



Molecular Typing and Antimicrobial Susceptibility of *Acinetobacter baumannii* Isolates in Kermanshah City with Pulse Field Gel Electrophoresis (PFGE)

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Authors' contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

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ABSTRACT

Background and Objective: *Acinetobacter baumannii* (*A. baumannii*) in the last decade has been identified as one of the opportunistic pathogens and an important cause of nosocomial infections. Molecular typing plays an important role in studying the epidemiology of *Acinetobacter*. The aim of this study was to investigate the genomic pattern which was performed by Pulse Field Gel Electrophoresis (PFGE) and antimicrobial susceptibility of the isolates from patients in various hospitals in the city of Kermanshah, the city which is located in the west of Iran.

Materials and Methods: 33 isolates of *A. baumannii* were collected from clinical samples in four general hospitals of Kermanshah. Isolates were identified by biochemical tests and API 20NE kit. The antimicrobial susceptibility of isolates was determined by Kirby-Bauer disk diffusion method. The clonal connection was estimated by PFGE and DNA patterns were analyzed by Gel compare II 6.5 software.

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Results: All isolates showed high-level of resistance, but resistance which was observed against Colistin and Minocycline was low, while no resistance to Polymyxin B and Tigecycline was observed. The PFGE analysis revealed the existence of 10 different genetic patterns among the 33 strains including: I (n = 12), II (n = 2), III (n = 4), IV (n = 3), V (n = 3), VI (n = 4), VII (n = 2). Clone I was the dominant clone. In terms of antibiotic resistance, no significant difference was observed among the different genetic patterns.

Conclusion: Isolates were obtained from a large variety of patterns genetically. This study could represent the wide range of isolates of *A. baumannii* that were gathered from different parts of the hospital. Diverse sources of infection may, therefore, appear to control these infections, according to various sources, which are not simple.

Keywords: Antimicrobial resistance; *Acinetobacter*; pulsed-field gel electrophoresis; Kermanshah.

1. INTRODUCTION

There are many infectious diseases transmitted to humans through the hospitals [1,2]. One of them is infectious pathogens *Acinetobacter baumannii* (*A. baumannii*). This is an important pathogen and an opportunistic bacteria that cause side effects in patients, especially patients in hospital intensive care unit [3,4]. The bacteria involved in infections such as ventilator-associated pneumonia, infections of urinary tract, bacteremia, meningitis and wound infection [4,5]. This organism lasted well on environmental conditions for a long time in the hospital environment through pollution levels, lead to the spread of infection in hospitals [5,6]. The ability of these organisms for achieving different mechanisms of resistance and the resistance of some strains to all common antibiotics are available and also the lack of antimicrobial drug effects are the most important causes of risk in this bacterium. The prevalence of multidrug-resistant (MDR) *A. baumannii* isolates increased in a recent decade. The MDR was defined resistant to three classes of antibiotics, including quinolones (ciprofloxacin), Cephalosporins (cefepime and ceftazidime) carbapenems and (imipenem and meropenem) and also resistant to carbapenem, called extensively-drug resistant (XDR) [2]. The frequency of *A. baumannii* in healthcare centers has increased around the world, so molecular typing plays an important role in the epidemiology of bacterial isolates typing. Therefore it seems useful to identify the type diversity in a region and to determine the dominant type in a period of time [4,7,8]. Pulsed-Field Gel Electrophoresis (PFGE) is one the common methods for separation and identification of bacteria that is used in epidemiologic bacteria typing [1,4]. *A. baumannii* in the past two decades has been identified as one of the nosocomial infections and thus causes mortality in hospitalized patients. Molecular

typing plays an important role in epidemiological studying of *Acinetobacter* in nosocomial infections [3,9,10]. The aim of this study was to determine the antimicrobial profile and investigate the genomic pattern of PFGE of the isolates from patients in various hospitals of Kermanshah.

2. MATERIALS AND METHODS

2.1 Bacterial Isolates

In this study, different clinical samples were collected during 2014-2015 (such as sputum, burn, wound, blood, and urine) from patients admitted to four hospitals of Kermanshah in the survey and after review of microbiology. *A. baumannii* isolates were identified by biochemical tests such as oxidase test, TSI, EMB, blood agar and chocolate agar and then confirmed by API 20 NE kit (BioMerieux, France) [2].

2.2 Antibiotic Susceptibility Test

Antimicrobial susceptibility test for 20 antibiotics was carried out by disk diffusion method on Mueller Hinton Agar (Merck, Germany) according to the recommendations of the Clinical and Laboratory Standards Institute (CLSI) [11]. The antibiotics tested were Ceftriaxone (30 µg), Ciprofloxacin (5 µg), Levofloxacin (5 µg), Meropenem (10 µg), Cefepime (30 µg), Amikacin (30 µg), Cefotaxime (30 µg), Ceftazidime (30 µg), Polymyxin B (300 units), Gentamicin (10 µg), Imipenem (10 µg), Mezlocillin (15 µg), Minocycline (30µg), Tetracycline (30 µg), Tigecycline (15 µg), Colistin (10 µg), Tobramycin (10 µg), Gatifloxacin (5 µg), Piperacillin (100 µg), Cotrimoxazole (1.25 µg) (Mast Group, UK).

Escherichia coli ATCC25922, *Pseudomonas aeruginosa* ATCC27853, and *Escherichia coli* ATCC35218 used as a control for antimicrobial susceptibility test.

2.3 Pulsed-field Gel Electrophoresis (PFGE)

The PFGE methods were done for isolates as described by Mohajeri et al. [3]. *A. baumannii* ATCC 19606 as the internal reference strain and Lambda Ladder PFGE Marker (NEB) were used in this study. Genome was digested by *Apal* (New England Biolabs, Ipswich, MA, USA) restriction enzyme. Following *Apal* digestion, the genomes were loaded into a 1% Low electroendosmosis agarose (Merck, Germany). Electrophoresis was performed using a CHEF MAPPER apparatus (Bio-Rad, USA) at conditions: temperature 14°C; voltage 6 V/cm; switch angle, 120°; switch ramp 2.2–35 s for 19 h. The Dice coefficient was used to calculate similarities, and the unweighted paired group method based on average linkages (UPGMA) was used for cluster analysis with Gel compare II version 7 (Applied Maths, St Martens-Latem, Belgium). When the pattern similarity was > 80% considered to be derived from a cluster (Fig. 1).

2.4 Statistical Analysis

Data were recorded and entered into an Excel file. Statistical analyses were performed using SPSS software (version 20). Variables were analyzed by chi-square test. A p-value of < 0.05 was set as the statistical significance of all analyses.

3. RESULTS

A total of thirty three clinical isolates of *A. baumannii* were collected from four hospitals of Kermanshah. Twenty (60.6%) strains from Imam Reza, 9 (27/3%) strains from Taleghani, 3(9%) Imam Khomeini and 1(3%) strains from Mohammad Kermanshah (Iran). All isolates were from hospitalized patients. The clinical samples were included Sputum and lung secretions (n=19, 57/6%), burn infections (n=7, 21/2%), urine (n=3, 9%), blood (n=2, 6%) and tissue (n=2, 6%). They were collected from female (14, 42/4%) and male (19, 57/6%).

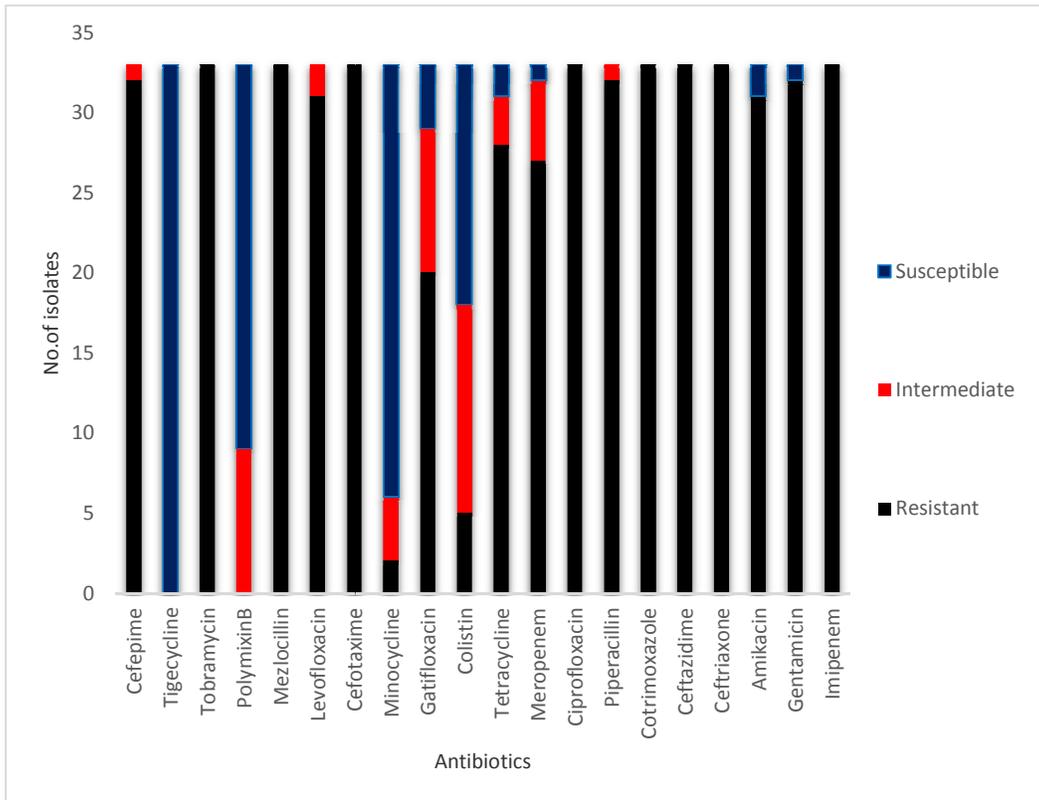


Fig. 1. Antimicrobial-susceptibility for *Acinetobacter baumannii* isolates

The disk diffusion susceptibility testing showed high-level of resistance to Imipenem (100%), Extended-spectrum cephalosporins (100%), Fluoroquinolones (>90%) and Aminoglycosides (>90%) as well as resistance to other antibiotics (Fig. 1). Resistance was observed against Colistin (15.2%). Resistance rates for Minocycline were low (6%), while no resistance to Polymyxin B and Tigecycline was observed. So there were effective antibiotics such as Polymyxin B, Colistin, Tigecycline and Minocycline on bacteria. MDR was defined resistant to three classes of antibiotics including cephalosporins (Cefepime and Ceftazidime), carbapenems (Imipenem and Meropenem), quinolones (Ciprofloxacin) and also resistant to carbapenem which is called XDR. MDR and XDR frequency were respectively (54.5% and 36.4%). Prevalence of MDR and XDR strains were higher in the ICU. According to the dendrogram, PFGE results suggested that the samples of *A. baumannii* collected from the patients hospitalized in hospitals of Kermanshah have 7 clones and 3 sub-clones. Amplitude of the clones were I [sub I (n=5), sub II (n=4), sub III (n=3)] and II (n=2), III (n=4), IV (n=3), V (n=3), VI (n=4), VII (n=2), respectively (Fig. 2). Three of the patterns were *sporadic and had no genetic relationship with the rest of the groups.

Clone I is the dominant clone with the most samples (12 samples). In the next rank, each of clones III and VI had 4 samples. Most of the isolates were collected from sputum and lung secretions* from the ICU section. Genetic pattern IV was observed only among isolates of *A. baumannii* isolated from ICU of Imam Reza Hospital. Isolated distribution is shown in terms of genetic pattern and sections of hospitals (Table 1). Genetic patterns had differences among the isolates *from Imam Khomeini hospital with strains isolated from Taleghani Hospital, but the same genetic patterns were observed among the isolates isolated from Imam Khomeini with Imam Reza hospital and strains isolated from hospital Reza with Taleghani Hospital. In terms of antibiotic resistance, no significant differences were observed among the different genetic patterns (Table 2).

4. DISCUSSION

Acinetobacter species, particularly *A. baumannii*, are important opportunistic pathogens cause of severe nosocomial infections, especially in intensive-care-unit (ICU) patients [12]. In this

study, the 70% of *Acinetobacter* spp. were isolated from the Intensive Care Unit (ICU) which is similar to the report of a previous research [13]. In our study, it is showed that the 57% of isolates are related to sputum, similar to * other studies [14]. These bacteria have a great tendency to acquire resistance to multiple classes of antimicrobials [15]. As our findings showed that 54% were MDR. There are increasing reports of multidrug-resistant *A. baumannii* outbreaks in clinical settings worldwide [16]. The isolates were susceptible to antibiotics: Tigecycline (100%), Polymyxin B (72%), Minocycline (81%), Colistin (45%), respectively. Only these four antibiotics can be used as effective drugs for the treatment of *A. baumannii* infections, but resistance from some of them observed. A high incidence of MDR strains were found in ICU wards in our study. It could be attributable to high usage of antimicrobials agents in ICU. These findings are in agreement with the result of other study in Iran [17]. Therefore, due to high resistance and easy riddance and the ubiquitous strains of *Acinetobacter*, as well as the ability to transfer resistance genes through genetic factors motile, such as plasmids and integrons in bacteria, the hospital should reduce the indiscriminate use of antibiotics in wide range [6]. MDR *A. baumannii* is an important pathogen that is related with outbreaks of nosocomial infections especially in ICU wards. The clonal study of hospital strains is very important in terms of understanding the epidemiology of these outbreaks and controlling nosocomial infection [9]. Today, many hospital infections are prevented to spread using different classification methods in hospital, and the economy and health of different societies has been improved thanks to different classification methods in hospitals [18]. As mentioned before, PFGE method was used by Schwartz as a tool for checking the DNA chromosomes of eukaryote organisms [19]. This method of classification is used for many bacteria including *A. baumannii*, *E. coli* and *Pseudomonas*. Although it is believed that the specification of nucleic acid sequence is the best method to determine the epidemiologic affinity of strains, but recently PFGE is mentioned as the golden standard method [20]. In this study, shown results of PFGE isolates of *A. baumannii* are collected from patients admitted to hospitals in Kermanshah which has ten genetic patterns that three of the them were examples of sporadic that had no genetic relationship with the rest of the group that these result are consistent with the result of Farahani et al. in Tehran [17].

Clone I was involved in the majority of outbreaks in ICU. Clone III and VI were the second most common patterns involved in outbreaks.

In study zhang and et al in China in 2010, the most common clones were relevant to the ICU in our study more strains clone I as the clone most common to the ICU [21]. Molecular typing revealed two features among genotypic patterns of *A.baumannii* isolates; first the isolates with genotypic diversity and the second isolates with similar genotypes. Polymorphism among isolates represents the different origin of isolates. Because of the easy and ubiquitous presence of *A. baumannii* isolates, as a result, these isolates are acquired more by patients from various

sources. In our study among thirty-three isolates were obtained ten genetic patterns that represents the genetic variation in a population of *A. baumannii*, in other studies on *Acinetobacter* including a study in Turkey on sixty-six isolates of *Acinetobacter* thirty-six clones were obtained [22], a study in twenty-nine samples were obtained from six genetic patterns [10].

Acinetobacter reviews seventeen samples in China was a third genetic pattern [23]. Studies have also shown *the genetic variation among isolates of *A. baumannii*. In this study, isolates with similar genetic patterns from a hospital may be due to the transfer of the hospital, particularly in ICU on patients, staff and contaminated equipment within the hospital and

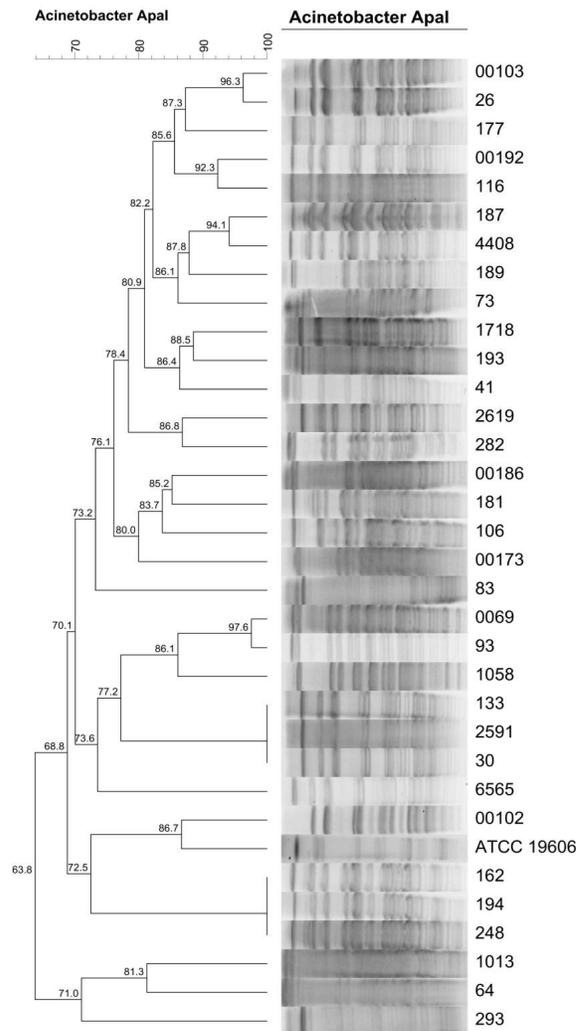


Fig. 2. Pulsed-field gel electrophoresis dendrogram of isolates of *Acinetobacter baumannii*. The pulsotypes were identified at a cut-off value of 80%

Table 1. The distribution of clones isolated among hospital wards

Clone	Number of isolates	Hospitals									
		A					B		C	D	
		ICU	Burn	Surgery	Infectious	Children	Burn	ICU	ICU	Burn	
I	12	4	2	2	0	0	0	0	3	1	
II	2	1	0	0	0	0	0	0	1	0	
III	4	1	0	0	1	0	0	0	2	0	
IV	3	3	0	0	0	0	0	0	0	0	
V	3	1	0	0	0	0	0	0	2	0	
VI	4	2	0	0	1	0	0	1	0	0	
VII	2	0	1	0	0	0	1	0	0	0	
Single colone	1	0	0	0	0	0	1	0	0	0	
Single colone	1	0	0	0	0	1	0	0	0	0	
Single colone	1	0	0	0	0	0	0	0	1	0	
Sum	33	12	3	2	2	1	2	1	9	1	

A=Imam Reza hospital; B=Imam Khomeini hospital; C=Taleghani hospital; D= Mohammad kermanshahi

Table 2. Comparison of PFGE pattern with antimicrobial susceptibility

Different antibiotics and wards	Clones										p
	I	II	III	IV	V	VI	VII	Single colone	Single colone	Single colone	
Minocycline	0	1(50%)	0	0	0	1(25%)	0	0	0	0	0.232
Gatifloxacin	6(50)	2(100)	3(75)	2(66.6)	1(33.3)	4(100)	1(50)	0	1(100)	0	0.480
Colistin	2(16.6)	0	0	1(33.3)	0	1(25)	1(50)	0	0	0	0.785
Tetracycline	8(66.6)	2(100)	3(75)	3(100)	3(100)	4(100)	2(100)	1(100)	1(100)	1(100)	0.987
Meropenem	9(75)	2(100)	4(100)	2(66.6)	3(100)	2(50)	2(100)	1(100)	1(100)	1(100)	0.747
PolymixinB	4(33.3)	0	0	1(33.3)	2(66.6)	1(25)	1(50)	0	0	0	0.806
MDR	5(41.6)	1(50)	2(50)	2(66.6)	2(66.6)	4(100)	1(50)	0	1(100)	0	0.579
XDR	2(16.6)	1(50)	2(50)	1(33.3)	2(66.6)	2(50)	1(50)	0	1(100)	0	0.652

in all clones except clone four, are isolated with 100% similarities in their genetic pattern indicates the different hospitals of the hospital by patients to other hospitals. Two of samples, the clones III the ICU of Taleghani hospital with antibiotic resistance pattern similar that reflects the commonality of their origin. Clones V and VI have a genetic relationship with respect to more samples of the two clones isolated from the ICU that could have been transferred from patient to patient.

5. CONCLUSION

In conclusion, the results of this study have shown that genetic patterns among *A. baumannii* isolated from Kermanshah hospitals are different, and in a hospital, genetic patterns links have caused the infection which in fact reflects the commonality of origin of genetic. It was also seen between different hospitals similarities in genetic patterns that may be due to the displacement of patients in hospitals. There are various genetic patterns represent the different genetic origins of isolates. While there may be varied sources of infection, it is important to control methods of disinfection of hospital hygiene by the patients and hospital staff in infection control, particularly in the ICU of the hospital for the duration of hospitalization and the severity of their disease, which are among the areas at risk top.

ETHICAL APPROVAL AND CONSENT

This work was approved (96515) by the research ethic committee of Kermanshah University of Medical Sciences (KUMS). In this way, all patients gave their written informed consent for participation.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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