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 *blaOXA-23* was found in 32 isolates, while *blaOXA-40, blaOXA-58, blaOXA-18*, *blaNDM-1*, and *blaIMP-9* 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 *blaOXA-23* gene; therefore, 64% of the isolates in this clone possessed *blaOXA-23* 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 MBLSs 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 electrophoresis (PFGE) are very useful in elucidating epidemiological relationships among isolates 

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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-51-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-51-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-23-like, bla_oxa-40-like (bla_oxa-24-like), and bla_oxa-58-like by multiplex reaction as well as the bla_nmdm-type, bla_nmdm-type, and bla_oxa-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 immunosorbtent 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 ≤ ODc non-adherent bacteria; ODc < OD ≤ 2 × ODc, weakly adherent bacteria; 2 × ODc < OD ≤ 4 × ODc, moderately adherent bacteria; 4 × 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 Apol (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 (Aplied 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-51-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’-GTACAAGGGATTCGGCATG-3                          | 569       |
| SIM-R                  | 5’-GTACAAGGGATTCGGCATG-3                          |           |
| NDMI-F                 | 5’-GAAGTTTCTGGGAGGCATGG-3                         | 497       |
| NDMI-R                 | 5’-CGAATGCTGGCAGCACATT-3                          |           |
| Oxa-51-like-F          | 5’-TATGCTTGGTACGGGCTTG-3                          | 353       |
| Oxa-51-like-R          | 5’-TGATGGAATCTACTTTTGG-3                          |           |
| Oxa-23-like-F          | 5’-GATGGGATGGGAACACGA-3                           | 501       |
| Oxa-24-like-F          | 5’-GATGGGATGGGAACACGA-3                           |           |
| Oxa-24-like-R          | 5’-GATGGGATGGGAACACGA-3                           |           |
| Oxa-58-like-F          | 5’-AGTATTGGGGCTTGTGCTG-3                          | 599       |
| Oxa-58-like-R          | 5’-AGTATTGGGGCTTGTGCTG-3                          |           |
| Imp-F                  | 5’-GATGGGATGGGAACACGA-3                           | 188       |
| Imp-R                  | 5’-GATGGGATGGGAACACGA-3                           |           |
| Vim-F                  | 5’-GATGGGATGGGAACACGA-3                           | 382       |
| Vim-R                  | 5’-GATGGGATGGGAACACGA-3                           |           |

Group 1 alleles

| Group1ompAF306         | 5’-GATGGGATGGGAACACGA-3                           | 355       |
| Group1and2ompAR660     | 5’-CAACTTTGATGGATTATC-3                           |           |
| Group1csuEF            | 5’-CTTTAACACATGACCTAC-3                           | 702       |
| Group1csuER            | 5’-TACACCCGGTCTGCTGCTG-3                          |           |
| Gp1OXA66F89            | 5’-GCGCTTCAAAATCTGATGTA-3                         | 559       |
| Gp1OXA66R647           | 5’-GCGCTTCAAAATCTGATGTA-3                         |           |

Group 2 alleles

| Group2ompAF378         | 5’-GAGCGCACTATGACCTAC-3                           | 343       |
| Group2and2ompAR660     | 5’-CAACTTTGATGGATTATC-3                           |           |
| Group2csuEF            | 5’-GCGCTTCAAAATCTGATGTA-3                         | 580       |
| Group2csuER            | 5’-GCGCTTCAAAATCTGATGTA-3                         |           |
| Gp2OXA69F69            | 5’-CATCGGATGTCATTGATGTA-3                         | 162       |
| Gp2OXA69R310           | 5’-GCGCTTCAAAATCTGATGTA-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 *csuE* and *bla*<sub>OXA-51-like</sub> fragments in the group 1 PCR (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.
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 blaOXA-51-like. The carbapenemase-encoding gene, blaOXA-23-like, was found in 32 isolates (64%). blaOXA-40-like (blaOXA-24-like), blaOXA-58-like, blaVIM-type, and blaIMP-type were found in 11 (22%), 1 (2%), 19 (38%), and 5 (10%) isolates, respectively. None of the isolates had positive PCR results for blaNDM-type and blaSIM-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 blaOXA-23-like gene, five isolates had blaOXA-23-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 blaOXA-23-like gene, 3 isolates had blaOXA-23-like +, VIM+ pattern and 1 isolate did not have resistance genes except for blaOXA-51-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 (20)           |
| 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)             |
| 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 (17.5)                             | 1 (25)                                 | 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)                             | 3 (60)                               | 1 (25)                                 | 0 (0)                                | 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...
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-like | Bla OXA24-like | VIM | IMP |
|------------|--------------------------------------|--------------------------------------|--------------------------------------------|-------------|----------------|----------------|-----|-----|
| Doripenem  | Resistant 19 (52.8) 7 (25.0) 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      |

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-23-like carbapenemase, 10 (8.1%) possessed bla OXA-24-like, and 1 (0.81%) bla OXA-58-like carbapenemase (31). The results of the current study showed that the carbapenemase-encoding gene, bla OXA-23-like, was found in 32 (64%) isolates and this finding was consistent with those of other studies reported from Iran. The bla OXA-40-like, bla OXA-24-like, bla OXA-58-like, bla VIM-type, and bla IMP-type genes
were found in II (22%), I (2%), 19 (38%) and 5 (10%) isolates, respectively. However, none of the isolates had positive PCR results for blaNDM-spp and blaOIM-spp (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 blaOXA-51-like (carbapenem resistance genes), showed high antibiotic resistance to all 3 antibiotics including meropenem, imipenem, and doripenem. None of the isolates had blaOXA-58-like and blaIMP-3pp.

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 blaOXA-23-like and blaOXA-24-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. Therefore, blaOXA-23-like gene; therefore, 64% of the isolates in this clone possessed blaOXA-23-like gene.

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Footnotes

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