, cefepime 30 μg, tobramycin 10 μg, amikacin 30 μg, tetracycline 30 μg, ciprofloxacin 5 μg, trimethoprim-sulfamethoxazole 1.25/23.75 μg, ceftazidime 30 μg, rifampin 5 μg, tigecycline 15 μg, aztreonam 30 μg and ampicillin-sulbactam (10/10 μg), (MAST, Group Ltd, Merseyside, UK). *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853 were used as control strains (20). Minimum inhibitory concentration (MIC) of imipenem, meropenem, colistin and tigecycline were determined by E-test strips (Liofilchem, Italy). Measures were obtained according to the CLSI guidelines. The US food and rug drug administration-approved criteria and Jones criteria were used for Enterobacteriacea and tigecycline breakpoint, respectively (21, 22).

3.3. Screening the metallo-β-Lactamase Producing Isolates

All isolates were screened for MBL production by an imipenem-EDTA (ethylene diamine tetra-acetic acid) double disk synergy and E-test MBL. Briefly, an overnight culture suspension of each sample was adjusted to a turbidity equivalent to 0.5 McFarland and inoculated on the surface of a Mueller-Hinton agar plate. Two 10 μg of imipenem disk (MAST, Group Ltd, Merseyside, UK) were placed on the plate 10 mm apart from edge to edge. Then 10 μL of 0.5 M EDTA solution (SinaClon, Iran) was directly added to one of them to obtain the desired concentration (750 μg). The plates were incubated at 35°C for 18 hours. After incubation, inhibition zones of the imipenem and imipenem-EDTA disks were measured and compared. If enlarged zone with imipenem-EDTA was 7 mm greater than the imipenem disk alone, it was considered as MBL positive revealing the inactivation of metallo-β-lactamase (class B) activity by EDTA (23, 24). Also an E-test MBL strip containing a double sided seven dilution range of imipenem (4 to 256 μg/mL) and imipenem (1 to 64 μg/mL) in combination with a fixed concentration of EDTA (Liofilchem, Italy) was used. The results were interpreted according to the manufacturer's instruction.

3.4. PCR Amplification of OXA and Metallo-β-Lactamase Genes

Multiplex PCR was performed to detect $bla_{\text{OXA-23-like}}$, $bla_{\text{OXA-24-like}}$ and $bla_{\text{OXA-58-like}}$ using specific primers as previously described (16). DNA template was obtained by boiling method (15). Each PCR reaction was performed in a final volume of 25 μL with 1x PCR buffer, 1 U Taq polymerase, 2 mM $MgCl_2$, 200 μM of dNTP (SinaClon, Iran), 0.2 μM of each primer (TAG, Copenhagen A/S Denmark) and 1 μL of template DNA. PCR conditions were programmed

in Mastercycler Eppendorf (Eppendorf, 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 final extension at 72°C for 6 minutes. PCR products were separated by electrophoresis on 1.5% agarose gel (SinaClon, Iran) and after staining with ethidium bromide, visualized under UV gel documentation system *Acinetobacter baumannii* reference strains including: NCTC 13304, NCTC 13302, 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 (16). For each gene, one amplicon was sequenced (Bioneer, South Korea) bla_{VIM}, bla_{IMP} and bla_{SPM} were sought by singleplex PCR and primers previously described (17, 18). Two clinical isolates of *P. aeruginosa* harbored bla_{IMP} and bla_{VIM} were sequenced using automated sequence analyzer (Bioneer, South Korea) and used as positive control to identify the genes. The DNA of bla_{SPM}-positive *P. aeruginosa* was purchased from Pasteur Institute of Iran and used as positive control in PCR reactions.

3.5. The rep-PCR

To investigate genotyping and identification of various clones, all isolates were subjected to rep-PCR with emphasis on carbapenem resistant isolates. Specific primers were used according to the previously described Bou et al. protocol (19). Template for PCR was extracted by phenol-chloroform method. Each reaction mixture was done in the total volume of 25 μL with 1x PCR buffer, 3.5 mM of $MgCl_2$, 300 μM of dNTP, 3% dimethyl sulfoxide (DMSO) (SinaClon, Iran), 0.5 μM of each primer (TAG, Copenhagen A/S, Denmark), and 1U of Taq polymerase and 1 μL of genomic DNA. Amplification conditions were as follows: 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. Products were separated by electrophoresis on 1.2% agarose gel (SinaClon, Iran); after staining with ethidium bromide, they were visualized under UV gel documentation system; then they were photographed and compared together by visual inspection (19). All fingerprints were observed by one observer. Snelling et al. protocol was used for classified various clones (11).

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 $bla_{\text{OXA-23-like}}$, HG937620 for $bla_{\text{OXA-24-like}}$ and HG937621 for $bla_{\text{OXA-51-like}}$.

3.7. Statistical Analysis

The results were analyzed using the SPSS version 16 to obtain frequencies and comparison among clones. Non-parametric chi-square test was used. A P value < 0.05 was considered statistically significant.

4. Results

4.1. Bacterial Isolates

Totally, 124 single-patient isolates were recovered. All isolates were positive for $bla_{\text{OXA-51-like}}$ and identified as *A. baumannii*. The rate of isolates from each ward and specimen are shown in Table 2.

4.2. Antimicrobial Susceptibility

The results of antimicrobial susceptibility test by disk diffusion method are shown in Table 3. Colistin and polymyxin-B were the most active agents against the tested isolates. According to the results of MICs by E-test, among 124 isolates, 97 (78.2%) were resistant to imipenem. However, meropenem resistance was observed in 91 (73.4%) of the studied isolates. Only one isolate (0.8%) was resistant to colistin and 123 isolates (98.2%) were susceptible to this antibiotic. No tigecycline resistant isolate was observed and 99 (79.8%) and 25 (20.2%) isolates were sensitive and intermediate to this antibiotic, respectively.

4.3. PCR Amplification of OXA Genes

Multiplex PCR analysis identified $bla_{\text{OXA-23-like}}$ in 83 (85.6%) of carbapenem resistant isolates. Six isolates (6.2%) contained $bla_{\text{OXA-24-like}}$ (Figure 1). All isolates were negative for $bla_{\text{OXA-58-like}}$. Eight isolates were carbapenem resistant but had only $bla_{\text{OXA-51-like}}$ and other studied isolates were negative in the other studied genes. No amplification products were obtained in carbapenem susceptible isolates.

4.4. Metallo-β-Lactamase E-Test

To study the MBL production, carbapenem resistant isolates were evaluated by double disk synergy (DDS) test and E-test MBL strips. Of the 97 carbapenem resistant isolates, 41 (42.3%) exhibited a > 7 mm inhibitory zone and were categorized as MBL producer. However 56 isolates (57.7%) were negative for MBL production. Results of MBL E-test showed that among 97 carbapenem resistant isolates, 77 (79.4%) were positive for metallo-β-lactamase production and 20 isolates (20.6%) were negative (Figure 2). PCR did not detect metallo-β-lactamase genes, including bla_{VIM}, bla_{IMP} and bla_{SPM}, among the studied isolates.

4.5. REP PCR

Among carbapenem resistant isolates, six clones (A - F) were observed which three of them were more prevalent (Figure 3). Genotype A was the most prevalent (P value < 0.001) and 30.9% (30 isolates) belonged to this genotype. The prevalence of other genotypes were as follows: 28 isolates (28.9%) belonged to clone B, 26 isolates (26.8%) to clone C, 10 isolates (10.3%) to clone D and 2 isolates (2.1%) to clone E. Clone F contained only one isolate (1%). Carbapenem susceptible isolates (27 isolates) had unique genotypes.

Table 1. Sequence of Primers Used in the Study

Primer	Forward Sequence 5' - 3'	Reverse Sequence 5' - 3'	Reference
$bla_{\text{OXA-51-like}}$	TAATGCTTTGATCGGCCTTG	TGGATTGCACTTCATCTTGG	(14)
$bla_{\text{OXA-23-like}}$	GATCGGATTGGAGAACCAGA	ATTTCTGACCGCATTTCCAT	(16)
$bla_{\text{OXA-24-like}}$	GGTTAGTTGGCCCCCTTAAA	AGTTGAGCGAAAAGGGGATT	(16)
$bla_{\text{OXA-58-like}}$	AAGTATTGGGGCTTGTGCTG	CCCCTCTGCGCTCTACATAC	(16)
bla_{IMP}	TCGTTTGAAGAAGTTAACGG	ATGTAAGTTTCAAGAGTGATGC	(17)
bla_{VIM}	GGTGTTTGGTCGCATATCGCAA	ATTCAGCCAGATCGGCATCGGC	(17)
bla_{SPM}	AAAATCTGGGTACGCAAACG	ACATTATCCGCTGGAACAGG	(18)
REP	REP-I, III: GCGCCGICATCAGGC	REP-II: ACGTCTTATCAGGCCTAC	(19)

Table 2. The Rate of *Acinetobacter baumannii* Species Isolated From Each Ward and Specimen

Ward	Rate of Isolates	Specimen	Rate of Isolates
ICU	74.2	Tracheal aspirate	57.3
Out patients	8.1	Cerebrospinal fluid	11.3
Neurosurgery	4	wound	10.5
Dermatology	4	Urine	8.1
Nephrology	3.2	Discharge	5.6
Orthopedic	2.4	Blood	3.2
Woman	2.4	Pleura	1.6
Surgery	0.8	Catheter	1.6
Neonatal	0.8	Eye infection	0.8

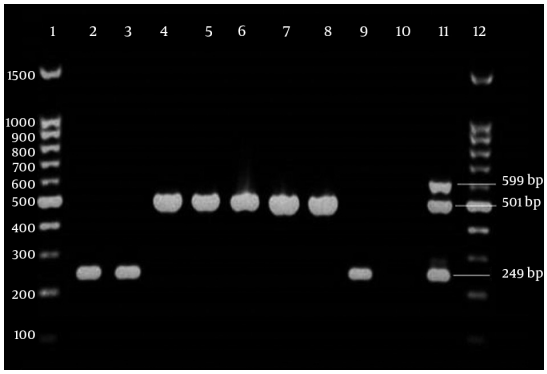
Abbreviation: ICU, intensive care unit.

Table 3. The Results of Antibiogram Analysis of *Acinetobacter baumannii* Isolates[a]

Antibiotic	Sensitive	Intermediate	Resistant
Imipenem	24.2	1.6	74.2
Meropenem	19.4	0.8	79.8
Ceftazidime	15.3	2.4	82.3
Cefepime	16.1	4	79.8
Ceftriaxone	1.6	12.1	86.3
Colistin	98.2	NA	0.8
Piperacillin	12.1	3.2	84.7
Piperacillin-tazobactam	16.9	1.6	81.5
Polymyxin-B	100	NA	NA
Gentamicin	28.2	4.8	66.9
Tobramycin	33.9	1.6	64.5
Amikacin	21	12.1	66.9
Tetracycline	21.8	12.1	66.1
Ampicillin-sulbactam	32.3	21.8	46
Ciprofloxacin	13.7	1.6	84.7
Trimethoprim-sulfamethoxazole	19.4	4.8	75.8
Rifampin	NA	3.2	96.8
Aztreonam	NA	4.8	95.2
Tigecycline (FDA)	6.5	58.1	35.5
Tigecycline (Jones)	45.2	50.8	4

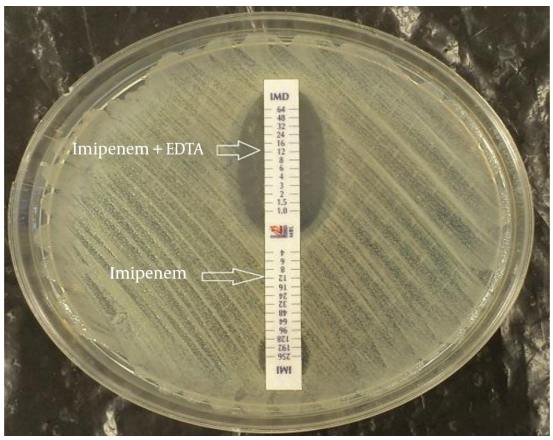
Abbreviation: NA, not available.
[a]Values are expressed as %.

Figure 1. Agarose gel Showing OXAs Genes Obtained by Multiplex PCR



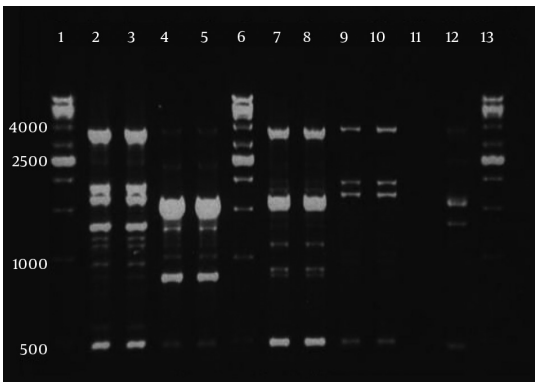
Lanes 1 and 12, 100 bp DNA ladder; lanes 2, 3 and 9, isolates with $bla_{\text{OXA-24-like}}$ in 249 bp; lanes 4 - 8, isolates with $bla_{\text{OXA-23-like}}$ in 501bp; lane 10, negative control (distilled water). lane 11, positive control *Acinetobacter baumannii* NCTC 13304, NCTC 13302 and NCTC 13305 were used as positive controls for $bla_{\text{OXA-23-like}}$, $bla_{\text{OXA-24-like}}$ and $bla_{\text{OXA-58-like}}$, respectively.

Figure 2. A MBL Producer *Acinetobacter baumannii* Isolate



An inhibitory zone is observed in Imipenem + EDTA compared to that of imipenem only.

Figure 3. An Agarose gel rep-PCR Product for the Resistant Isolates



For each clone, double fingerprints are shown. Lanes 1, 6, and 13, 1 kb DNA ladder; lanes 2 and 3, clone A; lanes 4, 5, clone B; lanes 7 and 8, clone C; lane 9 and 10, clone D; lane 11, negative control; lane 12, *A. baumannii* NCTC 12156 (ATCC 19606).

5. Discussion

In the current study most of the isolates (74.2%) were obtained from patients in ICUs and in accordance to other researches worldwide, the rate of infection caused by *A. baumannii* is high in ICUs (25, 26). Previously, it was reported that *A. baumannii* is more prevalent among endotracheal aspirate samples. Moreover, ventilator associated pneumonia is one of the frequent nosocomial infections caused by this organism (27-31). In accordance to the mentioned studies, most of the current study samples (57.3%) were isolated from tracheal aspirates. In the present study, antimicrobial susceptibility pattern showed that colistin and polymyxin-B are the most effective agents against *A. baumannii* isolates, *in vitro*. Colistin is the last line antimicrobial agent to treat multidrug resistant *A. baumannii* (26, 32). Although, colistin resistant isolates are reported globally (32), in the current study only 0.8% of isolates were colistin resistant.

This result indicates that colistin can be used to treat *A. baumannii* infections in the studied hospitals. Afterwards, among the tested antimicrobial agents, the highest rate of resistance was observed against rifampin; 96.8% and 3.2% of the isolates were resistant and intermediate to this antibiotic, respectively. No isolate was sensitive to rifampin; hence, it is suggested t that rifampin might be ineffective to treat *A. baumannii* infections in the studied hospitals. Carbapenems are successfully used to treat multidrug resistant *A. baumannii* infections; however, in recent years increase of carbapenem resistant *A. baumannii* isolates compromised their use (4, 26, 33). The emergence of carbapenem resistant *A. baumannii* is a global concern (4). In the current study the rates of resistance to imipenem by E-test and disk diffusion method were 78.2% and 74.2%, respectively. In addition, 79.8% and 73.4% of the isolates were resistant to meropenem by disk diffusion and E-test, respectively.

There is a discrepancy between carbapenems E-test and disk diffusion method. Based on E-test, 78.2% of the isolates were resistant to imipenem; while disk diffusion method showed that 74.2% of the isolates were resistance to imipenem. In contrast, meropenem E-test detected 73.4% of isolates as resistant; whilst, according to disk diffusion more isolates (79.8%) were recorded as meropenem resistant. However, authors could not elucidate this difference. In *A. baumannii*, the most common carbapenemase genes involved in carbapenem resistance are, $bla_{\text{OXA-23-like}}$, $bla_{\text{OXA-24-like}}$, $bla_{\text{OXA-58-like}}$ and $bla_{\text{OXA-143-like}}$ (26, 34). In the current study, multiplex PCR detected 85.6% of carbapenem resistant isolates carrying $bla_{\text{OXA-23-like}}$. The spread of OXA genes varies in different parts of the world and $bla_{\text{OXA-23-like}}$ is reported from 31% to 94% (7, 12, 35-39). The current study found that 6.2% of carbapenem resistant isolates harbor $bla_{\text{OXA-24-like}}$.

Some authors worldwide, reported the rate of $bla_{\text{OXA-24-like}}$ from 0 to 85.43% (7, 33, 36, 38-40). The results of the current study were consistent with those of other

studies and the findings for $bla_{\text{OXA-23-like}}$ and $bla_{\text{OXA-24-like}}$ were in the reported ranges. In contrast to other studies that reported the range of $bla_{\text{OXA-58-like}}$ from 2% to 84.92% (12, 35-38, 40), the current study could not find any isolates positive for $bla_{\text{OXA-58-like}}$. Albeit the reported co-existence of OXAs genes (29, 41, 42), coexistence between these genes was not observed in the current study and all *A. baumannii* isolates only had one of the $bla_{\text{OXA-23-like}}$ or $bla_{\text{OXA-24-like}}$ genes. In the current study, 42.3% and 79.4% of the isolates were MBL positive by DDs test and MBL E-test, respectively. Despite phenotypic tests, no bla_{IMP}, bla_{VIM} and bla_{SPM} genes were detected by PCR and the isolates were negative for these genes.

There are some possibilities about this phenomenon: 1) the MBL production may be false positive and due to bactericidal activity of EDTA, which may result in increased inhibitory zone and not associated with true MBL production (43); 2) MBL production my be true positive due to other MBL genes such as bla_{NDM} that were not investigated in the current study (44). Similar results are reported that *A. baumannii* isolates were MBL producer by phenotypic tests but no MBL encoding genes were detected (45, 46). It is reported that MBL E-test has good sensitivity for MBL detection and could detected MBL both chromosomally and plasmid mediated in aerobic and anaerobic bacteria (47).

According to the E-test results, it is possible that the current study isolates were true MBL producers. Interestingly the study found eight carbapenem resistant isolates that were negative for $bla_{\text{OXA-23-like}}$, $bla_{\text{OXA-24-like}}$, $bla_{\text{OXA-58-like}}$ and MBL genes and only harbored $bla_{\text{OXA-51-like}}$. Similarly, Nowak et al. reported that seven isolates of carbapenem resistant *A. baumannii* only had $bla_{\text{OXA-51-like}}$ (29). Carbapenem resistance in these isolates may be associated with other mechanisms such as: modification of penicillin binding proteins, loss of porins and decreased permeability, AmpC stable derepression or over expression of efflux pump (4, 8, 9).

It is noteworthy that, insertion of ISAba1 in upstream of $bla_{\text{OXA-51-like}}$ can lead to caebapenem resistance in *A. baumannii* (48). The relationship between harboring $\text{bla}_{\text{OXA-51-like}}$ and resistance to carbapenem in the eight isolates still need to be investigated.

The current study also aimed to investigate the clonality of *A. baumannii* isolates by rep-PCR. Genotypic comparison by rep-PCR revealed that carbapenem resistant isolates belonged to six clones. All clones were spread in the ICUs. Clone A was dominant (30.9%) and clone F had the lowest prevalence (1%). Clonal dissemination of carbapenem resistant *A. baumannii* was previously reported in different studies. It has been establish that multidrug resistant of *A. baumannii* isolates with similar genotype can disseminate among various wards, different hospitals and even among cities (4, 10, 12, 49-51).

In the current study, the vast majority of carbapenem resistant isolates (94/97, 96.9%) belonged to one of the four dominant genotypes indicating clonal dissemination of resistant isolates in the studied hospitals.

In conclusion, overall, the rate of carbapenem resistant isolates were high in the studied hospitals. Colistin and polymyxin-B were the effective antimicrobial agents, in vitro. Since in the current study $bla_{\text{OXA-58-like}}$ or MBL genes were not detected, it seems that carbapenem resistance is mostly related to $bla_{\text{OXA-23-like}}$ and $bla_{\text{OXA-24-like}}$, and these genes may play an important role in carbapenem resistance in the isolates. In addition, four clones of carbapenem resistant of *A. baumannii* isolates are disseminated in the two studied hospitals and clone A was dominant. In accordance to other studies, in the current investigation most of the resistant isolates belonged to four clones indicating clonal dissemination of *A. baumannii* in the studied hospitals and that effective infection control strategies are necessary to control the spread of these resistant isolates.

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Footnotes

Authors' Contribution: Study concept and design: Saeed Shoja, and Mojtaba Moosavian; acquisition of data: Saeed Shoja, and Soodabeh Rostami; analysis and interpretation of data: Saeed Shoja and Mohammad Amin Tabatabaiefar; drafting of the manuscript: Mojtaba Moosavian and Amir Peymani; critical revision of the manuscript for important intellectual content: Saeed Shoja, and Mojtaba Moosavian; statistical analysis: saeed shojia and Amir Peymani; administrative, technical, and material support: Saeed Shoja, Mojtaba Moosavian and Fariba Abbasi; study supervision: Saeed Shoja, and Mojtaba Moosavian.

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