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Clinical Isolates of *Acinetobacter baumannii* From Tehran Hospitals: Pulsed-field Gel Electrophoresis Characterization, Clonal Lineages, Antibiotic Susceptibility, and Biofilm-forming Ability

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Abstract

Background: *Acinetobacter baumannii* is known as a potential pathogen in hospitals and is responsible for the dramatic increase in carbapenem resistance in Iran in the recent years.
Objectives: The current study aimed at determining the genetic association of the isolates by the pulsed-field gel electrophoresis (PFGE) technique, identify international clones, evaluate biofilm formation ability and its relationship with antibiotic resistance.
Methods: In the current study, a total of 48 *A. baumannii* isolates were collected from 2 hospitals in Tehran, Iran, from 2010 to 2012. Isolates were subjected to antimicrobial susceptibility testing, determination of carbapenemase encoding genes, biofilm formation, and genetic relationships analysis.
Results: The obtained results demonstrated that the rate of resistance to carbapenem, meropenem, imipenem, and doripenem was 76%. The carbapenemase-encoding gene $bla_{OXA\text{-}23\text{-}like}$ was found in 32 isolates, while $bla_{OXA\text{-}40\text{-}like}$ ($bla_{OXA\text{-}24\text{-}like}$), $bla_{OXA\text{-}58\text{-}like}$, $bla_{VIM\text{-}type}$ and $bla_{IMP\text{-}type}$ were found in 11, 1, 19. and 5 isolates, respectively. When the lineage of the isolates was evaluated by the multiplex polymerase chain reaction (PCR), it was found that 28 isolates belonged to group 1 and 8 isolates to group 2. None of the isolates belonged to group 3. Twelve isolates could not be typed by this method. The study findings interestingly demonstrated that 13 isolates showed no biofilm formation. Data of biofilm formation also demonstrated that 28, 4, and 3 remaining isolates had weak, moderate, and strong biofilm formation, respectively. The pulsed field gel electrophoresis result revealed 11 unique clones.
Conclusions: International clone 1 was the most commonly identified clone in the current study. This clone was mostly associated with $bla_{OXA\text{-}23\text{-}like}$ gene; therefore, 64% of the isolates in this clone possessed $bla_{OXA\text{-}23\text{-}like}$ gene.

Keywords: *Acinetobacter baumannii*, Electrophoresis, Gel, Pulsed-Field, Biofilm

1. Background

Acinetobacter baumannii emerged as a highly troublesome pathogen for many institutions globally (1). Outbreaks of infections due to this organism are reported in neonatal intensive care units (NICUs) (2), medical and surgical wards, burn units, and are also associated with medical equipment and hands of personnel (3). The rate of antibiotic resistance is high (4). Multidrug-resistant (MDR) *A. baumannii* is frequently reported as endemic in certain hospital wards and responsible for numerous nosocomial outbreaks worldwide (4, 5). Metallo-beta-lactamases (MBLs) and oxacillinases (OXAs) are the 2 main groups of carbapenemases in *A. baumannii*. OXA-type beta-lactamases are responsible for the most common type of carbapenem resistance via enzymatic degradation (6). Although MBLs are not the predominant carbapenemases in *A. baumannii*, VIM, IMP, SIM, and NDM metallo-beta-lactamases contribute to the high-level resistance to carbapenems (7).

Infections can have serious repercussions for patient morbidity and mortality. Patients can acquire the infection from environmental sources or from other patients (8). *Acinetobacter* spp. frequently causes infections associated with medical devices. Biofilm formation is a well-known pathogenic mechanism in such infections (1). Various typing methods are employed in the epidemiological investigation of outbreaks due to *Acinetobacter* species. The most widely applied methods focus on variations in the phenotypic properties such as antimicrobial susceptibility, biotypes, serotypes, phage types, or cell envelope protein profiles (9). Many of these traditional typing procedures have insufficient reproducibility, type ability, and discriminatory power.

Molecular techniques such as plasmid typing, ribotyping, and analysis of genomic DNA by pulsed-field gel elec-

trophoresis (PFGE) and by arbitrarily primed polymerase chain reaction (PCR), which directly compare variations in the DNA of bacterial isolates, are applied to epidemiological investigations of *Acinetobacter* isolates (10, 11).

2. Objectives

The current study aimed at determining the susceptibility rate, PFGE characterization, clonal lineages, and biofilm formation in the clinical isolates of *A. baumannii* in Tehran hospitals, Iran.

3. Methods

3.1. Sample Collection and Bacterial Identification

A total of 48 clinical samples were collected from wound, trachea, urine, catheter, sputum, and burn from patients admitted to Tehran hospitals from 2010 to 2012. The isolates were identified as *A. baumannii* according to the analytical profile $bla_{OXA\text{-}51\text{-}like}$ index 20NE protocol (BioMerieux, France) and genetically confirmed by the presence of gene (12). Then, the isolates were stored at -70°C in nutrient broth plus 30% glycerol.

3.2. Antibiotic Susceptibility Testing

Antibiotic susceptibility was determined by disk diffusion method on Mueller-Hinton agar (Merck, Germany) according to the clinical and laboratory standards institute (CLSI) guidelines for the following antimicrobial agents: imipenem (10 μg), meropenem (10 μg), doripenem (10 μg), piperacillin (100 μg), gentamicin (10 μg), amikacin (30 μg), ciprofloxacin (5 μg), minocycline (30 μg), doxycycline (30 μg), and piperacillin-tazobactam (100/10 μg) (Mast Diagnostics, UK). *Escherichia coli* ATCC 25922, *Escherichia coli* ATCC 35218, and also *Pseudomonas aeruginosa* ATCC 27853 were used as controls (13).

3.3. PCR for Carbapenemase Genes

Genomic DNA was extracted using the boiling method. PCR was employed to detect $bla_{OXA\text{-}51\text{-}like}$, the *A. baumannii*-specific-gene) Table 1. (Isolates were also screened for the other major groups that confer resistance to carbapenems; ie, $bla_{OXA\text{-}23\text{-}like}$, $bla_{OXA\text{-}40\text{-}like}$ ($bla_{OXA\text{-}24\text{-}like}$), and $bla_{OXA\text{-}58\text{-}like}$ by multiplex reaction as well as the $bla_{NDM\text{-}type}$, $bla_{SIM\text{-}type}$, $bla_{IMP\text{-}type}$, and $bla_{VIM\text{-}type}$ by single PCR (TAG Copenhagenard, Denmark) (14) (Table 1). The PCR products were separated by 1% agarose gel electrophoresis stained with neutral red (Sigma Aldrich, Germany) and visualized under ultraviolet (UV) light.

3.4. Biofilm Formation

Biofilm formation was quantified by the microtiter plate assay method as described previously (15). Briefly, *A. baumannii* species were grown overnight in trypticase soy broth (TSB) (Merck, Germany) with 0.25% glucose at 37°C. The culture was diluted 1:50 in (TSB). Then, 200 mL of suspension was used to inoculate wells of sterile 96-well polystyrene microtiter plates, followed by incubation at 37°C for 72 hours. After 3 washes with phosphate buffered saline (PBS), any remaining biofilm was stained with crystal violet 1% (w/v) for 25 minutes and wells were washed again with PBS. The dye bound to the adherent cells was re-solubilized with 200 mL of ethanol/acetone (80:20, V/V) and the optical density (OD) was quantified at 570 nm using an enzyme-linked immunosorbent assay (ELISA) reader. Each assay was performed in triplicate. The adherence capabilities of the tested strains were classified into 4 categories; 3 standard deviations (SDs) above the mean OD of the negative control (broth only) was considered as the optical density cutoff (ODc). Isolates were classified as follows: $OD \leq ODc$ non-adherent bacteria; $ODc < OD \leq 2 \times ODc$, weakly adherent bacteria; $2 \times ODc < OD \leq 4 \times ODc$, moderately adherent bacteria; $4 \times ODc < OD$, strongly adherent bacteria (15).

3.5. Pulsed-Field Gel Electrophoresis

The collected isolates were analyzed by PFGE as described by Durmaz et al. (16). *Acinetobacter baumannii* ATCC 19606 was included for external reference. Genomic DNA was digested with *Apa*I (New England Biolabs). The lambda ladder PFGE marker (NEB, US) was used as a molecular size marker. The pulsed-field electrophoresis (Chef Mapper; Bio-Rad Laboratories, Hercules, CA, USA) was performed under the following conditions: temperature 14°C; voltage 6 V/cm; switch angle, 120°C; switch ramp 2.2 - 35 seconds for 19 hours. Gels were stained with ethidium bromide and patterns were visualized under UV light (BIO-RAD, USA). DNA banding patterns were analyzed using Bionumeric 7.0 software (Apllied maths NV, St-Martens-Latem Belgium).

3.6. PCR-Based Sequence Group Typing

Multiplex PCRs to identify groups (international clone) 1 to 3 organisms were performed by amplification of the *ompA*, *csuE*, and $bla_{OXA\text{-}51\text{-}like}$ genes using the primers listed in Table 1 (TAG Copenhagenard, Denmark).

PCR were performed under the following conditions: 94°C for 3 minutes, then, 30 cycles of 94°C for 45 seconds, 57°C for 45 seconds and 72°C for 1 minute, followed by a final extension at 72°C for 5 minutes. Identification of a strain as a member of group 1 or 2 required the amplification of all 3 fragments in the corresponding multiplex PCR,

Table 1 . Primers Used for Multiplex Polymerase Chain Reaction

Prime	Sequence	Size (bp)
SIM-F	5' - GTACAAGGGATTCGGCATCG-3	569
SIM-R	5' - GTACAAGGGATTCGGCATCG-3	
NDMI-F	5' - GAGATTGCCGAGCGACTTG-3	497
NDMI-R	5' - CGAATGTCTGGCAGCACACTT-3	
Oxa-51-like-F	5' - TAATGCTTTGATCGGCCTTG-3	353
Oxa-51-like-R	5' - TGGATTGCACTTCATCTTGG-3	
Oxa-23-like-F	5' - GATCGGATTGGAGAACCAGA-3	501
Oxa-23-like-R	5' - ATTTCTGACCGCATTTCCAT-3	
Oxa-24-like-F	5' - GGTTAGTTGGCCCCCTTAAA-3	246
Oxa-24-like-R	5' - AGTTGAGCGAAAAGGGGATT-3	
Oxa-58-like-F	5' - AGTATTGGGGCTTGTGCTG-3	599
Oxa-58-like-R	5'- CCCCTCTGCGCTCTACATAC-3	
Imp-F	5'- GAATAGAATGGTTAACTCTC-3	188
Imp-R	5'- CCAAACCACTAGGTTATC-3	
Vim-F	5' - GTTTGGTCGCATATCGCAAC -3	382
Vim-R	5'- AATGCGCAGCACCAGGATAG-3	
Group1 alleles		
Group1ompAF306	5'- GATGGCGTAAATCGTGGTA-3	355
Group1and2ompAR660	5' - CAACTTTAGCGATTTCTGG-3	
Group1csuEF	5' - CTTTAGCAAACATGACCTACC-3	702
Group1csuER	5' - TACACCCGGGTTAATCGT-3	
Gp1OXA66F89	5' - GCGCTTCAAAATCTGATGTA-3	559
Gp1OXA66R647	5' - GCGTATATTTTGTTTCCATTC-3	
Group2 alleles		
Group2omp AF378	5' - GACCTTTCTTATCACAACGA-3	343
GrouPland2ompAR660	5' - CAACTTTAAGCGATTTCTGC-3	
Group2csuEF	5' - GGCGAACATGACCTATTT-3	580
Group2csuER	5' - CTTCATGGCTCGTTGGTT-3	
Gp2OXA69F169	5' - CATCAAGGTCAAACTCAA-3	162
Gp2OXA69R330	5' - TAGCCTTTTTTCCCCATC-3	

and an absence of any amplification in the other multiplex PCR. Group 3 isolates were defined by the amplification of only the *ompA* fragment in the group 2 PCR, and the amplification of only the *csu*E, and $bla_{OXA\text{-}51\text{-}like}$ fragments in the group 1 PCR (17) (Table 1). The PCR products were separated by 1% agarose gel electrophoresis stained with neutral red (Sigma Aldrich, Germany) and visualized under UV light.

4. Results

4.1. Bacterial Strains

The Isolates were isolated from different clinical samples and wards of Loghman (No. 1) and Milad (No. 2) hospitals, described in Table 2.

4.2. Antibiotic Susceptibility Testing

The disk diffusion susceptibility testing revealed that 100% (n = 48) of the isolates were resistant to piperacillin.

Table 2. Clinical Samples and Different Wards of Hospital

Clinical Samples	Frequency of Samples (N)	Type of Wards	Frequency of Samples (N)
Wound	13	Intensive care unit	32
Trachea	24	Pediatric	1
Urine	3	Medical	7
Catheter	2	Surgery	5
Sputum	3	Ear, nose and throat	1
Burn	2	Emergency	2
Cerebrospinal fluid	1		

The rate of resistance to carbapenem was 76% (meropenem = 77%, imipenem =77%, and doripenem = 75%); among the other tested antibiotics, colistin and polymixin showed the higher rates of antimicrobial activities (isolates were 100% sensitive) (Table 3).

Minimum inhibitory concentration (MIC) result for meropenem ranged 0.5 to 256 μg/mL as follows: 10 isolates were sensitive, 3 isolates intermediate, and 35 isolates showed resistance. On the other hand, MIC results for imipenem ranged 0.5 to 64 μg/mL: 11 isolates were sensitive, 4 isolates intermediate, and 33 isolates resistant.

4.3. Prevalence of Carbapenemase-Encoding Genes

All 48 isolates were positive for $bla_{OXA\text{-}51\text{-}like}$. The carbapenemase-encoding gene, $bla_{OXA\text{-}23\text{-}like}$, was found in 32 isolates (64%). $bla_{OXA\text{-}40\text{-}like}$ ($bla_{OXA\text{-}24\text{-}like}$), $bla_{OXA\text{-}58\text{-}like}$, $bla_{VIM\text{-}type}$, and $bla_{IMP\text{-}type}$ were found in 11 (22%), 1 (2%), 19 (38%), and 5 (10%) isolates, respectively. None of the isolates had positive PCR results for $bla_{NDM\text{-}type}$ and $bla_{SIM\text{-}type}$.

4.4. Biofilm Formation

Among 48 isolates examined in the current study, 13 could not produce biofilm, 28 isolates had weak ability to form biofilm, 4 isolates showed moderate ability, and 3 isolates showed strong ability to form biofilm (Table 4).

4.5. Pulsed-Field Gel Electrophoresis Results

The PFGE results (Figure 1A) revealed 11 clonal complexes with cut off level 74%, and the most prevalent clonal complexes were E, F, and H. The clonal complex E was the most frequent, with 17 clinical isolates. Pulsotype I was sporadic and did not have close genetic similarity with the other groups (Figure 1).

4.6. PCR-Based Sequence Group Typing

All strains belonging to the group 1 of clonal complex yielded all 3 fragments in the group 1 PCR and none of the group 2 PCR; while strains belonging to group 2 gave the expected converse results. Twenty-eight isolates belonged to group 1 and 8 isolates belonged to group 2. None of the isolates belonged to group 3. Twelve isolates could not be typed by this method.

Of the 28 isolates belonging to group 1, eighteen isolates had $bla_{OXA\text{-}23\text{-}like}$ gene, five isolates had $bla_{OXA\text{-}23\text{-}like}$ +, VIM+ pattern. The pattern of imipenem resistance among the 28 isolates that belonged to group 1 was as follows: 20 isolates were resistant, 3 isolates had intermediate resistance, and 5 were sensitive; for meropenem: 20 isolates showed resistance, 2 isolates showed intermediate resistance, and 6 isolates were sensitive; for doripenem: 19 isolates were resistant, 2 isolates had intermediate resistance, and 7 isolates were sensitive. Of the 8 isolates that belonged to group 2, according to PCR-based sequence group method, 6 isolates had $bla_{OXA\text{-}23\text{-}like}$ gene, 3 isolates had $bla_{OXA\text{-}23\text{-}like}$ +, VIM+ pattern and 1 isolate did not have resistance genes except for $bla_{OXA\text{-}51\text{-}like}$; the pattern of resistance for all of the 3 evaluated antibiotics was the same in this group. Seven isolates were resistant to all antibiotics and 1 isolate had intermediate resistance (Tables 5 and 6).

5. Discussion

Acinetobacter baumannii is one of the main causes of nosocomial infections in the recent years (18). *Acinetobacter baumannii* is one of the most problematic organisms currently responsible for nosocomial infections, especially in intensive care units (ICUs) (19). Increasing antimicrobial resistance (20) and the ability of *A. baumannii* to survive on inanimate and dry surfaces are linked to the occurrence of periodic outbreaks observed in various hospitals (21). *Acinetobacter baumannii* is the most common isolate obtained from skin, blood, sputum, pleural fluid, and

Table 3. Resistance of *Acinetobacter baumannii* Isolates to the Tested Antibiotics

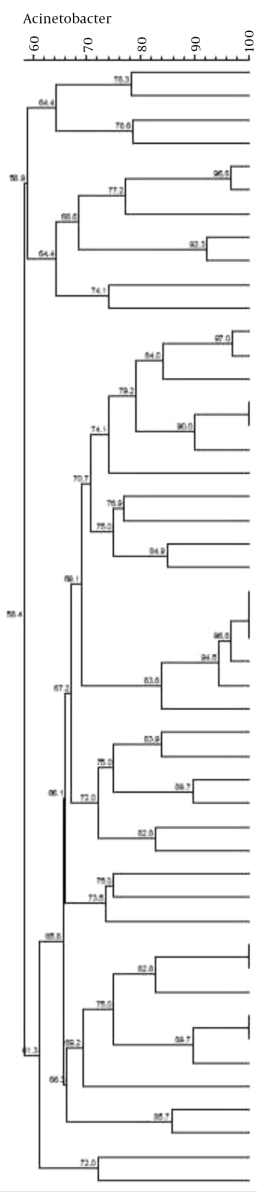
Antibiotic	Resistant, N (%)	Intermediate, N (%)	Susceptible, N (%)
Amikacin	33 (69)	0 (0)	15 (31)
Gentamicin	35 (73)	3 (6)	10 (21)
Trimethoprim-sulfamethoxazole	42 (88)	2 (4)	4 (8)
Ciprofloxacin	44 (92)	0 (0)	4 (8)
Ceftazidime	44 (92)	0 (0)	4 (8)
Cefepime	40 (84)	4 (8)	4 (8)
Cefotaxime	44 (92)	3 (6)	1 (2)
Colistin	0 (0)	0 (0)	48 (100)
Polymyxin B	0 (0)	0 (0)	48 (100)
Piperacillin-tazobactam	38 (79)	0 (0)	10 (21)
Piperacillin	48 (100)	0 (0)	0 (0)
Minocycline	4 (8)	12 (25)	32 (67)
Doxycycline	27 (56)	1 (2)	20 (42)
Meropenem	37 (77)	4 (8)	7 (15)
Imipenem	37 (77)	5 (10)	6 (13)
Doripenem	36 (75)	4 (8)	8 (17)

Table 4. Resistance of *Acinetobacter baumannii* Isolates to carbapenems, Based on the Ability to Form Biofilm

Antibiotic	Non-Biofilm, Forming (N = 13), N (%)	Weak Biofilm Forming (N =28), N (%)	Moderate Biofilm Forming (N = 4), N (%)	Strong Biofilm Forming (N =3), N (%)	Total, N (%)
Doripenem					
Resistant	8 (22.2)	23 (63.9)	2 (5.6)	3 (8.3)	36 (100)
Intermediate	1 (25)	2 (50)	1 (25)	0 (0)	4 (100)
Susceptible	4 (50)	3 (37.5)	1 (12.5)	0 (0)	8 (100)
Imipenem					
Resistant	10 (27)	22 (59.5)	2 (5.4)	3 (8.1)	37 (100)
Intermediate	1 (20)	3 (60)	1 (20)	0 (0)	5 (100)
Susceptible	2 (33.3)	3 (50)	1 (16.7)	0 (0)	6 (100)
Meropenem					
Resistant	9 (24.3)	23 (62.2)	2 (5.4)	3 (8.1)	37 (100)
Intermediate	2 (50)	1 (25)	1 (25)	0 (25)	4 (100)
Susceptible	2 (28.6)	4 (57.1)	1 (14.3)	0 (0)	7 (100)

urine (22). As many studies show, the organism is most frequently isolated from the respiratory tract than the other body organs among the ICU admitted patients (23, 24).

Acinetobacter baumannii is one of the most frequent causes of nosocomial infections throughout Tehran hospitals, especially in the ICU wards (23). Carbapenem resistance is recently increased by this bacterium; therefore, the current study evaluated the antibiotic sensitivity pattern, carbapenem resistance genes, the ability of biofilm formation, the genetic relationships and the lineages of the species isolated from Tehran hospitals. Most of the samples of the current study were obtained from the ICU wards. The samples were also collected from other wards in high abundance such as medical, surgery, emergency, ear, nose and throat (ENT), and pediatric. Although carbapenems, aminoglycosides, and fluoroquinolones are



Clonal Complex	I.C	Biofilm
A	-	Weakly
A	-	Weakly
A	1	Weakly
A	1	Weakly
B	2	Negative
B	-	Negative
B	1	Weakly
C	1	Weakly
C	2	Weakly
D	2	Moderate
D	-	Weakly
E	-	Weakly
E	-	Weakly
E	1	Weakly
E	1	Weakly
E	1	Negative
E	-	Weakly
E	1	Negative
E	-	Weakly
E	1	Moderate
E	1	Weakly
E	-	Weakly
E	1	Weakly
E	1	Weakly
E	1	Strong
E	1	Moderate
E	1	Weakly
E	1	Negative
F	1	Negative
F	1	Weakly
F	1	Negative
F	2	Negative
F	1	Weakly
F	2	Moderate
G	2	Moderate
G	-	Weakly
G	-	Weakly
H	1	Negative
H	1	Negative
H	1	Weakly
H	1	Strong
H	-	Weakly
H	2	Weakly
I	1	Moderate
J	1	Negative
J	1	Weakly
K	1	Negative
K	2	Strong

Figure 1. PFGE Clustering Analysis with Cut off Level 74% of 48 *A. baumannii* Strains, International Clones, and Biofilm Formation

used to treat *A. baumannii* infections in different medical settings, resistance to carbapenems is increasing in Iran (25-27).

A worldwide collection of 5127 *Acinetobacter* spp., collected from 2005 to 2009 from 140 hospitals of 32 countries in North America (17.1%), Europe (22.9%), Latin America (25.2%) and the Asia-Pacific region (34.8%) showed that overall non-susceptibility rate to imipenem and meropenem was 45.9% and 48.2%, respectively. However, the non-susceptibility percentage for imipenem and meropenem, increased from 27.8% and 37.5% in 2005 to 62.4% and 64.4% in 2009, respectively (28).

From 2008 to 2016, thirty-nine studies were performed in Iran on the antibiotic resistance of *A. baumannii* that more than half of them were performed in Tehran. Nowadays, the rate of carbapenem resistance has increased in Iran. By definition, MDR *A. baumannii* isolates are resistant to 3 or more agents of different antibiotic classes. In total, 7 studies revealed the presence of MDR isolates. Among the 7 studies, the rates of MDR ranged from 32.7% to 93% in the

Table 5. Resistance of *Acinetobacter baumannii* Isolates to the Tested Antibiotics, Based on International Clones and Beta-Lactamase Genes

Antibiotic	International Clone 1 (N = 28), N (%)	International Clone 2 (N = 8), N (%)	Other International Clones (N = 12), N (%)	Total, N (%)	Bla-$_{OXA23\text{-}like}$	Bla-$_{OXA24\text{-}like}$	VIM	IMP
Doripenem								
Resistant	19 (52.8)	7 (19.4)	10 (27.8)	36 (100)	27	6	16	4
Intermediate	2 (50)	1 (25)	1 (25)	4 (100)	2	1	0	1
Susceptible	7 (87.5)	0 (0)	1 (12.5)	8 (100)	3	4	3	-
Total					32	11	19	5
Imipenem								
Resistant	20 (54.1)	7 (18.9)	10 (27)	37 (100)	26	8	16	5
Intermediate	3 (60)	1 (20)	1 (20)	5 (100)	2	2	2	-
Susceptible	5 (83.3)	0 (0)	1 (16.7)	6 (100)	4	1	1	-
Total					32	11	19	5
Meropenem								
Resistant	20 (54.1)	7 (18.9)	10 (27)	37 (100)	26	6	16	5
Intermediate	2 (50)	1 (25)	1 (25)	4 (100)	3	2	1	-
Susceptible	6 (85.7)	0 (0)	1 (14.3)	7 (100)	3	3	2	-
Total					32	11	19	5

Table 6. Frequency of *Acinetobacter baumannii* Isolates in International Clones

Clonal Complex	International Clone 1 (N = 28)	International Clone 2 (N = 8)	Other International Clones (N = 12)	Total N
A	2	0	2	4
B	1	1	1	3
C	1	1	0	2
D	0	1	1	2
E	12	0	5	17
F	4	2	0	6
G	0	1	2	3
H	4	1	1	6
I	1	0	0	1
J	2	0	0	2
K	1	1	0	2
	28	8	12	48

584 isolated *A. baumannii* species sort by time: 2001 to 2007 (1 study, 50%), 2008 to 2009 (1 study, 66%), 2009 to 2010 (1 study, 83%), and 2010 to 2011 (4 studies, 32.7%, 74.9%, 93%, 94.4%) (29).

In a study by Karmostaji et al., in Iran during 2012, on 84 isolates of *A. baumannii*, 50 (59.52%) species were resistant to imipenem and 74 (88.09%) were resistant to meropenem (30). Also In another study by karmostaji et al., 54.47% of the isolates were resistant to amikacin, 67.47% to imipenem, and 84.55% of the isolates were resistant to meropenem. Among the 123 isolates, 100 (81.3 %) had an acquired $bla_{OXA\text{-}23\text{-}like}$ carbapenemase, 10 (8.1%) possessed $bla_{OXA\text{-}24\text{-}like}$, and 1 (0.81%) $bla_{OXA\text{-}58\text{-}like}$ carbapenemase (31). The results of the current study showed that the carbapenemase-encoding gene, $bla_{OXA\text{-}23\text{-}like}$, was found in 32 (64%) isolates and this finding was consistent with those of other studies reported from Iran. The $bla_{OXA\text{-}40\text{-}like}$ ($bla_{OXA\text{-}24\text{-}like}$), $bla_{OXA\text{-}58\text{-}like}$, $bla_{VIM\text{-}type}$, and $bla_{IMP\text{-}type}$ genes

were found in 11 (22%), 1 (2%), 19 (38%) and 5 (10%) isolates, respectively. However, none of the isolates had positive PCR results for $bla_{NDM\text{-}type}$ and $bla_{SIM\text{-}type}$ (29). The current study tried to evaluate the results of clonal lineage method with the results obtained by PFGE. In type E, which was the most frequent type in the current study, there were 17 isolates, 13 collected from ICU and 4 from other wards. All 17 isolates were collected in hospital No.1. The origin of 8 isolates was trachea, 5 isolates from wound, 2 isolates from sputum, 1 isolate from urine, and 1 isolate from catheter. Using clonal lineages as a typing method, it was shown that 12 isolates belonged to the group 1 and the method was unable to type 5 other isolates.

Three out of 48 isolates were strong biofilm producers, among which 1 isolate was from hospital No.1 and the other 2 from hospital No.2. Two of these isolates were collected from catheter in ICU and the others were from burn and general wards. These isolates belonged to 3 clonal complexes E, H, and K and by PCR-based sequence group typing method it was found that 2 isolates belonged to group 1 and the other belonged to group 2. All 3 isolates showed high resistance to carbapenem; therefore, MIC of these isolates for imipenem and meropenem were 32 to 128 μg/mL, respectively. All the isolates were MDR. Isolate 3, despite harboring just $bla_{OXA\text{-}51\text{-}like}$ (carbapenem resistance genes), showed high antibiotic resistance to all 3 antibiotics including meropenem, imipenem, and doripenem. None of the isolates had $bla_{OXA\text{-}58\text{-}like}$ and $bla_{IMP\text{-}type}$.

All 3 isolates, which were strong biofilm producers, showed resistance to all studied antibiotics. In a similar study conducted by Mahdian et al., 49% of isolates belonged to international clone 1 and 49% to international clone 2; also 74% and 49% of the isolates harbored $bla_{OXA\text{-}23\text{-}like}$ and $bla_{OXA\text{-}24\text{-}like}$, respectively; and 75% and 66% of the isolates were resistant to the imipenem and meropenem, respectively (27). In another study, 58% and 29% of the isolates belonged to international clones 2 and 1, respectively (32). In the study conducted by Kaliterena et al., in Croatia, 51% of the *A. baumannii* species belonged to class 1 and 27% to class 2 international clone, and the others belonged to other clones (33).

Despite the simplicity and cost-effectiveness of PCR-based sequence group typing method, it could not type all the isolates. However, PFGE as a method can type all isolates.

5.1. Conclusions

International clone 2 was the most commonly detected clonal lineage in the current study. Isolates that belonged to this clone were mostly associated with $bla_{OXA\text{-}23\text{-}like}$ gene; therefore, 64% of the isolates in this clone possessed $bla_{OXA\text{-}23\text{-}like}$ gene.

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Footnotes

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