AKADÉMIAI KIADÓ

Investigation of carbapenem resistant *Acinetobacter baumannii* ST2 in Iran

ALIAKBAR REZAEI, HOSSEIN FAZELI and JAMSHID FAGHRI*

Department of Microbiology, School of Medicine, Isfahan University of Medical Sciences, Isfahan, Islamic Republic of Iran

Acta Microbiologica et Immunologica Hungarica

68 (2021) 1, 20–26

DOI:
10.1556/030.2020.01164

Received: February 27, 2020 • Accepted: April 22, 2020
Published online: November 28, 2020

ORIGINAL RESEARCH PAPER

ABSTRACT

This study investigated carbapenem resistance among *Acinetobacter baumannii* isolated from respiratory specimens. Epidemiological relationship of the isolates was also evaluated. In this study, 81 respiratory specimens of *A. baumannii* from AL Zahra Hospital were confirmed by phenotypic and genotypic methods. Antimicrobial susceptibility was performed by disc diffusion method. Carbapenem resistance genes were identified by PCR. The isolates were typed by RAPD-PCR and multilocus sequence typing (MLST) methods. All isolates were resistant to imipenem and 80 isolates to meropenem. Frequency of oxacillinase genes was as follows: $bla_{\text{OXA-23}}$ gene was positive in 74 (91.3%), $bla_{\text{OXA-24}}$ gene in 50 (61.7%) and $bla_{\text{OXA-58}}$ was not found in any isolates. On the other hand 22 (27.2%) isolates contained $bla_{\text{IMP-1}}$, 3 (3.7%) isolates contained $bla_{\text{IMP-2}}$ gene, 5 (6.2%) isolates contained $bla_{\text{VIM-1}}$, 4 (5%) isolates had $bla_{\text{VIM-2}}$ and none of the isolates had $bla_{\text{SIM-1}}$ gene. RAPD-PCR typing identified 16 different patterns, with one pattern being the most frequent one in 26 isolates. In MLST 6 different sequence types were identified, the most predominant being ST2 belonging to clonal complex 2. The results of this study showed high resistance to carbapenems as well as high abundance of oxacillinase genes.

KEYWORDS

Acinetobacter baumannii, carbapenems, RAPD, MLST

INTRODUCTION

One of the most important nosocomial infections usually caused by endotracheal tube and mechanical ventilation is pneumonia. The incidence of this disease is 6–52% and is associated with high mortality and morbidity, especially in the intensive care unit [1].

Acinetobacter baumannii is a gram-negative opportunistic, oxidase negative and non-fermentative pathogen. It is one of the most important causes of nosocomial infections, especially in the intensive care unit around the world [2, 3]. Although it is capable of infecting different parts of the body such as bacteremia, surgical infection, urinary tract infection and secondary meningitis, the most important infection caused by these bacteria is respiratory tract infections [4]. Patients with a long-term stay in ICU and who are continuously using ventilators are more susceptible to respiratory infection with this bacterium [5].

Excessive consumption of imipenem and meropenem as the best treatment option for multidrug-resistant *A. baumannii*, resistance to these antibiotics is increasing widely. This increased resistance causes public concern because limited effective antibiotics are available against this organism in clinic departments [6, 7].

Different mechanisms have been identified for carbapenem resistance in these organisms. One of the most important one is carbapenemase production. The carbapenemases produced by these bacteria belong to A, B, and D classes of the Ambler classification. Class A possesses clavulanic acid-inhibited beta-lactamases such as KPC. Metallo-beta-lactamases (MBLs), such as bla_{IMP}, bla_{SIM}, and bla_{VIM}, belong to class B. Class D oxacillinases consist of six subclasses, which $bla_{\text{OXA-51}}$ exist innate in all *A. baumannii*. The genes of carbapenem resistance are usually transmitted through motile genetic elements such as plasmids and transposons [8, 9].

*Corresponding author.
E-mail: faghri@mui.ac.ir

Today, controlling and treating nosocomial infections caused by multidrug-resistant *A. baumannii* is one of the most important problems in the healthcare system. Epidemiological data can elucidate the origin of these infections. Also, monitor the isolates of *A. baumannii* may increase the prevention and control of infection and reduce transmission of the disease in the hospital system. Various techniques are available to increase our awareness of the geographical spread of *Acinetobacter*, including Multilocus sequence typing (MLST) and Random Amplification of Polymorphic DNA (RAPD) [10, 11]. MLST is a powerful procedure for examining the global epidemiology of *A. baumannii*. This is based on the conserved regions of seven house-keeping genes [12]. Epidemiological studies of clinical isolates of *Acinetobacter* worldwide have shown that this bacterium is highly diverse and that most infections occur by certain isolates. Epidemics around the world have been caused by 8 ICL (referred to as mlst clonal complex). The most prevalent are ICL1-3, which have been reported from most parts of the world [10, 13]. Other studies in Iran have shown that most of the identified STs in Iran belong to clonal complex 92 (belonging to ICL-2) [10, 14, 15]. This study was conducted due to the lack of sufficient information about the prevalence of carbapenem-resistant *A. baumannii* infection, the origin of these infections and the determination of common ST and antibiotic resistance patterns and production of metallo-beta-lactamase enzymes and genes involved in these resistances was conducted in Isfahan.

MATERIAL AND METHODS

Bacterial isolates

Eighty-one tracheal aspirates were isolated from different wards of al-Zahra hospitals in Isfahan from 2016 to 2017. Initial identification was performed by culture and biochemical tests and then final confirmation was conducted using PCR of the $bla_{\text{oxa-51}}$ gene which is inherent in *A. baumannii* [1, 2]. This study was evaluated and approved by the Ethics Committee of Isfahan University of Medical Sciences (project no. 395081).

Antibiotic susceptibility testing

Antibiotic susceptibility testing was performed using Kirby-Bayer method according to CLSI 2015 guidelines against imipenem (10 µg), meropenem (10 µg), cefepime (30 µg), ceftazidime (30 µg), ceftriaxone (30 µg), piperacillin-tazobactam (100/10 µg), gentamicin (10 µg), amikacin (30 µg), ciprofloxacin (5 µg), and tetracycline (30 µg) disks (MAST, Merseyside, UK). *Escherichia coli* ATCC 25922 were used as the control strain.

Phenotypic determination of MBLs

Combined disk diffusion test (CDDT) and Double Disk Synergy Test (DDST) methods were used to identify metallo-beta-lactamases.

Combined disk diffusion test (CDDT)

CDDT was performed by imipenem and meropenem (Mast Group, Merseyside, UK) alone and in combination with EDTA. Isolates that ≥7 mm inhibition zones of the imipenem-EDTA in comparison to imipenem disc alone were shown as MBL producers.

Double Disk Synergy Test (DDST)

In this method, imipenem disk and EDTA disk are used alone and close to each other and any expansion of the inhibition zone to the imipenem disk is considered as positive [3].

PCR for detection of antibiotic resistance genes

Total DNAs of the *A. baumannii* isolates were extracted by boiling method. The multiplex PCR test was performed for, $bla_{\text{OXA-51}}$, $bla_{\text{OXA-23}}$, $bla_{\text{OXA-24}}$, $bla_{\text{OXA-58}}$, and PCR test was done for $bla_{\text{IMP-1}}$, $bla_{\text{IMP-2}}$, $bla_{\text{VIM-1}}$, $bla_{\text{VIM-2}}$, and $bla_{\text{SIM-1}}$ genes. The names of the genes examined and the primers used for this purpose are listed in Table 1. The PCR test was performed in a total volume of 25 µL using Master Mix Amplicon (Denmark). PCR was performed in a thermocycler and the required temperature conditions were: initial denaturation at 95 °C for 3 min, 30 cycles of denaturation DNA with 94 °C for 1 min, specific annealing temperature each primer at 1 min (Table 1), extension at 72 °C for 2 min and final extension for 10 min at 72 °C. The PCR products were separated by electrophoresis in 1% agarose gel.

Random Amplification of Polymorphic DNA

RAPD analysis was performed as previously described [3]. Gel photograph of RAPD-PCR fingerprinting patterns were loaded to the CLIQS 1d program. DNA bands of patterns were signed and base pair (bp) Lengths were calculated by the program according to 100 bp DNA marker (Vivantis, Malesia) which was used as a control. After the calculation of bp lengths, dendrogram of RAPD-PCR patterns derived by the program. Patterns that showed 80% similarity were considered to be the same genotype.

Multilocus sequence typing

MLST was carried out on *A. baumannii*, relevant to Bartual et al. [16]. Seven conserved housekeeping genes were selected for sequencing and amplification based on their availability in GenBank, on prior studies of the phylogenetic relationships for the genus Acinetobacter (*gltA, gyrB, gdhB, recA, cpn60, gpi* and *rpoD*). The allelic numbers and sequence types (STs) were identified through the Pubmlst database. Clonal complexes (CCs) were formed by Sequence Types (STs) with five or more identical alleles by eBURST (version 3).

Statistical Analysis

The statistical analysis was performed using the software IBM SPSS Statistics version 25.0 (IBM Corp., USA). The

Table 1. List of primers used for amplification of resistance gene

Genes	Amplicon size (bp)	Sequences	Annealing temperature (°C)	Reference
bla_{OXA-23}	501	5′-GAT CGG ATT GGA GAA CCA GA-3′ 5′-ATT TCT GAC CGC ATT TCC AT-3′	50	[1–27]
bla_{OXA-24}	246	5′-GGT TAG TTG GCC CCC TTA AA-3′ 5′-AGT TGA GCG AAA AGG GGA TT-3′	50	[1]
bla_{OXA-51}	353	5′-CGG CCT TGTAA TGC TTT GAT-3′ 5′-TGG ATT GCA CTT CAT CTT GG-3′	50	[1]
bla_{OXA-58}	599	5′-AAG TAT TGG GGC TTG TGC TG-3′ 5′-CCC CTC TGC GCT CTA CAT AC-3′	50	[1]
bla_{IMP-1}	257	5-ACC GCA GCA GAG TCT TTG CC-3 5-ACA ACC AGT TTT GCC TTA CC-3	52	[23]
bla_{IMP-2}	678	5-GTT TTA TGT GTA TGC TTC C-3 5-AGC CTG TTC CCA TGT AC-3	58	[23]
bla_{VIM-1}	261	5-AGT GGT GAG TAT CCG ACA G-3 5-ATG AAA GTG CGT GGA GAC-3	61	[23]
bla_{VIM-2}	801	5-ATG TTC AAA CTT TTG AGT AAG-3 5-CTA CTC AAC GAC TGA GCG-3	54	[23]
bla_{SIM-1}	570	5-TAC AAG GGA TTC GGC ATC G-3 5- TAA TGG CTT GGT CCC ATG TG-3	66	[23]

association between the genes involved in carbapenem resistance and antibiotics profile of *A. baumannii* was evaluated by chi-square and Fisher's exact tests. The analysis was performed with a confidence level of 95%. *P* values <0.05 were considered statistically significant.

RESULTS

Bacterial isolates

Eighty-one clinical isolates of *A. baumannii* were isolated from the tracheal aspirate in al-Zahra hospital. Most of the isolates ($N = 63$, 77.8%) were obtained from intensive care units (ICUs) followed by inside ($N = 9$, 11.1%) and surgery ($N = 9$, 11.1%).

Antibiotic susceptibility testing

The highest susceptibility was detected by amikacin (11.1%) while the highest resistance rate was observed in imipenem and ertapenem (100%). The result of antibiotic susceptibility testing was shown in Table 2. Strains that are resistant to 3 or more classes of antibiotics are considered MDR strains. In this experiment 46 (56.8%) isolates were resistant to all antibiotics studied. The MDR pattern among *A. baumannii* strains is shown in Table 3.

MBL screening assays

Combined disk diffusion test (CDDT) and Double Disk Synergy Test (DDST) were performed on all isolates following results were obtained: 24 (29.6%) and 13 (16%) of isolates were positive, respectively.

PCR for detection of antibiotic resistance genes

Multiplex PCR was performed to investigate the oxacillinase genes including $bla_{\text{OXA-51}}$, $bla_{\text{OXA-23}}$, $bla_{\text{OXA-24}}$ and $bla_{\text{OXA-58}}$.

Table 2. Antimicrobial susceptibilities of the *A. baumannii* isolates

Antibiotics	Susceptible, No (0%)	Intermediate, No (0%)	Resistant, No (0%)
Imipenem	0 (0.0%)	0 (0.0%)	81 (100%)
Meropenem	1 (1.2%)	0 (0.0%)	80 (98.8%)
Ertapenem	0 (0.0%)	0 (0.0%)	81 (100%)
Cefepime	1 (1.2%)	2 (2.5%)	78 (96.3%)
Ceftriaxone	0 (0.0%)	2 (2.5%)	79 (97.5%)
Ceftazidime	3 (3.7%)	0 (0.0%)	78 (96.3%)
Piperacillin/ tazobactam	1 (1.2)	1 (1.2)	79 (97.5)
Tetracycline	5 (6.2%)	13 (16%)	63 (77.8%)
Ciprofloxacin	1 (1.2%)	0 (0.0%)	80 (98.8%)
Amikacin	9 (11.1%)	6 (7.4%)	66 (81.5%)
Gentamycin	4 (5%)	1 (1.2%)	76 (93.8%)

Table 3. Antibiotic Resistance Pattern to Antibiotic Classes in *Acinetobacter baumannii* isolates

Antimicrobial categories	Antimicrobial agents	Number of isolates (%)		
		Resistant	Intermediate	Susceptible
Carbapenems	imipenem	81 (100%)	0	0 (0.0%)
	meropenem	80 (98.8%)	0	1 (1.2%)
	ertapenem	81 (100%)	0	0 (0.0%)
Aminoglycosides	amikacin	66 (81.5%)	6 (7.4%)	9 (11.1%)
	gentamycin	76 (93.8%)	1 (1.2%)	4 (5%)
Cephalosporines	ceftriaxone	79 (97.5%)	2 (2.5%)	0 (0.0%)
	ceftazidime	78 (96.3%)	0	3 (3.7%)
	cefepime	78 (96.3%)	1 (1.2%)	1 (1.2%)
Fluoroquinolone	ciprofloxacin	80 (98.8%)	0	1 (1.2%)
Penicillin/ß-lactamase inhibitor	Piperacillin/tazobactam	79 (97.5)	1 (1.2)	1 (1.2)
Tetracycline	Tetracycline	116 (75.8%)	28 (18.3%)	9 (5.9%)

All isolates had $bla_{\text{OXA-51}}$ gene. The frequency of other oxacillinase genes was $bla_{\text{OXA-23}}$ and $bla_{\text{OXA-24}}$ positive in 74 (91.3%), 50 (61.7%) isolates, respectively. The $bla_{\text{OXA-58}}$ gene was not detected in any of the strains. 45 (55.5%) isolates had both $bla_{\text{OXA-23}}$ and $bla_{\text{OXA-24}}$ genes. 7 (8.6%) isolates had only $bla_{\text{OXA-51}}$ gene. All 81 isolates were evaluated for $bla_{\text{IMP-1}}$, $bla_{\text{IMP-2}}$, $bla_{\text{VIM-1}}$, $bla_{\text{VIM-2}}$ and $bla_{\text{SIM-1}}$ genes. Frequency of these genes was 22 (27.2%), 3 (3.7%), 5 (6.2%), 4 (5%) and 0, respectively. Three isolates were positive for $bla_{\text{IMP-1}}$ and $bla_{\text{IMP-2}}$ genes, simultaneously. Only one strain had both $bla_{\text{VIM-1}}$ and $bla_{\text{VIM-2}}$ genes together.

Random Amplification of Polymorphic DNA

All isolates were typed by this method. According to the dendrogram, the results of RAPD-PCR showed that the clinical isolates of Isfahan have 16 different genetic patterns. Pattern A with 26 isolates had the highest number of *A. baumannii* isolated from different parts of the hospital and then pattern D with 15 isolates showed the highest number. Overall, this method represented high homogeneity among *A. baumannii* isolates.

Multilocus sequence typing

Based on RAPD-PCR results, a total of 16 samples with different patterns were selected for MLST. A total of 7 different STs were observed. ST2 was found in 5 isolates and this was the the most common type. The second most common type was ST328, which was identified in three isolates. Table 4 shows the MLST results of these isolates.

DISCUSSION

A. baumannii causes a variety of nosocomial infections, including bacteremia, urinary tract infection, and secondary meningitis, but the predominant infection of this bacterium is pneumonia, which occurs in people who are admitted to the ICU and are under artificial respiration [3]. In the present study, high resistance to different antibiotics was observed, especially in carbapenems (imipenem and ertapenem 100%, meropenem 98.8%). Because carbapenem is used as a last-line antibiotic, high rates of resistance can be considered as a warning regarding multidrug-resistant *A. baumannii*, as this pathogen causes difficult to treat infections. A review of 3049 clinical isolates of *A. baumannii* 2001 to 2014 in Iran indicates that the rate of resistance to carbapenems has increased. Resistance to carbapenem was at the beginning (imipenem 51.1%, meropenem 64.3%) and reached the end of the study (imipenem 76.5%, meropenem 81.5%), indicating an increase in carbapenem resistance [4]. Studies show that over time, the rate of imipenem resistance in Iran is increasing, from 16.3% in 2007 to 100% in recent studies by Zanganeh et al. [5] and Salimi Zand et al. In Mashhad [6] the same study is included. The incidence of multidrug resistance in our study is high, which causes many problems in the treatment of nosocomial infections. In a different country, the difference in resistance to antibiotics can be seen. In that case, it comes from the environmental factors and patterns of use of antimicrobial agents, the type and number of samples, the type of study design and geographic area study. All isolates that were resistant to carbapenems except 4 had one gene encoding oxacillinases or metallo-beta-lactamases in addition to $bla_{\text{OXA-51}}$ gene. In a study of 131 isolates of *A. baumannii* in two Tehran hospitals between 2010 and 2013, 123 isolates had $bla_{\text{OXA-51}}$ genes, the prevalence of oxacillinase genes were $bla_{\text{OXA-23}}$ (93.3%), $bla_{\text{OXA-24}}$ (6.7%) and $bla_{\text{OXA-58}}$ (0%), respectively [7]. A study was conducted in the Gulf States (Saudi Arabia, United Arab Emirates, Kuwait, Qatar, Bahrain, and Oman) between 2011 and 2013, and 117 isolates of *A. baumannii* were examined, reducing the number of isolates that susceptible to imipenem and meropenem. All 117 isolates had $bla_{\text{OXA-51}}$ gene, 107 isolates (91%) had $bla_{\text{OXA-23}}$ gene, 5 isolates (4.3%) had $bla_{\text{OXA-24}}$ gene, and none of the isolates had $bla_{\text{OXA-58}}$ gene [28]. In the present study, the presence of metallo-β-lactamase enzymes was identified by CDT method in 24 (29.6%) isolates and by DDST method 15 (9.8%) isolates were reported. Out of 81 isolates, 24 (29.6) isolates showed metallo-β-lactamase genes by PCR. In a study conducted by Trash et al. in 2016 in Tehran on *A. baumannii* specimens, 45% of the samples were MBL-producing CDT phenotypic and the $bla_{\text{IMP-1}}$ gene in 10 isolates (3.5%) and the $bla_{\text{VIM-1}}$ gene in 34 isolates (1.18%) were

Table 4. Distribution of 16 clinical isolates of *A. baumannii* according to MLST profile, hospital ward and antibiotic resistant gene

Isolate	Allelic profile (*cpn-60, fusA, gltA, pyrG, recA, rplb, rpoB*)	ST	Hospital-ward	oxacillinase	Metallo-beta-lactamase
1	56-3-55-2-5-1-14	323	INS	bla_{OXA-23}	–
4	2-2-2-2-2-2-2	2	ICU	bla_{OXA-23}, bla_{OXA-24}	–
5	2-2-2-2-2-2-2	2	ICU	bla_{OXA-23}, bla_{OXA-24}	bla_{IMP-1}
10	56-3-6-2-28-1-29	78	ICU	bla_{OXA-24}	–
17	56-41-6-1-3-4-5	154	ICU	bla_{OXA-23}, bla_{OXA-24}	–
30	56-3-55-2-9-1-14	625	ICU	bla_{OXA-23}, bla_{OXA-24}	bla_{IMP-1}
36	25-3-6-2-28-1-29	78	ICU	bla_{OXA-24}	–
46	56-3-55-2-9-1-14	625	ICU	bla_{OXA-23}, bla_{OXA-24}	bla_{IMP-1}
66	2-2-2-2-2-2-2	2	ICU	bla_{OXA-23}, bla_{OXA-24}	–
78	2-2-2-2-2-2-2	2	ICU	bla_{OXA-23}, bla_{OXA-24}	bla_{IMP-1}
81	56-3-55-2-5-1-14	323	ICU	bla_{OXA-23}	–
54	2-2-2-2-2-2-2	2	ICU	bla_{OXA-23}, bla_{OXA-24}	–
18	56-1-1-25-5-1-2	328	SURG	bla_{OXA-23}, bla_{OXA-24}	–
36	56-1-1-25-5-1-2	328	SURG	bla_{OXA-23}, bla_{OXA-24}	–
74	56-1-1-25-5-1-2	328	ICU	bla_{OXA-23}, bla_{OXA-24}	–
27	1-3-2-25-9-1-5	1,035	ICU	bla_{OXA-23}, bla_{OXA-24},	bla_{IMP-1}, bla_{VIM-1}

positive [9]. In a study by Shanti et al. In India, 80% of *A. baumannii* isolates were phenotypically expressed MBL enzymes, and bla_{IMP} and bla_{VIM} genes were positive in 51% of isolates that phenotypically represented MBL enzyme. According to the results of this study, high resistance to carbapenems is primarily due to the high prevalence of oxacillinases, which showed high abundance among our isolates. Among the metallo-beta-lactamases, the highest prevalence was related to the bla_{IMP} gene, which showed a lower percentage compared to the oxacillinase genes. Our results show that oxacillinase gene plays a greater role in carbapenem resistance than metallo-beta-lactamase genes. This could be due to differences in the type of clinical sample obtained, the number of samples studied, the sampling method, the type of study performed, the geographical area and climatic conditions of the priority area in the administration of different antibiotics and the availability of antibiotics. Epidemiological knowledge of nosocomial infection is useful to develop effective strategies to control its spread. The use of molecular techniques such as PFGE and polymerase chain reaction-based typing is useful for tracking hospital epidemics [10]. 81 isolates were studied in 16 different clusters by RAPD method. Genetic variation in the present study is similar to other studies in Iran. In a study carried out by Azizi et al. on 96 samples in Tehran using m13 and DAF4 primers, 9 different clusters were identified, most isolated. Most isolates belonged to cluster A and other isolates were single [11]. In another study conducted by Zanganeh et al. in two hospitals of Shahid Motahari and Imam Hossein in Tehran, 60 isolates with 70% similarity coefficients in three clusters and 85% similarity coefficients in 10 different clusters were included [12]. In this study, according to the results of RAPD-PCR, a total of 16 samples were studied using MLST method. ST2 was the predominant type in this study, which was isolated from five specimens. The other STs isolated from Al-Zahra Hospital were ST328, ST323, and ST625, which were identified from 3, 2 and 2 isolates, respectively. In a study conducted by Hojabry et al. on samples isolated from two cities of Tehran and Tabriz using MLST method, the most common isolate was ST2 that was isolated from both Tehran and Tabriz. As well as the other common STs in this study were ST323 and ST328 that were observed in both studied cities which were similar to ours [13]. In another study conducted in Tehran, ST328 was identified in several specimens, which were also identified in our study [14]. In the study of Zowawi et al. on *A. baumannii* isolated from the Persian Gulf, seven different STs (ST195, ST208, ST229, ST436, ST450, ST452, ST499) were identified, but none of the STs were observed in our study. The specimens were ST195 and ST208 belonging to CC92, which is widespread worldwide and most of our study specimens belong to the same clonal complex [28]. Most of our isolates belong to the CC92 that is in ICL2. CC92 is widespread throughout Asia and is also widely spread worldwide. Overall, this investigation is similar to other previous studies in Europe, where most of the outbreaks are caused by carbapenem-resistant *A. baumannii* producing bla_{OXA-23} and belonging to International Clone 2 (ICL2).

CONCLUSION

The results of this study showed that resistance to different antibiotics, especially to carbapenems, in samples isolated from Isfahan is very high. According to the isolation of most specimens from the ICU as well as the weakness of the host immune system of patients in this ward, it seems that antibiotics should be revised in this section. The high abundance of oxacillinase genes in resistant isolates indicates the great role of these genes in generating these resistance patterns. Proper homology between RAPD and MLST typing methods was not observed. The isolates that showed the same typing in MLST method were clustered in RAPD-PCR method.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ACKNOWLEDGEMENTS

The authors would like to appreciate the Department of Microbiology of Isfahan University of Medical Sciences.

REFERENCES

[1] Gusatti CDS, Bertholdo LM, Otton LM, Marchetti DP, Ferreira AE, Corção G. First occurrence of blaOXA-58 in Acinetobacter baumannii isolated from a clinical sample in Southern Brazil. Braz J Microbiol 2012; 43(1): 243–6.

[2] Azizi O, Shahcheraghi F, Salimizand H, Modarresi F, Shakibaie MR, Mansouri S, et al. Molecular analysis and expression of bap gene in biofilm-forming multi-drug-resistant Acinetobacter baumannii. Rep. Biochem. Mol. Biol. 2016; 5(1): 62.

[3] Diancourt L, Passet V, Nemec A, Dijkshoorn L, Brisse S. The population structure of Acinetobacter baumannii: expanding multiresistant clones from an ancestral susceptible genetic pool. PloS One 2010; 5: 4.

[4] Falagas ME, Bliziotis IA, Siempos II. Attributable mortality of *Acinetobacter baumannii* infections in critically ill patients: a systematic review of matched cohort and case-control studies. Critical Care 2006; 10(2): R48.

[5] 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.

[6] Hagihara M, Housman ST, Nicolau DP, Kuti JL. In vitro pharmacodynamics of polymyxin B and tigecycline alone and in combination against carbapenem-resistant Acinetobacter baumannii. Antimicrob Agents Chemother 2014; 58(2): 874–9.

[7] Halaji M, Rezaei A, Zalipoor M, Faghri J. Investigation of class I, II, and III integrons among Acinetobacter Baumannii isolates from hospitalized patients in Isfahan, Iran. Oman Med J 2018; 33(1): 37.

[8] Hall GS. Bailey & Scott's diagnostic microbiology. 13th ed. American Society for Clinical Pathology; 2013.

[9] Hammerum AM, Hansen F, Skov MN, Stegger M, Andersen PS, Holm A, et al. Investigation of a possible outbreak of carbapenem-resistant Acinetobacter baumannii in Odense, Denmark using PFGE, MLST and whole-genome-based SNPs. J Antimicrobial Chemother 2015; 70(7): 1965–8.

[10] 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): 2121–6.

[11] Hojabri Z, Pajand O, Bonura C, Aleo A, Giammanco A, Mammina C. Molecular epidemiology of *Acinetobacter baumannii* in Iran: endemic and epidemic spread of multiresistant isolates. J Antimicrob Chemother 2014; 69(9): 2383–7.

[12] Huang G, Yin S, Gong Y, Zhao X, Zou L, Jiang B, et al. Multilocus sequence typing analysis of carba.penem-resistant *Acinetobacter baumannii* in a Chinese burns institute. Front Microbiol 2016; 7: 1717.

[13] Karah N, Sundsfjord A, Towner K, Samuelsen Ø. Insights into the global molecular epidemiology of carbapenem non-susceptible clones of Acinetobacter baumannii. Drug Resistance Updates 2012; 15(4): 237–47.

[14] Karmostaji A, Javadpour S, Davoodian P, Moradi N. In vitro activity of tigecycline and colistin against clinical isolates of Acinetobacter baumannii in hospitals in Tehran and Bandar-Abbas, Iran. Electronic physician 2014; 6(3): 919.

[15] Lee K, Chong Y, Shin H, Kim Y, Yong D, Yum J. Modified Hodge and EDTA-disk synergy tests to screen metallo-β-lactamase-producing strains of Pseudomonas and Acinetobactet species. Clin Microbiol Infect 2001; 7(2): 88–91.

[16] Bartua SG l, Seifert H, Hippler C. Development of a multilocus sequence typing scheme for characterization of clinical isolates of *Acinetobacter baumannii*. J Clin Microbiol 2005; 43: 4382–90.

[17] Mirnejad R, Mostofi S, Masjedian F. Antibiotic resistance and carriage class 1 and 2 integrons in clinical isolates of *Acinetobacter baumannii* from Tehran, Iran. Asian Pac J Trop Biomed 2013; 3(2): 140–5.

[18] Peleg AY, Seifert H, Paterson DL. Acinetobacter baumannii: emergence of a successful pathogen. Clin Microbiol Rev 2008; 21(3): 538–82.

[19] Rezaei A, Fazeli H, Halaji M, Moghadampour M, Faghri J. Prevalence of metallo-beta-lactamase producing *Acinetobacter baumannii* isolated from intensive care unit in tertiary care hospitals. Ann Ig 2018; 30(4): 330–6.

[20] Royer S, Faria ALS, Seki LM, Chagas TPG, Campos PAD, Batistão DWDF, et al. Spread of multidrug-resistant Acinetobacter baumannii and Pseudomonas aeruginosa clones in patients with ventilator-associated pneumonia in an adult intensive care unit at a university hospital. Braz J Inf Dis 2015; 19(4): 350–7.

[21] Saffari F, Monsen T, Karmostaji A, Azimabad FB, Widerström M. Significant spread of extensively drug-resistant Acinetobacter baumannii genotypes of clonal complex 92 among intensive care unit patients in a university hospital in southern Iran. J Med Microbiol 2017; 66(11): 1656–62.

[22] Sevillano E, Gallego L. Molecular techniques for detection and control of nosocomial infections caused by Acinetobacter baumannii. Science against microbial pathogens: communicating current research and technological advances. Spain: Formatex Research Center Badajoz; 2011. pp. 495–503.

[23] Tarashi S, Goudarzi H, Erfanimanesh S, Pormohammad A, Hashemi A. Phenotypic and molecular detection of metallo-beta-lactamase genes among imipenem resistant *Pseudomonas aeruginosa* and *Acinetobacter baumannii* strains isolated from patients with burn injuries. Arch Clin Inf Dis 2016; 11: 4.

[24] Turton JF, Woodford N, Glover J, Yarde S, Kaufmann ME, Pitt TL. Identification of *Acinetobacter baumannii* by detection of the $bla_{\text{OXA-51-like}}$ carbapenemase gene intrinsic to this species. J Clin Microbiol 2006; 44(8): 2974–6.

[25] Vincent J-L. Nosocomial infections in adult intensive-care units. The Lancet 2003; 361(9374): 2068–77.

[26] Yelken B, Erkasap N, Bayram B, Us T, Ceylan I, Özkurt M, et al. Epidemiology of Acinetobacter baumannii isolates from patients with severe sepsis in anesthesia intensive care unit/Anestezi Yogun Bakim Ünitesinde Agir Sepsisli Hastalardan Izole Edilen

Acinetobacter baumannii Izolatlarinin Epidemiyolojisi. FABAD J Pharm Sci 2011; 36(2): 63.

[27] Zangeneh Z, Eftekhar F. Study of class-D oxacillinase types in imipenem-resistant *Acinetobacter baumannii* clinical isolates by Rapd-Pcr. Iran J Public Health 2014; 43(2): 37.

[28] Zowawi HM, Sartor AL, Sidjabat HE, Balkhy HH, Walsh TR, Al Johani SM, et al. Molecular epidemiology of carbapenem-resistant Acinetobacter baumannii isolates in the Gulf Cooperation Council States: dominance of OXA-23-type producers. J Clin Microbiol 2015; 53(3): 896–903.