

# Molecular Characterization of Multidrug Resistant Strains of *Acinetobacter baumannii* Isolated from Intensive Care Units in West of Iran

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## ABSTRACT

**Introduction:** According to the results of various studies using phenotypic methods, the prevalence of Multidrug Resistant (MDR) *Acinetobacter baumannii* (*A. baumannii*) isolates has been increasing worldwide. Pulsed-Field Gel Electrophoresis (PFGE) technique is known as the gold standard method to determine clonal characterization of bacterial species, especially *A. baumannii*.

**Aim:** To determine the clonal relatedness and investigate the prevalence of integron classes 1 and 2 and genes encoding OXA-23 and 24 in *A.baumannii* isolates.

**Materials and Methods:** A cross-sectional study was conducted from November 2011 to January 2013. A total of 140 *A.baumannii* isolates collected from three hospitals of Kermanshah were considered out of which 75 ICU isolates were included in this study. Antibiotics susceptibility test was done by disk diffusion method. Polymerase Chain Reaction (PCR)

was performed in order to detect class 1 and 2 integrons and *bla*<sub>OXA-23</sub>-like, *bla*<sub>OXA-24</sub>-like genes. Isolates identified as MDR from a total of 75 Intensive Care Units (ICU) strains were subjected to genotyping for clonal relatedness.

**Results:** A total of 37 isolates among 75 ICU isolates were identified as MDR. The maximum drug resistance was observed against ceftriaxone, mezlocycline, cefotaxime, piperacilin, ciprofloxacin and imipenem. Frequency of Class 1 and Class 2 Integrons, *bla*<sub>OXA-23</sub>-like and *bla*<sub>OXA-24</sub>-like genes were 33(44%), 27(36%), 60(80%) and 14(18.6%) respectively. Four clusters with high level of similarity were obtained showing homogeneity among MDR isolates.

**Conclusion:** Significant correlation between presence of integrons and resistance to different classes of antibiotic was observed in this study. Monitoring of drug resistance using gene integrase PCR and *bla*<sub>OXA</sub> gene by cluster analysis is very important to plan specific infection control measures due to MDR *A. baumannii*.

**Keywords:** Ceftriaxone, Genotyping, Integron, OXA gene

## INTRODUCTION

*A.baumannii* has emerged as an important nosocomial pathogen in the healthcare setting. *Acinetobacter* has become a risk factor of infections in hospitals particularly Carbapenem Resistant *A.baumannii* (CRAB) which is an increasing problem in this area in recent years [1].

The carbapenem class has been considered as a choice for treating serious *A.baumannii* infections for many years [2]. According to the results of various studies using phenotypic methods, the prevalence of MDR *A.baumannii* isolates has been increasing worldwide and has become one of the most difficult pathogen to treat [2,3]. The frequency of MDR *A.baumannii* isolates increased between 2001- 2007 compared to 2010 to 2013 in Iran. [2,3] Resistance to carbapenem has shown a dramatic increase between 2010 and 2013 [3]. According to studies done by PCR-based molecular methods, resistant genes has greatly spread from 2001 to 2013 which indicates a quick gene transfer between isolates through mobile genetic elements or resistance transfer factor plasmids [3,4].

Mobile genetic elements including integrons, plasmids and transposons are the main resistance transfer factors in Gram-negative bacteria especially *A.baumannii*. A number of studies have shown that, there is a direct relationship between drug resistant isolates and the existence of chromosomal mobile elements [4,5].

The rate of *A.baumannii* resistance to imipenem is considerably lower than the rates found in the neighboring countries including Saudi Arabia (63%), United Arab Emirates (76%), Turkey (98%) and Pakistan (100%) compared to Iran [6]. Typing methods play an important role in assessing interspecies communication. Spread studies can be done easily using these methods and species

diversity can be identified [2,7]. Now-a-days, different typing methods such as PCR based methods {Enterobacterial Repetitive Intergenic Consensus (ERIC) and Random Amplified Polymorphic DNA (RAPD)}, Variable Number Tandem Repeat (VNTR), PFGE typing has been proposed to determine the common clones of *A.baumannii* [7,8].

PFGE technique is known as the gold standard method used to identify bacterial species, especially *A.baumannii* [2]. Although, it is arduous, required equipments are already available not only at Reference laboratories, but also at some of the advanced laboratories of the hospitals [9]. Knowledge of clonal outbreaks is of importance in epidemiological studies for *A.baumannii* infections [2]. Thus, the present study was conducted to determine clonal relatedness between MDR strains of *A.baumannii* isolates.

## MATERIALS AND METHODS

A cross-sectional study was conducted from November 2011 to January 2013. A total of 140 *A.baumannii* isolates from clinical samples (blood, sputum, wounds, urine, abdominal abscesses, synovial) identified using biochemical tests and kit API 20 NE, obtained from three hospitals of Kermanshah region, Iran was considered and of these only 75 *A.baumannii* ICU strains were included in this study. Isolates identified as MDR from these 75 ICU strains were subjected to genotyping (PFGE). The MDR determination was done according to the criteria as described previously [2]. Isolates resistant to at least three classes of anti-microbial agents (all penicillins and fluoroquinolones, cephalosporins, and aminoglycosides) were identified as MDR.

Kirby Bauer method was done according to the CLSI guidelines [10] for 20 antibiotics: levofloxacin (5 µg), gatifloxacin (5 µg),

ciprofloxacin (5 µg), tobramycin (10 µg), gentamycin (10 µg), tigecycline (15 µg), amikacin (30 µg), meropenem (10 µg), imipenem (10 µg), piperacilin (100 µg), mezlocycline (75 µg), cotrimoxazole (30 µg), polymyxine b (300 unit), colistin (10 µg), tetracycline (30 µg), minocycline (30 µg), cefepime (30 µg), cefotaxime (30 µg), ceftazidime (30 µg), ceftriaxone (30 µg).

**DNA extraction and PCR:** Bacterial DNA was extracted from *A. baumannii* isolates by boiling. PCR was carried out for amplification of genes in MDR isolates. Primers for Classes 1 and 2 Integron genes were designed as previously described by Mirnejad et al., [5] and *bla*<sub>OXA-23</sub>-like and *bla*<sub>OXA-24</sub>-like genes previously described by other authors [2].

**Pulsed-field gel electrophoresis analysis and dendrogram construction:** MDR strains were subjected to PFGE analysis using the methods as described by Mohajeri et al., [2]. The DNA banding patterns were analysed using Bionumeric 7.0 software (Applied maths NV, St-Martens-Latem Belgium). Cut off levels of 85 and 100% were applied to this dendrogram.

## RESULTS

Thirty seven isolates were MDR among 75 isolates. High resistance was observed to mezlocillin, ceftriaxone, cefotaxime (>90%) and imipenem, ceftazidime, levofloxacin, piperacilin (>80%) [Table/Fig-1] and susceptible to colistin, polymyxin B and tetracycline. Frequency of Class 1 and Class 2 Integrons, *bla*<sub>OXA-23</sub>-like and *bla*<sub>OXA-24</sub>-like were 33(44%), 27(36%), 60(80%) and 14(18.6%) respectively. Resistance to cephalosporins, carbapenemes, fluoroquinolones, penicillin and tetracycline were related to coexist in integrons and *bla*<sub>OXA</sub> genes [Table/Fig-1]. Significant correlation between presence of integron's and resistance to different of antibiotic classes was observed. A total of 13 (35.1%), 10 (27%) pulsotypes were positive for integron Classes 1, 2, and 32 (86.5%), 9 (24.3%) pulsotypes were positive for *bla*<sub>OXA-23</sub>-like and *bla*<sub>OXA-24</sub>-like in MDR isolates, respectively [Table/Fig-2]. Four clusters were

Antibiotic Class	Tested Members	Antibiotic			Integron-Positive <sup>1</sup> in MDR Isolates			OXA-Positive <sup>2</sup> in MDR Isolates		
		S	I	R	S	I	R	S	I	R
Fluoroquinolones	Levofloxacin	17	5	53	9	2	12	12	3	26
	Gatifloxacin	26	8	41	11	2	10	14	5	20
	Ciprofloxacin	10	0	65	3	0	20	8	0	33
Aminoglycoside	Tobramycin	34	6	35	10	2	11	21	3	17
	Gentamicine	14	0	61	8	0	15	14	0	27
	Tigecycline	73	1	1	19	2	2	38	1	1
	Amikacin	19	9	47	12	3	8	17	4	20
Carbapenem	Meropenem	13	5	57	3	4	16	1	3	36
	Imipenem	10	3	62	4	1	18	2	2	37
Spread range of penicillin	Piperacilin	9	0	66	7	0	16	11	0	30
	Mezlocycline	3	0	72	0	0	23	0	2	39
Sulfonamide	Cotrimoxazole	24	1	50	11	0	12	19	0	22
	Polymyxin B	65	0	10	21	0	2	35	0	6
	Colistin	67	0	8	21	0	2	37	0	4
Tetracycline	Tetracycline	13	1	61	3	0	20	12	0	29
	Minocycline	54	6	15	13	1	9	27	1	10
Cephalosporins	Cefepime	11	0	64	4	0	19	9	0	32
	Cefotaxime	4	0	71	0	0	23	0	0	41
	Ceftazidime	11	0	64	5	0	18	9	0	32
	Ceftriaxone	3	0	72	0	1	22	0	3	37

**[Table/Fig-1]:** Distribution of resistance in 75 clinical *A. baumannii* isolates with arrangement by antibiotic Classes, Integrons and OXA genes.

<sup>1</sup>Integron-positive isolates= Integron I + Integron II

<sup>2</sup>OXA- Positive= *bla*<sub>OXA-23</sub>-like + *bla*<sub>OXA-24</sub>-like

S: Susceptible

I: Intermediate

R: Resistant

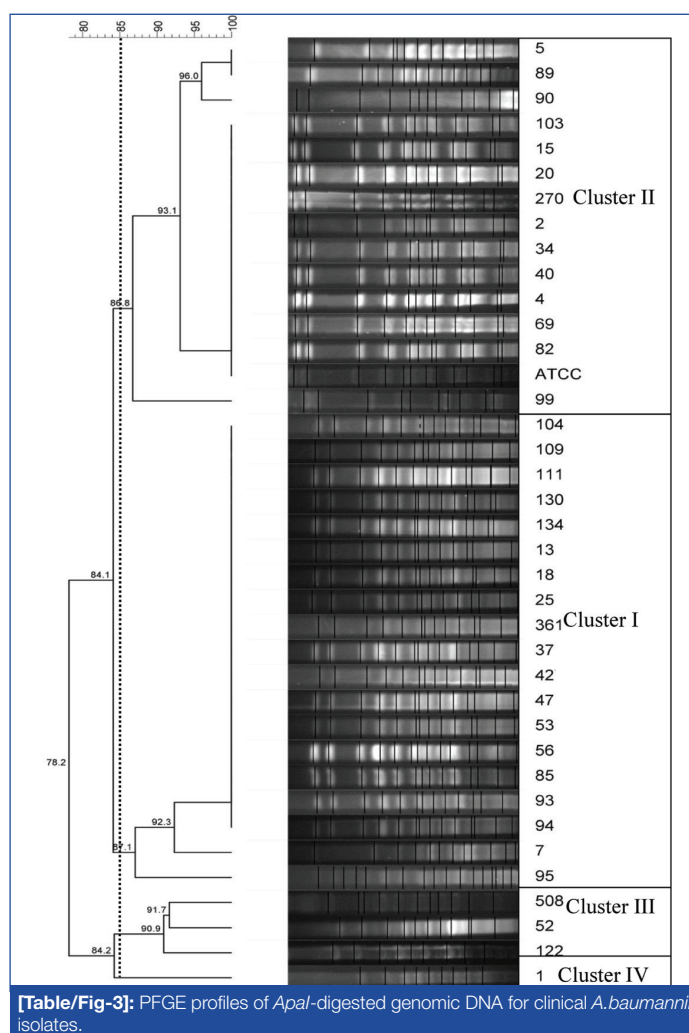
obtained through PFGE, cluster I and II were the most frequent isolates with 14 and 19 isolates and were present to the greatest extent, after them, there were cluster number three and four with three and one isolates, which were considered as a single clone [Table/Fig-3]. High homogeneity was observed among MDR isolates.

Pulsotypes	Integron 1		Integron 2		OXA-23		OXA-24		MBL <sup>1</sup>	
	N	P	N	P	N	P	N	P	N	P
Cluster I	13	6	11	8	3	16	14	5	4	15
Cluster II	8	6	12	2	2	12	12	2	2	12
Cluster III	2	1	3	0	0	3	1	2	0	3
Cluster IV	1	0	1	0	0	1	1	0	0	1
Total	37		37		37		37		37	

**[Table/Fig-2]:** Distribution of pulsotypes, classes 1 and 2 integron, *bla*<sub>OXA-23</sub>-like and *bla*<sub>OXA-24</sub>-like and resistance phenotypes among *A. baumannii* isolates.

N= Negative, P= Positive

<sup>1</sup>MBL= metallo-β-lactamase



**[Table/Fig-3]:** PFGE profiles of *Apal*-digested genomic DNA for clinical *A. baumannii* isolates.

## DISCUSSION

Antibiotic resistant genes and integrons in *A. baumannii* strains have emerged as a main problem in treatment of infections caused by these bacteria in recent times [11]. Infection by *A. baumannii* causes high incidence of morbidity and mortality among hospitalized patients [12]. In the present study, similar to Mirnejad et al., study, there was a significant correlation between presence of integrons and resistance to cephalosporins, ofloxacin, norfloxacin [5]. Class 1 and 2 integron are the most common among the *Acinetobacter* isolates and other clinical Gram negative bacteria [13-15]. A total of 63.5% of *A. baumannii* isolates contained class 1 Integrons in Koczura's and his colleagues' study [16] conducted in 2014, Poland and there were no class 2 integrons in isolates while both of these integrons were present in our study. A total of 53% of *Acinetobacter* isolates contained Class 1 or 2 or both in a study of

Japoni et al., conducted at 2011 in Shiraz and their results were the same as ours [17]. The number of Class 1 integrons was more than Class 2 in a study of Moammadi F et al., which lines with our study [18]. Frequency of Class 1 Integrons (92.5%) was reported more than Class 2 and MDR isolated were the most frequent isolates in a study of Peymani et al., [11]. In our study, 44% of isolates contained Class 1 Integrons and 36% contained Class 2. In contrast with Peymani's study, MDR isolates Class 1 Integrons were less frequent (35%). In contrast to our study, 42% of *A.baumannii* isolates contained Class 1 Integrons and 82% contained Class 2 in a study [5]. More than 50% of penicillins and cephalosporins resistant isolates contained integrons in another study [15]. Ceftriaxone and cefotaxime resistant isolates had the highest frequency of Class 1 Integrons. This is perhaps surprising since, resistance to antibiotic compounds is often resulted from point mutations by chromosomal element such as Insertion Sequence (IS) element. Tetracycline resistant isolates also consists of most number of Class 2 Integrons which were same as our results [Table/Fig-1]. PFGE is known as the gold standard genotypic technique to investigate the molecular epidemiology of bacteria especially for nosocomial infection outbreaks [2]. The frequency of *bla*<sub>OXA-23</sub>-like genes was reported 93% among MDR isolates in the previous study of Mohajeri et al., same as this study [2]. Isolates analysed by PFGE had more diversity in contrast with the present study [2]. *bla*<sub>OXA-23</sub>-like gene was introduced as class D Carbapenem's coding main factor similar to our results [19] conducted in 2007. *bla*<sub>OXA-23</sub>-like gene was also dominant among isolates of United States of America same as our study. Although, there were no *bla*<sub>OXA-24</sub>-like genes found in their study [20]. In this study, MDR *A. baumannii* clinical isolates showed lots of similarity (Between, 86.8-100%) suggesting the involvement of similar subtypes of the species in ICU infection. As previously reported, the ability of PFGE assay to determine main types and clusters association with infection can be used in outbreaks by nosocomial pathogens such as *A. baumannii*. Prevalence of *bla*<sub>OXA-23</sub>-like and integrons Classes 1 and 2 among MDR strains of *A. baumannii* are high in Kermanshah as indicated in this study.

## LIMITATION

One of the major limitations of this study was sample size and inadequate demographic information and underlying disease.

## CONCLUSION

Monitoring of drug resistance with use of coexist gene integrase PCR and *bla*<sub>OXA</sub> gene with cluster analysis are very important to plan specific infection control measures due to MDR *A.baumannii* in hospital settings. By PFGE analysis, we determined two main highly similar clusters (I and II) which stated genetic correlation between them.

Responsible patterns of infection prevalence in a hospital have genetic relationship and may have the same genetic source. Designing protection programs has a great importance such as existing infection control in different parts of the hospitals especially

in the ICU. Nevertheless, further studies need to be carried out to characterize other genetic elements with integrons in these isolates and determine clonal lineage with Multilocus Sequence Typing (MLST) methods.

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## REFERENCES

- [1] Mohajeri P, Sharbati S, Farahani A, Rezaei Z. Evaluate the frequency distribution of non adhesive virulence factors in carbapenemase producing *Acinetobacter baumannii* isolated from clinical samples in Kermanshah. *J Nat Sci Biol Med.* 2016;7(1):58-61.
- [2] Mohajeri P, Farahani A, Feizabadi MM, Noroji B. Clonal evolution multidrug resistant *Acinetobacter baumannii* by pulsed-field gel electrophoresis. *Indian J Med Microbiol.* 2015;33(1):87-91.
- [3] Moradi J, Hashemi FB, Bahador A. Antibiotic resistance of *Acinetobacter baumannii* in Iran: A systemic review of the published literature. *Osong Public Health Res Perspect.* 2015;6(2):79-86.
- [4] Cambrey G, Guerout AM, Mazel D. Integrons. *Annu Rev Genet.* 2010;44:141-66.
- [5] 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):140-45.
- [6] Woodford N, Ellington MJ, Coelho JM, Turton JF, Ward ME, Brown S. Multiplex PCR for genes encoding prevalent OXA carbapenemases in *Acinetobacter* spp. *Int J Antimicrob Agents.* 2006;27:351-53.
- [7] Rafei R, Pailhories H, Hamze M, Eveillard M, Mallat H, Dabboussi F, et al. Molecular epidemiology of *Acinetobacter baumannii* in different hospitals in Tripoli, Lebanon using *bla*<sub>OXA-51</sub>-like sequence-based typing. *BMC Microbiol.* 2015;15:103.
- [8] Bartual SG, Seifert H, Hippler C, Luzon MA, Wisplinghoff H, RodriguezValera F. Development of a multilocus sequence typing scheme for characterization of clinical isolates of *Acinetobacter baumannii*. *J Clin Microbiol.* 2005;43:4382e90.
- [9] Khosravi AD, Mehrabzadeh RS, Farahani A, Jamali H. Molecular identification of clinical isolates of mycobacterium fortuitum by Random Amplified Polymorphic DNA (RAPD) polymerase chain reaction and ERIC PCR. *J Clin Diagn Res.* 2015;9(12):DC01-5.
- [10] Clinical and Laboratory Standards Institute, Performance standards for antimicrobial susceptibility testing; Twentieth informational supplement, CLSI document M100-S20. Clinical and Laboratory Standards Institute, Wayne, Pennsylvania, USA, 2014.
- [11] Peymani A, Farajnia S, Nahaei MR, Sohrabi N, Abbasi L, Ansarin K, et al. Prevalence of Class 1 integron among multidrug resistant *Acinetobacter baumannii* in Tabriz, northwest of Iran. *Pol J Microbiol.* 2012;61(1):57-60.
- [12] Mohajeri P, Farahani A, Feizabadi MM, Ketabi H, Abiri R, Najafi F. Antimicrobial susceptibility profiling and genomic diversity of *Acinetobacter baumannii* isolates: A study in western Iran. *Iran J Microbiol.* 2013;5(3):195-202.
- [13] Lavakhamseh H, Mohajeri P, Rouhi S, Shakib P, Ramazanadeh R, Rasani A, et al. Multidrug resistant *Escherichia coli* strains isolated from patients are associated with Class 1 and 2 Integrons. *Chemotherapy.* 2016;61(2):72-76.
- [14] Martinez-Freije P, Fluit AC, Schmitz FJ, Grek VS, Verhoef J, Jones ME. Class 1 integrons in Gramnegative isolates from different european hospitals and association with decreased susceptibility to multiple antibiotic compounds. *Journal of Antimicrobial Chemotherapy.* 1998;42(1):689-96.
- [15] Taherikalani M, Maleki A, Sadeghifard N, Mohammadzadeh D, Soroush S, Asadollahi P, et al. Dissemination of Class 1, 2 and 3 integrons among different multidrug resistant isolates of *Acinetobacter baumannii* in Tehran hospitals, Iran. *Pol J Microbiol.* 2011;60(2):169-74.
- [16] Koczura R, Przystlakowska B, Mokracka J, Kaznowski A. Class 1 integrons and antibiotic resistance of clinical *Acinetobacter calcoaceticus-baumannii* complex in Poznan, Poland. *Curr Microbiol.* 2014;69(3):258-62.
- [17] Japoni S, Japoni A, Farshad S, Ali AA, Jamalidoust M. Association between existence of integrons and multi-drug resistance in *Acinetobacter* isolated from patients in southern Iran. *Pol J Microbiol.* 2011;60(2):163-68.
- [18] Moammadi F, Arabestani M, Safari M, Roshanai G, Alikhani M. Prevalence of Class 1, 2 and 3 integrons among extensive drug resistance *Acinetobacter baumannii* strains isolated from intensive care units in Hamadan, west province, Iran. *Iran J Med Microbiol.* 2014;8(3):8-14.
- [19] Mendes RE, Bell JM, Turnidge JD, Castanheira M, Jones RN. Emergence and widespread dissemination of OXA-23, -24/40 and -58 carbapenemases among *Acinetobacter* spp. in Asia-Pacific nations: report from the SENTRY Surveillance Program. *J Antimicrob Chemother.* 2009;55:59-63.
- [20] Srinivasan VB, Rajamohan G, Pancholi P, Stevenson K, Tadesse D, Patchanee P. Genetic relatedness and molecular characterization of multidrug resistant *Acinetobacter baumannii* isolated in central Ohio, USA. *Ann Clin Microbiol Antimicrob.* 2009;8:8-10.

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