

## Molecular Genotyping of *Acinetobacter baumannii* Species Isolated from Patients in Tehran, Iran, by Repetitive Element PCR Fingerprinting

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### KEYWORDS

Acinetobacter;  
Antibacterial Resistance;  
REP-PCR

### ABSTRACT

**Background & objective:** *Acinetobacter baumannii* is an opportunistic pathogen with high pathogenic and antibiotic-resistance potential and is also considered as one of the main nosocomial agents, specifically in the intensive care units (ICUs). It is highly important to use molecular biology methods in the epidemiological studies, determine the source of infection, and understand the relationships and distributional patterns of pathogens. Therefore, the current study aimed to determining the similar molecular types in the *A. baumannii* species isolated from patients in Tehran, Iran, by the repetitive element PCR fingerprinting (REP-PCR) method.

**Methods:** A total of 350 clinical samples were collected from patients admitted to different hospital in Tehran, assessed to identify *Acinetobacter* spp., based on the special culture media and biochemical test results. The resistance of isolates was evaluated against 11 different antibiotics. The cefepime and ceftazidime were assessed by the minimum inhibitory concentration (MIC) method, based on serial dilutions. The genome of isolated strains was extracted using the modified boiling method and amplified in REP-PCR technique using specific primers.

**Results:** In the current study, out of 120 isolates of *Acinetobacter* spp., 100 (76.9%) were identified as *A. baumannii*, mostly from ICUs and infectious diseases wards. The isolates of *A. baumannii* in the current study mostly showed antimicrobial resistance against cefepime and ceftazidime, and had the highest sensitivity to polymyxin B. About 70% of *A. baumannii* isolates in the current study were resistant to 3 or more antibiotics. According to dendrogram analyses, the patterns were classified to A- I with the maximum population (36%) of group A. All genotypes of *Acinetobacter* spp. in the current study showed resistance against carbapenems and aminoglycosides.

**Conclusion:** High similarities between the isolates in the current study indicated the high distribution of *A. baumannii* species in the hospitals of Tehran.

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### Introduction

In recent years, due to prolonged hospitalization and also increasing daily use of invasive methods such as cardiovascular catheters and ventilators in the diagnostic procedures and treatment of patients, risk of nosocomial infections increased (1-4). Nosocomial infections increase the costs, duration of hospitalization, transmission of infections to other patients, and mortality. Different microorganisms such as *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Esch-*

*erichia coli*, *Enterococcus* spp., and *Acinetobacter baumannii*, cause nosocomial infections with different dissemination patterns in the countries (2,4). *Acinetobacter baumannii* is an opportunistic pathogen with high pathogenicity and antibacterial resistance, which was one of the main causes of nosocomial infections, especially in the intensive care units (ICUs), within the last 3 years (5-7). Recently, there is dramatic increase in the use of different molecular methods such as pulsed-field gel electrophoresis (PFGE),

enzymatic cuts techniques, analysis of plasmids, and typing based on PCR in the epidemiological evaluations, finding the source of infection, and understanding the relationships and dissemination patterns of the pathogens (8,9).

Molecular methods based on PCR, used for typing purposes are more stable and less dependent on growth factors. Such methods are time-effective and are used in finding the relationships between the microbial isolates and putting strains in specific groups. There are different bacterial typing PCR-based methods such as random amplification of polymorphic DNA (RAPD)-PCR, arbitrarily primed (AP)-PCR, PCR-restriction fragment length polymorphism (RFLP), and repetitive element PCR fingerprinting (REP-PCR); each one includes advantages and disadvantages. Among the mentioned methods, the ones evaluating the repetitive elements in bacterial genome are widely used, due to the stability of such elements during evolution. One of the methods used for the sequencing purposes is REP-PCR. Methods such as enterobacterial repetitive intergenic consensus (ERIC)-PCR, 23SARDRA, 16SARDRA and etc. have more differentiating abilities and are also time-effective, compared to PCR-RFLP and PFGE methods (10-12). Therefore, in the current study the REP-PCR technique was used for the molecular typing purposes in *A. baumannii* species isolated in Tehran, Iran.

## Materials and Methods

### Samples and species

In the current study, a total of 350 clinical samples of blood, urine, wound, trachea, etc., were collected from patients admitted to different hospitals in Tehran (Imam Khomeini, Baqiyatallah, Milad, etc.) from 2014 to 2015. The collected samples were transferred to the laboratory in brain-heart infusion (BHI) broth (Merck company, USA) .

### Isolation of *Acinetobacter baumannii*

The clinical samples were cultured on blood agar and MacConkey agar (Merck company, USA) and incubated for 24 hours at 37°C. Then, the cultures were evaluated using specific media and biochemi-

cal tests such as urease, OF (oxidation-fermentation), SIM (sulfide, iron, motility), MR/VP (methyl red and Voges-Proskauer), in addition to catalase, oxidase, and Simmons citrate tests as well as growth at 37°C and 42°C to identify different species of *Acinetobacter*.

### Susceptibility testing based on the disc diffusion method

To determine the antimicrobial-resistant phenotypes, the disc diffusion method was used according to the guidelines of the Clinical and Laboratory Standards Institute (CLSI) (13). In the current study, 11 different antibiotic disks, purchased from MAST Company (Mast Diagnostics, Mast group Ltd., Merseyside, UK), and were used as follows:

cefepime (30µg), ceftriaxone (30µg), amikacin (30µg), imipenem (10µg), piperacillin-tazobactam (110µg), meropenem (10µg), gentamicin (12µg), tobramycin (10µg), tetracycline (30µg), ampicillin-sulbactam (20 µg), and polymyxin B (300 µg).

To assess the quality of test, *E. coli* ATCC 25922 and *A. baumannii* ATCC 19606 were used as negative and positive controls respectively (13).

### Minimum inhibitory concentration testing

According to CLSI (2014) guidelines, a minimum concentration of an antibiotic, which inhibits bacterial growth, is referred to MIC. In the current study, MIC was assessed for Cefepime and Ceftazidime, based on the macro dilution method (13).

### DNA extraction and PCR amplification

Genomic content of all *A. baumannii* isolates were extracted by the modified boiling method. For this purpose, a loopful of pure culture was dissolved in 100 µL of saline solution and, then placed in a boiling water bath at 100°C for 20 minutes and immediately centrifuged in microtubes at 12 000 g for 15 minutes. The supernatant contained bacterial DNA, which its purity was assessed on 0.8% agarose gel and the spectrophotometric method (14). The PCR reaction was performed in a total volume of 25 µL including 12 µL of 2X Master Mix (Ampliqon III

Ltd., Denmark, containing 20 mM dNTP, 1.5 mM MgCl<sub>2</sub>), 2 μL (10 ngr/ μL) of DNA pattern, 1 μL of primer containing 10 pmol of each primer (reverse: 5'-IIIGCGCCGICATCAGGC-3'; forward: 5'-ACGTCTTATCAGGCCTAC-3') and double distilled water to reach a total volume of 25 μL. PCR product was electrophoresed on 1% agarose gel and, then, using GelClust software, DNA segments were analyzed based on Dice algorithm (distance matrix analysis), and the clones clustering was conducted by UPGMA

## Results

In the current study, out of 120 species of *Acinetobacter* isolated from 350 hospitalized patients, 100 (76.9%) isolates were identified as *A. baumannii*, 13 (11%) as *A. lwoffii*, and 7 (6%) as other species of *Acinetobacter*. Out of 100 isolated species, 40 were obtained from ICUs, 30 from infectious diseases wards, 20 from emergency rooms, and 10 from other wards. Among all *A. baumannii* species isolated in the current study, 40 (40%) were obtained from blood samples, 27 (27%) from tracheas, 12 (12%) from wounds, 8 (8%) from urine samples, and 13 (13%) from unknown sources.

The highest antibiotic resistance in *A. baumannii* isolates was observed against cefepime, ceftriaxone, and amikacin, respectively; the lowest resistance was also reported against polymyxin B (Table 1). About 70% of *A. baumannii* isolates showed resistance against 3 or more antibiotics. Also, results of the current study indicated that none of the isolates was resistant against all antibiotics and all of them showed

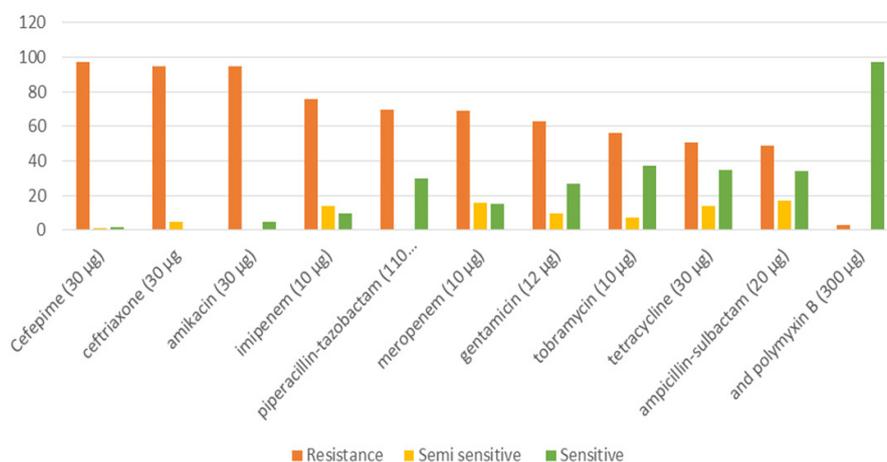
susceptibility at least to 1 antibiotic.

Among the isolated species in the current study, MIC for ceftazidime and cefepime were  $\geq 128$  μg/mL in 84% and 91% of the isolates, respectively.

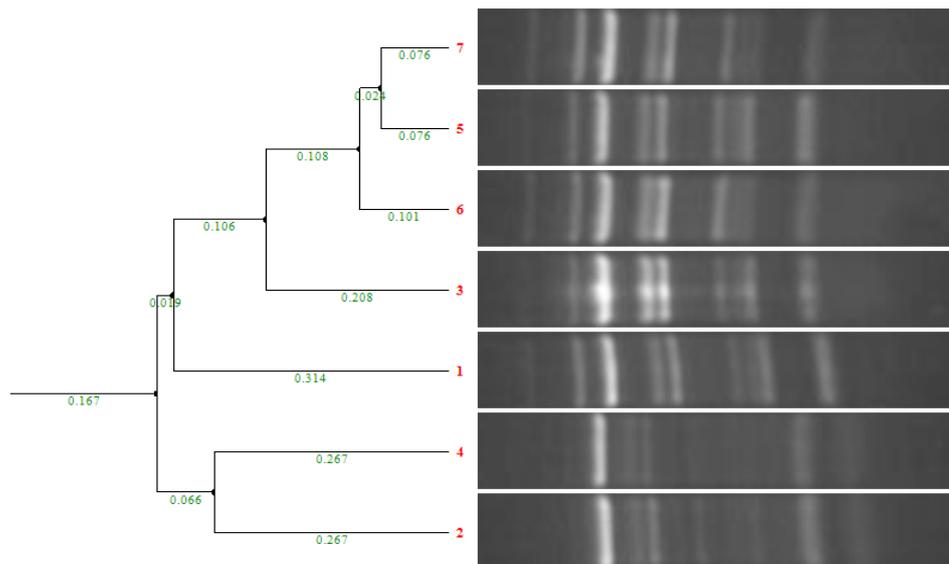
Using REP1 and REP2 primer pairs, the presence of palindromic repeat sequences was assessed by REP-PCR method. All 100 isolates of *A. baumannii* were assessed by this method and accordingly, 9 to 15 PCR amplifications were illustrated. According to dendrogram analysis and evaluation of segments obtained from the electrophoresis of REP-PCR products on 1% gel agarose, 9 different patterns were obtained, which were categorized from A to I (chart 1). Evaluation of PCR patterns indicated that 36% of the isolates were belonging to group A, 4% to group B, 8% to group C, 2% to group D, 10% to group E, 2% to group F, 8% to group G, 20% to group H, and 10% to group I. Size of the segments ranged from -4 to 0.2 kb.

To determine the patterns, only the bands < 3 kb or more >0.2 kb were used. After the analysis of patterns by the visual method using GelClust software, dendrogram was constructed and accordingly, 9 genotypes were obtained. All genotypes of *Acinetobacter* spp. showed resistance against carbapenems and aminoglycosides in the current study.

Results of the current study showed that *A. baumannii* species isolated from patients admitted to Imam Khomeini, Milad, and Rasoul Akram hospitals had the same genotype and the species isolated from Baqiyatallah hospital had a different genotype, compared to other ones.



**Figure 1.** The frequency of antibiotic resistance among the *A. baumannii* isolates



**Chart 1.** Dendrogram constructed based on REP-PCR amplifications of *A. baumannii* isolates based on their similarity coefficient (Dice coefficient).

## Discussion

*Acinetobacter baumannii* is one of the pathogens involved in nosocomial infections, which its cases increase annually (5, 6). Similar to the results of the studies by Constantiniu et al. and Khalatabadi et al., which reported the frequency of *A. baumannii* 75% and 80%, respectively (15,16); the frequency of *A. baumannii* isolates was shown 83% in the current study, which was higher than those of other species of *Acinetobacter*. Resistance of *A. baumannii* against most of the routine antibiotics caused severe problems in controlling these bacteria. The current study was designed as *A. baumannii* is one of the main causes of recent nosocomial infections, especially in ICUs, its number is increasing, and it shows resistance to different antibiotics in different hospitals. Today, dissemination of resistance genes through plasmid transmission among bacteria is a severe problem in successful treatment of infections (17-21). According to the results of the current study, *A. baumannii* was mostly isolated from ICUs in the studied hospitals by 76%. It was also indicated that the antibiotic resistance was high among the *A. baumannii* species isolated from hospitals; even about 70% of *A. baumannii* isolates were the multiresistant strains (19, 20). The isolated species of *A. baumannii* mostly showed resistance to cefepime, ceftriaxone, and amikacin. Results of the study by Kartbika and Rahar on the resistance pat-

tern of these bacteria were consistent with those of the current study (21,22), but in contradiction with the results of Ayan et al., which can be due to the different study design and time (23). According to the results of antibiogram in the current study, polymyxin B was the only antibiotic that was most effective against all *A. baumannii* isolates. Similarly, Mak et al., also reported polymyxin B as an effective antibiotic on *A. baumannii* (24).

Recently, using of different molecular methods is of great importance in the epidemiological evaluations, determining the source of infection, and understanding the dissemination pattern and associations of bacteria. The molecular methods are usually performed based on REP-PCR, which are less dependent on the bacterial growth variables and are more sustainable. Such methods are time-effective and are used in finding the relationships between the microbial isolates and putting strains in specific groups. Families of repeated sequences are available in the genomic content of different groups of bacteria, which are useful in genotyping purposes (25,26). Repetitive element sequence based -PCR is one of the most useful methods in the sequencing of such segments. This method benefits from higher differentiating ability, compared with PCR-ERIC, ARDRA16S, and 23SARDRA, and is also time-effective compared with RFLP-PCR and PFGE methods (27). Evaluation of 120 genomic con-

tents of *A. baumannii* by REP-PCR showed 9 clones among the species isolated from different hospitals in Tehran. Most of the *A. baumannii* isolates (36%) were attributed to genotype A. In the study by Misbah et al., in Malaysia, 109 isolates of *A. baumannii* were categorized in 9 genotypes using REP-PCR method and most of the isolates attributed to 2 genotypes, which was almost consistent with the results of the current study (27). Most of the *A. baumannii* species in genotype A isolated from trachea and blood samples, which indicated that application of invasive instruments such as trachea and cardiovascular catheters during the therapeutic procedures may play a role in the dissemination of *A. baumannii* clones. In a study in China in 2009 on 49 isolates of *A. baumannii* using REP-PCR method, the isolates were categorized in 9 genotypes, out of which 2 genotypes were identified in 3 hospitals, 2 other genotypes in 1 hospital, and another genotype in 2 hospitals (28). Results of the study indicate a cross of *A. baumannii* in different hospitals, which was consistent with those of the current study, based on the dendrogram analysis on the same genotype among 3 hospitals in Tehran. Genotyping assessments indicated significant similarities among *A. baumannii* isolates in the current study. Bacterial species isolated from Baqiyatallah hospital in Tehran had various genotypes, different from the genotypes identified in other studies, which could be due to particular patients.

### Conclusion

High similarities among the studied species indicated the dissemination of *A. baumannii* species isolated from different hospitals in Tehran. Dissemination of the bacteria can be done by physicians, personnel of the hospital, and medical equipment. Increase in the antibacterial resistance of *A. baumannii*, in addition to the obtained fingerprinting pattern, emphasized the need to design programs to apply more control and supervision over the hygiene standards in the hospitals.

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### Conflicts of interest

The authors declared no conflict of interest.

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